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Review Article

Open Access

Predictive factors of clinical assays on hydroxychloroquine for COVID-19 mortality during the first year of the pandemic: a meta-synthesis

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Abstract:

Background: The COVID-19 pandemic led to a violent debate about the efficacy of a repurposed drug hydroxychloroquine (HCQ) and a new broad-spectrum antiviral (remdesivir) and about randomized controlled trials (RCTs) and observational studies. To understand conflicting results in the literature, we performed a meta-synthesis to determine whether intrinsic qualitative criteria within studies may predict apparent efficacy or ineffectiveness of HCQ and remdesivir.

Methodology: Predictive criteria were identified through critical review of studies assessing HCQ and remdesivir for COVID-19 mortality from March to November 2020. Multiple correspondence analysis, comparative meta-analysis, and predictive value were used to explore and identify criteria associated with study outcomes.

Results: Among the 61 included studies, potential conflict of interest, detailed therapeutic protocol, toxic treatment (overdose or use in contraindicated patients), known centers and doctors, and private data computing company were the most predictive criteria of the direction of effect of the studies. All 18 observational studies evaluating HCQ and reporting detailed therapeutic protocol without conflict of interest were Pro. Potential conflict of interest was a perfect predictor for remdesivir efficacy. RCTs were associated with HCQ inefficacy and potential conflict of interest. The most predictive criteria were validated and allowed perfect classification of 10 additional studies.

Conclusion: In therapeutic trials on COVID-19, the major biases predicting the conclusions are not methodology nor data analysis, but conflict of interest and absence of medical expertise. The thorough search for declared or undeclared and direct or indirect conflict of interest, and medical expertise should be included in the quality criteria for the evaluation of future therapeutic studies in COVID-19 and beyond. A new checklist evaluating not only methodology but also conflict of interest and medical expertise is proposed.

Keywords: COVID-19; Hydroxychloroquine; Remdesivir; Meta-analysis; Conflict of interest; Clinical expertise; Methodology; Simpson's paradox effect; Checklist

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Facteurs prédictifs des tests cliniques sur l'hydroxychloroquine pour la mortalité du COVID-19 au cours de la première année de la pandémie: une méta-synthèse

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Résumé:

Contexte: La pandémie de COVID-19 a conduit à un violent débat sur l'efficacité d'un médicament réutilisé, l'hydroxychloroquine (HCQ) et un nouvel antiviral à large spectre (remdesivir) et sur les essais contrôlés randomisés (ECR) et les études observationnelles. Pour comprendre les résultats contradictoires de la littérature, nous avons effectué une méta-synthèse pour déterminer si les critères qualitatifs intrinsèques des études peuvent prédire l'efficacité ou l'inefficacité apparente de l'HCQ et du remdesivir.

Méthodologie: Des critères prédictifs ont été identifiés grâce à un examen critique des études évaluant l'HCQ et le remdesivir pour la mortalité due au COVID-19 de mars à novembre 2020. Une analyse des correspondances multiples, une méta-analyse comparative et une valeur prédictive ont été utilisées pour explorer et identifier les critères associés aux résultats de l'étude.

Résultats: Parmi les 61 études incluses, les conflits d'intérêts potentiels, le protocole thérapeutique détaillé, le traitement toxique (surdosage ou utilisation chez des patients contre-indiqués), les centres et médecins connus et la société informatique privée étaient les critères les plus prédictifs de la direction de l'effet des études. Les 18 études observationnelles évaluant l'HCQ et rapportant un protocole thérapeutique détaillé sans conflit d'intérêt étaient Pro. Un conflit d'intérêts potentiel était un prédicteur parfait de l'efficacité du remdesivir. Les essais randomisés étaient associés à l'inefficacité des HCQ et à un conflit d'intérêts potentiel. Les critères les plus prédictifs ont été validés et ont permis une classification parfaite de 10 études supplémentaires.

Conclusion: Dans les essais thérapeutiques sur COVID-19, les principaux biais prédisant les conclusions ne sont pas la méthodologie ni l'analyse des données, mais le conflit d'intérêts et l'absence d'expertise médicale. La recherche approfondie des conflits d'intérêts déclarés ou non, directs ou indirects et de l'expertise médicale doit être incluse dans les critères de qualité pour l'évaluation des futures études thérapeutiques dans le COVID-19 et au-delà. Une nouvelle liste de contrôle évaluant non seulement la méthodologie mais aussi les conflits d'intérêts et l'expertise médicale est proposée.

Mots-clés: COVID-19; Hydroxychloroquine; Remdesivir; Méta-analyse; Conflit d'intérêt; Expertise clinique; Méthodologie; L'effet paradoxe de Simpson; Liste de contrôle

Introduction:

In the COVID-19 episode, one of the greatest scientific scandals of all time occurred (1) with the rapid retractions of two major publications in most famous journals (2,3). In the meantime, a considerable debate has emerged on a broad-spectrum antiviral drug candidate recently developed by a biopharmaceutical company, which was finally considered ineffective in lowering mortality among hospitalized patients with COVID-19 (4), a few days after the European Commission purchased two billion Euros worth of this drug. The putative efficacy of this drug was mainly published in the most famous medical journals, some of whose articles were only small non-comparative series (5,6). On the other hand, more than 180 publications have been made on hydroxychloroquine (HCQ), with censorship effects such as refusal to examine the publications, including ours (7), even though it was the largest mono-centric series in the world. All publications showing a positive effect of HCQ have been published in journals that until then were not the scientific leaders in the field. All this was done in an unprecedented financial context, since the broad-spectrum antiviral drug candidate, whose futility was finally shown (4), was the subject of unprecedented speculation on a pharmaceutical product and therefore the financial stakes were colossal (1, 6).

Furthermore, conflicts of interest at all levels have been neglected; that of the government, politicians, scientific advisors, appointees (5), and that of the journals and the publishers themselves, whose funding is often common with that of the pharmaceutical industry,

and who receive advertising from the pharmaceutical industry (1,5). Conflicts of interest of authors are often neglected, without being penalized in scientific journals, despite the evidence of bias (8,9). Finally, conflicts of interest of reviewers are neglected, given that the milieu of people who conduct therapeutic trials is very commonly affected by conflicts of interest, as shown for infectious disease academics (9).

In this context, an objective analysis of published data requires the establishment of new criteria, which are independent of these pressures, in order to have certain reliability. The absence of such criteria leads to variability in meta-analyses (10,11). Finally, quantitative meta-analysis, and apparently therapeutic trial specialists, take less account of current medical practice and care, and the risk of bias related to pharmaceutical company influence, but rather focus on methodologies commonly recommended by pharmaceutical companies. For instance, randomized control trials (RCTs) are not superior to observational studies (12, 13) so that there is no transcendental methodology in therapeutic trials. Multicentric RCTs only reflect one perspective, which is not universal (12,13), and which is more in line with the needs of the pharmaceutical industry than with the reality of practice, including in episodes of acute infection epidemics.

Overall, it seemed essential to list all the evaluation criteria for scientific studies, whether comparative, randomized or not, to assess their quality not from a medical-political point of view (5), and to consider the classifications obtained, depending on whether certain criteria are retained or excluded. The basic elements of the clinical description

have led to profound errors in the interpretation of the data, such as the lack of stratification of patients according to severity, which is also an error related to people who no longer practice or have never practiced medicine, and who make a single entity of a disease that has different stages, different degrees of severity, and different potential risks of mortality.

In this context, conflicting studies on HCQ and remdesivir provide an opportunity to identify intrinsic criteria of studies associated with their qualitative results (treatment is deleterious or beneficial). Indeed, quantitative meta-analysis is not appropriate when direction of effect is not consistent among studies, as is the case for HCQ and remdesivir. Such qualitative meta-synthesis may help identify qualitative criteria not included in the current guidelines or checklists to improve future research in COVID-19 and beyond.

Material and methods:

Inclusions of studies: Search strategy

The global strategy to identify new evaluation criteria is detailed in Supplementary Data. Briefly, the keywords "hydroxy-chloroquine", "HCQ", "chloroquine", "coronavirus", "COVID-19", "SARS-CoV-2", and "remdesivir" were entered into PubMed, MedRxiv, Google Scholar and Google search engines on

studies published in English Language from March to November 11, 2020. Only the death outcome was considered, so studies without any death were not eligible. We reviewed studies evaluating the effects of chloroquine derivatives and remdesivir against SARS-CoV-2 in groups of COVID-19 patients as compared to control groups of patients who did not receive any experimental treatment.

Identification of characteristics and criteria

The criteria are summarized in Table 1 and detailed in the Supplementary Data. Some of these criteria have already been identified in previous works (14,15) and have been completed, as we observed critical pitfalls in studies assessed for the present work.

Multiple Correspondence Analysis

The Multiple Correspondence Analysis (MCA) is a statistically based visualization method that allows the user to graphically represent and analyze the associations among categorical variables (16). The basic idea behind our approach was; (i) to use MCA to construct synthetic quantitative variables that represent the studies, their characteristics, and their criteria (Table 1) on a two-dimensional plane, and (ii) to identify clusters of studies that shared the same criteria and characteristics. MCA was performed with the R software and the FactoMineR package (17).

Table 1: Twenty quality criteria proposed to assess future clinical therapeutic studies in infectious diseases

Proposed quality criteria		Conflict of interest	
1.	Potential conflict of interest	•	Work funded by a company with a conflict of interest
		•	At least one author compensated by a company with a conflict of interest (received fee) declared by this author, or not declared (identified through transparency websites and/or internet investigations)
		•	A private data computing company (see definition below)
2.	Private data computing company	•	A for-profit company collecting, aggregating, and computing data in "Big Data" studies (with frequent unclear funding)
3.	Undeclared funding and conflict of interest	•	Funding of the work not mentioned.
		•	A conflict of interest not declared by an author but found through transparency websites (dollarfordocs, eurosfordocs) or other means (through internet investigations).
		•	An indirect funding through a shell company by a company with a conflict of interest
Centers and doctors' identification			
4.	Known centers and doctors	•	Recruiting centers and investigating doctors who directly take care of patients in the clinical unit (at least one by center) are identified
Clinical expertise			
5.	Patients without confirmation of diagnosis by a microbiological test are excluded	•	A patient is considered infected only if the infection is confirmed in the laboratory (PCR, blood culture, serology). Clinical or CT-scan definition not sufficient
6.	Detailed standard of care (SoC)	•	The standard care of patients with or without experimental treatment is reported (including criteria for admission, vital monitoring, initial check-up, anticoagulants, oxygenotherapy...). This standard care is likely

		<ul style="list-style-type: none"> to influence outcome in a greater extent than the experimental treatment itself. Change over time of standard of care should be reported
7.	Detailed therapeutic protocol	<ul style="list-style-type: none"> With at least most frequent contraindications assessed, dosage, and duration
8.	Treatment not toxic	<ul style="list-style-type: none"> Dosage is usual (not overdosed) and known to be well tolerated, treatment is effectively not used in patients with contra-indications
9.	Treatment monitoring	<ul style="list-style-type: none"> Side effects are reported Critical (serious) side effects are reported (death, organ failure). If any death were related to experimental treatment, it should be mentioned Interruption of experimental treatment because of side effect
10.	Untreated group is not treated	<ul style="list-style-type: none"> Group without experimental treatment does not receive another specific treatment
11.	At least one main author is a clinical expert-in-the-field	<ul style="list-style-type: none"> At least one author directly takes care of patients and is specialized in this care (for a respiratory viral disease, this includes an infectious disease specialist, an internal medicine specialist or a pneumologist)
12.	Confounding role of previous health status (at least age) is ruled out	<ul style="list-style-type: none"> Previous health status should be assessed (at least age) and controlled for. This could be achieved using comorbidity score (Combined Charlson score). Previous health status should not be different at baseline and/or approaches should be used to control it (matching, multivariate analyses). Authors should provide evidence that this confounding has been controlled (for instance, age and comorbidities after matching are shown and not different)
13.	Confounding role of severity (at least vital parameters) is ruled out	<ul style="list-style-type: none"> Initial severity should be assessed (at least vital parameters) and controlled for. This could be achieved using severity score (NEWS score). Initial severity should not be different at baseline and/or approaches should be used to control it (matching, multivariate analyses). Authors should provide evidence that this confounding has been controlled (for instance, initial severity after matching is shown and not different)
14.	Different stages of the disease are not mixed	<ul style="list-style-type: none"> Different treatment could be associated with different effect at different stages of the disease. Results should be stratified by stage of the disease (for instance outpatient, non-severe or severe inpatient or early versus late) according to previous knowledge of the disease
Methodology		
15.	Identification of observational and interventional studies	<ul style="list-style-type: none"> Observational studies may be a case/control (dead/alive) or exposed/unexposed (treated/untreated). In this case, covariables are adjusted by matching, propensity score approaches or multivariate analysis. Interventional studies may be randomized studies, and theoretically the patient's situation is comparable
15.1.	Among observational studies, identification of electronic ("Big Data") versus clinical studies	<ul style="list-style-type: none"> Studies should be classified as 'electronic' or 'big data' studies when conducted on electronic medical records extracted by public-health specialists and epidemiologists who did not care for COVID-19 patients themselves. Conversely, studies should be classified as 'clinical studies' when the authors are physicians who cared for COVID-19 patients themselves
15.2.	Among interventional studies, identification of megatrials	<ul style="list-style-type: none"> Large-scale interventional trials including several centers (usually > 10)
16.	Identification of monocentric and multicentric studies, and center effect is evaluated in multicentric studies	<ul style="list-style-type: none"> Multicentric observational (including "Big Data" studies) and interventional (including megatrials) studies are sensitive to Simpson's paradox effect. Therefore, in multicentric studies, adjusted results should be reported for each center, using forest plot. Summary effect calculation should use random effects models since experimental conditions are inevitably different among different centers recruiting human patients. Indeed, in contrast to mouse lines in environmentally controlled cages (where fixed effect model could be used), standard of care and human populations are always genetically, environmentally, and behaviorally different between centers.

17.	Objective is objective and invariant	•	The main outcome is objective, independent of human subjectivity and context (death, viral load) and should not change during study
18.	Number of events and total sample size mentioned for each group in each center	•	This may improve verifiability
Interpretation and conclusions			
19.	Conclusions do not neglect a 25% difference in risk of death (in whole population or any subgroup)	•	An observation of a relevant change in mortality risk in the whole population or in any secondary analysis (subgroup, etc...) should be reported and discussed, regardless of significance. In this case, the authors should calculate the number of participants that would be needed to significantly confirm the effect observed in the relevant group and, if data from similar studies are available, conduct a meta-analysis to eliminate a lack of statistical power
20.	Clinical relevance should be evaluated	•	When a difference is observed regardless of significance, the number needed to treat (NNT) to prevent an event in the specified risk group should be reported. Assessment of clinical relevance may also include the years of life lost (YLL), years lived with disability (YLD) and disability adjusted life years (DALY)
21.	Unexpected findings may be reported	•	When a non-prespecified effect is observed and clinically relevant, it should be analyzed. For instance, a clinically relevant effect in a specific subgroup
Data sharing			
Additional criterion: Data should be shared within 12 months		•	Data sharing may improve verifiability

Predictive value

In a qualitative meta-synthesis approach, we evaluated the predictive value of the presence or absence of the identified criterion on the positive (Odds Ratio for mortality < 1; identified as Pro regardless of significance) or negative (OR ≥ 1; identified as Con) outcome of the included studies. The association of the presence or absence of each criterion with Pro or Con was tested using a two-sided Fisher exact test. A p-value < 0.05 was considered significant.

Meta-analysis and heterogeneity

To confirm the qualitative approach, when applicable, a comparative meta-analysis was performed with a random effects model using Comprehensive Meta-Analysis (CMA, RRID: SCR_012779) v3 (Biostat, Englewood, NJ, USA) as recommended by Borenstein et al., (18). The most adjusted effect size, reflecting the greatest control for potential confounding factors, was extracted. When propensity score matching was used, the number of matched patients was included in quantitative analysis. Heterogeneity was considered substantial when $I^2 > 50\%$. A p-value < 0.05 was considered significant. To identify which criteria were associated with a significant difference in summary effect, the Q-value and its p-value were reported, and criteria were ranked according to Q-value.

Validation of predictive criteria

The most predictive criteria were validated on an independent data set of 10 addi-

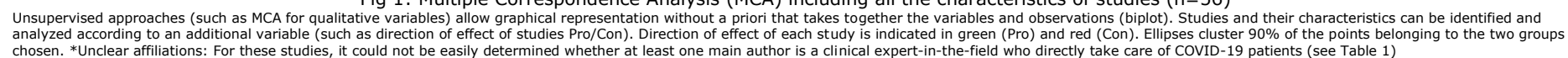
tional studies (validation set) not included in the group of studies used for criteria identification (training set). The performance of the selected criteria was assessed by comparing the direction of effects of each additional study with the direction of the effect of the majority (>50%) of studies with identical criteria in the training set.

Results:

Multiple Correspondence Analysis

Unsupervised analysis (Fig 1) of HCQ studies evidenced three clusters. First, mega-trials and RCTs were associated with most prestigious medical journals with highest impact factor, unclear affiliations of authors, absence of laboratory confirmation of diagnosis, toxic treatment (overdose or use in contraindicated patients), unexpected results not reported and conclusions neglecting a 25% decrease in the risk of mortality.

A second cluster regrouped Big Data studies that were associated with private data computing company of unknown financing (and therefore a likely existence of a conflict of interest), a potential conflict of interest, unknown centers and doctors, undeclared funding and conflict of interests, and absence of detailed therapeutic protocol and treatment monitoring. These studies were also associated with the absence of an expert in the field among the authors and a role of previous health status and severity not ruled out (confounding by indication).



Conversely, monocentric studies were associated with absence of potential conflict of interest, an author expert in the field, a detailed therapeutic protocol, a detailed treatment monitoring, and standard care reported. This cluster was associated with Andorra, China, Egypt, France, Iran, Italy, Mexico, and Spain. These studies were mainly observational (but not "Big Data" studies), with a laboratory confirmation of the diagnosis, the different stages of disease kept separate, role of severity ruled out, centers and doctors clearly reported with at least one author expert in the field. These studies were associated with 2 journals; American Journal of Tropical Medicine and Hygiene, and International Journal of Antimicrobial Agents.

Predictive value

Among the 6 studies on the broad-spectrum antiviral drug candidate recently dev

eloped by a biopharmaceutical company, both positive and negative predictive values of potential conflict of interest with remdesivir were 100%. All 5 studies with a conflict of interest declared or not declared were in favor of remdesivir, the only study without conflict of interest reported no benefit with remdesivir.

Among the 56 studies on HCQ, the following criteria were associated with a predictive value > 50% for HCQ efficacy (Table 2); detailed treatment protocol (84%), at least one of the main authors expert in the field (affiliated in infectious diseases, internal medicine or pneumology) (76%), control for severity (at least oxygen) (75%), centers and doctors who took care of patients are identified (73%), diagnosis formally confirmed (PCR or serology-based diagnosis) (69%) and control for health status (at least age) (63%).

Conversely, the following criteria were associated with a predictive value significantly

Table 2: Predictive value of each criterion for the issue of clinical assays for HCQ

Proposed criteria	Con HCQ	Pro HCQ	p-value*
	n (%)	n (%)	
Potential conflict of interest (n=15)	11 (73.3)	4 (26.7)	0.001
No potential conflict of interest (n=41)	9 (21.9)	32 (78.1)	
Detailed therapeutic protocol (n=25)	4 (16.0)	21 (84.0)	0.011
Absence of detailed therapeutic protocol (n=31)	16 (51.6)	15 (48.4)	
Toxic treatment (n=4)	4 (100.0)	0	0.013
Non-toxic treatment (n=52)	16 (30.8)	36 (69.2)	
Known centers and doctors (n=41)	11 (26.8)	30 (73.2)	0.030
Unknown centers and doctors (n=15)	9 (60.0)	6 (40.0)	
Private data computing company (n=3)	3 (100.0)	0 (0.0)	0.041
No private data computing company (n=53)	17 (32.1)	36 (67.9)	
Declared Funding COI (n=47)	14 (29.8)	33 (70.2)	0.056
Undeclared funding COI (n=9)	6 (66.7)	3 (33.3)	
Observational (n=47)	14 (29.8)	33 (70.2)	0.056
Not observational (n=9)	6 (66.7)	3 (33.3)	
Role of severity ruled out (n=32)	8 (25.0)	24 (75.0)	0.090
Role of severity not ruled out (n=24)	12 (50.0)	12 (50.0)	
Big data (n=22)**	11 (50.0)**	11 (50.0)**	0.092
No big data (n=34)	9 (26.5)	25 (73.5)	
Number of events and total mentioned for each group (n=40)	17 (42.5)	23 (57.5)	0.13
Number of events and total not mentioned for each group (n=16)	3 (18.8)	13 (81.3)	
Standard care reported (n=9)	1 (11.1)	8 (88.9)	0.136
Standard care not reported (n=47)	19 (40.4)	28 (59.6)	
Treatment monitoring (n=19)	4 (21.1)	15 (78.9)	0.143
Absence of treatment monitoring (n=37)	16 (43.2)	21 (56.8)	
Lab confirmed diagnosis (n=42)	13 (30.9)	29 (69.1)	0.198
No lab confirmed diagnosis (n=14)	7 (50.0)	7 (50.0)	
Monocentric (n=18)	4 (22.2)	14 (77.8)	0.2326
Multicentric (n=38)	16 (42.1)	22 (57.9)	
One author expert in the field (n=29)	7 (24.1)	22 (75.9)	0.345
No author expert in the field (n=20)	8 (40.0)	12 (60.0)	
Different stages mixed (n=21)	9 (42.9)	12 (57.1)	0.405
Different stages not mixed (n=35)	11 (31.4)	24 (68.6)	
Unexpected results reported (n=48)	16 (33.3)	32 (66.7)	0.437
Unexpected results not reported (n=8)	4 (50.0)	4 (50.0)	
Conclusions neglect a 25% decrease in mortality (n=12)	3 (25.0)	9 (75.0)	0.506
Conclusions do not neglect a 25% decrease in mortality (n=44)	17 (38.6)	27 (61.4)	
Megatrial (n=6)**	3 (50.0)**	3 (50.0)**	0.6553
Not a megatrial (n=50)	17 (34.0)	33 (66.0)	
Role of previous health status ruled out (n=45)	17 (37.8)	28 (62.2)	0.728
Role of previous health status not ruled out (n=11)	3 (27.3)	8 (72.7)	
Untreated group with specific treatment (n=2)	1 (50.0)	1 (50.0)	1.000
Untreated group without specific treatment (n=54)	19 (35.2)	35 (64.8)	
Death as a clear outcome (n=47)	17 (36.2)	30 (63.8)	1.000
Death not a clear outcome (n=9)	3 (33.3)	6 (66.7)	

*: Two-sided p-value (Fisher's exact test). n = 56 studies. **Note that for Big Data and megatrials, 50% of studies are Pro and 50% are Con, respectively. This illustrates the Simpson's paradox: when mixing different centers with different standards of care, the prevalence of the outcome (death) in the overall population (treated and untreated) may be very different between centers. If the proportion of treated and untreated patients is different between centers, the observed effect corresponds to a center effect (difference in population and standard of care) and the real effect of the intervention remains unobserved. Future multicentric studies should report effect by center using forest plot.

Table 3. Observational studies with a detailed therapeutic protocol without potential conflict of interest

Study name	Inpatients/ Outpatients/ Both	Country	Pro/Con HCQ
Alberici, Kidney International, 2020	Both	Italy	Pro
Arshad, Int J Infect Dis, 2020	Inpatients	USA	Pro
Ashraf, MedRxiv, 2020	Inpatients	Iran	Pro
Ayerbe, Intern Med Emerg, 2020	Inpatients	Spain	Pro
Catteau, Int J Antimicrob Agents, 2020	Inpatients	Belgique	Pro
Davido, Int J Antimicrob Agents, 2020	Inpatients	France	Pro
Derwand, Int J Antimicrob Agents, 2020	Outpatients	USA	Pro
Di Castelnuovo, Eur J Intern Med, 2020	Inpatients	Italy	Pro
Guerin, Asian J Med Health, 2020	Outpatients	France	Pro
Lagier, Trav Med Infect Dis, 2020	Both	France	Pro
Lauriola, Clinical Transl Sci, 2020	Inpatients	Italy	Pro
Lecronier, Critical Care, 2020	ICU	France	Pro
Membrillo de Novales, Preprints, 2020	Inpatients	Spain	Pro
Mikami, J Gen Intern Med, 2020	Inpatients	USA	Pro
Nachega, Am J Trop Med Hyg, 2020	Inpatients	Congo	Pro
Paccoud, Clin Infect Dis, 2020	Inpatients	France	Pro
Sulaiman, MedRxiv, 2020	Outpatients	Saudi Arabia	Pro
Yu, Sci Chi Life Sci, 2020	ICU	China	Pro

All these 18 studies were in favor of a HCQ efficacy (100% predictive value). ICU: Intensive care unit

greater than 50% for HCQ inefficacy; private data computing company (100%), toxic treatment (100%), potential conflict of interest with remdesivir (73%), and undeclared funding or conflict of interest (66%). The difference of predictive value according to each criterion was significant for potential conflict of interest ($p=0.001$), lack of detailed therapeutic protocol ($p=0.011$), toxic treatment ($p=0.013$), unknown centers and doctors not known ($p=0.03$), and private data computing company ($p=0.041$).

The 18 observational studies with a detailed therapeutic protocol and without a potential conflict of interest had a 100% predictive value for HCQ efficacy (Table 3). Considering the odds ratio, apart from toxic treatment and private data computing companies (perfect predictors), conflict of interest was the strongest predictor of HCQ inefficiency (OR=9.8, 95% CI=2.50-38.2, Fig 2).

Comparative meta-analysis

Among these 18 studies, 17 provided quantitative results available for meta-analysis with a significant beneficial effect ($n=17$, OR=0.60, 95% CI=0.52 - 0.70, $p=6.7 \times 10^{-12}$). This was not related to an isolated aberrant study as shown by one-study-removed meta-analysis (Supplementary Fig 1). Combination of HCQ with azithromycin (AZ) was associated with a significant beneficial effect compared to HCQ monotherapy ($n=5$ comparisons with HCQ-AZ in all patients, 0.36, 0.21-0.63/ $n=9$ with HCQ but not AZ in any patient, 0.68, 0.56-0.82/ Q -value = 4.41, $p=0.036$).

Comparative meta-analysis with ranking by Q -value confirmed that potential conflict of interest, including private data computing company, was the criterion associated with the greatest and most significant difference in summary effect (Supplementary Table 1). Effect of HCQ on mortality was beneficial ($n=43$, 0.75, 0.66-0.84, $p=6.3 \times 10^{-7}$) or

deleterious ($n=19$, 1.15, 1.07-1.23, $p=1.1 \times 10^{-4}$) when an absence or a presence of a potential conflict of interest was found, respectively (Fig 3).

Neglecting a non-significant but relevant decrease in mortality

We found 6 studies observing a decrease in the risk of mortality greater than 25% but this finding was not analyzed nor mentioned because; (i) it was not significant (underpowered studies) or (ii) thought not to be relevant to the outcome of the study (Supplementary Data). We have already commented on these two frequent issues (19). Strikingly, the day-28 mortality was halved in a French RCT (20) suspended and closed after the publication of Mehra et al., (2). The difference was clinically relevant since the number of patients needed to prevent 1 death was 25 (mortality at day 28 of 4.8% in HCQ and 8.9% in untreated). If the planned enrollment had been included (1300 patients), if the observed tendency were confirmed, the difference would have been significant [(31/650 (4.8%) versus 58/650 (8.9%), OR 0.55, two-sided Mid- p exact test $p=0.003$)].

Validation of predictive criteria

Finally, we validated the identified criteria by analysing 10 additional studies not included in the first analysis. All 10 studies were correctly classified as favorable or unfavorable based on the most significant criteria (Supplementary Table 3). All the 3 Con studies were associated with potential conflict of interest and were from the same country (USA), while the other 7 Pro studies were from Brazil, China, France, Mexico and Spain. This was consistent with MCA (Fig 1). In agreement with predictive value analysis and comparative meta-analysis (Figs 2 & 3), potential conflict of interest was a perfect predictor in this validation data set.

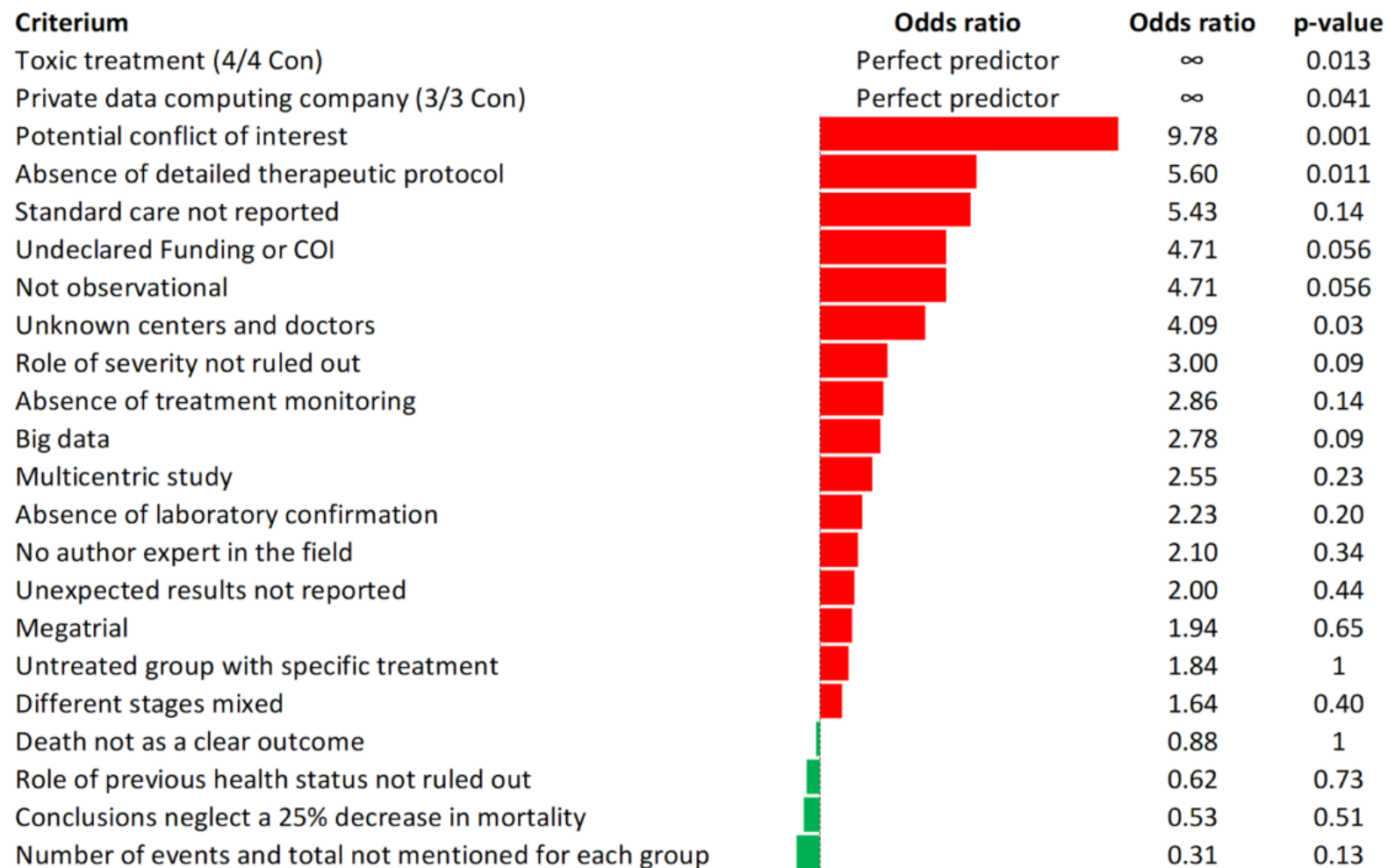


Fig 2: Predictive factors of clinical assays during COVID-19

Con: Odds Ratio ≥ 1 for mortality. These odds ratios were calculated based on data provided in Table 2. Odds ratio could not be calculated when a criterium was a prefect predictor of HCQ inefficacy (division by zero)

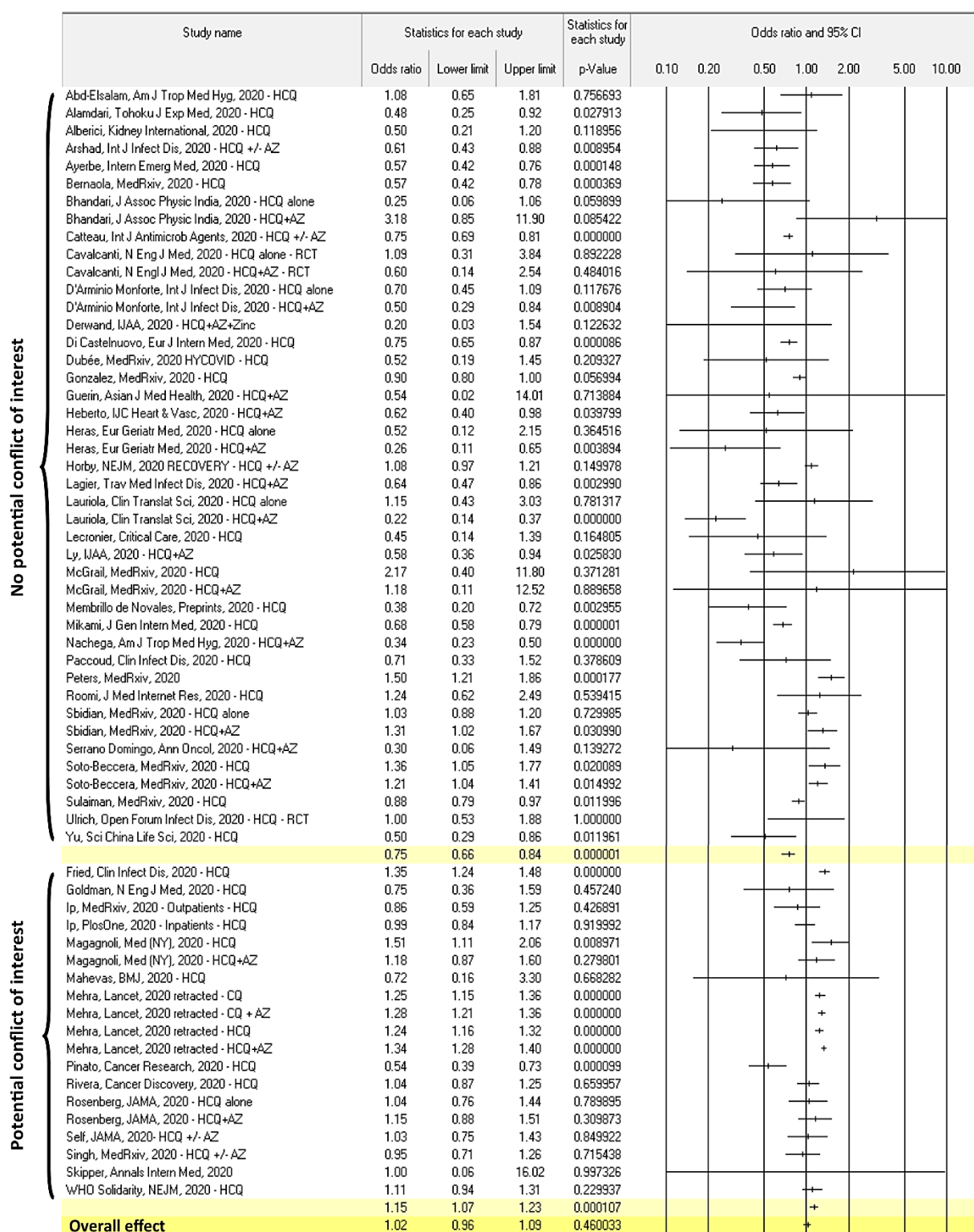


Fig 3: HCQ meta-analysis according to potential conflict of interest
95% CI: 95% confidence interval. Random effects model

Discussion:

There is a conflict in the evaluation of therapeutics for infectious diseases between several public health specialists and methodologists who recommend multicentric randomized controlled trials (RCTs), which are mainly used by the pharmaceutical industry, and

observational studies performed by medical doctors. More recently, a third source of comparative analysis has been the analysis of large data (Big Data) collected automatically in health care centers. Interestingly in infectious diseases currently, 83% of IDSA recommendations are not based on RCTs (21), although considered the "gold standard". More-

over, RCTs require significant funding, and the pharmaceutical industry's willingness to demonstrate efficacy or non-inferiority is under pressure of conflict of interest because those who pay and analyze have a well-known and long-evaluated chance of having biased results in favor of the products they finance (8). In addition, the company's provision of the compound to be tested is usually subject to possible censorship, as approval of the submitted work is required (22). That may lead to dissimulate negative results (22).

The methodology of analysis used here is to our knowledge unique. Pharmaceutical industry is a major actor directly or indirectly influencing authors with conflicts of interest, declared or not. Potential conflict of interest has a predictive value of 74% against HCQ in the training set (whereas 78% of the studies with no link to this company was in favor of HCQ) and 100% in the validation set. This work also made it possible to identify the target journals of the studies in which the remdesivir producer or its partners played an important role.

Concerning Big Data, this is a new problem. In some Big Data studies, data acquisition is directly financed by the pharmaceutical industry with a conflict of interest against HCQ (23,24). In another Big Data study reporting a beneficial effect of remdesivir and a deleterious effect of HCQ, a direct conflict of interest was declared by several authors (25). Companies such as Surgisphere, two papers of which had to be retracted (2,3), have unknown funding, something that should have been required from the publisher. One may question if data companies (2,24,26) have received funding since these "Big Data" studies also clearly have a predictive value in favor of remdesivir (24) and to the disadvantage of HCQ (2,24,26). This suggests that potential conflict of interest must be sought well beyond the mere declaration of conflict of interest by authors or direct funding of studies. Conversely, individual monocentric studies focusing on HCQ have multiplied and are associated with the success of HCQ.

These three elements (potential conflict of interest, private data computing company, and multi- or mono-centric studies) can predict the outcome of the meta-analysis based on the choices that will be made to retain certain studies. Number of studies were conducted ignoring the very basis of inclusions at the medical level i. e. clinical signs found in this disease (not yet reported in acute respiratory infection in general) such as anosmia and ageusia, and pulmonary embolisms, are not in the clinical diagnostic criteria. On the other hand, some studies have been published without even confirmatory biological tests (27, 28), which for infectious diseases is a regression that has no equivalent. Finally, in most

cases the evaluation of treatments in the different stages of the disease should correspond to different therapeutic options, and this is often not evaluated.

All in all, this crisis highlighted very different therapeutic evaluation strategies. The considerable weight of the pharmaceutical industry on the results of therapeutic trials is clear (1,5,8,29,30). Meta-analyses allow small studies to be analyzed and multicenter studies should report results by center so that investigators can ensure validity, and to avoid the Simpson effect (31). A French megatrial (32), recruiting patients in 32 French sites, and an international megatrial recruiting patients in 405 hospitals in 30 countries (4) did not stratify by region of inclusion. Since the number of patients included per center (very low number of inclusions in some regions (32) and the effect for each center was not reported and may have been highly variable, both these megatrials are likely to be biased by the Simpson's effect.

Most of the criteria identified in this work (checklist detailed in Table 1) are new, are not part of the usual quality checklists (STROBE, CONSORT or PRISMA – see Supplementary Table 2) and may be useful for future critical review. This comeback to independent clinical and microbiological expertise is the best lesson to be learned from the global scandal we have witnessed, for the greatest benefit of patients.

Author contributions:

MM wrote the first draft of the manuscript (MS), revised the different versions of the MS, performed statistical analysis and analyzed all data, PD analyzed data, performed systematic research on the field and revised different versions of the MS, EC performed systematic research on the field, analyzed data and revised different versions of the MS, SC analyzed data, performed statistical analysis and revised different versions of the MS, YR performed systematic research on the field, analyze data, performed statistical analysis and revised the different versions of the MS, PB performed systematic research on the field, analyze data, performed statistical analysis and revised the different versions of the MS, and DR designed the work and revised the different versions of the MS.

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Conflicts of interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Our group used widely available generic drugs distributed by many pharmaceutical companies.

Supplemental Information

Supplemental information can be accessed online at: www.afrcem.org/supplementarymaterials

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Copyright AJCEM 2022: <https://dx.doi.org/10.4314/ajcem.v23i1.2>**Review Article****Open Access****Invasive fungal infections and COVID-19: a review**^{1,2,3}Fayemiwo, S. A., and ^{*4}Adegboro, B.¹Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Nigeria²Medical Mycology Unit, Infectious Diseases Institute, College of Medicine, University of Ibadan, Nigeria³Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health,
School of Biological Sciences, University of Manchester, Manchester, United Kingdom⁴Department of Medical Microbiology and Immunology, Nile University of Nigeria, Abuja, Nigeria*Correspondence to: boazadegboro@gmail.com and boaz.adegboro@nileuniversity.edu.ng**Abstract:**

Invasive fungal diseases (IFDs) are major causes of morbidity and mortality among hospitalized patients all over the world with a global prevalence of 15%. Since the first case of COVID-19 was reported on February 27, 2020, in Nigeria, it had been discovered across all geopolitical zones in Nigeria. As the medical community confronts the ongoing COVID-19 pandemic, determining whether patients infected with SARS-CoV-2 develop fungal complications, especially invasive aspergillosis, is crucial. This review aimed to highlight the fungal co-infections that might be associated with SARS-CoV-2 infection, and modalities for their diagnosis, prevention, and management, with the view to reducing the high mortality associated with these infections.

Keywords: Fungal infections; opportunistic; candidiasis; mucormycosis; aspergillosis

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Infections fongiques invasives et COVID-19: une revue^{1,2,3}Fayemiwo, S. A., et ^{*4}Adegboro, B.¹Département de microbiologie médicale et de parasitologie, Hôpital universitaire, Ibadan, Nigéria,²Unité de mycologie médicale, Institut des maladies infectieuses, Faculté de médecine, Université d'Ibadan, Nigéria³Division de l'infection, de l'immunité et de la médecine respiratoire, Faculté de biologie, médecine et
santé, École des sciences biologiques, Université de Manchester, Manchester, Royaume-Uni⁴Département de microbiologie médicale et d'immunologie, Université du Nil du Nigéria, Abuja, Nigéria*Correspondance à: boazadegboro@gmail.com et boaz.adegboro@nileuniversity.edu.ng**Résumé:**

Les maladies fongiques invasives (MFI) sont des causes majeures de morbidité et de mortalité chez les patients hospitalisés partout dans le monde avec une prévalence mondiale de 15 %. Depuis que le premier cas de COVID-19 a été signalé le 27 février 2020 au Nigeria, il avait été découvert dans toutes les zones géopolitiques du Nigeria. Alors que la communauté médicale est confrontée à la pandémie de COVID-19 en cours, il est crucial de déterminer si les patients infectés par le SRAS-CoV-2 développent des complications fongiques, en particulier l'aspergillose invasive. Cette revue visait à mettre en évidence les co-infections fongiques qui pourraient être associées à l'infection par le SRAS-CoV-2, et les modalités de leur diagnostic, prévention et gestion, en vue de réduire la mortalité élevée associée à ces infections.

Mots-clés: Infections fongiques; opportuniste; candidose; mucormycose; aspergilloses**Introduction:**

Invasive fungal diseases (IFDs) are major causes of morbidity and mortality among hospitalized patients all over the world, with a

global prevalence of 15% (1). An outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread globally to affect countries in all the continents of the world.

The first case of COVID-19 in Nigeria was reported on February 27, 2020 in Lagos after an Italian who came into the country from Italy two days earlier presented with signs and symptoms remarkable for the disease. Most patients infected with SARS-CoV-2 suffer a self-limited viral infection or no symptoms, but a significant minority have severe acute respiratory distress (ARDS) with other symptoms that necessitate hospitalization (2-5), and patients who recover from severe COVID-19 are also likely to face long-term sequelae.

One of the complications observed in COVID-19 cases is secondary infection. The immune system of the body becomes depleted when the burden of SARS-CoV-2 increases, leading to increase in the risk of fungal infection. Most immunocompetent patients who develop severe forms of COVID-19 have more than one underlying conditions such as chronic obstructive pulmonary disease, hypertension, diabetes or chronic kidney disease (6), but none of these predisposing factors generally are associated with increased risk for fungal infections. Some researchers have raised concerns on the occurrence of invasive fungal diseases among the hospitalized patients with COVID-19 and expressed opinion that fungal co-infections associated with COVID-19 globally might be missed or misdiagnosed (7,8).

Several risk factors including ICU admission, corticosteroid therapy, intubation/mechanical ventilation, underlying respiratory disease, and cytokine storm, have been attributed with invasive fungal infections (IFIs) and high mortality among COVID-19 patients in the clinical setting (8). The most common fungi causing IFIs are the yeasts, *Candida* spp and *Cryptococcus* spp, with *Candida* spp being the most frequently encountered. The commonly isolated moulds are *Aspergillus* spp and *Fusarium* spp. Fungi pathogens especially *Aspergillus flavus*, *Candida glabrata* and *C. albicans* were recovered from respiratory sample cultures in one study (2), however, the role of *Candida* as a respiratory pathogen is doubtful even among critically ill patients and could be regarded as a colonizer. In a study, Yang et al., (9) found *A. flavus* and *A. fumigatus* from two of the seven patients with hospital-acquired pneumonia identified among 52 critically ill patients admitted to ICU in Wuhan, China. In another retrospective study conducted in Wuhan in two different hospitals concerning 85 deadly COVID-19 cases, 9 patients were reported to have positive fungal culture from sputum in 33.3% of cases, with 8 (9.4%), 3 (3.5%) and 2 (2.4%) patients receiving voriconazole, fluconazole and caspafungin (10) respectively. Interestingly, Antinori and coworkers (11), reported

a high rate of candidaemia (6.9%) among 43 patients treated with tocilizumab, a recombinant humanized anti-human IL-6 receptor monoclonal antibody that has been suggested to be active against the cytokine storm described in patients with severe COVID-19.

Aspergillus co-infection has been increasingly reported in severe influenza pneumonia resulting in acute respiratory distress syndrome (ARDS) (12). Influenza virus causes alveolar epithelial and endothelial damage together with impaired mucociliary activity, while pathologic findings in COVID-19 pneumonia include pulmonary oedema, hyaline membrane formation, multinucleated syncytial cells with atypical enlarged type II pneumocytes (13). The diagnosis of invasive pulmonary aspergillosis (IPA) in ICU patients is considered difficult for several reasons and even if available algorithms are applied, they show variable and generally low performance with sensitivities ranging from 23 to 85% and specificities from 70 to 80% (14, 15). The diagnosis of IPA was made with a median of 5 days post-ICU admission and with an overall mortality of 67% in all patients hospitalized in the ICU. *Aspergillus fumigatus* was cultured in most cases from tracheal aspirate of bronchoalveolar lavage, and galactomannan antigen performed on serum was positive only in 23.1% of cases. Secondary or hospital-acquired infections have been reported in 5.1% to 38.9% among Chinese patients and in 4.8% to 27.4% of patients in the Western countries, but all the data appeared biased by the limited follow-up, especially for those patients hospitalized. Invasive aspergillosis is a well-described complication of severe influenza pneumonia (14, 15), but many intensivists seem to overlook this superinfection (6).

As the medical community confronts the ongoing COVID-19 pandemic, determining whether patients infected with SARS-CoV-2 develop fungal complications, especially invasive aspergillosis, is crucial. To provide effective treatment, COVID-19 patients with *Candida*, *Aspergillus* and other opportunistic fungal infections would require a comprehensive diagnostic work-up for early detection (7). This review is aimed at highlighting fungal co-infections that might be associated with COVID-19, and modalities for their diagnosis, prevention, and management, with the view to reducing the high mortality associated with these infections.

Methodology:

Online database searches for relevant publications were conducted on PubMed and Google Scholar. Six search strategies were used

to retrieve the publications with each of the fungal diseases associated with COVID-19 searched separately with the terms 'candidiasis', 'aspergillosis', 'mucormycosis', 'histoplasmosis', 'coccidioidomycosis', 'pneumocystis', and COVID-19. Two hundred and ten, 2430, 655, 685, 430, and 2300 articles were respectively retrieved for candidiasis, aspergillosis, mucormycosis, histoplasmosis, coccidioidomycosis, pneumocystis, and COVID-19 on Google scholar, and 159, 62, 60, 50, 8 and 4 articles on PubMed from 2020 to date. In all, 6961 articles (6712 from Google Scholar and 249 from PubMed) were retrieved.

Upon applying filter, 113 publications (82 from Google Scholar and 31 from PubMed) were assessed for relevance. The content of the relevant articles including the title, abstracts, and full texts were carefully reviewed by the authors. After eliminating duplicate articles (n=58), a total of 55 articles were deemed eligible. References of the eligible articles were scrutinized to find additional articles that might not have been included in the results of these search strategies. Five additional articles fell into this category, giving a total of 60 articles for the review as shown in Fig 1. The University Ethics Committee approval was not applicable in this review article.

Results and Discussion:

Clinical and laboratory data of fungal infections in COVID-19 patients

Candidiasis and COVID-19

Candida species especially *Candida albicans* are normal flora of human beings, and the main cause of invasive fungal infections, with a very high mortality rate. Candidemia and candidosis are increasingly being recognized as complications of severe COVID-19 (16), and the risk of invasive and mucocutaneous candidiasis in patients with severe COVID-19 cannot be over-emphasized. Some of these patients might have been treated with broad-spectrum anti-bacterial drugs, undergone parenteral nutrition and invasive examinations, or accompanied with prolonged neutropenia and other immune impairment factors (7).

In the face of the distinct immune hyperexcitability in COVID-19, outstanding defects in immune cells required for host immunity to *Candida* have not been reported (16). In one of the series of case studies reported early in the epidemic, it was observed that there was an increased risk for critically ill COVID-19 patients to develop *Candida* co-infection that increased the mortality rates. In another study,

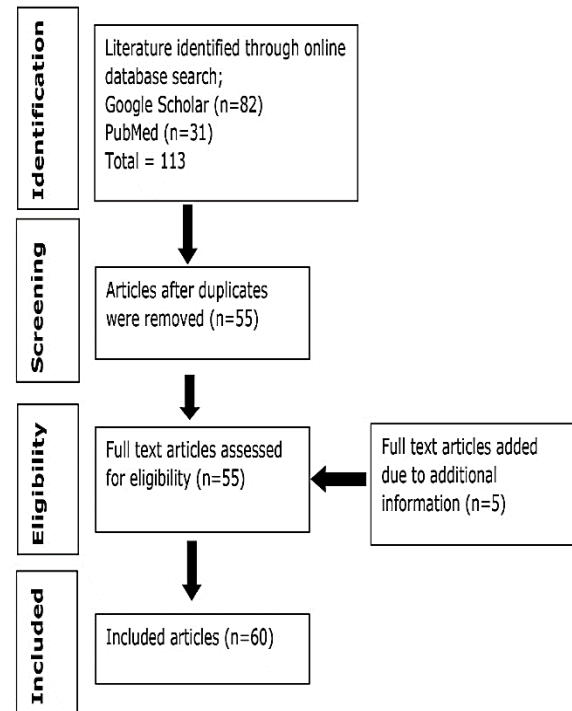


Fig 1: Process of selection of publications (PRISMA guide) for the review

several putative cases of oropharyngeal candidiasis among hospitalised patients in intensive care units (ICU) were discovered (17). Oral manifestations especially candidiasis has been observed in many patients with coronavirus disease 2019 (COVID-19), however, it is not too clear if the manifestation is due primarily to COVID or secondary to concurrent systemic diseases (18). In Spain, four cases of candidaemia were reported among 88 co-infections and super-infections, while in a related study in Italy, 21 cases of candidaemia in patients with COVID-19 were reported, with a higher incidence of candidaemia in COVID-19 patients compared with cohort history in the hospital. (19,20) Some of these patients experienced lymphocytopenia, had cytokine storms, used corticosteroids and were intubated (8). Increased age was also reported to be significantly associated with fungal infections in COVID-19 patients (21).

Candidaemia can easily be diagnosed with available culture and non-culture diagnostic methods. These include mannan and anti-mannan IgG tests, *C. albicans* germ tube antibody (CAGTA), 1,3-β-D Glucan (BDG), MALDI-TOF and PCR-based assays (17,22,23) (Fig 2). *Candida albicans* is still the commonest *Candida* species followed by *C. glabrata*, *C. parapsilopsis*, *C. tropicalis*, and *C. krusei* (7). Patients with

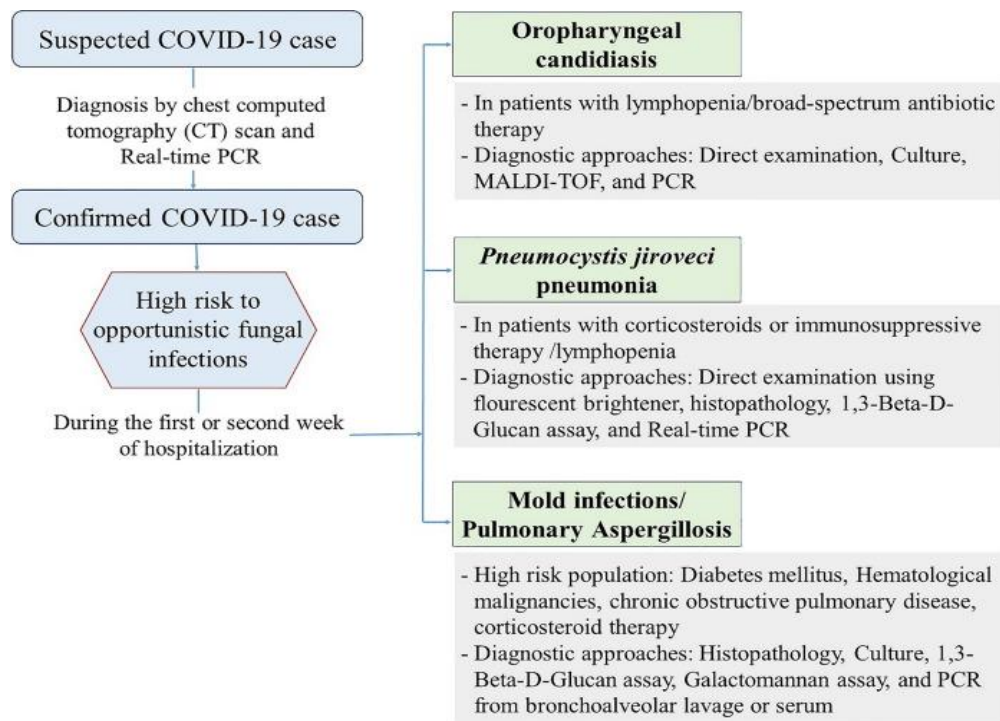


Fig 2: Clinical and diagnosis data of opportunistic fungal infections among COVID-19 patients
Adapted from Salehi et al., (17)

confirmed candidaemia can be treated with echinocandins (caspafungin, micafungin and anidulafungin), azoles (fluconazole, voriconazole, itraconazole), and amphotericin B and its liposomes. To improve the outcome of COVID-19 patients on admission in the hospital, early recognition of *Candida* infection and appropriate therapy should be instituted (24). There should be increased awareness as well as the routine screening of COVID-19 patients to reduce the diagnostic challenges of candidaemia (16).

Aspergillosis and COVID-19

In those with high risk factors, invasive aspergillosis could be an important cause of life-threatening infection among COVID-19 patients. The potential risk factors for the patients include glucocorticoid use, chronic obstructive pulmonary disease (COPD), prolonged neutropaenia, allogeneic haematopoietic stem cell transplant (allo-HSCT), solid organ transplant (SOT), inherited immunodeficiencies, haemopoietic malignancy (HM), cystic fibrosis (CF) and others (25, 26). At the initial stage of the pandemic fewer than 5% of patients with COVID-19 are treated in intensive care units (ICUs) due to respiratory failure. Recently, it has been observed that rates of invasive aspergillosis are high among patients with severe influenza admitted to the ICU (14, 27). Influenza pneumonia in critically ill patients can be complicated by invasive pulmonary aspergillosis (IPA) due to the viral destruction of

bronchial mucosa, facilitating invasion of *Aspergillus* species, and that compromise the host defenses to *Aspergillus* (28,29). Influenza-associated aspergillosis can be diagnosed within an average of three days after ICU admission, with its associated high mortality (14). In a case-series of patients studied in New York, USA, an estimated 14-30% of hospitalized patients diagnosed with COVID-19 developed severe respiratory failure requiring intensive care (30). In another study conducted in Brazil, 5.4% of the patients with IFI were positive for different *Aspergillus* species such as *A. fumigatus*, *A. flavus*, *A. penicilliioides* and *A. niger* (31).

The diagnosis of invasive aspergillosis (IA) in COVID-19 patients could be challenging because of lack of suitable specimen acquisition and consensus suitable methods. However, it requires a combination of microbiologic, histopathologic, and radiological evidence. Some of the invasive procedures such as lung biopsy or bronchoalveolar lavage (BAL) might be contraindicated in patients with severe respiratory failure and coagulation disorders (7,32). Most of these tests make use of bronchoalveolar lavage (BAL) and serum for the confirmation of acute invasive aspergillosis. The galactomannan index (GMI) and PCR-based assays can be used for the detection of *Aspergillus* in BAL, serum, and occasionally in sputum of COVID-19 patients (33,34). Definitive confirmation can be by ident-

ification of the characteristic acute angle branching septate hyphae of *Aspergillus* spp or histologic findings of the Grocott-Gomori's methenamine-silver stain (GMS) and Periodic Acid Schiff (PAS) stains of fixed tissues (7). However, due to the diagnostic challenges and lack of specificity in the classification of probable, putative, or possible COVID-19 associated pulmonary aspergillosis (CAPA), aspergillosis might be over diagnosed (35,36). COVID-19 patients with confirmed aspergillosis of any form can be treated with azoles (voriconazole, itraconazole, posaconazole and esaconazole), amphotericin B and its liposomes derivatives, and echinocandins such as micafungin and caspofungin (37,38).

Mucormycosis and COVID-19

Mucormycosis, an opportunistic infection caused by *Mucorales* has been responsible for increasing cases of devastating rhino-orbito-cerebral infection in susceptible COVID-19 patients (39). The predominant form of mucormycosis observed in COVID-19 patients was rhino-orbital, followed by pulmonary, gastrointestinal and rhino-cerebral (40). COVID-19 patients who also have premorbid conditions such as diabetes mellitus, hematological malignancies, prolonged neutropenia, and increased use of glucocorticoid are more likely to develop mucormycosis. COVID-19 induces microvascular thrombosis and endothelitis in the vascular beds. which may result in the infarction of the infected tissues that may compound the angio-invasive impact of mucormycosis (39,41). In a cross-sectional study conducted in Iran on biopsy-proven mucormycosis patients with confirmed COVID-19, the mean interval time between COVID-19 and development of mucormycosis was 7 days in patients with favourable risk factors compared with mean interval time of 8 and 11 days between diagnosis of COVID-19 and clinical presentations of oropharyngeal candidiasis and pulmonary aspergillosis respectively (42,43).

In another systematic review of cases reported worldwide and in India, Awadhesh Kumar Singh et al., (44) reported that cases of rhino-orbital mucormycosis were being encountered in more and more numbers amidst people with COVID 19, particularly in India. They reported on 101 cases, with 82 from India, and 19 from other parts of the world, and an overall mortality of 30.7%. The most frequent presenting features were involvement of nose and sinuses (88.9%) and rhino-orbital infection (56.7%). The co-morbidities were diabetes mellitus (80%), corticosteroid intake (76.3%), and diabetic ketoacidosis (14.9%). They concluded that COVID-19 in association with pre-

existing diabetes mellitus and administration of corticosteroids combined to worsen the prognosis of COVID-19 patients and increased the probability of acquiring mucormycosis.

The diagnosis of mucormycosis can be suspected with the observation of non-septate or pauci-septate *Mucorales* hyphae on direct microscopy or florescent brighteners. This can be confirmed with the appearance of non-septate hyphae invading tissues after staining with GMS and HE stains (45). Definitive identification of the organisms can be done to species level by culture on supportive media giving characteristics whitish cottony colonies that changes to grayish black colonies with time. Other form of diagnosis include PCR and sequencing as well as Magnetic Resonance Imaging (MRI) (40). Management of COVID-19 patients with confirmed mucormycosis include complete surgical treatment and early administration of systemic antifungal therapy. The antifungal treatment includes amphotericin B lipid complex, liposomal amphotericin B and posaconazole as first line drugs in combination with isavuconazole. Posaconazole can be given as prophylaxis to those with high-risk factor and in graft versus-host disease (46).

Histoplasmosis and COVID-19

Histoplasmosis is caused by *Histoplasma capsulatum*, a dimorphic thermal fungus that mainly affects people who are immunocompromised such as HIV patients (47). It can present in the form of flu-like symptoms, or as different manifestations of disseminated histoplasmosis (DH). There is a paucity of information on the co-infection of COVID-19 with histoplasmosis. Predisposing factors to histoplasmosis co-infection with COVID-19 include advanced HIV infection with CD4⁺ count of <200 cells/mm³ and resident in endemic areas (48,49). Fernando et al., (49) reported a case of disseminated histoplasmosis in a 36 year old female in Argentina who developed COVID-19 during hospital care. The patient had advanced HIV infection as the predisposing factor to the development of histoplasmosis in the first place. She presented with papule-like lesions in the right malar region and in the proximal area of the nose and erythematous lesion with slight central ulceration in the central palate. Chest computed tomography (CT) also revealed a bilateral and diffuse micronodular interstitial pattern, compatible with miliary histoplasmosis. After being treated for histoplasmosis, the patient's initial fever subsided, but later developed a second bout of fever, with laboratory investigations helping the diagnosis of COVID 19 (49).

Recently, Cafardi et al., (50) reported

two cases of opportunistic fungal infections in patients with COVID-19. One of them was infected by *H. capsulatum*, whose diagnosis was made by complement fixation test for yeast phase and mycelial phase histoplasma antigens. The underlying factors were a history of hypertension and chronic obstructive pulmonary disease. The diagnosis in these cases were made possible through blood culture, serology (antigen detection in urine and serum) and histological staining methods like Giemsa, Wright, and Gomori-Grocott (GMS). Management of COVID-19 patients with histoplasmosis include administration of antifungal drugs such as amphotericin B deoxycholate and itraconazole (51).

Coccidioidomycosis and COVID-19

Coccidioidomycosis is a respiratory infection caused by inhalation of fungal spores of *Coccidioides* in dust (52). It is a soil-dwelling dimorphic fungus, which spores are found in hot and arid environments. A meta-analysis showed that coccidioidomycosis outbreaks frequently occur among workers in construction and agricultural sectors who were regularly exposed to dust (52). They also carried a higher risk factor for illness and death from viral respiratory infections including COVID-19 (52-55). The wide range of symptoms of coccidioidomycosis such as cough, fatigue, and breathlessness are also common among patients with COVID-19. These may cause diagnostic challenges especially in endemic areas. In two cases reported, a man and a woman, the main predisposing factor discovered was being residents in endemic areas (40). Other risk factors that are associated with co-infections of coccidioidomycosis and COVID-19 include older age, immunosuppression, DM and smoking (52). Early management of cases and assessment of the risk factors for severity as well as regular follow-up visits to monitor symptoms can be crucial to mitigating severe diseases. Prompt initiation of antifungal treatments reduces the chances of unnecessary use of antimicrobial drugs and resolves symptoms more effectively (52).

Pneumocystis and COVID-19

In COVID-19 patients who are critically ill with high level of lactate dehydrogenase (LDH) especially in those undergoing immunosuppressive or corticosteroid therapies, *Pneumocystis jirovecii* pneumonia (PJP) was suspected based on radiological signs (17). However, the researcher's observations were not supported by mycological, immunological, and molecular assays. Remarkably, lymphopenia was the main laboratory finding in 85% of critically ill patients with COVID-19 (17). Since lymphocytes play a decisive role in maintaining immune homeo-

stasis and defensive response against microbial invasion throughout the body, it might be hypothesized that inadequate lymphocyte count may be a key factor contributing to secondary fungal infections such as PJP in COVID-19 patients (17).

Pathophysiological factors of invasive fungal infections in COVID-19

The following may contribute to super-infection by mycoses in patients with COVID 19; (i) during the process of respiratory infection by SARS CoV-2, there is severe damage to the lung parenchyma resulting in microthrombi formation in the vasculature, and hyaline membrane formation and infiltration of the interstitium by lymphocytes (56,57); (ii) there are immunologic abnormalities associated with SARS CoV-2 infection which include high levels of cytokines, that can lead to immunosuppression, a predisposing condition to IFI (7,8); (iii) the use of mechanical ventilation can predispose to development of opportunistic fungal infections (58); and (iv) the use of dexamethasone in treating COVID-19 patients who are on mechanical ventilation, which though has been shown to reduce mortality rate and length of hospital stay, may however lead to immunosuppression, predisposing these patients to super-infection by fungi such as *Aspergillus* and *Coccidioides* (58-60).

Diagnostic challenges of invasive fungal infections in developing countries

In developing countries, the diagnosis of fungi infections is based on direct or histopathological microscopy and fungal cultures. Molecular assays, antigen testings for galactomannan and 1,3- β -D-glucans, and other modern diagnostic assays for further differentiation of fungi are rarely available in these regions.

Conclusion:

There are great similarities between respiratory symptoms of COVID-19 and chronic pulmonary diseases caused by fungal infections. The presence of co-morbidities such as HIV infection, DM, corticosteroid administration increases the risk of mortality from COVID 19 and the possibility of super-infection with the invasive mycoses. It is recommended that all COVID-19 patients with co-morbidities as exemplified, who require respiratory support be routinely screened for pulmonary mycotic infections using sensitive laboratory methods. Employers and public health officials should mitigate exposure to dust, other risk factors and SARS-CoV-2 by promoting the use of face masks and social dist-

cing practices.

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Copyright AJCEM 2022: <https://dx.doi.org/10.4314/ajcem.v23i1.3>**Mini Review****Open Access****Leptospirosis: a need for increased awareness and improved laboratory testing**

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601 S. Martin Luther King Jr. Drive, Winston-Salem, North Carolina 27110, USA
Correspondence to: wishonl@wssu.edu; 336-750-8339 (work); 336-253-3677 (cell)**Abstract:**

While leptospirosis is currently described as an emerging pathogen, there have likely been numerous cases worldwide each year for centuries. Hurricanes and other flooding events contribute to its spread through rodent urine, many cases of which go undiagnosed. This is especially problematic in developing countries where laboratory techniques may be out of date. There are over 100 cases per year in the United States of America, but millions of cases occur worldwide annually. Caused by many different species of fastidious, spiral-shaped *Leptospira*, it is difficult and slow to culture. Strides have been made to improve culture techniques in order to reduce the time to grow this genus of bacteria. Greater understanding of this disease by laboratorians, physicians, and other healthcare workers and improved laboratory identification techniques will help increase diagnoses and decrease morbidity and mortality of leptospirosis.

Keywords: leptospirosis, hurricanes, emerging pathogen, zoonosis

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Copyright 2022 AJCEM Open Access. This article is licensed and distributed under the terms of the Creative Commons Attribution 4.0 International License [rel="license" href="http://creativecommons.org/licenses/by/4.0/">](http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution and reproduction in any medium, provided credit is given to the original author(s) and the source. Editor-in-Chief: Prof. S. S. Taiwo**Leptospirose: un besoin de sensibilisation accrue et d'amélioration des tests de laboratoire**

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Correspondance à: wishonl@wssu.edu; 336-750-8339 (travail); 336-253-3677 (portable)**Résumé:**

Alors que la leptospirose est actuellement décrite comme un agent pathogène émergent, il y a probablement eu de nombreux cas dans le monde chaque année depuis des siècles. Les ouragans et autres inondations contribuent à sa propagation par l'urine de rongeurs, dont de nombreux cas ne sont pas diagnostiqués. Ceci est particulièrement problématique dans les pays en développement où les techniques de laboratoire peuvent être dépassées. Il y a plus de 100 cas par an aux États-Unis d'Amérique, mais des millions de cas surviennent chaque année dans le monde. Causée par de nombreuses espèces différentes de *Leptospira* fastidieux en forme de spirale, sa culture est difficile et lente. Des progrès ont été faits pour améliorer les techniques de culture afin de réduire le temps de croissance de ce genre de bactéries. Une meilleure compréhension de cette maladie par les laboratoires, les médecins et les autres professionnels de la santé et l'amélioration des techniques d'identification en laboratoire contribueront à augmenter les diagnostics et à réduire la morbidité et la mortalité de la leptospirose.

Mots clés: leptospirose, ouragans, pathogène émergent, zoonose

Introduction:

Leptospirosis is caused by a variety of *Leptospira* species resulting in flu-like symptoms in addition to jaundice, red eyes, and rash (1). Some patients recover from this flu-like illness while others at first appear to recover but their symptoms then worsen after recovery. In some cases, liver or kidney damage, meningitis, respiratory distress, and even death occurs. About 10% of those with leptospirosis develop the severe form, and in those cases, the fatality rate is 5-15% (1).

Leptospirosis is the most widespread zoonotic infection in the world and is most common in the tropics and subtropics (2). It is considered emerging since there is increasing contact between humans and rodents that carry the pathogen (3). Human invasion of the natural habitats of zoonotic carriers and climate change, which results in local environmental changes, are believed to have altered the transmission rates of *Leptospira* sp (2,3). Many cases follow hurricanes and other flooding events and are due to exposure of open wounds during cleanup efforts. More awareness is needed of the disease since many cases are likely missed. Fewer cases can be missed through increasing accessibility of laboratory testing.

Epidemiology of leptospirosis

In the United States, just over 100 cases are reported each year and about half of those are in Puerto Rico (1). The largest outbreak ever reported in the US was in 1998 where about 775 people were exposed to the disease, with 110 infected. Most of those infected were triathletes exposed at events held at Madison, Wisconsin and Springfield, Illinois a couple of weeks apart (4). The median age of the victims was 35 years, 76% were male, 66% sought medical care, and 32% were hospitalized. There was an outbreak of 81 U.S. marines training at the Jungle Warfare Training Center in Okinawa in 2014, likely due to drinking of stagnant water (5). An outbreak had previously occurred there among marines in 1987.

Leptospirosis was reported in Puerto Rico long before being reported after Hurricane Maria in 2017, especially correlating with periods of rainfall. This is likely because animal urines do not have the opportunity to saturate into the soil and are instead carried along in floodwaters to people who may not be wearing protective clothing and thus, become exposed (6). In 1996, leptospirosis was reported in Puerto Rico after Hurricane Hortense in September of that year. Four patients were sus-

pected to have dengue fever, but tested negative, instead, they tested positive for *Leptospira* IgM antibodies. This caused the researchers to do a before and after comparison of dengue-negative *Leptospira* testing, and they found that 4 of 72 antisera from patients before the hurricane and 17 of 70 antisera from patients after the hurricane were positive for leptospirosis. The average positive patient was 34 years of age and male. It was then suggested that the disease is underreported due to absence of optimal testing, lack of physician awareness, and the similarity of symptoms to dengue. In this study, while many symptoms were similar to both dengue and leptospira, such as headache, rash, fever, and body pain, symptoms most specific to leptospira were eye and joint pain, diarrhea, and jaundice.

Just a couple of years later, Hurricane Mitch hit Honduras in 1998 and the incessant rains led to outbreaks of leptospirosis [7]. One study used Ellinghausen-McCullough-Johnson-Harris (EMJH) media and microscopic agglutination test (MAT) to determine the presence of pathogen in 68 samples, 24 of the samples had reactive MAT analysis and were speciated for *Leptospira*, the most common being *Leptospira icterohaemorrhagiae*. Most of the patients' reported rodents in or around their homes or contact with stagnant water, and many also claimed contact with their pets. Two cases of leptospirosis were reported among 48 people tested following a flood at a university in Oahu in 2004 (8), caused by a stream that overflowed following heavy rains. The first case, a 56-year-old patient had broken skin, and later blisters on his feet, while wading in the floodwaters and became ill with fever, chills, nausea, and vomiting about 10 days later. A few days later he had tremors, visual flashes, and poor balance. The second patient was a 27-year-old graduate student of the first patient and received a laceration to his foot during cleanup efforts. His symptoms began with nausea, vomiting, diarrhea, headache, fever, and chills 10 days after the flood. The second patient improved without treatment, while the first patient was hospitalized and later improved.

Leptospirosis became increasingly discussed recently due to Hurricane Maria. A debate pursued on whether or not cases of death from *Leptospira* sp. that occurred more than a month after the hurricane could be attributed to the storm itself (9,10). About 26 deaths occurred due to *Leptospira* sp. within 6 months of the hurricane and only 4 were officially attributed to the disease if going by the one-month criteria. Several families argue that their family members were part of the hurricane clean-up and

were not given prophylactic measures, protective gear, or warned about the disease and died days after the arbitrary one-month reporting cut-off.

Several recent studies have shown the worldwide abundance of the disease. One report reviewed 80 studies on the incidence of leptospirosis in 34 countries with some of the highest estimates of morbidity and mortality found in South and Southeast Asia, Oceania, Latin America, and East Sub-Saharan Africa (11). The study estimated 1.03 million cases and 58,900 deaths worldwide annually from leptospirosis, with many cases and deaths in males between the ages of 20 and 49 years. It was estimated in another study that almost 3 million disability adjusted life years (DALYs) are lost annually due to the greater than one million annual cases of leptospirosis, with males carrying around 80% of this burden (12). Additionally, most of this burden falls in developing countries of South and Southeast Asia, Western Pacific, Central and South America, and Africa.

Although leptospirosis is listed as endemic in Southeast Asia, Central and South America, the Caribbean, and Oceania, it was listed as probable endemic in Malaysia (13). A recent study indicated that Malaysia had 3,665 and 4,457 laboratory confirmed cases in 2012 and 2013, respectively. The overall age-standardized incidence rate was 29.02 per 100,000 and the most common age group was age 19 years or less, making up 23.3%. The male to female ratio was 2.6:1. Students, agriculture-based, and plantation workers were among those most affected. The overall fatality rate was 1.47%. Due to the results of the study, the authors stated that this is an emerging health concern in Malaysia and should be well-controlled.

Although it is suggested that the disease is emerging, it undoubtedly has existed far longer than the study indicates. In a similar study, it was reported that Mexico had 1,547 cases of leptospirosis between 2000 and 2001, with 198 recorded deaths (14). While findings did not indicate higher morbidity in males, the mortality was higher in males. Of total deaths from the disease, 61.1% were males during this time period. Whereas the case fatality rate was lower for the study in Malaysia than in many others, the cases in Mexico had a case fatality rate of 12.8%.

Laboratory identification of *Leptospira*

Identification of *Leptospira* sp in the laboratory does not occur by using traditional methods of plating on blood, MacConkey, or

chocolate agar plates. These spiral-shaped Gram-negative bacteria are fastidious and grow slowly even on broth media. Culture of this pathogen requires special nutrient media not included in the normal battery of media used in laboratories, which can be EMJH base media or whole blood or plasma (15,16). Special media consisting of sodium pyruvate, superoxide dismutase, and/or fetal bovine serum has also been suggested (15).

The need for special culture requirements helps to explain why many cases are likely missed. Physicians would have to specifically order this test since the organism will not grow on routine media used when spinal fluid, urine, or blood cultures are ordered (17). The diagnosis is also missed often because the spirochete cannot be stained by the common Gram stain. A more sensitive stain must be used, such as immunofluorescent, immunohistochemical, or silver stain, which will have to be specifically ordered by the physician. The test ordered when a physician suspects a patient with leptospirosis, is often performed by a serological method such as enzyme-linked immunosorbent assay (ELISA) or MAT. These serological tests can be problematic for identification since during the early stages of the infection, antibodies may not be present in adequate numbers, resulting in false negatives.

Polymerase chain reaction (PCR) assay is increasingly being used to identify *Leptospira* and is usually positive from 5 to 15 days after the onset of disease (18). *Leptospira* serovars included in the database of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (18,19) has been shown to accurately and precisely identify multiple species of this pathogen. Many laboratories do not have the serovars included in their databases, since test for leptospirosis is not a routinely ordered test. However, including these serovars in MALDI-TOF may increase the likelihood of physicians ordering this test, but many developing countries where the disease is endemic do not have the financial resources for MALDI-TOF analyzer.

Susceptibility testing has not been traditionally performed on *Leptospira* sp. since culturing is time-consuming and currently there are no challenges of antibiotic resistance in leptospirosis. However, experimental attempts have been made to design appropriate growth media for detection of resistance that may occur in *Leptospira* population in the future. One such experimental study determined that the use of Noble agar base, in addition to EMJH base and enrichment with sodium pyruvate, 10% rabbit serum and increased CO₂ increased the growth

of *Leptospira* sp. better than other media tested (20). The other media tested were mixtures of bacteriological agar and various concentrations of rabbit serum as well as increasing CO₂ at various concentrations (or not increasing it at all). Using this newly concocted *Leptospira* Vanaporn Wuthiekanun (LVW) agar, E-test could be performed after 7 days, with 2 days incubation as above, and 5 more days in ambient air at 30°C. The authors felt this was a more rapid, simple, and cost-effective test that could be more widely used in tropical developing countries.

The disk diffusion susceptibility method was soon validated using this same LVW agar (21). Azithromycin, ceftriaxone, ciprofloxacin, doxycycline, gentamycin, and penicillin-G were all tested on 83 human species of *Leptospira*, which showed that all the species were sensitive to the 6 antibiotics, with large zones of inhibition. The median growth time on the LVW agar was 5 days, with some taking only 3 days and others taking up to 12 days to reach 4+ growth. This indicates that the newly adapted LVW agar could potentially be used to perform Kirby-Bauer testing on *Leptospira* if resistance arise.

The most recent study on improving *Leptospira* growth media was based on PCR analysis of the genome of the organism from culture growth, to determine what ingredients would best be included (22). After the results of genome analysis were obtained, the authors then included different ingredients into the culture media to determine which ingredient mixtures would result in the fastest growth. A mixture, dubbed M12, was found to grow various pathogenic and non-pathogenic species of *Leptospira* within 2 days, with the characteristic ring-like white layer. M12 media has specific compositions of Na₂HPO₄·2H₂O, KH₂PO₄, tryptone, yeast extract, KCl, aspartic acid, sodium pyruvate, glucose, MgCl₂ anhydrous, CaCl₂·2H₂O, NH₄Cl, thiamine HCl, sodium acetate, NaHCO₃, casein, and water, at pH of 7.2. To have produced growth media with the ability to rapidly grow *Leptospira* sp. is a vast improvement that could lead to marked decreases in turnaround times for diagnoses as well as susceptibility testing if and when necessary. Further experiments with this media certainly seem warranted as does its use in identification in patient samples.

Conclusion:

Models that predict the effects of global warming on hurricanes vary (23-25). Some models report that global warming will increase the strength while others predict a weakening.

Either way, financial spending at coastlines is ever-increasing, indicating that cleanup after hurricanes will continue to increase (25). This increase will mean more cases of leptospirosis. Additionally, humans are continuing to encroach on natural lands where animals live and whose urine is present in the soil. The need for physicians, nurses, and laboratory workers to be aware of and understand the disease will ever be necessary, in order to reduce mortality and morbidity from leptospirosis in people working in and around floodwaters. Additionally, in areas with hurricane and flooding activity, especially in developing countries, there is need to increase laboratory testing abilities for identifying *Leptospira* sp. Countries that cannot afford more expensive molecular methods of identification should at minimum have updated culture methods in place that can more rapidly identify this deadly pathogen.

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**Review Article****Open Access****A review of the implications of Lactic Acid Bacteria and Bifidobacteria in human and animal diseases**

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Abstract:

Lactic acid bacteria (LAB) and Bifidobacteria are taxonomically distinct groups of bacteria with proven biotechnological properties such as anti-cancer, immune-stimulating, anti-microbial, maintenance of normal flora balance, probiotics, anti-inflammatory, vaccine carriers, among others. However, studies have implicated some of them, including the ones under the European Food Safety Authority (EFSA) qualified presumption of safety in fatal human and veterinary diseases. We performed online database searches of publications on Google, Google Scholar and PubMed using the criteria, "lactic acid bacteria, bifidobacteria as causative agents of human, animal diseases". Data generated showed LAB across genera and Bifidobacteria either primarily or opportunistically involved in diseases of both immuno-competent and immuno-depressed humans and animals. The members of lactobacilli such as *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus oris*, *Lactobacillus gasseri* and *Leuconostoc mesenteroides*, were mainly implicated in nosocomial infections, endophthalmitis, neonatal meningitis, and bacteraemia while *Lactobacillus delbrueckii* and Bifidobacteria, specifically, *Bifidobacterium longum*, *Bifidobacterium breve*, and *Bifidobacterium animalis* were implicated in urinary tract infections (UTIs), necrotizing pancreatitis, fatal pulmonary infections, sepsis, and epidural abscess. The animal diseases, neonatal sepsis in foal, was caused by *Weissella confusa* while the fish pathogen, *Lactococcus garvieae* caused various zoonotic cases such as acute acalculous cholecystitis in human. In conclusion, this review showed the up-to-date reports on LAB and Bifidobacteria implicated in serious humans and animal diseases.

Keywords: Lactic acid bacteria; Bifidobacteria; Human; Animal; Diseases; Probiotics

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Un examen des implications des bactéries lactiques et des bifidobactéries dans les maladies humaines et animales

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Résumé:

Les bactéries lactiques (LAB) et les bifidobactéries sont des groupes de bactéries taxonomiquement distincts avec des propriétés biotechnologiques prouvées telles que anticancéreuses, immunostimulantes, antimicrobiennes, maintien d'un équilibre normal de la flore, probiotiques, anti-inflammatoires, porteurs de vaccins, entre autres. Cependant, des études ont impliqué certains d'entre eux, y compris ceux relevant de la présomption qualifiée de sécurité de l'Autorité européenne de sécurité des aliments (EFSA) dans les maladies humaines et vétérinaires mortelles. Nous avons effectué des recherches dans des bases de données en ligne de publications sur Google, Google Scholar et PubMed en utilisant les critères «bactéries lactiques, bifidobactéries en tant qu'agents responsables de maladies humaines et animales». Les données générées ont montré des

bactéries lactiques à travers les genres et les bifidobactéries impliquées principalement ou de manière opportuniste dans les maladies des humains et des animaux immuno-compétents et immunodéprimés. Les données générées ont montré des bactéries lactiques à travers les genres et les bifidobactéries impliquées principalement ou de manière opportuniste dans les maladies des humains et des animaux immuno-compétents et immunodéprimés. Les membres des lactobacilles tels que *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus oris*, *Lactobacillus gasseri* et *Leuconostoc mesenteroides* ont été principalement impliqués dans les infections nosocomiales, l'endophtalmie, la méningite néonatale et la bactériémie tandis que *Lactobacillus delbrueckii* et les bifidobactéries, en particulier, *Bifidobacterium longum*, *Bifidobacterium breve* et *Bifidobacterium animalis*, ont été impliquées dans les infections des voies urinaires (IVU), la pancréatite nécrosante, les infections pulmonaires mortelles, la septicémie et l'abcès épidermique. Les maladies animales, la septicémie néonatale chez le poulain, ont été causées par *Weissella confusa* tandis que l'agent pathogène du poisson, *Lactococcus garvieae*, a causé divers cas de zoonoses telles que la cholécystite calculée aiguë chez l'homme. En conclusion, cette revue a montré les rapports à jour sur les bactéries lactiques et les bifidobactéries impliquées dans les maladies humaines et animales graves.

Mots-clés: Bactéries lactiques; Bifidobactéries; Humain; Animale; Maladies; Probiotiques

Introduction:

Lactic acid bacteria (LAB) are composed of thirteen genera of Gram-positive bacteria that include *Carnobacterium*, *Lactobacillus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus*, *Enterococcus*, *Lactococcus*, *Pediococcus*, *Paralactobacillus*, *Streptococcus*, *Tetragenococcus*, *Vaginococcus* and *Weissella* (1). They are divided into two groups based on their metabolic end products from glucose or other hexose sugars. Those that produce solely lactic acid as major product are known as homofermenters while those producing equal molar amounts of lactate, CO₂ and ethanol are designated heterofermenters (1). The genus *Bifidobacterium* on the other hand, has the ability to produce both lactic acid and short chain fatty acids. It is not grouped directly under LAB because they are strict anaerobes, do not produce gas during growth and have GC content of about 55-67 mol% (2).

Over the years, LAB and indeed Bifidobacteria have been proven to be safe for human and animal consumption through various experiments and scientific procedures hence, they have assumed the 'Generally Regarded As Safe' (GRAS) status (3). To this end therefore, many studies have demonstrated the beneficial roles played by these set of bacteria such as; lactose digestion, immune stimulating, diarrhoea treatment and prevention (4), production of useful industrial and medical products (5,6), probiotics and growth promoters in livestock, substituting for antibiotics (7,8), anti-inflammatory (9), anti-cancers (10), and as vaccine carriers (11,12). In spite of these track records, LAB and Bifidobacteria have been implicated in many human and animal diseases. In this study, we reviewed various published articles reporting the involvement of selected genera of LAB and Bifidobacteria that have been regarded to be safe, in human and animal diseases.

Methodology:

Online literature searches were conducted on Google search engine, PubMed and Google Scholar using the following words as criteria; "lactic acid bacteria, bifidobacteria as causative agents of human and animal diseases" to generate results for a traditional narrative review. These criteria were further refined to "diseases caused by probiotic lactic acid bacteria or bifidobacteria" to remove unwanted publications. Due to paucity of publications in the literature, the searches were not specified to cover particular periods.

A total of 85 published articles were retrieved from the database searches and evaluated based on credibility of sources, key concepts, and theories. Twenty-one publications were excluded after they were deemed to be irrelevant, leaving a total of 64 articles for the review.

Results:

The summary of Lactic acid bacteria and Bifidobacteria implicated in human and animal diseases is shown in Table 1.

Leuconostoc

These are Gram positive, facultative anaerobic coccobacilli which may be misidentified as *Enterococcus* or *Streptococcus* by routine biochemical testing (13). They have been reported as emerging pathogens in nosocomial outbreaks, immunosuppression and vancomycin resistance (14–19). Singh et al., (20) reported a case of acute endophthalmitis caused by *Leuconostoc* spp. after intravitreal bevacizumab injection in an 86-years old immunocompetent female. Damasceno et al., (21) also reported isolation of *Leuconostoc mesenteroides* in the blood and vitreous cultures of a patient who presented 48 hours after uncomplicated intravitreal injection of ranibizumab. Two other sets of researchers (22,23) have previously reported *Leuconostoc* associated endophthalmitis in

Table 1. Summary of Lactic acid bacteria and Bifidobacteria implicated in human and animal diseases

LAB/Bifidobacteria	Disease	Reference
<i>Lc. mesenteroides</i> , <i>L. rhamnosus</i> , <i>L. fermentum</i> , <i>L. paracasei</i> , <i>Lc.</i> <i>oris</i> , <i>L. gasseri</i> , <i>L. iners</i> , <i>L.</i> <i>salivarius</i>	Nosocomial infections	(14-19,27)
<i>Lc. mesenteroides</i>	Endophthalmitis	(20)
<i>L. rhamnosus</i> , <i>P. acidilactici</i> , <i>B.</i> <i>longum</i> , <i>B. breve</i> , <i>B. animalis</i>	Neonatal meningitis, Bacteraemia, Necrotizing pancreatitis, Sepsis, Epidural abscess, Fatal pulmonary infections	(28-30,55,71,75-79)
<i>L. delbrueckii</i> , <i>L. jensenii</i> , <i>Bifidobacterium</i> spp.	Urinary tract infections	(37,38,74)
<i>Weissella confusa</i>	Systemic infections Neonatal sepsis in foal Pneumonitis	(49) (50) (60)
<i>Lactococcus garvieae</i>	Endocarditis Acute acalculous cholecystitis Post-operative osteomyelitis abscess Infected prosthetic joint	(53,64-66) (56) (57) (58)

Lc = Leuconostoc; L = Lactobacillus; LAB = Lactic acid bacteria; B = Bifidobacterium; P = Pediococcus

immuno-competent patients following uncomplicated phacoemulsification surgery.

Lactobacillus

These are non-spore forming, aerotolerant LAB comprising of more than 237 species and sub-species (www.bacterio.net/lactobacillus.htm). Lactobacilli are rarely associated with diseases in immunocompetent people except in the presence of risk factors and underlying conditions such as diabetes mellitus, pre-existing structural heart defects, cancer, and antibiotic therapy (23,24) where they cause endocarditis, bacteraemia, neonatal meningitis, liver abscess, pulmonary infections, pyelonephritis, meningitis, post-partum endometritis and chorioamnionitis (25,26). In a reported case of bacteraemia in Argentina between 2012 and 2017 by Roca et al., (27), *Lactobacillus rhamnosus* was most commonly isolated followed by *L. fermentum*, *L. paracasei*, *L. oris*, *L. gasseri*, *L. iners* and *L. salivarius*.

In premature infants, cases of infections caused by probiotic *L. rhamnosus* have been reported such as late onset sepsis (LoS) following a laparotomy and bacteraemia after tube feeding with the bacterium (28-30). It seems the potential for probiotics to cause sepsis is greater in immuno-deficient neonates and this was recently supported by a report of sepsis in humans (31). In adults, endocarditis caused by *Lactobacillus* spp is not uncommon and this occurred in patients who had dental extractions or gingival bleeding after toothbrushing (32), after colonoscopy (33) and in haemorrhagic telangiectasia (34). Also associated with Lactobacilli

is bacteraemia especially when probiotics are consumed by patients undergoing haematopoietic stem cell transplantation and HIV-infection (31,35).

Other cases such as meningitis where *Lactobacillus* spp was isolated from blood and CSF in a neonate, and in a 10-year-old neutropaenic child with acute leukaemia, were suspected to be from the mothers' genital tracts. Meningoencephalitis in a 63-year-old man with metastatic planoepithelial lung cancer have been hypothesized to be due to direct bacterial (*Lactobacillus*) dissemination from the gastrointestinal tract (36). *Lactobacillus delbrueckii* and *L. jensenii* have been reported to cause urinary tract infections (chronic pyuria and pyelonephritis) in women (37,38).

Studies on virulence of *Lactobacillus* spp have implicated *L. rhamnosus* and *L. paracasei*, which are widely used as probiotics, to be virulent, for instance, some strains can aggregate human platelets (39, 40). *Lactobacillus rhamnosus* has been linked more frequently with infections than other lactobacilli (41,42). Apart from their propensities to cause infections, *Lactobacillus* have been demonstrated as reservoirs of antibiotic resistance genes and possess ability to transfer them. For instance, resistance to tetracycline, erythromycin, clindamycin and chloramphenicol have been acquired by food-borne lactobacilli (43, 44). Also, in *L. ingluviei*, *L. amylophilus* and *L. amylophilus*, resistance genes on mobile genetic elements with potential for horizontal transfer, have been reported by Campedelli et al., (45).

Weissella

Although the bacteria under this genus have several strains used for biotechnological and probiotic purposes, some have been reported to possess inherent abilities as pathogens. Abriouel et al., (46) found several virulence determinants such as collagen adhesins, aggregation substances, mucus binding proteins and haemolysin, including several antibiotic resistance encoding genes in some species in an *in-silico* analyses of their whole genome sequences. The species of *Weissella* include, *W. ceti*, *W. cibaria*, *W. confusa*, *W. halotolerans*, *W. hellenica*, *W. koreensis*, *W. oryzae*, *W. paramesenteroides* and *W. thallandensis*. Of all *Weissella*, only *W. confusa*, *W. cibaria* and *W. viridescens* have been isolated from human clinical specimens (47,48) while *W. confusa* has been documented as a cause of systemic infection in healthy primates (49) and neonatal sepsis in a foal (50). In humans, most isolated *W. confusa* infections were from immunocompromised patients (51,52), which may be occasioned by organ transplant, long term use of steroids, chronic renal insufficiency, and diabetes mellitus (53,54). The common human infections caused by *W. confusa* are bacteraemia (55), endocarditis (53), post-operative osteomyelitis (56), abscess (57), and prosthetic joint infections (58).

Pediococcus

These bacteria have been infrequently isolated from the human respiratory tract, stool, urine and blood of immunocompromised patients, and in patients with malignancy, cardiovascular, lung diseases, and diabetes mellitus (13,59), while *Pediococcus acidilactici* specifically has been implicated in pneumonitis and bacteraemia in a pregnant woman, and septicaemia caused by vancomycin resistant strain (60). In a fatal case of necrotizing cellulitis of the abdominal wall secondary to the rupture of a retroperitoneal stromal tumor in a 60-years old Caucasian male patient, *Pediococcus pentosaceus* strain resistant to vancomycin, teicoplanin, trimethoprim and kanamycin was isolated from blood, subcutaneous, and peritoneal specimens (61).

Lactococcus

Lactococcus species are mainly associated with infective endocarditis, hepatic abscess and hip prosthetic infections (62,63). In particular, *L. garvieae* which is reputed to be a fish pathogen has been implicated in zoonosis following consumption of raw fish by humans, causing diseases such as acute acalculous cholecystitis, endocarditis (64-66), and knee periprosthetic infection in a 79 years old male with multiple comorbidities,

who had a habit of eating perch fish (67). *Lactococcus lactis* subsp *lactis* was isolated from samples of facial cellulitis and apical periodontitis. This bacterium was resistant to multiple antibiotics. Eiji et al., (68) reported virulence genes in *L. garvieae* from different sources using comparative genomic analysis and recently, this bacterium isolated from diseased rainbow trout was reported to have carried important virulence genes such as haemolysins 1,2,3, NADH oxidase, phosphoglucomutase, adhesins, superoxide dismutase, enolase, among others (69,70).

Bifidobacteria

These are strictly anaerobic commensals that colonize the oro-gastrointestinal tract and are said to rarely cause invasive infections. However, they have been demonstrated to be implicated in fatal bacteraemic infections especially *B. longum*, *B. breve* and *B. animalis* in both immunocompromised and immunocompetent hosts, and in patients with gastrointestinal tract related conditions (71). Also, they have been estimated to constitute 0.5-3.0% of anaerobic blood culture isolates (31,72), and until 2015, only 15 adult cases were reported in the literature (73). Bifidobacteria have been implicated in cases of necrotizing pancreatitis, sepsis, epidural abscess, fatal pulmonary infection, dental caries and urinary tract infections (74-79).

Esaassen et al., (71) reported 98 putative virulence genes among 15 *Bifidobacterium* isolates, such as iron and magnesium transport, adhesin, toxin secretion, immune invasive, stress proteins, among others. Also reported is the ability of Bifidobacteria to harbour antibiotic resistance genes including those on mobile genetic elements. These genes, found mainly in *B. animalis* were confirmed by high resolution molecular analysis, to have been acquired through horizontal gene transfer (80,81) with heavier presence of Bifidobacteria in guts of antibiotic treated adults and children when compared to non-antibiotic treated persons.

Discussion:

This review was necessitated by the historic accolade of 'Generally Regarded As Safe' (GRAS) status given to LAB and Bifidobacteria, which has placed them as probiotics or "good" bacteria and alternative therapeutics without side effects. Their biotechnological properties have been demonstrated and reported in various literatures. Although, there exist quite a few published data on the subject of LAB and disease causation, this review calls for continuous surveillance of these bacteria as regards their implications in human and veterinary diseases.

In this review, it was revealed that

the common denominator is that these bacteria are always involved in opportunistic infections especially in immuno-compromised patients or in patients on long hospitalization with underlying conditions such as diabetes mellitus, cancers, or with use of steroids and antibiotic therapy (14-19,23,24,27), and the commonest LAB are of *Lactobacillus* species, while Bifidobacteria are sparsely reported as pathogenic (37,38,74). The ability of Lactobacilli and Bifidobacteria to acquire and disseminate antibiotic resistance through mobile genetic elements should be taken seriously especially in this era of global problems of antimicrobial resistance (AMR). Also, Lactobacilli are resident flora of human gastrointestinal tract (GIT), and with their consumption as adjuncts in fermented food products, they can be means through which the AMR genes are transferred to GIT commensals. Surveillance efforts should be geared up in fermented foods and products supplemented with LAB and Bifidobacteria probiotics, and such surveillance should include annotation of all functional genes in the genome of these bacteria.

The animal diseases were reported more commonly in fish and this was caused mainly by *Lactococcus* species especially *L. garvieae* and as a matter of fact, this LAB is reputed for fish disease (64-66), although with few exceptions in humans who have direct contact with fish and certain marine animals.

Conclusion:

Although Lactic acid bacteria from different niches are generally regarded as safe by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO), they may play either primary or opportunistic pathogenic roles, as reported by different studies over time in causality of serious diseases of human and in veterinary setting, irrespective of the host immune status, and they can additionally carry transmissible antibiotic resistance genes.

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Original Article

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Nasopharyngeal temperature probes: is South Africa's current decontamination process adequate?

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Abstract

Background: The standard practice in many institutions incorporates nasopharyngeal probes for temperature monitoring in patients undergoing general anaesthesia. Current disinfection guidelines for these devices are not clear and they are poorly adhered to. In South Africa, these temperature probes are reused and subjected to unstandardized decontamination processes. This study sought to investigate nasopharyngeal temperature probes as possible source for cross-contamination, and assess the efficacy of current disinfection practices for these probes.

Methodology: This was an analytical double-blind randomized study of 4 different disinfection protocols for 48 nasopharyngeal temperature probes. The probes were randomized to disinfection protocols that included water wash, dry wipe, hibitane® and cidex® wash. After decontamination by the respective protocol, the probes were aseptically placed in nutrient broths, manually agitated and removed, and the broths were then inoculated onto blood agar plates. After 48 hours of aerobic culture incubation at 37°C, plates were examined for growth and bacteria identified using automated bioMérieux Vitek-2 microbial identification system. Chi square and logistic regression analyses were used to assess bacterial contamination rates of the disinfected probes, in order to infer the efficacy of the decontamination processes.

Results: Of the 48 nasopharyngeal temperature probes disinfected by the different protocols, 22 (45.8%) had bacterial contamination, with frequency of isolation for coagulase negative staphylococci (44%), *Bacillus cereus* (20%), *Staphylococcus aureus* (10%), *Enterobacter cloaca* (7%), *Pseudomonas aeruginosa* (4%), *Pseudomonas fluorescens* (3%), *Acinetobacter baumannii* (3%), amongst other bacterial species. Dry wipe, and water and soap methods, had statistically significant higher contamination rates of 83.3% and 66.7% than hibitane® and cidex®, with 25.0% and 8.3% respectively ($X^2=17.69$, $p<0.0001$). The odds of contamination when water-wipe was used as a cleaning method was 6 times (OR=6.000; 95% CI=1.018-35.374, $p=0.048$) that of hibitane® method while the odds for dry-wipe was 15 times (OR=15.000, 95% CI=2.024-111.174, $p=0.008$). No statistically significant difference was observed in the contamination rates between cidex® and hibitane® disinfection methods (OR=0.273, 95% CI=0.024-3.093, $p=0.294$).

Conclusion: These data shows that nasopharyngeal temperature probes are possible source of cross-contamination and pathogen transmission due to inadequacy of the decontamination processes for these temperature probes.

Keywords: nasopharyngeal probe; cross-contamination; decontamination; hibitane®; cidex®; infection control

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Sondes de température nasopharyngées: le processus de décontamination actuel en Afrique du Sud est-il adéquat?

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Résumé:

Contexte: La pratique standard dans de nombreux établissements incorpore des sondes nasopharyngées pour la surveillance de la température chez les patients subissant une anesthésie générale. Les directives de désinfection actuelles pour ces appareils ne sont pas claires et elles sont mal respectées. En Afrique du Sud, ces sondes de température sont réutilisées et soumises à des procédés de décontamination non standardisés. Cette

étude visait à étudier les sondes de température nasopharyngées comme source possible de contamination croisée et à évaluer l'efficacité des pratiques de désinfection actuelles pour ces sondes.

Méthodologie: Il s'agissait d'une étude analytique randomisée en double aveugle de 4 protocoles de désinfection différents pour 48 sondes de température nasopharyngées. Les sondes ont été randomisées dans des protocoles de désinfection comprenant un lavage à l'eau, un essuyage à sec, un lavage à l'hibitane® et au cidex®. Après décontamination par le protocole respectif, les sondes ont été placées de manière aseptique dans des bouillons nutritifs, agitées et retirées manuellement, et les bouillons ont ensuite été inoculés sur des plaques de gélose au sang. Après 48 heures d'incubation de culture aérobie à 37°C, les plaques ont été examinées pour la croissance et les bactéries identifiées à l'aide du système d'identification microbienne automatisé bioMérieux Vitek-2. Des analyses du chi carré et de régression logistique ont été utilisées pour évaluer les taux de contamination bactérienne des sondes désinfectées, afin de déduire l'efficacité des processus de décontamination.

Résultats: Sur les 48 sondes de température nasopharyngées désinfectées par les différents protocoles, 22 (45,8%) présentaient une contamination bactérienne, avec fréquence d'isolement pour les staphylocoques à coagulase négative (44%), *Bacillus cereus* (20%), *Staphylococcus aureus* (10%), *Enterobacter cloaca* (7%), *Pseudomonas aeruginosa* (4%), *Pseudomonas fluorescens* (3%), *Acinetobacter baumannii* (3%), parmi d'autres espèces bactériennes. Les méthodes d'essuyage sec et d'eau et de savon avaient des taux de contamination statistiquement plus élevés de 83,3 % et 66,7 % que l'hibitane® et le cidex®, avec respectivement 25,0% et 8,3% ($\chi^2=17,69$, $p<0,0001$). Le risque de contamination lorsque l'essuyage à l'eau était utilisé comme méthode de nettoyage était 6 fois (OR=6,000; IC à 95%=1,018-35,374, $p=0,048$) celui de la méthode hibatane® tandis que le risque pour l'essuyage à sec était de 15 fois (OR=15,000, IC à 95%=2,024-111,174, $p=0,008$). Aucune différence statistiquement significative n'a été observée dans les taux de contamination entre les méthodes de désinfection cidex® et hibatane® (OR=0,273, IC à 95 % = 0,024-3,093, $p=0,294$).

Conclusion: Ces données montrent que les sondes de température nasopharyngées sont une source possible de contamination croisée et de transmission d'agents pathogènes en raison de l'insuffisance des processus de décontamination de ces sondes de température.

Mots-clés: sonde nasopharyngée; contamination croisée; décontamination; hibatane®; cidex®; contrôle d'infection

Introduction:

The recommendation of the American Society of Anaesthesiologists for temperature monitoring is that "every patient receiving anaesthesia shall have temperature monitored when clinically significant changes in body temperature are intended, anticipated or suspected" (1). As a consequence of this recommendation, temperature monitoring is considered standard of care in most general anaesthesia procedures. The most frequently used temperature monitor is the nasopharyngeal temperature probe. The international infection control guidelines recommend high-level disinfection for these semi-critical devices (2). High-level disinfection requires removal of any physical material by means of washing the probe, bathing the device in disinfectant for a specified period of time and concluding with the rinsing of residual disinfectant. This ideal is often not realized in resource-constrained facilities.

Anaesthesia equipment, as a source of cross-contamination has previously been explored. Investigations into the infectious potential of laryngoscope blades and handles as well as bronchoscopy equipment encompass the bulk of this literature (3–8). The nasopharyngeal probe has not previously been investigated as a vehicle for pathogen. In contrast to the laryngoscope, another proven source of cross-contamination with minimal contact time with mucosal surface, the nasopharyngeal temperature probe remains in situ

for the duration of the procedure. The risk to patient health and safety with nasopharyngeal temperature probe may prove greater than the established risk with routine laryngoscope usage.

There are concerns regarding the decontamination of these devices, adding to the notion of infectivity. Samuel et al., (9) reported in their study that recommended infection control practices were not strictly adhered to in South Africa, and identified the current decontamination practices for nasopharyngeal temperature probes to include; (i) washing with soap and water; (ii) dry wipe; (iii) washing with water then bathing in 4% chlorhexidine (hibitane®), and (iv) washing with water followed by bathing in 2.4% glutaraldehyde (cidex®). None of these methods align with the national guidelines, however cidex® decontamination aligns with international high-level disinfection method.

The potential of anaesthesia devices to serve as vehicle for pathogen transmission is well documented, however the nasopharyngeal temperature probe has not been previously investigated. Based on this fact and the knowledge of inappropriate decontamination processes, it is postulated that temperature probe may as act as a source of cross-contamination. We therefore sought to investigate the nasopharyngeal temperature probe as a vehicle for pathogen transfer, and assess the efficacy of the current decontamination practices in our hospital.

Materials and method:

Study setting and ethical approval

The study was conducted at the Tygerberg Hospital theatre complex, Cape Town, South Africa between February and June 2019. Ethical approval was obtained from Stellenbosch University Health Research and Ethics Committee (HREC S17/03/057). Microbiologist aides were enlisted for the study, and the research was conducted in accordance with the Helsinki Declaration.

Study design and protocol

This was an analytical double-blind study of 48 nasopharyngeal temperature probes used on adult patients at the theatre complex of the hospital, randomized into four decontamination procedures; group 1: washing with soap and water; group 2: dry wipe; group 3: alcohol-based decontamination by washing first with water followed by bathing in hibitane® for a period of 5 minutes; and group 4: washing first with water followed by bathing in cidex® for a period of 5 minutes. Randomization was performed by a computer-generated program, allowing for 12 probes in each group. Children and patients with nasal or oropharyngeal pathology were excluded from the study.

The used nasopharyngeal probes were decontaminated based on the randomization group. Theatres were assigned sealed instructions detailing the cleaning process to be followed, and anaesthesia assistants executed the assigned decontamination instructions as received in concealed envelope. The study was conducted between February and June, 2019.

Laboratory procedure

The nasopharyngeal probes were first cultured by immersing 5-8 cm of the probes in test tubes containing nutrient broth under sterile condition by a single data collector and immediately transferred to the laboratory. Each test tube was marked with a study number. No patient demographic details were collected, and both investigator and laboratory staff were blinded to the decontamination method.

In the laboratory, the nutrient broths containing the immersed probes were manually agitated, removed, and the broths inoculated onto a prepared blood agar plates in

Petri dishes. The plates were incubated aerobically at 37°C for 48 hours. Microbial identification was done using the automated Vitek-2 microbial identification system (bioMérieux, Marcy-l'Étoile, France). Contamination, in the context of this study, was reported as any isolate of microbial growth.

Statistical analysis of data

Contamination rates were calculated for each decontamination process. Logistic regression and Chi-square analyses were used to compare contamination rates between the decontamination procedures, and $p < 0.05$ was considered statistically significant.

Results:

As depicted in Table 1, of all the 48 nasopharyngeal temperature probes randomized into 4 decontamination procedures, 22 (45.8%) had bacterial contamination, with dry wipe and water and soap methods, having statistically significant higher contamination rates of 83.3% and 66.7% than hibitane® and cidex®, with 25.0% and 8.3% respectively ($\chi^2 = 17.69$, $p < 0.0001$).

Binary logistic regression model showed in Table 2, a statistically significant difference between water and dry-wipe methods in comparison to the hibitane® method, with these two methods having significantly higher contamination rates, and therefore inferior to hibitane® as decontamination methods. The odds of contamination when water-wipe was used as a cleaning method was 6 times (OR = 6.000; 95% CI = 1.018-35.374, $p = 0.048$) that of hibitane® method, while the odds for the dry-wipe was 15 times (OR = 15.000, 95% CI = 2.024-111.174, $p = 0.008$). No statistically significant difference was observed between the cidex® and hibitane® decontamination methods (OR = 0.273, 95% CI = 0.024-3.093, $p = 0.294$).

Fig. 1 shows the frequency distribution of bacterial isolates recovered from cultures of decontaminated probes and these include; coagulase negative staphylococci (44%), *Bacillus cereus* (20%), *Staphylococcus aureus* (10%), *Enterobacter cloaca* (7%), *Pseudomonas aeruginosa* (4%), *Pseudomonas fluorescens* (3%), *Acinetobacter baumannii* (3%), amongst other bacterial species.

Table 1: Contamination rates of nasopharyngeal temperature probes with respect to decontamination methods

Decontamination method of probe	Contamination rate		χ^2	p value
	Not Contaminated (%)	Contaminated (%)		
Hibitane® (n=12)	9 (75.0)	3 (25.0)	17.79	<0.0001*
Water-wipe (n=12)	4 (33.3)	8 (66.7)		
Dry wipe (n=12)	2 (16.7)	10 (83.3)		
Cidex® (n=12)	11 (91.7)	1 (8.3)		
Total (n=48)	26 (54.2)	22 (45.8)		

* = statistically significant; χ^2 = Chi square

Table 2: Logistic regression for the test of association between decontamination methods and contamination rates of nasopharyngeal probes

Decontaminants	Estimate	S. E.	Wald	df	p value	OR	95% CI for OR	
							Lower	Upper
Hibitane® (ref.)			13.582	3	0.004			
Water-wipe	1.792	0.905	3.918	1	0.048	6.000	1.018	35.374
Dry-wipe	2.708	1.022	7.021	1	0.008	15.000	2.024	111.174
Cidex®	-1.299	1.239	1.100	1	0.294	0.273	0.024	3.093
Constant	-1.099	0.667	2.716	1	0.099	0.333		

S. E = standard error; OR = odds ratio; CI = confidence interval; ref = reference

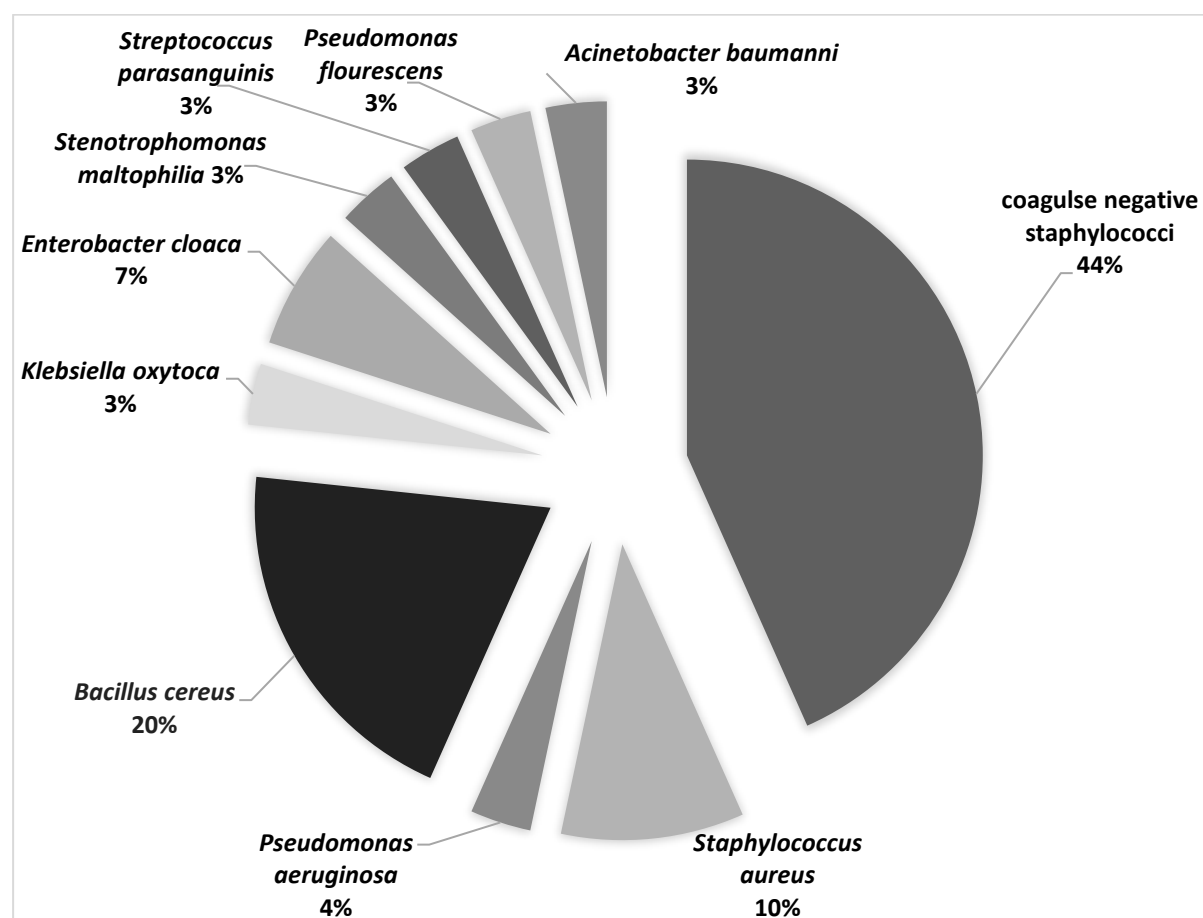


Fig 1: Frequency distribution of bacterial isolates from decontaminated nasopharyngeal temperature probe cultures

Discussion:

It is considered an international standard to monitor temperature in patients receiving anaesthesia. Perioperative thermoregulation and temperature monitoring are vital, as it alerts the anaesthesia practitioner to hypothermia or hyperthermia, because extremes of temperature are associated with grave systemic complications (10). Theatre complexes both locally and internationally have indicated that nasopharyngeal probes are the most commonly used perioperative temperature monitor (11).

The South African Society of Anaesthesiologists (SASA) published infection control guidelines in 2014 recommending sterilization of nasopharyngeal temperature probes, and multiple probes be available in each theatre (12). As the national infection control guidelines propose sterilization of nasopharyngeal temperature probes (12), majority of theatre complex call for the application of heat as sterilization techniques, including processes such as autoclaving (steam sterilization) and gas sterilization. However, concern exists regarding the malfunction of temperature probes when exposed to high temperature sterilization methods. This sentiment was shared amongst temperature probe manufacturers, and many of them advocated for single use of these devices.

The nasopharyngeal temperature probe is considered a semi-critical device as it is a device that comes into contact with mucosal membranes. International literature regarding semi-critical devices advocates for high-level disinfection processes. These ideals and recommendations put forward by the various bodies have proven to be a difficult benchmark in resource-constrained environments. Non-compliance to national and international infection control guidelines (10,12,13), lack of institutional decontamination protocols and miseducation (9), have led to the use of non-standardized and non-recommended cleaning practices for nasopharyngeal temperature probes. Our study investigated these practices and sought to ascertain evidence-based recommendations for the decontamination process of nasopharyngeal temperature probes.

The results of our study confirmed the inefficiency of some current cleaning practices and confirmed that decontaminated nasopharyngeal temperature probes can indeed be a vehicle for pathogen transmission. Statistical analyses by Chi square and logistic regression of our data showed some current decontamination protocols as being ineffective. Dry wipe and water-wash techniques particularly performed poorly, with decontamination success rates of only 16% and 33% respectively. Hibitane achieved decontamination success

rate of 75% but not surprisingly, was outperformed by cidex® with 91.7% decontamination success rate.

In light of potential probe malfunction with heat sterilization and rapid patient turnover, developing countries view high-level disinfection as an attractive alternative in decontamination of these potentially contaminable probes. Summation of the tested methods indicates the usefulness of hibitane® and cidex®, as high-level disinfection practices. Cidex® provides particular benefit as it has a wide spectrum of activity against bacteria, viruses and fungi, in addition to proven potent action against *Mycobacterium tuberculosis* (14). Some researchers have reported that the distinction between sterilization and high-level disinfection may be theoretical. Muscarella (15), reviewed these techniques in light of semi-critical instruments and surmised that high-level disinfection was not associated with higher infection rate than sterilization (16).

Nasopharyngeal temperature probe as potential vehicle for pathogen transfer has not been previously explored. Historically, literatures focusing on anaesthesia equipment (4,5, 17-19) have apparently neglected nasopharyngeal temperature probe, with greater focus on laryngoscopes and endoscopic equipment as well as anaesthesia workstation. However, our study showed that these devices are proven cross-contaminators to both patient and staff. In a closely-related study of decontamination procedure for nasal endoscopes with water and soap, alcohol-based wash and cidex® immersion, only cidex® immersion strategy was effective against all inoculated organisms (8). The findings of this study are congruent with our current study.

Aerobic microbial growth in this study showed that 42% of all the probes were contaminated, particularly in the water-wash and dry-wipe groups. Bacteria isolated from contaminated probes in order of frequency were coagulase negative staphylococci (44%), *Bacillus cereus* (20%), *Staphylococcus aureus* (10%), *Enterobacter cloaca* (7%), *Pseudomonas aeruginosa* (4%), amongst others. With the exception of *Bacillus cereus*, all the cultured organisms pose significant infectious risk, contributing wholly or in part to certain postoperative morbidity and mortalities.

Assessment of postoperative complications was not the objective of this study, however the high contamination rates of the decontaminated probes and the types of pathogens cultured raise serious concern, especially when one considers the incidence of immune impairment amongst the population serviced in Africa, coupled with the immunosuppressive effects of surgery and anaesthesia on the host immune system (20-23). Patients

with HIV/AIDS, diabetes mellitus and various oncological and autoimmune conditions are particularly at risk of infection from the use of with these ineffective cleaning procedures (24). Although, limited by small sample size, the present study highlights nasopharyngeal temperature probes as possible source of cross-contamination, and cautions against the use of non-standardized decontamination processes.

Conclusion:

A high theatre demand, heavy patient burden and financial constraints are important considerations when reviewing the non-compliance with infection control guidelines. These factors have led to application of non-recommended cleaning techniques which pose significant threat to patient health and safety. The findings of our study show that decontaminated nasopharyngeal temperature probes can indeed be a source of cross-infection and pathogen transmission, due to inadequacy of the decontamination processes for these temperature probes. The study demonstrates a greater than 90% decontamination rate following the use of cidex®, a practice in keeping with international literature which supports high-level disinfection for these semi-critical devices.

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Conflict of interest:

Authors declare no conflict of interest

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Original Article

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High faecal carriage of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-PE) among hospitalized patients at Sylvanus Olympio Teaching Hospital, Lomé, Togo in 2019

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Abstract:

Background: Extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-PE) are a global health concern, associated with increased morbidity and mortality. Even in the absence of infections, colonization by these pathogens is still a great threat because of the risk of cross transfer among hospitalized patients. Faecal carriage of ESBL-PE remained poorly documented in Africa. This study aimed to determine faecal carriage rate of ESBL-PE, factors associated with carriage, and antimicrobial susceptibility of the strains among hospitalized patients at Sylvanus Olympio Teaching Hospitals (CHU SO) in Lomé, Togo.

Methodology: This was a cross-sectional study of 105 randomly selected hospitalized patients between September and November 2019. Socio-demographic and clinical data as well as rectal swabs were collected after obtaining the consent of the selected participants. Rectal swabs were cultured on selective bromocresol purple (BCP) lactose agar containing 6µg/l ceftazidime, for isolation of Enterobacteriaceae. Identification of each isolate was performed using Uriselect 4 medium and API 20E. Antibiotic susceptibility of the bacterial isolates was performed by the Bauer-Kirby agar disc diffusion test and interpreted according to CASFM-EUCAST recommendations.

Results: The faecal carriage rate of ESBL-PE among selected hospitalized patients was 80.9% (85/105). *Escherichia coli* was the most frequent bacteria 69.5% (73/105), followed by *Klebsiella pneumoniae* 22.8% (24/105). The antibiotic profile of ESBL producing *Escherichia coli* showed resistance to amoxycillin+clavulanic acid (72.6%), ticarcillin+clavulanic acid (82.2%), piperacillin+tazobactam (30.1%), cefoxitin (30.1%) ciprofloxacin (84.9%), levofloxacin (76.7%), nalidixic acid (83.6%), chloramphenicol (26.0%), gentamicin (49.3%), sulfamethoxazole-trimethoprim (86.3%), imipenem (5.5%), and ertapenem (21.9%). All (100%) isolates were sensitive to amikacin and fosfomycin. None of the characteristics or risk factors assessed was significantly associated with faecal carriage of ESBL-PE.

Conclusion: Faecal carriage rate of ESBL-PE in these hospitalized patients was very high, but no factor was associated with carriage of ESBL-PE among the study population. Implementation of infection control measures, and surveillance are needed to limit the spread of these resistant pathogens within CHU SO healthcare facilities.

Keywords: Faecal carriage; ESBL; Enterobacteriaceae; hospitalized patients; Togo

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Fort taux de portage fécal d'Entérobactéries productrices de bêta-lactamases à spectre élargi (E-BLSE) chez les patients hospitalisés au CHU Sylvanus Olympio de Lomé, Togo en 2019

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Résumé:

Contexte: Les entérobactéries productrices de bêta-lactamases à spectre élargi (E-BLSE) constituent un problème mondial de santé associé à une morbidité et une mortalité accrue. Même en l'absence d'infections, la colonisation par ces pathogènes reste une grande menace en raison du risque de transfert croisé entre les patients hospitalisés. Le portage fécal d'E-BLSE est peu documenté en Afrique. Cette étude vise à estimer le taux de portage fécal des E-BLSE, à déterminer les facteurs associés à leur portage et la sensibilité aux antibiotiques des souches chez les patients hospitalisés au Centre Hospitalier Universitaire Sylvanus Olympio (CHU SO) de Lomé, Togo.

Méthodologie: Il s'est agi d'une étude transversale portant sur 105 patients hospitalisés sélectionnés au hasard entre Septembre et Novembre 2019. Les données sociodémographiques, cliniques et des prélèvements rectaux par écouvillonnage ont été collectés, après obtention du consentement des participants. Pour l'isolement des entérobactéries, les écouvillonnages rectaux ont été ensemencés sur une gélose sélective (BCP) contenant 6 µg/l de ceftazidime. L'identification de chaque isolat s'est faite en utilisant le milieu Uriselect 4 et la galerie API 20E. L'étude de la sensibilité aux antibiotiques des isolats bactériens a été réalisée par la technique de diffusion des disques en milieu gélosé (Kirby-Bauer) et interprétée selon les recommandations du CASFM-EUCAST.

Résultats: Le taux de portage fécal des E-BLSE chez les patients hospitalisés était de 80,9% (85/105). *Escherichia coli* a été la bactérie la plus isolée 69,5% (73/105), suivie de *Klebsiella pneumoniae* 22,8% (24/105). Les souches d'*Escherichia coli* productrices de BLSE ont montré une résistance à l'amoxycilline+acide clavulanique à (72,6%), ticarcilline+acide clavulanique (82,2%), pipéracilline+tazobactam (30,1%), céfoxitine (30,1%) ciprofloxacine (84,9%), lévofloxacine (76,7%), acide nalidixique (83,6%), chloramphénicol (26,0%), gentamicine (49,3%), sulfaméthoxazole-triméthoprim (86,3%), imipénem (5,5%) et ertapénème (21,9%). Tous les isolats (100%) étaient sensibles à l'amikacine et à la fosfomycine. Nous n'avons pas retrouvé des caractéristiques ou des facteurs de risque associés au portage fécal des E-BLSE.

Conclusion: Le taux de portage fécal des E-BLSE chez ces patients hospitalisés était très élevé, mais aucun facteur n'était associé à leur portage. La mise en œuvre de mesures de contrôle et de prévention des infections et la surveillance sont nécessaires pour limiter la propagation de ces pathogènes résistants au sein du CHU SO voire des établissements de santé.

Mots-clés: Portage fécal; BLSE; Entérobactéries; patients hospitalisés; Togo

Introduction:

Extended spectrum β -lactamase producing Enterobacteriaceae (ESBL-PE) are among the most important pathogens causing infections in both the community and hospital settings in recent years (1,2). Even in the absence of infection, colonization with the extended-spectrum β -lactamase (ESBL) producing bacteria is a strong cause for concern (3,4). Among them, *Klebsiella pneumoniae* and *Escherichia coli* represent the most frequently isolated ESBL-PE worldwide but the ESBLs are also identified in several other bacterial species (5).

In the hospital settings, gastrointestinal carriage of ESBL is the main reservoir of these organisms, and is associated with high risk for developing self and cross infections (6). This is a global issue because infections caused by ESBL producing clinical isolates are associated with high mortality, increased length of hospital stays and health care cost, and longer duration of antibiotic therapy, when compared to infections caused by non-ESBL producing pathogens (7). Furthermore, ESBL-PE pose significant therapeutic challenge in daily management of infectious diseases due to their resistance to additional

classes of antibiotics, thereby reducing the effectiveness of alternative antimicrobial regimens (8,9). The co-existence of multiple ESBLs in the same clinical strain including AmpC beta-lactamases, carbapenemases and other antibiotic resistance plasmid determinants further creates therapeutic challenges (10).

Faecal carriage of ESBL-PE has been increasingly reported worldwide over the last decade, with the highest ESBL-PE carriage rates being reported in Asia (11), while carriage rates are lower in Europe and North America (12,13). Some studies in Africa have investigated and reported faecal carriage rates of ESBL-PE in the community and in hospitalized patients in Chad, Mauritania, Cameroon, Ethiopia, and Burkina Faso (14-18).

In Togo, some researchers have described the phenomenon of bacterial resistance due to ESBLs in the hospital environment from laboratory stains but carriage of ESBL among patients have not been investigated (19,20). The objectives of this study therefore, are to investigate faecal carriage of ESBL-PE among hospitalized patients, determine factors associated with their carriage, and perform antimicrobial suscepti-

bility on isolated ESBL-PE strains in Sylvanus Olympio Teaching Hospital in Lomé, Togo.

Materials and method:

Study area

The Sylvanus Olympio Teaching Hospital (CHU SO) is located in Gulf 4 municipality of big Lomé autonomous district (1.5 million inhabitants). It is a national benchmark establishment, with a capacity of 1,138 beds. CHU SO is the largest University affiliated teaching hospital in Togo, where patients from different parts of the country are referred for further management. The various technical wards are functionally and administratively organized into seven departments; medicine and medical specialties, surgery and surgical specialties, paediatrics, gynaecobstetrics, laboratories, medical imaging, preventive medicine and public health. The samples were analysed at the bacteriology laboratory of the hospital.

Study design and participants

This was a cross-sectional study conducted from September to November 2019 on 105 randomly selected volunteer hospitalized patients after obtaining their consent to participate in the study. Inclusion criteria for study participants were all hospitalized patients including children and adult patients who have been admitted for ≥ 48 hours, and who consented to participate in the study. Participants were selected from eight hospitalization wards; military flag, medicine, neurology, polyvalent reanimation, gynaecology and obstetrics, traumatology, visceral surgical, and paediatrics.

Ethical approval

Ethical approval for the study was obtained from the Scientific and Ethical Committee of the Health Research Ethics Committee (048/2019/CBRS) of Health Ministry of Togo. Written informed consent of each adult participant and from parent or guardian of children were obtained. The confidentiality of data obtained was assured.

Data and sample collection

Socio-demographic data and risk factors were collected from patients using a self-administered questionnaire. The socio-demographic information collected included age, gender, place of residence, and education level, while information on risk factors for carriage of ESBL included length of hospitalization, hospitalization in the previous year, and antimicrobial use in the previous three months before the study. Rectal sample was collected from each participant using a cotton swab, and transported to the laboratory within 30 minutes of collection.

Bacteriological analysis

Briefly, each rectal swab was placed in an enrichment broth (thioglycolate broth) and incubated for 5 hours at 37°C. A loop-full culture from enrichment broth was streaked onto selective BCP lactose agar containing 6 µg/l cefotaxime, and incubated aerobically at 37°C for 18-24 hours.

ESBL-producing strains of Enterobacteriaceae which grow on selective BCP agar were selected according to their morphological appearance and re-isolated on Uriselect 4 medium for identification while non-ESBL-producing strains will not grow on this selective medium. *Escherichia coli* appeared pink in colour on Uriselect medium. Other isolates were identified to species level using commercial API 20E gallery (BioMérieux, France).

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing of Enterobacteriaceae isolates was performed using the Kirby Bauer disk diffusion method on Mueller Hinton agar following standard zone size interpretative criteria recommended by the 2019 CASFM-EUCAST (European Committee of Antibiotic Susceptibility Testing) (21). Four identical colonies from overnight culture on Mueller-Hinton (MH) medium was picked using sterile Pasteur loop and emulsified in 5 ml of sterile normal saline and the inoculum density was adjusted to equal that of 0.5 McFarland turbidity standards ($\sim 1.5 \times 10^8$ CFU/ml). Using a sterile swab, the inoculum was spread on Mueller Hinton agar and allowed to dry. Antimicrobial discs (Oxoid, UK) were placed on the medium 30 mm apart and 15 mm away from the edge of the plate, and incubated at 37°C for 24 hours to obtain a lawn culture growth. The inhibition zone diameter (in mm) was measured with a calibrated ruler and interpreted according to the recommendations of 2019 EUCAST (21).

The isolates were tested against a panel of 21 antimicrobials in different classes that are commonly used to treat human bacterial infections, and includes; ampicillin 10µg, ticarcillin 75µg, amoxicillin+clavulanic acid 30 µg, ticarcillin+clavulanic acid 85µg, cefoxitin 30µg, piperacillin 30µg, piperacillin+tazobactam 36µg, ceftazidime 10µg, ceftriaxone 30 µg, aztreonam 30µg, cefepime 30µg, imipenem 10µg, ertapenem 10µg, amikacin 15µg, gentamicin 10µg, chloramphenicol 30µg, sulfamethoxazole-trimethoprim 25µg, nalidixic acid 30µg, ciprofloxacin 5µg, levofloxacin 5 µg, and fosfomycin 200µg. A standard reference strain of *Escherichia coli* ATCC25922 (which is sensitive to all the tested antimicrobial agents) was used as control strain.

Detection of ESBL by double disk method

All isolated strains of Enterobacteriaceae were tested for ESBL production by the

double disk method (21). Combination disks of amoxicillin+clavulanic acid (20+10µg) or ticarcillin+clavulanic acid (75+10µg) was applied to the center of Mueller-Hinton agar that has been inoculated with the test strain. Ceftazidime (10µg) and cefepime (30µg) disks frame the ticarcillin+clavulanic acid disk 30 mm apart from each other and 15 mm from the edge of the plate. After 18-24 hours of incubation at 37°C, isolate that showed increase of ≥ 5 mm in the zone of inhibition of the combination disks in comparison to that of the ceftazidime and cefepime disk was considered an ESBL. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control strains, respectively.

Statistical analysis of data

Data were entered into Excel software. Statistical analysis was performed using SPSS software (IBM SPSS Statistics 21, Armonk, NY). Chi-square test at 5% level of significance was used to assess the prevalence differences. Multivariate logistic regression analyses were performed to investigate the risk factors associated with ESBL-PE carriage including calculation of the Odds ratio (OR) and 95% Confidence Interval (CI). A threshold of p value < 0.05 was considered significant for multivariate analyses, and also if OR is > 1 and value of 1 is not included in the CI range.

Results:

Socio-demographic and clinical characteristics of study participants

Of the total of 105 participants, 46 (43.8%) were males while 59 (56.2%) were females. The age of the participants ranged from 1 month to 80 years with a median age of 40 years. A total of 49 (46.7%) participants had secondary school education, 65 (61.9%) had been hospitalized for more than 7 days, 14 (13.3%) had been hospitalized in the year preceding current hospitalization, and 47 (44.8%) had used antibiotics within 3 months preceding hospitalization (Table 1).

Carriage rate of Enterobacteriaceae isolates

Escherichia coli (69.5%, $n=73$), followed by *K. pneumoniae* (22.9%, $n=24$) and *Enterobacter cloacae* (3.8%, $n=4$) were the most frequently isolated Enterobacteriaceae

from the rectal specimens (Fig. 1). The carriage rate of ESBL-producing Enterobacteriaceae was 80.9% ($n=85$) of the 105 hospitalized patients while 19.1% ($n=20$) were carrier of non-ESBL-producing Enterobacteriaceae. In 19 (22.4%) patients who carried ESBL-PE, association of two strains (21.2%) and three ESBL bacteria was found, with *E. coli* and *K. pneumoniae* association being the most frequent (Table 2).

Carriage rate of ESBL-PE according to the hospitalization department

The carriage rate of ESBL-PE by ward in the hospital is shown in Table 3. Carriage rates were highest among patients in polyvalent intensive care unit (100%) and in the traumatology and medicine wards, with over 90% ESBL-PE carriage rate each.

Factors associated with carriage of ESBL-PE

Statistical analysis in Table 1 showed that no socio-demographic characteristic (gender, age group, educational level) or risk factor (length of hospitalization, previous hospitalization, use of antibiotics in preceding 3 months), was significantly associated with carriage of ESBL-PE in the study population ($p>0.05$ for all analyses).

Resistance profiles of isolates

The antimicrobial resistance profile of ESBL-producing *E. coli*, *K. pneumoniae*, and *E. cloacae* showed that they were 100% resistant to ampicillin, ticarcillin, piperacillin, ceftazidime, ceftriaxone, aztreonam and cefepime. *Escherichia coli* isolates showed high resistance of over 70% to amoxycillin+clavulanic acid (72.6%), ticarcillin+clavulanic acid (82.2%), sulfamethoxazole - trimethoprim (86.3%), nalidixic acid (83.6%), ciprofloxacin (84.9%) and levofloxacin (76.7%). However, resistance rate of *E. coli* isolates was less than 50% to piperacillin+tazobactam (30.1%), ceftazidime (30.1%), chloramphenicol (26.0%), gentamicin (49.3%), imipenem (5.5%), and ertapenem (21.9%). *Klebsiella pneumoniae* isolates showed high resistance of over 50% to piperacillin+tazobactam (66.7%), ciprofloxacin (91.7%), levofloxacin (79.2%), nalidixic acid (54.2%), chloramphenicol (54.2%), gentamicin (95.8%), and sulfamethoxazole-trimethoprim (88.9%), but low resistance to ceftazidime (20.8%). However, both *E. coli* and *K. pneumoniae* isolates were 100% sensitive to amikacin and fosfomycin (Table 4).

Table 1: Socio-demographic and clinical characteristics of participants with respect to carriage of Enterobacteriaceae

Characteristics	ESBL carriers n (%)	Non-ESBL carriers n (%)	p value	χ^2	OR (95% CI)
Age group (years)					
< 20	21 (25.0)	3 (15.0)	0.6437	3.366	NA
20-29	11 (13.0)	3 (15.0)			
30-39	11 (13.0)	1 (5.0)			
40-49	13 (15.0)	5 (25.0)			
50-59	10 (12.0)	4 (20.0)			
≥ 60	19 (22.0)	4 (20.0)			
Gender					
Female	47 (55.0)	12 (60.0)	0.8956	0.01721	0.8246 (0.3058 - 2.223)
Male	38 (45.0)	8 (40.0)			
Education level					
Less than Secondary	44 (52.0)	12 (60.0)	0.6780	0.1723	0.7154 (0.2656 - 1.927)
Secondary and above	41 (48.0)	8 (40.0)			
Length of hospitalization					
≤ 7 days	29 (34.0)	11 (55.0)	0.1404	2.174	0.4237 (0.1576 - 1.139)
> 7 days	56 (66.0)	9 (45.0)			
Previous hospitalization in preceeding year					
No	75 (88.0)	16 (80.0)	0.5424	0.3712	1.875 (0.5217 - 6.739)
Yes	10 (12.0)	4 (20.0)			
Antimicrobial use in the previous 3 months					
No	45 (53.0)	13 (65.0)	0.4679	0.5269	0.6058 (0.2200 - 1.668)
Yes	40 (47.0)	7 (35.0)			

χ^2 =Chi square; OR=Odds Ratio; NA=Not Applicable; n=no of participants; CI=Confidence Interval; ESBL=Extended Spectrum Beta Lactamase

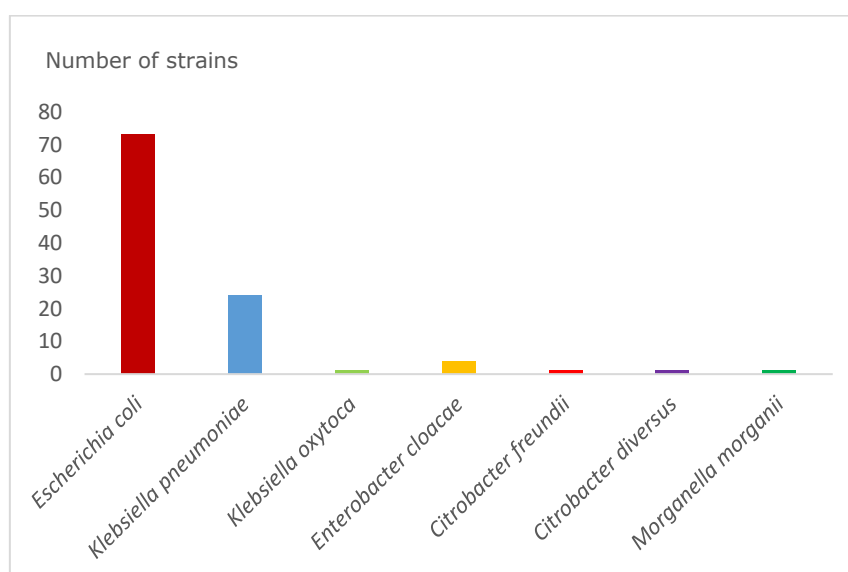


Fig 1 : Frequency distribution of Enterobacteriaceae isolates among hospitalized patients at Sylvanus Olympio Teaching Hospital in Lomé, 2019

Table 2: Multiple Enterobacteriaceae isolates in hospitalized patients at Sylvanus Olympio Teaching Hospital, Lomé, 2019

Bacteria	N	Percentage
<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i>	10	11.8
<i>Escherichia coli</i> , <i>Escherichia coli</i>	3	3.5
<i>Escherichia coli</i> , <i>Enterobacter cloacae</i>	1	1.2
<i>Escherichia coli</i> , <i>Morganella morganii</i>	1	1.2
<i>Escherichia coli</i> , <i>Citrobacter diversus</i>	1	1.2
<i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i>	1	1.2
<i>Klebsiella pneumoniae</i> , <i>Citrobacter freundii</i>	1	1.2
<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter cloacae</i>	1	1.2

N=no of hospitalized patients

Table 3: Distribution of ESBL-PE carriage rate in hospitalized patients at Sylvanus Olympio Teaching Hospital in 2019

Ward/unit	ESBL producing Enterobacteriaceae			
	Negative		Positive	
	N	%	N	%
Military	6	46	7	54
Medicine	1	8.3	11	91.7
Neurology	4	30.8	9	69.2
Polyvalent reanimation	0	0	7	100
Gynaecology	3	20.0	12	80.0
Traumatology	1	7.1	13	92.9
Visceral surgical	2	16.7	10	83.3
Paediatric	3	15.8	16	84.2

N=no of hospitalized patients; ESBL Extended Spectrum Beta-Lactamase

Discussion:

Colonisation with ESBL-PE is a risk factor for ESBL-PE infections, which are associated with difficulty in initiating effective treatment, prolonged hospitalization, increased healthcare costs, morbidity and mortality (22). Our study recorded high faecal carriage rate (80.9%) of ESBL-PE in hospitalized patients in Lomé, Togo, which agrees with reports of high intestinal carriage of ESBL-PE in sub-Saharan Africa countries such as Burkina Faso (70%), Chad (51%), and Tanzania (59.7%) (14,18,23,24). Desta et al., (17) in Ethiopia and Kurz (15) in Rwanda also reported high colonisation rates of 52% and 50% respectively in their studies. The findings of our study and those of others could be explained by high antibiotic consumption that favors selection of antibiotic resistant bacteria (ARB) and/or high rate of in-hospital acquisition of ARB that then colonize the patient's intestinal tract (26,27). Several factors can contribute to high ARB selection in low-income countries, particularly from poor drug quality or inadequate posology, long treatment, increase antimicrobial use by healthcare professionals, unskilled practitioners, auto-medication (because antibiotics can be purchased without prescription), poor hygiene, resulting in the spread of resistant bacteria, and inadequate surveillance programs (28-30).

Escherichia coli was the most frequently isolated ESBL-PE with 69.5% followed by *K. pneumoniae* 22.9%, which is similar to

the findings from a study conducted in Chad (14) which reported ESBL-producing *E. coli* of 69% and *K. pneumoniae* of 21%, and another study by Desta et al., (17) in Ethiopia, which reported 68% for *E. coli* and 32% for *K. pneumoniae*. Our attempt at investigating factors associated with faecal carriage of ESBL-PE among the subjects revealed that none of the characteristics or risk factors assessed was significantly associated with carriage. This finding differs from those of previous studies conducted in France (31) in which length of hospitalization and high antimicrobial consumption were identified risk factors, and that of Rubio-Perez in Spain (26) who also reported that previous hospitalization and antibiotic treatment were risk factors associated with faecal carriage of ESBL-PE among their patients.

In our study, most ESBL-PE isolates were resistant to three or more classes of antimicrobials, which included mainly quinolones, aminoglycosides (except amikacin), and co-trimoxazole (trimethoprim-sulfamethoxazole). We noticed high resistance rates of *E. coli* isolates to sulfamethoxazole-trimethoprim, nalidixic acid and fluoroquinolones but low resistance rates to chloramphenicol (26.0%) and complete susceptibility (100%) to amikacin and fosfomycin. Cross-resistance of ESBLs to other drug classes such as aminoglycosides and fluoroquinolones has been previously documented (32,33). Findings from our study corroborated the Ethiopia study (17), which reported high co-resistance pattern to the to the antibiotic classes in all

Table 4: Resistance profile of isolated strains from hospitalized patients in Sylvanus Olympio Teaching Hospital in Lomé, 2019

Antimicrobial class	Antimicrobial disk	Percentage of isolates resistant		
		<i>Escherichia coli</i> (n = 73)	<i>Klebsiella pneumoniae</i> (n = 24)	<i>Enterobacter cloacae</i> (n = 4)
Beta – lactams	Ampicillin (10µg)	100	100	100
	Amoxicillin-Clavulanic acid (20-10µg)	72.6	0	100
	Ticarcillin (75µg)	100	100	100
	Ticarcillin-Clavulanic acid (75-10µg)	82.2	0	100
	Piperacillin (30µg)	100	100	100
	Piperacillin-Tazobactam (36µg)	30.1	66.7	75
	Cefoxitin (30µg)	30.1	20.8	75
	Ceftazidime (10µg)	100	100	100
	Ceftriaxone (30µg)	100	100	100
	Cefepime (30µg)	100	100	100
	Aztreonam (30µg)	100	100	100
	Imipenem (10µg)	5.5	8.3	25
	Ertapenem (10µg)	21.9	29.2	50
Aminoglycosides	Amikacin (30µg)	0	0	0
	Gentamicin (10µg)	49.3	95.8	100
Sulfonamides and associates	Sulfamethoxazole-Trimethoprim (27.75/1.25µg)	86.3	100	100
Phenicol	Chloramphenicol (30µg)	26.0	54.2	75
Quinolones and Fluoroquinolones	Nalidixic Acid (30µg)	83.6	54.2	25
	Ciprofloxacin (5µg)	84.9	91.7	50
	Levofloxacin (5µg)	76.7	79.2	25
Other family	Fosfomycin (200µg)	0	0	25

tested isolates.

We also noticed resistance to carbapenems; imipenem (5.5%) and ertapenem (21.9%). Desta et al., (17) in Ethiopia similarly reported resistance to carbapenem in their study. Resistance to carbapenems can be explained by the increased use of this newer generation antimicrobials for the treatment of ABR infections caused by ESBL-PE in hospitals in the event of a therapeutic impasse. Treatment of severe infections caused by ESBL producing *E. coli* and *K. pneumoniae* in hospitalized patients rely on carbapenems, which are the last resort antibiotics reserved

for treatment of life-threatening infections. Infections caused by carbapenemase-producing Enterobacteriaceae (CPE) are most difficult to manage and are associated with high mortality rates (34).

Patients colonized with ESBL are at increased risk for invasive infections compared with non-colonized patients (35). If hand hygiene is not applied as recommended, these resistant bacteria can spread from one patient to another through contaminated hands of healthcare workers. In the hospital setting, identification of patients colonized or infected with ESBL and adoption of preven-

tive measures such as hand hygiene, and isolation in a single-patient room, is important to prevent cross-transmission and reduce morbidity and healthcare costs (36). Result from our study provides a baseline data necessary to conduct larger studies involving molecular characterization of resistant strains to identify the source, types and the pattern of spread of antimicrobial resistance in Sylvanus Olympio Teaching Hospital.

Conclusion:

This study revealed high faecal carriage rate of ESBL-PE among hospitalized patients in Sylvanus Olympio Teaching Hospital, Lome, Togo, especially among those admitted into polyvalent reanimation unit. None of the characteristics or risk factors assessed was significantly associated with faecal carriage of ESBL-PE. Routine infection prevention strategies such as rational use of antimicrobial agents, compliance with hand hygiene practices and surveillance of antimicrobial resistance (AMR) are urgently needed to prevent and control the spread of AMR pathogens in healthcare facilities.

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Original Article

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Faecal carriage of multi-drug resistant Enterobacteriaceae in hospitalized children at University Teaching Hospital Sylvanus Olympio of Lomé, Togo

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Abstract:

Background: High prevalence of infections and associated antibiotherapy may put children at increased risk for development of multidrug-resistance (MDR), mostly to bacterial infections. The objective of this study therefore was to determine the prevalence of gastrointestinal carriage of MDR Enterobacteriaceae among hospitalized children in the Paediatric department of Sylvanus Olympio University Hospital, Lomé, Togo.

Methodology: A descriptive cross-sectional study was carried out on randomly selected hospitalized children in the Paediatric wards of the hospital from November 03 to November 10, 2020, after obtaining informed consent from their parents/guardians. Rectal swabs and stool samples were collected from each participant and cultured for isolation of members of the family Enterobacteriaceae on Hektoen enteric agar containing 4 µg/L cefotaxime, which was incubated aerobically at 37°C for 24 hours. The isolates were identified using in-house biochemical tests. Antibiotic susceptibility test (AST) of each isolate to a panel of antibiotics including ertapenem and imipenem was done by the disc diffusion method and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints version 2020 V.1.1. ESBL production was detected by the double-disc synergy test of amoxicillin and clavulanic acid, and resistance to carbapenem was inferred by resistance to ertapenem and imipenem discs in the AST. Multi-drug resistance (MDR) was defined as resistance to at least three families of antibiotics. Statistical analysis of data was carried out on Excel 2010 and EPI INFO 7.2 and *p* value < 0.05 was considered to be statistical significance.

Results: A total of 70 hospitalized children during the study period were randomly recruited with an average age of 4 years 3 days and a range of 1 day to 18 years. The male gender was predominant (54.3%) with a M: F ratio of 1.2. Samples were culture positive in 85.7% (60/70) and a total of 72 species of Enterobacteriaceae were isolated in 93.3% (56/60) of these cultures. *Escherichia coli* was the most frequently isolated species (56.9%, 41/72). More than 90% (65/72) of the isolates were resistant to ampicillin, 58.3% (42/72) to third generation cephalosporins, 59.7% (43/72) to fourth generation cephalosporins, 43% (31/72) to aminoglycosides, 55.6% (40/72) were multi-drug resistant, 48.6% (35/72) were ESBL-producing strains, and 6.9% (5/72) were carbapenem resistant. Eighty-three percent (29/35) of ESBL-producing and all the carbapenem resistant isolates (5/5) were recovered from children on antibiotic therapy. The prevalence of ESBL-producing phenotypes among culture-positive children on antibiotic treatment (72.5%, 29/40) was significantly higher than among culture-positive children not on antibiotic treatment (20.0%, 4/20), indicating that antibiotic therapy was significantly associated with carriage of MDR isolates (OR=10.545, 95% CI=2.882-38.590, *p*=0.0002).

Conclusion: The high faecal carriage rate of MDR Enterobacteriaceae, which are all ESBL-producing strains, in this study is worrying. There is urgent need to develop measures to monitor and limit the spread of these MDR organisms in children and the community in Togo.

Keywords: children; Enterobacteriaceae; multidrug-resistance; faecal carriage; Togo

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Portage d'entérobactéries résistantes aux antibiotiques chez des enfants hospitalisés au CHU Sylvanus Olympio de Lomé, Togo

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Résumé:

Contexte: La prévalence élevée des infections associée à une antibiothérapie excessive peuvent exposer les enfants à un risque accru de développer des bactéries multirésistantes (BMR). L'objectif de cette étude était de déterminer la prévalence du portage digestif des entérobactéries multirésistantes aux antibiotiques chez les enfants hospitalisés dans le service de pédiatrie du CHU Sylvanus Olympio, Lomé, Togo.

Méthodologie: Une étude transversale descriptive a été réalisée sur des enfants hospitalisés et sélectionnés au hasard dans le service de pédiatrie du CHU Sylvanus Olympio du 03 Novembre au 10 Novembre 2020. Après avoir obtenu le consentement éclairé des parents/tuteurs, des écouvillons rectaux et des échantillons de selles ont été prélevés sur chaque participant et cultivés pour l'isolement des entérobactéries sur la gélose Hektoen contenant 4 µg/L de céfotaxime. Le milieu ensemencé a été incubé en aérobie à 37°C pendant 24 heures. Les isolats ont été identifiés à l'aide de tests biochimiques internes. Le test de sensibilité aux antibiotiques (Antibiogramme par la méthode de diffusion sur disque) de chaque isolat a fait appel à un panel d'antibiotiques comprenant l'ertapénème et l'imipénème a été effectué. Les résultats ont été interprétés selon les recommandations du Comité Européen sur les Tests de Sensibilité aux Antimicrobiens (EUCAST) version 2020 V.1.1. La production de BLSE a été détectée par le test de synergie à double disque d'amoxicilline et d'acide clavulanique, et la résistance aux carbapénèmes a été déduite par la résistance aux disques d'ertapénème et d'imipénème dans l'AST. La multirésistance aux antibiotiques (BMR) a été définie comme une résistance à au moins trois familles d'antibiotiques. L'analyse des données a été réalisée sur Excel 2010 et EPI INFO 7,2 et la valeur $p < 0,05$ a été considérée comme statistiquement significative.

Résultats: Un total de 70 enfants hospitalisés au cours de la période d'étude ont été recrutés au hasard avec un âge moyen de 4 ans 3 jours avec des extrêmes de 1 jour à 18 ans. Le sexe masculin était prédominant (54,3%) avec un rapport M: F de 1,2. Les échantillons étaient positifs en culture dans 85,7% (60/70) et un total de 72 espèces d'entérobactéries ont été isolées dans 93,3% (56/60) de ces cultures. *Escherichia coli* était l'espèce la plus fréquemment isolée (56,9%, 41/72). Plus de 90% (65/72) des isolats étaient résistants à l'ampicilline, 58,3% (42/72) aux céphalosporines de troisième génération, 59,7% (43/72) aux céphalosporines de quatrième génération, 43% (31/72) aux aminosides, 55,6% (40/72) étaient multirésistants. Par ailleurs, 48,6% (35/72) étaient des souches productrices de BLSE et 6,9% (5/72) étaient résistants aux carbapénèmes. Quarante-trois pour cent (29/35) des isolats producteurs de BLSE et tous les isolats résistants aux carbapénèmes (5/5) ont été isolés chez des enfants sous antibiothérapie. La prévalence des phénotypes BLSE chez les enfants à culture positive sous traitement antibiotique (72,5%, 29/40) était significativement plus élevée que chez ceux à culture positive ne bénéficiant pas d'une antibiothérapie (20,0%, 4/20), indiquant que l'antibiothérapie était significativement associée au portage d'isolats de BMR (OR=10,545, IC à 95%=2,882-38,590, $p=0,0002$).

Conclusion: Le taux de portage fécal élevé d'entérobactéries BLSE, multirésistantes chez les enfants hospitalisés, dans cette étude est préoccupant. Il est urgent de développer des mesures pour surveiller et limiter la propagation de ces BMR chez les enfants et la communauté au Togo.

Mots-clés: enfants; Entérobactéries; multirésistance; transport fécal; Togo

Introduction:

Antibiotic resistance is a natural phenomenon but misuse of antimicrobial drugs in humans and animals can accelerate the process (1). Antibiotic resistance is now one of the most serious threats to global health, food security and development. It can affect anyone, at any age and in any country (1). Infections caused by multidrug-resistant (MDR) bacteria are a major public health problem worldwide. According to the 2014 World Health Organization (WHO) published report, the proportion of resistance of

Escherichia coli to third generation cephalosporins (3GC) reached 70% in the WHO Africa region, 82% in Europe, and up to 95% in the South-East Asia, and the proportion of resistance to fluoroquinolones was up to 98% (2). Resistance to amoxicillin or ampicillin has also remained very high at 100% of isolated strains (3,4).

Resistance rates of various bacterial pathogens progress over time and vary from one country to another (5,6). Data on the intestinal carriage rate of resistant bacteria in children, who are mostly at risk due to frequent infection and antibiotherapy,

are lacking in Togo. The objective of this study therefore is to determine the faecal carriage rate of MDR Enterobacteriaceae among children in the Paediatric hospital setting at the Sylvanus Olympio University Hospital in Lomé, Togo.

Materials and method:

Study setting and design

The study was conducted in the Paediatric Department and the Microbiology Laboratory of the CHU-Sylvanus Olympio (CHU-SO) of Lomé, Togo. It was a descriptive cross-sectional study conducted during the period November 03 to November 10, 2020.

Study participants and sample collection

All children hospitalized without any digestive pathologies in the Paediatric department of the hospital whose parents gave informed consent, were randomly recruited into the study. In newborns, infants and younger children, rectal swabs were used to collect samples, while for older children and adolescents, stool cultures were collected into sterile jar containers. A pre-tested survey form was used to collect demographic (age, gender) and clinical data (diagnosis, use of antibiotic, type of antibiotic therapy, duration of hospitalization at time of collection, hospitalization unit etc).

Microbiological culture isolation & bacterial identification

All the rectal and stool samples were immediately seeded on Brain Heart Infusion (BHI) broth and incubated for 5 hours at 37°C to improve bacteriological yield (7). After this enrichment phase, 100 µL of the broth was transferred to Hektoen enteric agar (Oxoid, UK) supplemented with 4 µg/L cefotaxime and incubated at 37°C for 24 hours. Predominant colonies of different morphotypes were identified to species level by using in-house biochemical test panels including triple sugar iron (TSI) agar, sulfur-indole-motility, Simmon citrate agar and urease tests.

Antibiotic susceptibility test (AST)

Antimicrobial susceptibility test (AST) was performed on each isolate by the agar disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints version 2020 V.1.1 recommendations. Antibiotic discs used include; penicillins (ampicillin, amoxicillin + clavulanic acid, piperacillin, ticarcillin), cephalosporins (cefalotin, cefoxitin, cefotaxime, ceftazidime, cefepime), carbapenem (imipenem, ertapenem), monobactam (aztreonam), flu-

oroquinolones (nalidixic acid, ciprofloxacin, ofloxacin), aminoglycosides (amikacin, gentamicin), phenicol (chloramphenicol), sulfonamide (trimethoprim) and fosfomycin. *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Detection of ESBL production

ESBL production was detected by the presence of synergy between the third generation cephalosporins and the inhibitor (clavulanic acid) (8–10). In case of high-level cephalosporinase production, the combined double-disc synergy test was performed using cloxacillin-supplemented agar medium.

Statistical analysis

Statistical analysis of data was carried out on Excel 2010 and EPI INFO 7.2 software. Qualitative variables were compared using Chi-square or Fisher's exact test (when an expected value was less than 5) while continuous variables were compared using Students' 't' test. A *p* value < 0.05 was considered to be statistical significance.

Results:

Socio-demographic and clinical characteristics of the children

A total of 70 children were included, the average age of the patients was 4 years 3 days with a range of 1 day to 18 years. Infants (age < 1 years) were the most frequently represented age group (31.4%, *n* = 22). Male to female ratio was 1.2. Majority of the children were hospitalized in the intensive care units (ICU), with average length of hospitalization of 43 days, and a range of 1 day to 360 days. More than half of the children (57.1%, *n* = 40) were on antibiotic therapy, and the most commonly used antibiotic was the combination of cephalosporin and gentamicin 67.5% (*n* = 27).

Frequency distribution of bacteria isolates:

Out of the 70 samples analyzed, 60 (85.7%) cultures were positive and 10 (14.3%) were negative. Enterobacteriaceae were isolated in 93.3% (56/60) of the 60 positive cultures, and 72 species of Enterobacteriaceae were isolated with *Escherichia coli* being the most frequently isolated species (57.0%) followed by *Klebsiella pneumoniae* (26.0%) (Fig.1).

Antibiotic susceptibility patterns of isolates

High resistance rates of all isolated species (>80%) were obtained against ampicillin, followed by third generation cephalosporins (58.3%), while low resistance rate was exhibited to imipenem (Table 1). The

prevalence of multi-drug resistance (MDR) among the Enterobacteriaceae isolates was 55.6% (40/72), 48.6% (35/72) were ESBL-producing, and 6.9% (5/72) were resistant to carbapenems.

The frequency distribution of resistance phenotypes of the isolates is shown in Table 2 with the wild type being the most represented in all isolated species. Other resistance phenotypes were also observed with differences in frequency distribution in children on antibiotic therapy from children not on antibiotic therapy (Table 3). ESBL-producing phenotype was the most represented resistance phenotype among the children (35/60, 58.3%) followed by carb-

apenem resistant phenotype (6/60, 10.0%). The prevalence of ESBL-producing phenotypes among culture-positive children on antibiotic treatment (72.5%, 29/40) was significantly higher (OR=10.545, 95% CI= 2.882 - 38.590, $p=0.0002$) than among culture-positive children not on antibiotic treatment (20%, 4/20), indicating that antibiotic therapy was significantly associated with carriage of MDR isolates (Table 3).

Factors influencing carriage of MDR strains

None of the factors such as gender, age group, or length of hospitalization was significantly associated with the carriage of MDR bacteria (Table 4).

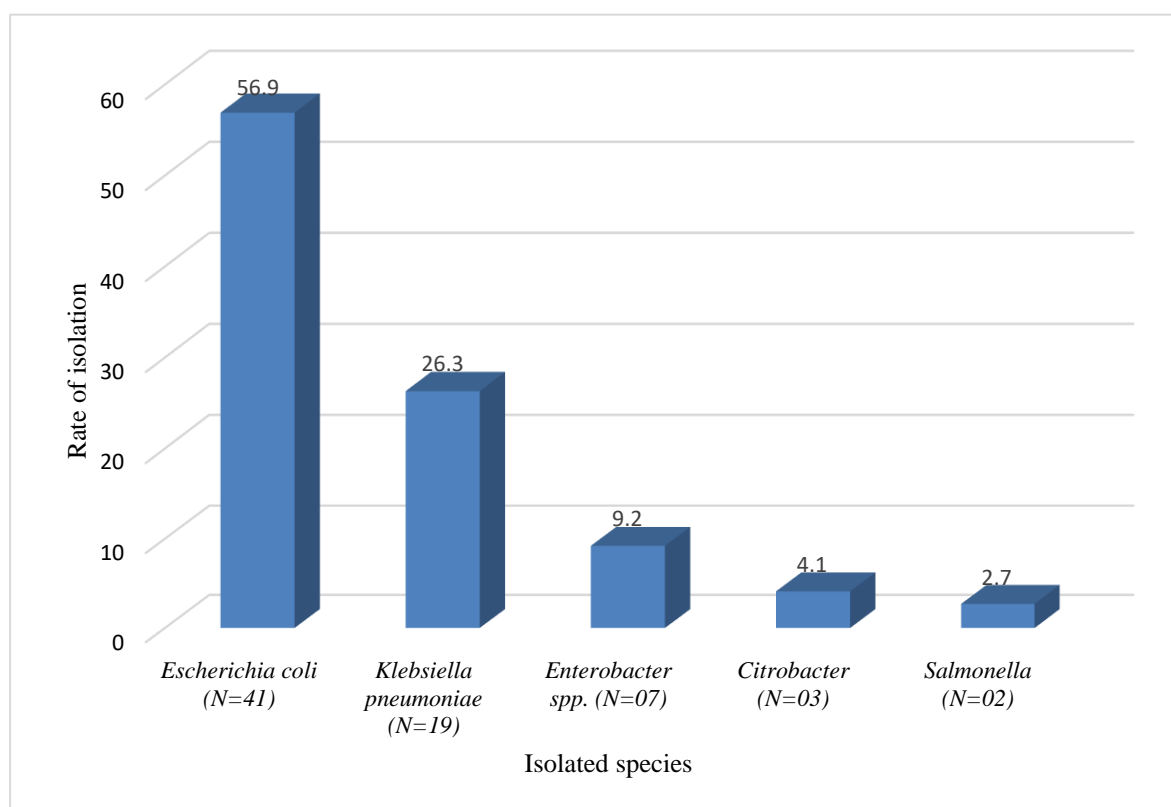


Fig. 1: Frequency of Enterobacteriaceae isolates from hospitalized children

Table 1: Antibiotic susceptibility patterns of Enterobacteriaceae isolates

Antibiotics		Isolated Enterobacteriaceae species				
		<i>Escherichia coli</i> n=41 (%)	<i>Klebsiella pneumoniae</i> n=19 (%)	<i>Enterobacter</i> n=7 (%)	<i>Citrobacter</i> n=3 (%)	<i>Salmonella spp</i> n=2 (%)
Ampicillin	R	35 (85.4)	18 (94.8)	7 (100)	3 (100)	2 (100)
	S	6 (14.6)	1 (5.3)	0	0	0
Amoxicillin+ clavulanic acid	R	8 (19.5)	7 (36.8)	3 (42.9)	1 (33.3)	1 (50.0)
	S	33 (80.4)	12 (63.2)	7 (51.1)	2 (66.7)	1 (50.0)
Piperacillin+ tazobactam	R	15 (36.6)	5 (26.4)	4 (51.1)	0	1 (50.0)
	S	26 (63.4)	14 (73.7)	3 (42.9)	3 (100)	1 (50.0)
Ceftriaxone	R	23 (85.4)	11 (57.9)	5 (71.4)	2 (66.7)	1 (50.0)
	S	18 (44.6)	8 (42.2)	2 (28.6)	1 (33.3)	1 (50.0)
Ceftazidime	R	23 (56.0)	11 (57.9)	4 (51.1)	1 (33.3)	1 (50.0)
	S	18 (43.9)	8 (42.2)	3 (42.9)	2 (66.7)	1 (50.0)
Cefepime	R	23 (85.4)	11 (57.9)	5 (71.4)	2 (66.7)	1 (50.0)
	S	18 (44.6)	8 (42.2)	2 (28.6)	1 (33.3)	1 (50.0)
Cefoxitin	R	10 (24.3)	3 (15.8)	7 (100)	1 (33.3)	1 (50.0)
	S	31 (75.6)	16 (84.3)	0	2 (66.7)	1 (50.0)
Imipenem	R	3 (7.3)	0	1 (14.2)	0	1 (50.0)
	S	38 (92.6)	19 (100)	6 (85.7)	3 (100)	1 (50.0)
Gentamicin	R	15(36.9)	8 (42.1)	5 (71.4)	2 (66.7)	1 (50.0)
	S	26 (63.4)	11 (57.9)	2 (28.6)	1 (33.3)	1 (50.0)
Ciprofloxacin	R	20 (48.8)	10 (52.7)	3 (42.9)	1 (33.3)	1 (50.0)
	S	21 (51.3)	9 (47.4)	4 (51.1)	2 (66.7)	1 (50.0)

R= Resistant; S = Sensitive; n = number

Table 2: Frequency of resistance phenotypes of Enterobacteriaceae isolates on antimicrobial susceptibility tests

Resistance phenotypes	<i>Escherichia coli</i> n=41 (%)	<i>Klebsiella pneumoniae</i> n=19 (%)	<i>Enterobacter</i> n=7 (%)	<i>Citrobacter</i> n=3 (%)	<i>Salmonella spp</i> n=2 (%)
Wild type	7 (17.1)	7 (36.9)	1 (14.3)	1 (33.3)	0
ESBL+ Carbapenems	1 (2.4)	0	2 (28.5)	0	1 (50.0)
ESBL+ Quinolone	10 (24.4)	7 (36.9)	1 (14.3)	0	0
ESBL (Synergic picture)	5 (12.2)	0	1 (14.3)	0	0
ESBL+ Aminoglycosides	2 (4.9)	3 (15.7)	1 (14.3)	1 (33.3)	0
Carbapenems	1 (2.4)	0	0	0	0
Aminoglycosides	2 (4.9)	0	0	0	0
Quinolone	2 (4.9)	0	0	1 (33.3)	0
Other resistance to beta lactams	11 (26.8)	2 (10.5)	1 (14.3)	0	1 (50.0)

ESBL = Extended Spectrum Beta-Lactamase

Table 3: Distribution of resistance phenotype of Enterobacteriaceae isolates in culture-positive children with respect to antibiotic treatment

Resistance phenotypes of isolates	Children on antibiotic therapy	Children not on antibiotic therapy	Total	OR ⁺	95% CI ⁺	p value ⁺
Wild types	4	12	16	10.545	2.882 – 38.590	0.0002*
ESBL + Carbapenems	4	0	4			
ESBL + Quinolone	15	3	18			
ESBL (Synergic picture)	3	1	4			
ESBL + Aminoglycosides	7	0	7			
Carbapenems	1	0	1			
Aminoglycosides	2	0	2			
Quinolone	3	1	4			
Other resistance to β -lactam	1	3	4			
Total	40	20	60			

ESBL=Extended Spectrum Beta-Lactamase; OR=Odds ratio; CI=Confidence Interval; +=Frequency of ESBL producing phenotype among culture-positive children on antibiotic treatment (n=29/40, 72.5%) was compared with culture-positive children not on antibiotic treatment (4/20, 20.0%) using Chi square test; *=statistically significant at $p < 0.05$

Table 4: Socio-demographic and clinical characteristics of children with respect to carriage of multi-drug resistant Enterobacteriaceae

Characteristics	No of children with multi-drug resistant bacteria (%)	No of children without multi-drug resistant bacteria (%)	X ² (or OR ⁺)	95% CI	p value
Gender					
Male	16 (50.0)	15 (53.6)	0.8667 ⁺	0.3138 - 2.394	0.8017
Female	16 (50.0)	13 (46.4)			
Age group (years)					
<1	13 (40.6)	6 (21.4)	4.362	NA	0.1129
1-10	17 (53.1)	16 (57.1)			
10-18	2 (6.3)	6 (21.4)			
Length of hospitalization (days)					
≤ 7	20 (62.5)	12 (42.9)	2.222 ⁺	0.7881 - 6.261	0.1946
> 7	12 (37.5)	16 (57.1)			

X²=Chi square; OR⁺ = odds ratio; CI = confidence Interval; NA = Not Applicable

Discussion:

Clinically significant levels of resistance to various antibiotic classes have developed in most Gram-negative bacteria, although, there is marked geographic variation in the prevalence of resistance. Historically, many forms of antibiotic resistant organisms emerged in and were disseminated from healthcare facilities in high-income settings, but in more recent times, hospitals in low and middle-income countries (LMICs) appear to have been the origin and amplifiers of several forms of resistance. Many factors such as weak antibiotic stewardship, limited infection control resources, and overuse of antibiotics promote the emergence and spread of antibiotic resistant organisms in hospitals, especially where there are no microbiological diagnostic services for the identification of resistance. Due to high antibiotic therapy requirement in children as a result of frequent childhood infections, they are at high risk for emergence and reservoir of multidrug resistance. This is one of the reasons for determining the prevalence of gastrointestinal carriage of multi-drug resistant Enterobacteriaceae (MDR-E) among hospitalized children in the Paediatric department of Sylvanus Olympio University Hospital in Lomé, Togo.

The average age of children in our

study population was 4 years 3 days, infants were the most represented (31.4%), and the male gender was predominant, with a male to female ratio of 1.2. Our findings are comparable to those reported by Pessinaba et al., (7) in Togo in their study of 81 children under 5 years of age, of which 64 (79.0%) were infants, and male to female ratio was 1.4, which is close to the figure reported in our study. The average length of hospitalization in our study was 43 days with a range of 1 day to 360 days. A study in Switzerland reported an average length of hospitalization of 25.5 days (8). The longer hospitalization observed in our study could be explained by the inclusion of children hospitalized in the oncology unit, with long-term hospitalization. During hospitalization, 60.0% (n=42/70) of children were on antibiotic therapy. In the Switzerland study (8), higher number of children (86.9%) were on antibiotic therapy. In another similar study conducted in France (9), 40.5% of the children were on antibiotic treatment and in 41.2% of cases, the antibiotic was a third-generation cephalosporin (3GC). Our study found similar percentage of 3GC use.

In our study, *E. coli* was the most frequently isolated bacteria (57%, n=41/72) followed by *K. pneumoniae* (26%, n=19/72). This agrees with the findings of previous

studies conducted in Togo from 2015 to 2016 on intestinal carriage of ESBL Enterobacteriaceae (10), where *E. coli* was the most frequently isolated strain, and another study conducted from 2010 to 2019 on the prevalence of MDR Enterobacteriaceae (10), which reported *E. coli* (64.6%) as the most frequently isolated strain, followed by *K. pneumoniae* (22.25%). Our findings are also comparable to other studies in Burkina Faso (11), Mali (12) and France (9) reporting *E. coli* as the most frequently isolated strain followed by *K. pneumoniae*. In contrast to our study, the studies conducted in Guinea-Bissau (13) and Switzerland (8), had predominance of *K. pneumoniae*, followed by *E. coli*.

More than 90% (n=65) of the bacterial isolates in our study were ampicillin resistant, which agrees with the report of a similar study in Chad in 2017 (14), which reported 96% resistance to amoxicillin. Fifty-eight-point three percent (n=42) of the isolates were resistant to 3GCs and 59.7% (n=43) to fourth-generation cephalosporins (4GCs). The study by Sadji et al., (10) reported 38.9% resistance to 3GCs in children 0-15 years of age, and Pessinaba et al., (7) reported resistance to 3GCs by all the isolates and all *K. pneumoniae* isolates were resistant to 4GCs in their study. Isendahl et al., (13) in Guinea-Bissau reported resistance rate to 3GCs of over 94% for *E. coli* and over 97% for *K. pneumoniae*.

Resistance of 43% of the Enterobacteriaceae isolates to aminoglycosides was reported in our study with *K. pneumoniae* (42.1%) and *E. coli* (36.5%). Resistance to quinolones was higher than aminoglycoside, with 52.6% for *K. pneumoniae* and 48.7% for *E. coli*. In a similar study in Togo, 80% and 94% of isolates recovered from cultures were resistant to quinolones and aminoglycosides respectively (10). A study in Mali (12) reported resistance of 36-70% to aminoglycosides and 20-50% to quinolones. In the Guinea-Bissau study (13), *E. coli* isolates were more resistant to quinolones (81%) than to aminoglycosides (43.7%). The results of these studies are similar to ours. More than 90% of the isolates in our study were resistant to cotrimoxazole. In 2017, resistance of about 80% to co-trimoxazole was reported in Chad (14). This observation could be explained by the increase in the prevalence of resistance over time. Fortunately, all of our isolates were sensitive to colistin, which is a cheering news in combating antibiotic resistance in our communities, although colistin is a relatively toxic antibiotic that is only used as one of the antibiotics of "last resort" for the treatment of serious Gram-negative bacterial infections, where other antibiotics have failed.

The prevalence of MDR Enterobacteriaceae (MDR-E) in our study was 55.6% (n=40/72), 48.6% (n=35/72) were ESBL-producing strains, and 6.9% (n=5/72) were carbapenem resistant strains. A study in Togo covering the period 2010-2019 reported a MDR prevalence rate of 34.8% (10). The high prevalence of faecal carriage of MDR-E isolates among the children in our study is worrying, because carriage of these pathogens is a risk factor for subsequent infections with MDR pathogens.

The most frequent resistance mechanism was ESBL production in 48.6% of our isolates. In Togo, many studies conducted in the general population have reported ESBL-E prevalence rates of 22.4% in 2011 (15), and 60.4% in 2015-2016 among children (10). These results lead us to conclude that ESBL-producing strains are expanding in our populations. In other African countries, studies conducted in Burkina Faso (11), Mali (12), Guinea-Bissau (13), Chad (14) and Senegal (15,16) reported ESBL prevalence rates ranging between 32% and 34%. In Burkina Faso, Ouédraogo et al., (11) reported 24% ESBL carriage rate among children in Paediatrics setting. In Europe, a study carried out in France reported a prevalence rate of 6.7% in 2012 (9), which increased sharply, with a carriage rate of 10.2% among community children in 2015 in another French study (17). However, the difference between the prevalence rates in studies from Europe and those from Africa shows that ESBL-producing strains are more widespread in the African populations.

In our study, 85.7% (30/35) of the ESBL-producing strains were isolated from patients on antibiotic therapy. The study conducted in 2012 on children in nursery (9), and many other studies (10-18) had reported that children who were on antibiotic treatment or had received antibiotic treatment less than 3 months previously, were those who carried strains resistant to 3GCs or strains that produced ESBLs. In fact, in our study, statistical analysis showed a significantly higher carriage rate in culture-positive children on antibiotic treatment, who were more than 10 times likely to carry ESBL-producing strains, than culture-positive children not on antibiotic treatment (OR=10.545, 95% CI=2.882-38.590, $p=0.0002$).

There was no significant association between carriage of MDR-E and factors such as gender, age group and length of hospitalization in our study. None of the previously cited studies reported association between gender and age of patients, with carriage of MDR-E, and these agree with the findings of our study. Contrary to our study however, Sadji (10) reported age as a factor influencing

the carriage of MDR-E, in which subjects aged 45 years and above were 1.7 times more likely to carry MDR-E in their study.

Conclusion:

The results of our study showed a worrying high level of faecal carriage of MDR-E, which were all ESBL-producing strains, in the children population. Antibiotic treatment was the only factor significantly associated with carriage of MDR-E in the study. There is urgent need to develop measures to monitor and limit the spread of these MDR organisms in children and the community in Togo.

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Copyright AJCEM 2022: <https://dx.doi.org/10.4314/ajcem.v23i1.8>**Original Article****Open Access****Outcomes of tuberculosis treatment in a tertiary health facility in north-central Nigeria**

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Abstract:

Background: Tuberculosis (TB) remains a major public health concern despite being a curable and preventable disease. The treatment of TB using a cocktail of drugs over a period of six months under the directly observed treatment short-course strategy has led to a reduction in cases but is plagued by some challenges that leads to unsuccessful or poor outcomes, which can ultimately result in spread of infections, development of drug resistance and increase in morbidity and mortality. The objectives of this study are to determine outcomes of TB treatment in Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria and the factors that may be associated with the outcomes.

Methodology: This was a retrospective study using the medical records of patients who were registered for TB treatment over a five-year period between 2016 to 2020. Data from TB registers including demographic and relevant clinical information, and treatment outcomes, were extracted into a structured data extraction format, and analysed with SPSS version 21.0 software package. Univariate and bivariate analyses were conducted, and Chi square test was used to determine association between TB outcomes and independent variables at 95% confidence interval and $p < 0.05$ was considered as the significant value.

Results: Records of 1,313 patients were studied, 744 (56.7%) were males while 569 (43.3%) were females. The age range of the patients was ≤ 1 year - 96 years, with a mean age of 30 ± 16.7 years. Most were pulmonary TB cases (88.8%, $n=1,166$), newly diagnosed (95.5%, $n=1,254$), and human immunodeficiency virus (HIV) negative at the time of TB diagnosis (63.7%, $n=837$). Eight hundred and seven (61.5%) patients had successful treatment, and 34% ($n=446$) had unsuccessful outcomes made of 'loss to follow-up' 25.8% ($n=339$), deaths 7.8% ($n=102$) and treatment failure 0.4% ($n=5$), while 2.3% ($n=30$) were transferred out and 2.3% ($n=30$) removed from TB register. Treatment success rate was significantly higher in patients with pulmonary TB ($p=0.0024$), residents in Lafia LGA ($p=0.0005$), those treated in 2016 ($p=0.0006$) and bacteriologically confirmed cases ($p < 0.0001$), while death rate was significantly lower among patients who were HIV-negative at the time of TB diagnosis ($p < 0.0001$).

Conclusion: TB treatment success rate in this study fell short of the WHO average rate. High rates of 'loss to follow-up' and deaths in this study is a wake-up call to all stakeholders in the facility and the State to put in place measures to reduce poor outcomes of TB treatment.

Keywords: tuberculosis; treatment outcomes; directly observed treatment short course; factors

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Résultats du traitement de la tuberculose dans un établissement de santé tertiaire du centre-nord du Nigéria

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Résumé:

Contexte: La tuberculose (TB) reste un problème majeur de santé publique bien qu'il s'agisse d'une maladie curable et évitable. Le traitement de la tuberculose à l'aide d'un cocktail de médicaments sur une période de six mois dans le cadre de la stratégie de traitement de courte durée sous observation directe a conduit à une réduction des cas, mais est entravé par certains défis qui conduisent à des résultats infructueux ou médiocres, ce qui peut finalement entraîner propagation des infections, développement de la résistance aux médicaments et augmentation de la morbidité et de la mortalité. Les objectifs de cette étude sont de déterminer les résultats du traitement de la tuberculose à l'hôpital spécialisé Dalhatu Araf, Lafia, État de Nasarawa, Nigéria et les facteurs qui peuvent être associés aux résultats.

Méthodologie: Il s'agissait d'une étude rétrospective utilisant les dossiers médicaux des patients qui ont été enregistrés pour un traitement contre la tuberculose sur une période de cinq ans entre 2016 et 2020. Les données des registres de la tuberculose, y compris les informations démographiques et cliniques pertinentes, et les résultats du traitement, ont été extraites dans un format d'extraction de données, et analysé avec le progiciel SPSS version 21.0. Des analyses univariées et bivariées ont été menées, et le test du Chi carré a été utilisé pour déterminer l'association entre les résultats de la TB et les variables indépendantes à un intervalle de confiance de 95% et $p < 0,05$ a été considéré comme la valeur significative.

Résultats: Les dossiers de 1 313 patients ont été étudiés, 744 (56,7%) étaient des hommes tandis que 569 (43,3%) étaient des femmes. La tranche d'âge des patients était ≤ 1 an - 96 ans, avec un âge moyen de $30 \pm 16,7$ ans. La plupart étaient des cas de TB pulmonaire (88,8%, $n=1166$), nouvellement diagnostiqués (95,5%, $n=1254$) et négatifs pour le virus de l'immunodéficience humaine (VIH) au moment du diagnostic de TB (63,7 %, $n=837$). Huit cent sept (61,5%) patients ont eu un traitement réussi, et 34% ($n=446$) ont eu des résultats infructueux constitués de «perte de suivi» de 25,8% ($n=339$), de décès de 7,8% ($n=102$) et échec du traitement 0,4% ($n=5$), tandis que 2,3% ($n=30$) ont été transférés et 2,3% ($n=30$) retirés du registre TB. Le taux de réussite du traitement était significativement plus élevé chez les patients atteints de TB pulmonaire ($p=0,0024$), les résidents de Lafia LGA ($p=0,0005$), ceux traités en 2016 ($p=0,0006$) et les cas confirmés bactériologiquement ($p < 0,0001$), tandis que le taux de mortalité était significativement plus faible chez les patients séronégatifs au moment du diagnostic de TB ($p < 0,0001$).

Conclusion: Le taux de réussite du traitement antituberculeux dans cette étude était inférieur au taux moyen de l'OMS. Les taux élevés de « pertes de vue » et de décès dans cette étude sont un signal d'alarme pour toutes les parties prenantes de l'établissement et de l'État afin de mettre en place des mesures pour réduire les mauvais résultats du traitement de la tuberculose.

Mots-clés: tuberculose; résultats du traitement; traitement de courte durée sous observation directe; facteurs

Introduction:

Tuberculosis (TB) is a chronic but curable and preventable infection that has remained a major cause of morbidity and mortality in many developing countries of the world (1). Tuberculosis is caused by the bacterium, *Mycobacterium tuberculosis*, which is spread by infected droplets (1). TB primarily occurs in the lungs but infections can spread outside the lungs, resulting in extra pulmonary infections which can affect any system in the body (1). Everyone who comes in contact with TB infected droplets is at risk of developing the infection but only about 5-10% of infected people will become ill with the disease (1). Several factors are known to contribute to the risk of active TB disease including conditions which lead to compromised immunity such as human immunodeficiency virus (HIV) infection, diabetes mellitus, and conditions that damage to the lungs such as

tobacco smoking (1). TB affects people of all ages with morbidity and mortality higher in those with underlying conditions and compromised immunity such as HIV, malnutrition, and diabetes (1).

The World Health Organization (WHO) reports that all regions of the world are affected by TB. However, 30 countries have been identified as high burden countries for TB, accounting for 87% of the total global burden of TB in 2019 (1-3). The TB high burden countries are found mostly in Africa and Asia with few in Eastern Europe and the Americas. Nigeria accounts for 4.4% of the global TB burden (2). An estimated 10 million people were reported to have fallen ill with TB in 2019 (2). Of these, 1.4 million deaths were recorded, 15% of which occurred in children and about 208,000 in people living with HIV (2,3). People living with HIV (PLHIV) are 18 times more likely to develop TB than those who are HIV negative. The wide availability of anti-

retroviral drugs (ARVS) and the early commencement of ARVs has led to a decline in morbidity and mortality from TB in people living with HIV (PLHIV) (4,5).

TB is curable with a combination of drugs (6). The WHO global strategy for control of TB involves the use of the Directly Observed Treatment Short course (DOTS) strategy where a provider observes the administration of drugs over a short course of about 6 months (6). Treatments are provided at no cost to the patients in all countries through the WHO global initiative to stop TB supported by various international partners (6). The DOTS strategy in Nigeria was adopted in 2003 and aimed at ensuring adherence to treatment thus reducing the rates of relapse, failure and overall favorable outcomes for the patient and achievement of epidemic control (6,7).

During the course of treatment, different outcomes can occur. These outcomes are influenced by several factors among which include coexisting diseases such as HIV and DM (7). Successful completion of treatment will lead to cure which can be bacteriologically or clinically confirmed whereas those who do not complete treatment can have several unsuccessful outcomes, including loss to follow up, treatment failure and death (8,9). These unsuccessful outcomes ultimately result in morbidity or mortality in the patient as well as unfavorable epidemiologic outcomes resulting in infection spread (8, 9).

Several studies have been conducted on treatment outcomes for TB in different states in Nigeria with different factors identified as contributing to the outcomes (10,11). This current study seeks to have a State and facility specific data to help in fashioning out facility specific interventions to improve TB treatment outcomes for patients accessing treatment in our facility. The overall aim of this study is to assess the treatment outcome of patients treated for TB in Dalhatu Araf Specialist Hospital, Lafia, and the specific objectives are to assess outcome of TB patients who registered for TB treatment in the facility, and identify factors associated with the treatment outcomes.

Materials and method:

Study area:

The study was carried out in Dalhatu Araf Specialist Hospital, located in Lafia, Nasarawa State in north-central Nigeria. Lafia is located in Nasarawa South senatorial zone, one of the three senatorial zones of the State. The other senatorial zones are Nasarawa North and West. Other local government areas (LGAs) in

Nasarawa South senatorial zone include Doma, Awe, Keana and Obi. The State is bounded by Benue State in the south, Taraba State in the east, Plateau and Kaduna States in the north and Kogi State and the Federal Capital territory (FCT) in the west. The Dalhatu Araf Specialist hospital is a tertiary health facility located in Lafia, the State capital and offers both tertiary and secondary health services to people from the State and the neighboring States.

Study population and design:

The study utilized data of patients (both adults and children) who were registered for TB treatment in the TB treatment unit of the facility. The study was a retrospective design utilizing secondary data from TB treatment registers.

Sampling method and selection criteria:

TB patients who presented for treatment from January 2016 to December 2020 and captured in the TB register, were considered for the study. All TB patients with documented treatment outcomes were included while patients with more than one documented treatment outcomes were excluded

Ethical consideration:

Approval for the study was obtained from Dalhatu Araf Specialist Hospital (DASH) Research Ethics Committee. Confidentiality of data was ensured as no identifiable information of the patients was collected. Registers were handled carefully and with utmost confidentiality by the researchers during data extraction. All registers were kept in a locked cabinet when not in use. All data were imputed in a password protected personal computer accessible only to the researcher. No consent was sought for the study because there was no contact with patients and extracted patients' data did not carry any identifiers.

Data collection:

Data were collected from the DOTS TB treatment registers over a five-month period (February-June 2021). The register was developed by the National TB and Leprosy Control program (NTBLCP) of the Nigerian Federal Ministry of Health. Data collected include; (i) demographic variables (age, gender and local government/State of residence); (ii) clinical variables such as site of TB (pulmonary or extra pulmonary); (iii) category of TB (newly diagnosed, relapse, treatment after failure, treatment after 'loss to follow-up'); (iv) methods of TB diagnosis (sputum smear microscopy, GeneXpert, chest radiographs, biopsy, clinical suspicion); (v) HIV serostatus (HIV positive, HIV negative,

HIV positive on anti-retroviral drugs) and; (vi) treatment outcome variables such as treatment success (cured or completed treatment), treatment failure, death, lost to follow-up, transferred out, removed from the register or not evaluated.

The definition of terms referred to in TB treatment outcomes are; (i) cure, in a TB patient who was bacteriologically (sputum smear) positive at diagnosis and sputum smear negative in the last month of treatment and at least one previous occasion; (ii) treatment completed in a TB patient who completed treatment but did not meet criteria for cure or failure; (iii) treatment success in a TB patient whose outcome was cure or treatment completed; (iv) treatment failure in any TB patient who was smear positive at 5 months or later during treatment; (v) death of TB patient from any cause while on TB treatment; (vi) loss to follow-up in TB patient whose treatment was interrupted for 2 or more consecutive months; (vii) transferred out in a TB patient for whose outcome is not known due to transfer to other facility, and therefore not evaluated; (viii) removed from TB register in a patient from whom *Mycobacterium tuberculosis* was detected with rifampicin (RIF) resistance at any point and were moved to the second line register; and (ix) unsuccessful outcome in those who had treatment failure, lost to follow-up or died.

Data processing and statistical analysis:

Data were analysed with the Statistical Package for Social Sciences (SPSS) version 20.

Analysis was carried out using descriptive and bivariate statistics, and Chi square test was used to determine relationships between variables, with p value <0.05 considered statistically significant.

Results:

A total of 1,313 TB patients were studied, age range <1 year-96 years and mean age 30.24 ± 16.7 years, with 62% ($n=814$) between age 21-50 years. Children aged ≤ 10 years constituted 12.3% ($n=162$) and 27.5% ($n=361$) were ≤ 20 years old. There were more males ($n=744$, 56.7%) than females ($n=569$, 43.3%) with a male to female ratio of 1.3:1. Most ($n=1037$, 79.0%) of the study population were resident in Lafia LGA (Table 1).

A total of 1,166 (88.8%) patients had pulmonary tuberculosis (PTB) while 147 (11.2%) had extrapulmonary TB, 1254 (95.5%) were newly diagnosed, and 32 (2.4%) were relapse cases. Most ($n=820$, 62.5%) of the patients were bacteriologically confirmed by sputum smear or GeneXpert at diagnosis, while 484 (36.7%) had their diagnosis by biopsy, radiological or clinical means. Most ($n=837$, 63.7%) of the patients were HIV-negative at the time of TB diagnosis (Table 2). A total of 807 (61.5%) had successful treatment outcome while 446 (34%) had unsuccessful outcomes such as treatment failed (0.4%, $n=5$), 'loss-to-follow-up' (25.8% $n=339$), death (7.8%, $n=102$), and 30 (2.3%) patients transferred out while another 30 (2.3%) were removed from the TB register (Table 3).

Table 1: Distribution of TB patients by demographic characteristics

Variable	Frequency	Percentages
Gender		
Male	744	56.7
Female	569	43.3
Total	1313	100
Age group category (years)		
1-10	162	12.3
11- 20	199	15.2
21-30	376	28.6
31-40	304	23.2
41-50	134	10.2
51-60	66	5.0
61-70	43	3.3
71-80	22	1.7
81-90	3	0.2
Total	1313	100
LGA/State of residence		
Lafia LGA in Nasarawa South senatorial zone	1037	79
Other LGAs in Nasarawa South	218	16.6
LGAs in Nasarawa North	31	2.4
LGAs in Nasarawa West	13	1.0
Neighboring States and FCT	13	1.0
Other States	1	0.1
Total	1313	100

Table 2: Distribution of TB patients by clinical characteristics

Variable	Frequency	Percentages
Site of TB		
Pulmonary TB	1166	88.8
Extra pulmonary TB	147	11.2
Total	1313	100
Category of TB		
Newly diagnosed	1254	95.5
Relapse	32	2.4
Treatment after failure	10	0.8
Treatment after loss to follow-up	1	0.1
Other previously treated TB patient	5	0.4
Transfer-in	11	0.8
Total	1313	100
Method of TB diagnosis		
Bacteriologically Confirmed by sputum smear or Gene Xpert	820	62.5
Radiograph, Biopsy or Clinical	484	36.9
Method unknown	9	0.7
Total	1313	100
HIV status at time of TB diagnosis		
Positive	432	32.9
Negative	837	63.7
Unknown	44	3.4
Total	1313	100
Calendar year patient registered for treatment		
2016	269	20.5
2017	286	21.8
2018	235	17.9
2019	338	25.7
2020	185	14.1
Total	1313	100

Table 3: Distribution of patients by treatment outcomes

Treatment outcomes	Frequency	Percentage
Treatment success (cured or completed)	807	61.5
Treatment failed	5	0.4
Died	102	7.8
Lost to follow-up	339	25.8
Transferred out	30	2.3
Removed from TB register	30	2.3
Total	1313	100

Four hundred and fifty-eight (61.6%) of 744 males and 349 (61.3%) of 569 females had treatment success, which shows no significant difference between the two genders (OR 1.009, 95% CI 0.8066-1.263, $p=0.9799$). Treatment success rate was also not significantly associated with age groups of patients ($X^2=3.617$, $p=0.0572$). However, 663 (63.9%) of 1037 residents of Lafia LGA had treatment success, compared to 144 (52.2%) of 276 residents from other LGAs and neighboring States, which shows significantly higher treatment success rate in residents of Lafia LGA (OR 1.625, 95% CI 1.243-2.125, $p=0.0005$) (Table 4).

As shown in Table 5, treatment success rate of 62.9% (734/1166) in pulmonary TB patients was significantly higher than success rate of 49.7% (73/147) in extrapulmonary TB cases (OR = 1.722, 95% CI = 1.220-2.431, $p=0.0024$). This may be partly accounted for by the significantly lower rates of 'loss to follow up' (LTFU) of 24.3% (283/1166) in pulmonary TB than 38.1% (56/147) in extrapulmonary TB cases (OR=0.5208, 95% CI = 0.3638 - 0.7457, $p=0.004$).

There was no statistically significant difference in the treatment success rate between newly diagnosed TB cases and other TB treatment categories (OR 0.6891, 95% CI 0.3913-1.215, $p=0.2461$). Treatment success rate was also not significantly associated with HIV status at the time of diagnosis ($X^2=4.326$, $p=0.1150$), however, death rate was significantly higher ($X^2=39.165$, $p<0.0001$) among patients who were HIV positive at the time of TB diagnosis (14.1%, 61/432) than those with unknown HIV status (11.4%, 5/44), and HIV negative patients (4.3%, 36/837).

Treatment success rate was significantly higher ($X^2=19.131$, $p<0.0001$) in TB cases with bacteriological confirmation of TB at diagnosis

Table 4: Distribution of TB treatment outcomes of patients by their demographic variables

Variable	Successful outcome n (%)	Treatment failed n (%)	Died n (%)	LTFU n (%)	T/O n (%)	Removed from Register (n %)
Gender						
Male (n=744)	458 (61.6)	4 (0.5)	51 (6.9)	199 (26.7)	13 (1.7)	19 (2.6)
Female (n=569)	349 (61.3)	1 (0.2)	51 (8.9)	140 (24.6)	17 (2.9)	11 (1.9)
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.8)	30 (2.3)	30 (2.3)
OR	1.009	3.070	0.7475	1.119	0.5775	1.329
95% CI	0.8066-1.263	0.3420-27.550	0.4988-1.120	0.8709-1.437	0.2781-1.199	0.627-2.817
p value	0.9799	0.5466	0.1901	0.4148	0.1922	0.5759
Age group (years)						
1-10 (n=162)	87 (53.7)	0	20 (12.3)	49 (30.2)	3 (1.9)	3 (1.9)
11-20 (n=199)	132 (66.3)	0	17 (8.5)	46 (23.1)	2 (1.0)	5 (2.5)
21-30 (n=376)	259 (68.9)	1 (0.3)	19 (5.1)	85 (22.6)	6 (1.6)	6 (1.6)
31-40 (n=304)	184 (60.5)	2 (0.7)	31 (10.2)	71 (23.4)	8 (2.6)	8 (2.6)
41-50 (n=134)	74 (55.2)	1 (0.7)	6 (4.5)	45 (33.6)	4 (2.9)	4 (2.9)
51-60 (n=66)	31 (46.9)	0	7 (10.6)	24 (36.4)	1 (1.5)	3 (1.9)
61-70 (n=43)	24 (55.8)	1 (2.3)	1 (2.3)	13 (30.2)	3 (6.9)	1 (2.3)
71-80 (n=22)	11 (50.0)	0	2 (9.1)	6 (27.3)	3 (13.6)	0
81-90 (n=3)	2 (66.7)	0	1 (33.3)	0	0	0
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.8)	30 (2.3)	30 (2.3)
X ² (for trend)	3.617	NA	18.176	NA	NA	NA
p value	0.0572		0.2965			
LGA/State of residence						
Lafia LGA in Nasarawa South (n=1037)	663 (63.9)	3 (0.3)	78 (7.5)	248 (23.9)	19 (1.8)	26 (2.5)
Other LGAs in Nasarawa South (n=218)	121 (55.5)	2 (0.9)	15 (6.9)	71 (32.6)	6 (2.8)	3 (1.4)
LGAs in Nasarawa North (n=31)	16 (51.6)	0	7 (22.5)	5 (16.1)	3 (9.7)	0
LGAs in Nasarawa West (n=13)	3 (23.1)	0	1 (7.7)	8 (61.5)	0	1 (7.7)
Neighboring States/FCT (n=13)	4 (30.8)	0	1 ((7.7)	6 (46.2)	2 (15.4)	0
Other States in Nigeria (n=1)	0	0	0	1 (100)	0	0
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.8)	30 (2.3)	30 (2.3)
OR ⁺	1.625	0.3975	0.8540	0.6390	0.4496	1.749
95% CI ⁺	1.243-2.125	0.06606-2.392	0.5294-1.378	0.4787-0.8529	0.2113-0.9566	0.605-5.055
p value ⁺	0.0005*	0.6215	0.6024	0.0029*	0.0573	0.4129

n = frequency; % = percentage; LTFU = Lost to follow up; T/O = Transferred Out; OR = Odds Ratio; CI = Confidence Interval; X² = Chi square;* = statistically significant at $p < 0.05$; + = bivariate analysis was performed by comparing Lafia LGA with other LGAs/neighboring States merged together

(65.9%, 541/820) than those with clinical/radiological diagnosis (54.1%, 262/484) and unknown method of diagnosis (55.6%, 4/9). This observation may be partly due to the lower rate of 'lost to follow up' (LTFU) of 22.5% (187/820) in those with bacteriological confirmation compared to 30.6% (148/484) in those with clinical/radiological diagnosis and 55.6% (4/9) in those with unknown method of diagnosis ($X^2=11.244$, $p=0.0036$). Treatment success rate was significantly higher in 2016 (71.4%, 192/269) than the other years ($X^2=19.522$, $p=0.0006$), which may partly be due to the fact that 'loss to follow up' (LTFU) rate was significantly lower in 2016 (17.8%, 48/269) than the other years ($X^2=26.660$, $p<0.0001$).

Discussion:

The introduction of DOTS for TB treatment was a novel strategy that improved the overall outcomes for patients treated for TB especially in developing countries (7,8). However, the DOTS strategy is being challenged by many issues such as poor adherence, treatment default and failure (7,8). In this study, the finding of treatment success rate of 61.5% in

the TB patients on treatment is similar to the report from another facility in north-central Nigeria which reported 67% of TB patients had successful treatment outcomes (10). Similar findings were also reported in other studies from East Africa with rates of successful treatment outcomes between 60% and 67% (12,13). This similarity in findings may be due to the fact that these studies are all from developing countries with similar socioeconomic characteristics. Many studies from Africa however reported lower rates of treatment success from 25% to 56% (14-16). The low treatment success rate in one study was due to a high rate of transfer out of over 60% to other facilities as reported by Biadlegne et al., in 2013 (14).

Our finding of 61.5% treatment success rate is however still lower than the rates reported in studies from other regions of the world including Asia and Europe with over 70% of TB patients having successful treatment outcomes (16-18). Higher rates of treatment success in developed nations of Europe and Asia may be due to more advanced health care services than obtained in the developing and often resource poor countries of Africa including Nigeria. The

Table 5: TB treatment outcomes of patients in relation to clinical variables

Variable	Successful treatment n (%)	Treatment failed n (%)	Died n (%)	LFTU n (%)	T/O n (%)	Removed from Register n (%)
Site of TB						
Pulmonary TB (n=1166)	734 (62.9)	4 (0.3)	91 (7.8)	283 (24.3)	27 (2.3)	27 (2.3)
Extra pulmonary TB (n=147)	73 (49.7)	1 (0.7)	11 (7.5)	56 (38.1)	3 (2.0)	3 (2.0)
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.8)	30 (2.3)	30 (2.3)
OR	1.722	0.5026	1.047	0.5208	1.138	1.138
95% CI	1.220-2.431	0.05577-4.529	0.5460-2.006	0.3638-0.7457	0.3408-3.799	0.340-3.799
p value	0.0024*	0.5316	0.8909	0.004*	0.8336	0.8336
TB treatment category						
Newly diagnosed (n=1254)	766 (61.1)	4 (0.3)	100 (7.9)	326 (25.9)	29 (2.3)	27 (2.2)
Relapse (n=32)	23 (71.9)	0	0	7 (21.9)	1 (3.1)	3 (9.4)
Treatment after failure (n=10)	6 (60.0)	1 (10.0)	1 (10.0)	2 (20.0)	0	0
Treatment after loss to follow up (n=1)	0	0	1 (100)	0	0	0
Other previously treated TB patient (n=5)	5 (100)	0	0	0	0	0
Transfer-in (n=11)	7 (63.6)	0	0	4 (36.4)	0	0
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.9)	30 (2.3)	30 (2.3)
OR ⁺	0.6891	0.1856	2.470	1.243	1.373	0.4108
95% CI ⁺	0.3913-1.213	0.02041-1.688	0.5939-10.269	0.6630-2.331	0.1837-10.261	0.120-1.395
p value ⁺	0.2461	0.5515	0.2998	0.5978	0.7563	0.3044
Method of TB diagnosis						
Bacteriologically confirmed by sputum smear or Gene Xpert (n=820)	541 (65.9)	5 (0.6)	46 (5.6)	187 (22.8)	20 (2.4)	21 (2.6)
Radiologic, Biopsy or Clinical (n=484)	262 (54.1)	0	56 (11.6)	148 (30.6)	10 (2.0)	8 (1.7)
Method of diagnosis not recorded (n=9)	4 (55.6)	0	0	4 (55.6)	0	1 (11.1)
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.9)	30 (2.3)	30 (2.3)
χ^2	19.131	NA	NA	11.244	NA	4.286
p value	< 0.0001*			0.0036*		0.1173
HIV status at time of TB diagnosis						
Positive (n=432)	249 (57.6)	0	61 (14.1)	107 (24.8)	9 (2.1)	6 (1.4)
Negative (n=837)	532 (63.6)	5 (0.6)	36 (4.3)	222 (26.5)	21 (2.5)	21 (2.5)
Unknown (n=44)	26 (59.1)	0	5 (11.4)	10 (22.7)	0	3 (6.8)
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.9)	30 (2.3)	30 (2.3)
χ^2	4.326	NA	39.165	0.6853	NA	5.792
p value	0.1150		< 0.0001*	0.7099		0.0553
Year patient was registered for TB treatment						
2016 (n=269)	192 (71.4)	2 (0.7)	22 (8.2)	48 (17.8)	5 (1.9)	0
2017 (n=286)	180 (62.9)	0	24 (8.4)	72 (25.2)	4 (1.4)	6 (2.1)
2018 (n=235)	135 (57.4)	1 (0.4)	20 (8.5)	55 (23.4)	12 (5.1)	12 (5.1)
2019 (n=338)	185 (54.7)	2 (0.6)	18 (5.3)	120 (35.5)	3 (0.9)	10 (2.9)
2020 (n=185)	115 (62.2)	0	18 (9.7)	44 (23.8)	6 (3.2)	2 (1.1)
Total (n=1313)	807 (61.5)	5 (0.4)	102 (7.8)	339 (25.9)	30 (2.3)	30 (2.3)
χ^2	19.522	NA	4.207	26.660	13.321	NA
p value	0.0006*		0.3787	< 0.0001*	0.0098*	

n = Frequency; %=percentage; LFTU = Lost to follow up; T/O = Transferred Out; OR = Odds Ratio; CI = Confidence Interval; χ^2 = Chi square;

*=statistically significant at $p < 0.05$; + = bivariate analysis was performed by comparing newly diagnosed TB with other TB treatment categories merged together; NA= not applicable (analysis not possible due to value of zero in some cells)

average Nigerian national TB treatment success rate was reported to be about 86% in 2016 (7). The lower rate of treatment success in our study may be due to our facility being a referral center with patients being referred from primary and secondary facilities, often with severe illness for diagnosis and once diagnosis is made and patients are discharged from care, they return to the facilities they were referred from.

This study found that patients who defaulted their treatment or were lost to follow-up (25.8%) constituted majority of those with unsuccessful outcomes. This rate is slightly higher than the rates reported from some studies from Nigeria including one from the same region

as ours where the default rate was 17%-18.5% (10,16). Similarly, studies elsewhere in Africa reported even lower rates of loss to follow-up (12,19). The loss to follow-up in our study may be due to our facility being a referral center, thus, patients tend to return to their referred facilities and places of residence often without appropriate documentation. The low transfer out rate of 2.3% in our study may be a further testament to this. The high rate of death (7.8%) among TB patients in our study is similar to the report of a study from Jos, northcentral Nigeria (10), but lower than the death rates reported in studies from southern Nigeria by Bamidele et al., (11) and Umeokonkwo et al., (16). How-

ever, the rate in our study is higher than those reported from other regions of Africa and Europe (12,17). Many patients who are referred from primary and secondary healthcare facilities to our center for diagnosis and treatment are in most cases severely ill and present late, which may thus contribute to the high death rate reported in our study. A low treatment failure rate of 0.4% in our study is similar to rates from other studies in Africa (12,14).

Our study found that both genders had similar treatment outcomes, which contrast with most reports, where males were more likely to have poorer treatment outcomes (12,15,18). However, the study by Gertie and Alemnew (20) in 2020, reported that females had poorer outcomes than males. Although, treatment success rate appeared higher in the age groups 11-20 years (66.3%), 21-30 years (68.9%) and 81-90 years (66.7%), compared to other age groups in our study, the statistical analysis using Chi-square by trend did not show significant difference between the age groups with respect to treatment success ($X^2=3.617$, $p=0.0572$) or deaths ($X^2=18.176$, $p=0.2965$). This contrasts the findings from other studies that reported poorer outcomes in older age groups (15,19).

Treatment success rate was higher in pulmonary TB cases, those with bacteriological confirmation of TB at diagnosis, and cases resident in Lafia LGA, which may partly be as a result of the significantly lower rates of 'lost to follow up' (LTFU) in these categories of TB patients. However, we did not observe any significant difference in the outcome between newly diagnosed TB and other TB treatment categories (OR 0.6891, 95% CI 0.3913-1.215, $p=0.2461$). HIV positivity at the time of TB diagnosis is a known factor associated with poor TB treatment outcome, but treatment success in our study was not significantly associated with HIV status at the time of TB diagnosis, however, independently, death rate was significantly higher in TB patients who were HIV positive at the time of diagnosis, which agrees with study reports from Africa, Asia and Europe of high mortality associated with TB-HIV co-infections (12,13,15,20).

Overall, this study found significantly higher treatment success rate, and lower rates of loss to follow up and deaths in patients with pulmonary TB, residents in Lafia LGA, patients treated in 2016, patients bacteriologically confirmed, and patients who were HIV-negative at the time of diagnosis, which agrees with many other studies that have reported similar signifi-

ficant associations of some of these variables with TB treatment success outcome (10,13,18, 20).

Conclusion:

The finding of treatment success rate of 61.5% among TB patients in our facility falls below the national and WHO average figure. This finding together with the high rates of loss to follow up and death, is a wake-up call to all stakeholders in our facility and the State to put in place necessary measures to reduce these poor outcomes of DOTS TB treatment strategy.

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Copyright AJCEM 2022: <https://dx.doi.org/10.4314/ajcem.v23i1.9>**Original Article****Open Access****Malaria rapid diagnostic test positivity rate among febrile patients seen at the Paediatric emergency unit of a tertiary care facility***¹Obu, D. C., ¹Asiegbu, U. V., ¹Okereke, B. E., ¹Ukoh, U. C., ²Ujunwa, F. A., ¹Afeji, C. O.,
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Ebonyi State, Nigeria*Correspondence to: obudora@yahoo.com; +2348037511272**Abstract:****Background:** Malaria, a life-threatening parasitic disease transmitted to humans by the female Anopheles mosquito is one of the infectious causes of fever in children. In Nigeria, malaria remains one of the most important health problems, accounting for 25% of infants and 30% of under-five mortalities. The objective of this study was to determine the prevalence of malaria among febrile children presenting at the children's emergency room (CHER) of a tertiary health facility in Abakaliki using a malaria rapid diagnostic test (mRDT).**Methodology:** This was a retrospective study that involved children presenting with fever in CHER over a 3-year period. A total of 1,273 febrile children below 18 years of age were tested with a malaria rapid diagnostic test (mRDT) kit during this period. Medical records of the patients were assessed to retrieve information such as age, gender, and clinical diagnoses. Data were analyzed using SPSS version 25.**Results:** A total of 707 (55.5%) were males and 883 (69.4%) were below 5 years of age. The overall prevalence of malaria by the mRDT test was 26% (n=331). Uncomplicated malaria, 283 (22.2%) was the commonest clinical diagnosis made while the least was malnutrition 3 (0.2%). Children aged 10-<18 years were predominantly affected as mRDT positivity rate was significantly higher in children age group 10-<18 years (40.4% 76/189) than other age groups ($X^2=44.76$, $p<0.001$). Similarly, the rate was significantly higher (OR 9.625, 95% CI 7.233-12.808, $p<0.0001$) in children with the clinical diagnosis of malaria (55.2%, 235/426) than those with the clinical diagnosis of other illnesses (11.3%, 96/847), and significantly higher (OR 0.19, 95% CI 0.1186-0.3043, $p<0.0001$) among those clinically diagnosed with complicated (79.7%, 114/143) than those with uncomplicated malaria (42.8%, 121/283).**Conclusion:** There is a high prevalence of malaria among febrile children presenting at the CHER of Alex Ekwueme Federal University Teaching Hospital Abakaliki. Children age group 10-<18 years were predominantly affected. The use of mRDT should be encouraged both as a screening and diagnostic tool with a protocol such that febrile children who have positive results are confirmed as having malaria while those with negative results are further evaluated with microscopy.**Keywords:** children; malaria; prevalence; rapid diagnostic test; emergency room

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Copyright 2022 AJCEM Open Access. This article is licensed and distributed under the terms of the Creative Commons Attribution 4.0 International License <http://creativecommons.org/licenses/by/4.0/>, which permits unrestricted use, distribution and reproduction in any medium, provided credit is given to the original author(s) and the source. Editor-in-Chief: Prof. S. S. Taiwo**Taux de positivité des tests de diagnostic rapide du paludisme chez les patients fébriles vus à l'unité d'urgence pédiatrique d'un établissement de soins tertiaires***¹Obu, D. C., ¹Asiegbu, U. V., ¹Okereke, B. E., ¹Ukoh, U. C., ²Ujunwa, F. A., ¹Afeji, C. O.,
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Résumé:

Contexte: Le paludisme, une maladie parasitaire mortelle transmise à l'homme par le moustique femelle *Anopheles*, est l'une des causes infectieuses de fièvre chez les enfants. Au Nigeria, le paludisme reste l'un des problèmes de santé les plus importants, représentant 25% des nourrissons et 30% des décès des moins de cinq ans. L'objectif de cette étude était de déterminer la prévalence du paludisme chez les enfants fébriles se présentant à la salle d'urgence pour enfants (CHER) d'un établissement de santé tertiaire à Abakaliki à l'aide d'un test de diagnostic rapide du paludisme (mRDT).

Méthodologie: Il s'agit d'une étude rétrospective portant sur des enfants présentant de la fièvre en CHER sur une période de 3 ans. Au total, 1 273 enfants fébriles de moins de 18 ans ont été testés avec un kit de test de diagnostic rapide du paludisme (mRDT) au cours de cette période. Les dossiers médicaux des patients ont été évalués pour récupérer des informations telles que l'âge, le sexe et les diagnostics cliniques. Les données ont été analysées à l'aide de SPSS version 25.

Résultats: Au total, 707 (55,5 %) étaient des hommes et 883 (69,4%) avaient moins de 5 ans. La prévalence globale du paludisme par le test mRDT était de 26% (n=331). Le paludisme non compliqué, 283 (22,2%) était le diagnostic clinique le plus fréquemment posé alors que le moins était la malnutrition 3 (0,2%). Les enfants âgés de 10 à <18 ans étaient principalement touchés, car le taux de positivité du mRDT était significativement plus élevé chez les enfants du groupe d'âge 10-<18 ans (40,4% 76/189) que les autres groupes d'âge ($X^2=44,76$, $p<0,001$). De même, le taux était significativement plus élevé (OR 9,625, IC à 95% 7,233-12,808, $p<0,0001$) chez les enfants ayant reçu un diagnostic clinique de paludisme (55,2%, 235/426) que ceux ayant reçu un diagnostic clinique d'autres maladies (11,3%, 96/847), et significativement plus élevés (OR 0,19, IC à 95% 0,1186-0,3043, $p<0,0001$) parmi les patients cliniquement diagnostiqués avec un paludisme compliqué (79,7%, 114/143) que ceux avec un paludisme non compliqué (42,8%, 121/283).

Conclusion: Il existe une prévalence élevée de paludisme chez les enfants fébriles se présentant au CHER du CHU Alex Ekwueme d'Abakaliki. Les enfants du groupe d'âge 10-<18 ans étaient principalement touchés. L'utilisation du mRDT devrait être encouragée à la fois comme outil de dépistage et de diagnostic avec un protocole tel que les enfants fébriles qui ont des résultats positifs sont confirmés comme ayant le paludisme tandis que ceux qui ont des résultats négatifs sont évalués plus avant par microscopie.

Mots-clés: enfants; paludisme; prévalence; test de diagnostic rapide; urgences

Introduction:

Febrile illnesses in children are worrisome to caregivers and are one of the major reasons for hospital visits in the Paediatric department in developing countries. At times, it creates anxiety among caregivers leading to presentation to the Children's emergency room (CHER) (1). Infectious causes of febrile illnesses could be viral, bacterial, fungal, or parasitic agents. Malaria a life-threatening disease caused by the plasmodium parasite which is transmitted to humans by the female *Anopheles* mosquito is present in 99 countries and territories. The disease is of serious public health concern in sub-Saharan Africa especially among children and pregnant women (2). Children under five years of age are highly vulnerable to malaria and face dire consequences such as severe malaria if they are not promptly and adequately treated (3).

The 2019 world malaria report indicated that 228 million malaria cases and 405,000 malaria deaths occurred worldwide. A documented 93% of the estimated cases and 94% of the global malaria deaths occurred in the African continent. Nigeria accounts for the highest burden of malaria cases and death (4). It is estimated that about 25% of the global malaria cases and 24% of all malaria deaths occurred in Nigeria in 2018 (4). Children less than five years were the most vulnerable accounting for 67% (272,000) of global malaria deaths. In Nigeria, malaria remains one of the most important health problems, accounting for 25% of infant mortality, 30% of under

five mortality, and 11% of maternal mortality (5).

One of the key malaria control strategies in an area with a high burden of malaria is early diagnosis and prompt and effective treatment of childhood fevers caused by malaria (6). The signs and symptoms obtained from patients by physicians play a key role in the clinical diagnosis of malaria. Clinical diagnosis is still used for the therapeutic care of most febrile individuals by physicians in several malaria-endemic regions despite some reported cases of its imprecision as early malarial clinical features vary and are nonspecific hence, its sole utilization becomes quite challenging and unreliable (7). Therefore, clinical diagnosis of malaria without laboratory support may lead to misdiagnosis and wrong treatment (8).

The Nigerian malarial control policy recommends universal diagnostic testing by microscopy or malaria rapid diagnostic tests in all suspected cases and the use of artemisinin-based combination therapy (ACT) for the treatment of malaria (9). Microscopy requires a laboratory setup and a trained microscopist, and constant availability of electricity which limits its applicability as both requirements are lacking in many health care settings. In these settings, therefore, the malaria rapid diagnostic test (mRDT) provides a parasite-based rapid, early, and accurate diagnosis of malaria (10). It requires a few drops of blood, little expertise, and takes only 5-15 min to perform. It is thus suitable for usage in malaria-endemic areas, settings with limited health perso-

nnel and facilities, and during outbreaks. Its usage has been reported to significantly reduce referrals and inpatient length of hospital stay (11).

Despite efforts of the malaria control program in the past decade, malaria remains a primary cause of morbidity and mortality globally (12). Studies have shown malaria prevalence of 30.5% and 83.1% in Nigerian children (13,14). Onwe et al., (15) once studied the prevalence of malaria parasitemia among children aged 0-15 years in Abakaliki using microscopy as a diagnostic method and reported a prevalence rate of 24.1%. The present study was aimed to determine the prevalence of malaria among febrile children presenting at CHER of Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) to provide updated data on malaria epidemiology and surveillance.

Materials and method:

Study location and setting

The study was conducted at the Alex Ekwueme Federal University Teaching Hospital Abakaliki, a tertiary institution created by a merger of the former Federal Medical Center Abakaliki and Ebonyi State University Teaching Hospital Abakaliki to a Federal Teaching Hospital in December 2011. It is one of the tertiary health facilities located in Abakaliki metropolis, Ebonyi State. Ebonyi State belongs to the southeast geopolitical zone bounded in the north by Benue State, in the west by Enugu State, in the east by Cross River State, and in the south by Abia State. It occupies a land area of 5,953 square kilometers and situated between longitude 7°30' and 8°30'E and between latitude 5°40' and 6°54'N.

The vegetation of the State is predominantly tropical rainforest with a mean annual rainfall of 2100 mm. The rainy season starts from April to October and the dry season from October to March (16). The state lies entirely in the Cross River plains with frequent floods during the rainy season, resulting from poor drainage systems, stagnant streams, and ponds that predispose the state to the menace of mosquito infestations and malaria endemicity. About 75% of the state population is engaged in subsistent agrarian economic activities especially rice farming which also produces breeding sites for mosquitoes (16).

Study design and ethical approval

This was a retrospective study of febrile children who presented in CHER from November 2016 to October 2019. A total of 1,273 children under the age of 18 years out of 5,568 who attended the CHER with a history of fever were tested for malaria with a finger prick mRDT kit. Ethical approval for the study

was obtained from the Human Research and Ethical Committee of the hospital.

Malaria rapid diagnostic test

Parasitological diagnosis of malaria was done in-house in the Paediatrics department's side laboratory using the SD Bioline Malaria Ag P.f (HRP-II)TM device (05FK50 Standard Diagnostics, Inc. Gyeonggi-do, Republic of Korea). This is a rapid qualitative test based on lateral flow immunochromatography in cassette format which detects *Plasmodium falciparum*-specific protein and histidine-rich protein II (HRP-II) antigen in human whole blood. The device contains a membrane strip that is pre-coated with mouse monoclonal antibodies specific for HRP-II of P.f-colloid gold conjugate that reacts with *P. falciparum* antigen in the specimen. The antigen in the blood moves along the membrane chromatographically to the test region 'P.f' and forms a visible line as the antibody-antigen-antibody gold particle complex. The manufacturer's performance ratings of the kit for detecting *P. falciparum* in blood are sensitivity of 99.7% and specificity of 99.5%.

The protocol for testing was contained in a standard operating procedure (SOP) and a bench aid was always pasted on the wall of the laboratory for a quick reference. About 5µl of blood, collected aseptically through a finger prick from each patient, was transferred to the sample well of the mRDT cassette while drops of chase buffer were applied to the other well in line with the manufacturer's instruction. Test results were interpreted after the recommended waiting period of 15 minutes. A test was interpreted to be positive if both control and patients' sample lines turn red, and negative if only the control line turned red. Results were interpreted to be invalid if the patient's test line turned red, but the control line was not visible. Invalid results were repeated immediately with another kit. Internal quality control was carried out on each batch of mRDT kit supplied to the laboratory by using malaria-positive blood samples from clinical cases that were also confirmed by microscopy.

Data collection

The medical registers of data of all children who presented with fever and who had mRDT tests done were retrieved. Information obtained included age, gender, and clinical diagnoses by admitting physicians, and the results of mRDT conducted on the patients. Clinical diagnosis of malaria was based on the presence of features of malaria such as the history of fever or presence of elevated temperature, vomiting, loss of appetite, body weakness, bitter taste in the mouth, and others. Confirmation of malaria was done by

the presence of a positive mRDT in such study participants.

Statistical analysis:

Data analysis was performed using the SPSS® Statistics program, version 25 (IBM Corporation, Armonk, NY, USA). Descriptive statistics (frequency and percentages) were calculated for categorical variables. Chi-square test was used to determine the association between mRDT test result and patients' variables. A p-value of <0.05 was considered statistically significant.

Results:

Socio-demographic characteristics of study participants:

A total of 1,273 (22.8%) febrile children out of 5,568 children that presented at the CHER during the 3-year study period were tested for the presence of malaria parasite (*P. falciparum*) using mRDT kit. Seven hundred and seven (55.5%) were males and 883 (69.4%) were below 5 years of age (Table 1).

Table 1: Socio-demographic characteristics of the study participants

Variables	Frequency (%)
Gender	
Male	707 (55.5)
Female	566 (44.5)
Age group (years)	
0-<5	883 (69.4)
5-<10	201 (15.80)
10-<18	189 (14.8)
Total	1273 (100)

Clinical diagnosis in febrile children presenting in CHER

The commonest initial clinical diagnosis made among the febrile children presenting in CHER was malaria (33.4%, 426/1273), with uncomplicated malaria (22.2%, 283/1273) and complicated malaria (11.2%, 143/1273), while the least common diagnosis was malnutrition (0.2%, 3/1273). Other diagnoses included malignancies, otitis media,

syncope, helminthiasis, food poisoning, viral hepatitis, and diabetic ketoacidosis (Table 2).

Table 2: Clinical diagnosis of the study participants

Variable	Frequency (%)
Uncomplicated malaria	283 (22.2)
Complicated malaria	143 (11.2)
Gastroenteritis	238 (18.7)
Upper respiratory tract infection	151 (11.9)
Sepsis	85 (6.7)
Bronchopneumonia	42 (3.3)
Sickle cell crisis	42 (3.3)
Febrile convulsion	30 (2.4)
Enteric fever	22 (1.7)
Meningitis	17 (1.3)
Urinary tract infection	13 (1.0)
Heart failure	13 (1.0)
Asthma	8 (0.6)
Shock	8 (0.6)
Anaemia	5 (0.4)
Malnutrition	3 (0.2)
Others	170 (13.4)
Total	1273 (100.0)

Result of malaria RDT in febrile children

A total of 331 (26%) febrile children with a clinical diagnosis of malaria (complicated or uncomplicated) tested positive to mRDT (Table 3). The mRDT was positive in 55.2% (235/426) of all the children with malaria, [42.8% (121/283) in children with uncomplicated malaria was significantly lower than 79.7% (114/143) in children with complicated malaria (OR 0.19, 95% CI 0.1186-0.3043, $p<0.0001$)]. The positivity rate of mRDT was also significantly higher (OR 9.625, 95% CI 7.233-12.808, $p<0.0001$) in children with a diagnosis of malaria (55.2%, 235/426) than in children with a diagnosis of other illnesses (11.3%, 96/847).

For the age group of the children, mRDT positivity rate was significantly higher ($\chi^2=44.76$, $p<0.0001$) in the age group 10-<18 years (40.4%, 76/189) than in the age groups 5-<10 years (36.3%, 73/201) and 0-<5 years (20.6%, 182/883). However, mRDT positivity rate was not significantly different between males (25%, 177/707) and females (27.2%, 154/566) children (OR 0.8935, 95% CI 0.6948-1.149, $p=0.4156$).

Table 3: Association between age, gender, clinical diagnosis, and outcome of malaria Rapid diagnostic test

Variable	Results of mRDT n (%)		X ²	p value
	Positive	Negative		
Age group (years)				
0-<5	182 (20.6)	701 (79.4)	44.76	<0.001*
5-<10	73 (36.3)	128 (63.7)		
10-<18	76 (40.4)	112 (59.6)		
Gender				
Male	177 (25.0)	530 (75.0)	0.6627	0.4156
Female	154 (27.2)	412 (72.8)		
Initial clinical diagnosis				
Uncomplicated malaria	121 (9.5)	162 (12.7)	384.53	<0.001) *
Complicated malaria	114 (9.0)	29 (2.3)		
Upper respiratory tract infection	15 (1.2)	136 (10.7)		
Febrile convulsion	14 (1.1)	16 (1.3)		
Gastroenteritis	12 (0.9)	226 (17.8)		
Sepsis	12 (0.9)	73 (5.7)		
Sickle cell crisis	6 (0.5)	36 (2.8)		
Meningitis	5 (0.4)	12 (0.9)		
Enteric fever	4 (0.3)	18 (1.4)		
Bronchopneumonia	3 (0.2)	39 (3.1)		
Heart failure	3 (0.2)	10 (0.8)		
Urinary tract infection	3 (0.2)	10 (0.8)		
Malnutrition	1 (0.08)	2 0.2)		
Shock	0 (0.0)	8 (0.6)		
Asthma	0 (0.0)	8 (0.6)		
Anaemia	0 (0.0)	5 (0.4)		
Others	18 (1.4)	152 (11.9)		
Total	331 (26.0)	932 (74.0)		

* = statistically significant

Discussion:

The study revealed an overall malaria prevalence of 26.0% among febrile cases seen in CHER. This is not unexpected because our study area is in a malaria-endemic region. Also, Abakaliki is a rice-producing center in Nigeria where farmers deliberately flood farmlands to enhance rice yields. This practice is known to enhance mosquito breeding (17). This was supported by the findings of Onwe *et al.* (15) who also found a prevalence of 24.1% in an earlier study. Another study in the northern region of Nigeria where rice is also

produced, reported a similar prevalence of 26.4% (18). The finding was comparable to reports of 22.1% in Ghana (19) and 22.0% in Gabon (1) but lower than 75.77% in Benin (20) and 83.1% in Imo State (14). A study conducted in India reported a prevalence of 36.6% (21). These variations in malaria prevalence may be due to the age of study participants, diagnostic methods, and differences in sensitivities of mRDT kits used. The studies in Nigeria, Benin, and India involved only under 5 children who are more prone to malaria attack and may have accounted for the high prevalence reported (18,20,21).

The present study alongside studies done in India used mRDT for diagnosis which serves as a screening tool with varying sensitivity and specificity to malaria parasites while other studies done in Abuja, and Anambra States in Nigeria used microscopy which is regarded as the 'gold standard' test for malaria diagnosis. Different socioeconomic statuses of study participants and/or plasmoidal transmission rates, as well as a decrease in free distribution of impregnated bed nets in addition to its utilization and awareness campaigns on malaria, are also possible contributors. This emphasizes the need to intensify the usage of indoor residual spray, insecticide-treated nets, and other interventions to sustain a reduction in transmission of malaria parasites and the burden of malaria as this study showed an obvious decline in malaria prevalence rate among children.

In this study, children age group 10- <18 years had the highest prevalence of malaria, with 40.4% positivity for *P. falciparum* on mRDT. This may be attributed to repeated exposure to the parasite in these children due to frequent mosquito bites caused by staying out late at night and playing with their body exposed most times. This observation agrees with the findings of a study conducted in the same locale (16). On the contrary, the findings of researchers in different parts of the world reported age groups below five years to be most affected (1,22-24). Differences in child-rearing practices and sociocultural factors may account for the variation. Gender was not associated with malaria positivity in our study, which agrees with reports obtained by other researchers (23,25,26). However, some studies conducted in Benin reported that males had a significantly higher risk of malaria (20, 24).

There was significant association between clinical diagnosis and the result of mRDT in the sense that mRDT positivity rate was significantly higher ($p < 0.0001$) in children who had clinical diagnosis of malaria (55.2%) than those who had clinical diagnosis of other illnesses (11.2%), and was also significantly higher ($p < 0.0001$) among those clinically diagnosed with complicated malaria (79.7%) than those with uncomplicated malaria (42.8%). This suggests a reliable clinical sense of judgement about the ability of clinicians to diagnose malaria, and may also indicate increased diagnostic sensitivity of mRDT in complicated malaria, probably from increased parasite antigens from heavy parasitaemia. However, because of the possibility of missed diagnosis of uncomplicated malaria in some children which may progress to complicated malaria with a higher risk of mortality, such febrile children should benefit from mRDT testing. Some researchers had observed that mRDTs had significantly higher sensitivity and diag-

nostic accuracy than clinical diagnosis (27). Therefore, mRDTs should be used to complement microscopy or used alone when expert microscopy is unavailable.

Our study was limited by the fact that it was a retrospective analysis of records, as such, the information obtained was more limited and could not show determinants of malaria among study participants. Furthermore, the accuracy of the mRDTs and results generated could have been affected by several factors including transport and storage of kits and parasite density of a sample.

Conclusion:

This study showed a high prevalence of malaria among febrile children seen in the Children Emergency Room (CHER) of Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA). Children age group 10- <18 years were predominantly affected. Malaria rapid diagnostic test positivity was significantly associated with clinical diagnosis of malaria than clinical diagnosis of other illnesses, and was more sensitive in the diagnosis of complicated than uncomplicated malaria. The use of mRDT should be encouraged both as a screening and diagnostic tool with a protocol such that febrile children who have positive results are confirmed as having malaria while those with negative results are further evaluated with microscopy.

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Conflict of interest:

Authors declare no conflict of interest

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Original Article

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Phytochemical and antibacterial activity of *Mangifera indica* Linn (Mango) bark and leaf extracts on bacteria isolated from domestic wastewater samples

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Abstract:

Background: Wastewaters generated from ubiquitous use of water in daily human activities often contains various pathogenic microorganisms, which may contaminate surface or ground waters when released indiscriminately into the environment. Consumption of natural water resources polluted by such contaminated wastewaters may compromise public health and decrease the populations of aquatic organisms in such water bodies. *Mangifera indica* (mango) plants have been widely used as remedy for treatment of a wide range of water borne ailments. This study was therefore conducted to identify bacteria contaminating wastewaters from domestic sources and to determine the antibacterial potentials of mango bark and leaf extracts against them.

Methodology: Wastewater samples were obtained from the wash areas of five randomly selected female hostels in Bowen University, Iwo, Osun State, Nigeria. Bacteria in the wastewater samples were isolated by standard aerobic cultures and identified using conventional biochemical test schemes. The antimicrobial activities of the methanol extracts of *M. indica* leaf and bark, and a standard antibiotic (tetracycline), were determined by the modified disc diffusion test. Phytochemical analysis of the extracts was determined by standard method, and the active compounds in them were analyzed by FT-IR spectroscopy using Agilent technologies FT-IR spectrometer at a scan range of 4,000-600 cm⁻¹.

Results: The bacterial species isolated from the wastewater samples included *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, with *E. coli* the most frequent (35.7%) and *K. pneumoniae* the least frequent (7.1%). Leaf extract (30µg) of *M. indica* exerted high antimicrobial activity against *Salmonella* spp (inhibition zone diameter of 15mm) while the bark extract exerted high antimicrobial activity against *P. aeruginosa* (inhibition zone diameter of 13mm). Comparatively, tetracycline (30µg) exerted highest antimicrobial activity against *Salmonella* spp (inhibition zone diameter of 18mm) but no activity against *P. aeruginosa* (inhibition zone diameter of 0 mm). The FT-IR analysis confirmed the presence of several functional groups with antimicrobial potentials such as flavonoids, alkaloids, tannins, saponins, phenols, and phytosterols.

Conclusion: These results indicate the antibacterial potential effects of *M. indica* leaf and stem bark extracts against some bacterial isolates, and thus may be recommended for biological treatment of water contaminated by wastewater sources.

Keywords: antimicrobial; phytochemical; bioactivity; *Mangifera indica*; bacteria; wastewater

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Activité phytochimique et antibactérienne d'extraits d'écorce et de feuilles de *Mangifera indica* Linn (Mangue) sur des bactéries isolées d'échantillons d'eaux usées domestiques

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Résumé:

Contexte: Les eaux usées générées par l'utilisation omniprésente de l'eau dans les activités humaines quotidiennes contiennent souvent divers micro-organismes pathogènes, qui peuvent contaminer les eaux de surface ou souterraines lorsqu'ils sont rejetés sans discernement dans l'environnement. La consommation de ressources en eau naturelles polluées par de telles eaux usées contaminées peut compromettre la santé publique et diminuer les populations d'organismes aquatiques dans ces plans d'eau. Les plantes *Mangifera indica* (mangue) ont été largement utilisées comme remède pour le traitement d'un large éventail de maladies d'origine hydrique. Cette étude a donc été menée pour identifier les bactéries contaminant les eaux usées d'origine domestique et pour déterminer les potentiels antibactériens de l'écorce et des extraits de feuilles de manguier contre elles.

Méthodologie: Des échantillons d'eaux usées ont été prélevés dans les zones de lavage de cinq foyers pour femmes sélectionnés au hasard à l'Université Bowen, Iwo, État d'Osun, Nigéria. Les bactéries présentes dans les échantillons d'eaux usées ont été isolées par des cultures aérobies standard et identifiées à l'aide de schémas de tests biochimiques conventionnels. Les activités antimicrobiennes des extraits au méthanol des feuilles et de l'écorce de *M. indica* et d'un antibiotique standard (tétracycline) ont été déterminées par le test de diffusion sur disque modifié. L'analyse phytochimique des extraits a été déterminée par une méthode standard, et les composés actifs qu'ils contiennent ont été analysés par spectroscopie FT-IR à l'aide du spectromètre FTIR Agilent technologies à une plage de balayage comprise entre 4000-600 cm⁻¹.

Résultats: Les espèces bactériennes isolées des échantillons d'eaux usées comprenaient *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp, *Pseudomonas aeruginosa* et *Klebsiella pneumoniae*, avec *E. coli* le plus fréquent (35,7%) et *K. pneumoniae* le moins fréquent (7,1%). L'extrait de feuille (30µg) de *M. indica* a exercé une activité antimicrobienne élevée contre *Salmonella* spp (diamètre de la zone d'inhibition de 15mm) tandis que l'extrait d'écorce a exercé une activité antimicrobienne élevée contre *P. aeruginosa* (diamètre de la zone d'inhibition de 13mm). En comparaison, la tétracycline (30µg) a exercé la plus forte activité antimicrobienne contre *Salmonella* spp (diamètre de la zone d'inhibition de 18mm) mais aucune activité contre *P. aeruginosa* (diamètre de la zone d'inhibition de 0 mm). L'analyse FT-IR a confirmé la présence de plusieurs groupes fonctionnels à potentiel antimicrobien tels que les flavonoïdes, les alcaloïdes, les tanins, les saponines, les phénols et les phytostérols.

Conclusion: Ces résultats indiquent les effets potentiels antibactériens des extraits de feuilles et d'écorce de tige de *M. indica* contre certains isolats bactériens, et peuvent donc être recommandés pour le traitement biologique de l'eau contaminée par des sources d'eaux usées.

Mots-clés: antimicrobien; phytochimique; bioactivité; *Mangifera indica*; bactéries; les eaux usées

Introduction:

The common saying goes 'water is life'. This same indispensable element that has been recognized as a source of life may also well be termed a source of sickness and death, especially in developing countries such as Nigeria. Water sources may become easily contaminated and polluted due to their adoption for ubiquitous purposes especially in rural and semi-urban settings, where adequate treatment facilities and/or chemicals may not be readily available, accessible, or functional.

It is an indisputable fact that water is essential to the major life processes of plants and animals. Its occurrence and utilization in a vast array of biological processes such as photo synthesis, transpiration, excretion, growth, reproduction, and so on underscores the crucial importance of this element. An adequate, safe, and accessible supply must therefore be available to all. Improving access to safe drinking water and other purposes like domestic, indus-

trial and agricultural purposes can result in significant benefits to public health.

The major microorganisms in wastewater effluents are viruses, bacteria, fungi, protozoans and helminthes (1). Water-borne infections may have serious consequence for the immunologically compromised individuals (2). Bacteria in wastewaters are usually from faecal droplets of individuals with acute disease or carriers, which can be pathogenic with capacity to cause infection and allergies, and can easily contaminate and disseminate in the soil. The possibility of contamination of underground water sources from indiscriminate release of wastewater is a very grim reality in developing countries and this in turn, may have grave impact on the public health status of the populace who are highly dependent on these water sources to meet their daily domestic needs.

The use of plant extracts has long been exploited in folk medicine for therapeutic antimicrobial and antioxidant properties. Natural

bioactive compounds have also been found to interfere with development of cancers (3). On the other hand, the efficacy of synthetic antimicrobial agents has been mitigated due to the continuing emergence of drug resistant microorganisms and the adaptations by microbial pathogens to commonly used antimicrobials (4). This therefore necessitates the continuous exploration of local plant resources that may be exploited to address the health and environmental challenges where such plant resources exist.

Mangifera indica (Mango) is a fruit crop which is the most widely cultivated and economically important species of the family Anacardiaceae, a family of many tropical species (5). The phytotherapeutic effects of Mango plant parts have been well documented. Abdul et al., (6) reported that leaf extracts of *M. indica* demonstrated notable inhibitory effects on antibiotic sensitive and multi-drug resistant *Salmonella* Typhi. Disegha and Akani (7) also reported that *M. indica* extracts were effective as anti-fungal agents in the treatment of infectious diseases caused by *Candida albicans* and *Aspergillus* spp. Mango plant parts have traditionally been widely used as herbal remedy for a wide range of ailments due to their association with many pharmacological effects (8).

Fourier-transform infrared (FT-IR) spectroscopy is a high-resolution analytical tool used to identify the chemical constituents of compounds and elucidate structural components (3). This study was conducted using FT-IR spectroscopy to screen for the functional groups of extracts of the leaf and bark of *M. indica*. In addition, the antibacterial activities of the phytochemical components of this plant were tested on bacteria isolated from wastewaters.

Materials and method:

Collection and preparation of plant materials

Fresh leaves and barks of *M. indica* (Mango) were collected from tree stands located within Bowen University campus, Iwo, Nigeria, and identified at the herbarium of the Department of Botany, University of Ibadan, Ibadan, Nigeria. The plant samples were air-dried at room temperature for 10 days to remove the residual moisture. The dried leaves were then ground to powder using an electrical blender while the bark portions were first pulverized in a mortar before using a mechanical grinder. The pulverized plant material was then stored in sterile air-tight containers before use.

Extraction of mango leaf and bark for phytochemical screening

Methanol, ethanol, hexane and distilled water were selected for extracting the plant materials for phytochemical screening. The coarse powder (100g) was soaked in 250ml of each solvent for 48 hours and filtered using Whatman No. 1 filter papers. Soxhlet apparatus was used to obtain crude extracts from the solvent by evaporating the extracts to dried form. Pulverized samples of leaf and bark were extracted using methanol at room temperature using the cold maceration method by soaking 100g of each sample in 500ml of methanol for 48 hours. Each extract sample was evaporated using a rotatory evaporator with Stuart digital water bath (RE300DB) at 45°C under reduced pressure. Phytochemical constituents were screened for tannins, alkaloids, saponins, glycosides, flavonoids, phenols, protein and phyto-sterols according to the method described by Prabhavathi et al., (9).

FT-IR spectrum analysis

The extracts of *M. indica* stem bark and leaf were loaded onto FT-IR spectroscopy and the spectroscopy results were recorded on an Agilent Technology FT-IR spectrometer (Cary 630) at a scan range between 4,000–600 cm^{-1} .

Collection of wastewater samples

Wastewater samples were collected into sterile sample bottles from the wash areas of five randomly selected ladies' hostels (UPE, SADLER, NH1, NH2 and NH3) in Bowen University (BU), Iwo, Nigeria. They were then placed aseptically inside new polythene bags and transported to the microbiology laboratory of the University for analyses.

Physico-chemical analysis of the wastewater samples

The pH of the wastewater samples was tested using the Test 2 waterproof digital pH meter. Prior to measurement, standard calibration procedure was done for the pH meter using standard buffer solutions. Turbidimetric method (10) was used to determine the sulphate (SO_4^{2-}) content, and acid colorimetric method was used to determine the phosphate (PO_4^{3-}) anions level in the wastewater samples.

Isolation of bacteria

Bacteria were isolated from the wastewater samples using the ten-fold serial dilution method (11). Different dilutions were plated onto Petri dishes containing sterilized nutrient agar (Lab M) until pure cultures were obtained.

Pure cultures of the bacterial isolates were identified based on their colony morphology, Gram stain reaction, and conventional biochemical tests such as citrate utilization, indole, sugar fermentation, starch hydrolysis, methyl red and Voges-Proskauer tests, in accordance with the Bergey's manual of systematic bacteriology (12).

Test bacteria for antimicrobial assay

Pure cultures of the following bacteria were selected for antimicrobial assay; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella sp.*, and *Proteus mirabilis*. Biochemical tests were used to confirm the identity of the isolates according to the method described by Cheesbrough (13). The organisms were sub-cultured on nutrient agar (NA) plates and then stored on nutrient agar slants at 4°C until use.

Antimicrobial assay

The modified agar disc diffusion of Bauer et al., (14) was used for antimicrobial assay. Whatman No. 1 filter paper was cut using a paper perforator into discs measuring 4 mm in diameter and sterilized in an autoclave. A stock solution of the Mango extracts was prepared by dissolving 100 mg of each extract in 1 ml of methanol and kept in the fridge until use. The stock solution was diluted (x100) at the point of working to obtain the working solution using the formula $C_1V_1 = C_2V_2$, where C_1 =concentration of stock solution, V_1 =volume of stock solution, C_2 =concentration of working solution and V_2 =volume of working solution. From the working solution, a fixed volume of 0.03ml (equivalent to 0.03mg or 30µg) of each extract was carefully used to load (impregnate) each disc and the impregnated discs were placed in Petri dishes approximately 5 mm apart, taking precautions that the tip was in slight contact with the sterilized disc using a micro pipette. All impregnated discs were fully dried in a container.

Mueller Hinton agar (Lab M) was prepared under standard conditions. Inoculum of pure cultures of each bacteria isolate was prepared and standardized by adjusting its turbidity to match 0.5 McFarland standard, giving inoculum size of approximately 1×10^8 CFU/ml.

The plates were streak-inoculated with a sterile swab stick dipped into the inoculum. The prepared discs were applied with a sterile forcep onto the surface of the seeded agar plates, along with standard tetracycline (30 µg) disc as comparative control. The plates were incubated aerobically for 48 hours at 37°C. Antimicrobial activity was determined by measuring the diameter (in millimetres) of the zone of inhibition with a calibrated ruler. The antimicrobial assay for the extracts and tetracycline control was carried out in duplicate, and the average of the two replicated results was calculated for each isolate.

Results:

Five bacteria isolates were obtained from the wastewater samples. Fig. 1 shows the relative frequency of occurrence of the bacteria isolates from the wastewater samples. All isolated bacteria were Gram-negative rods and identified as *Escherichia coli*, *Proteus mirabilis*, *Salmonella sp.*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The results obtained from the physico-chemical test carried out on the water samples are shown on Table 1.

Table 1: Physico-chemical parameters of wastewater samples

Parameters	pH	PO ₄ ³⁻ conc. WL 470 nm	SO ₄ ²⁻ conc WL 425 nm
Sample A (UPE)	7.0	-0.062	0.553
Sample B (SADLER)	6.5	0.114	1.225
Sample C (NH1)	7.3	-0.060	0.583
Sample D (NH2)	7.5	0.270	0.249
Sample E (NH3)	8.0	0.040	0.730

PO₄³⁻=Phosphate concentration; SO₄²⁻=Sulphate concentration; WL=Wavelength

The pH values of the wastewater samples ranged from 6.5-8.0. Wastewater from hostel NH2 (sample D) had the highest phosphate concentration (0.270) while wastewater from UPE hostel (sample A) had the lowest concentration (-0.062), and wastewater from NH1 hostel (sample C) had the lowest BOD level of 110mg/L. The results of sulphate concentration test showed that wastewater sample from SADLER hostel (sample B) had the highest concentration (1.225) while wastewater from NH2 hostel (sample D) had the lowest concentration (0.249).

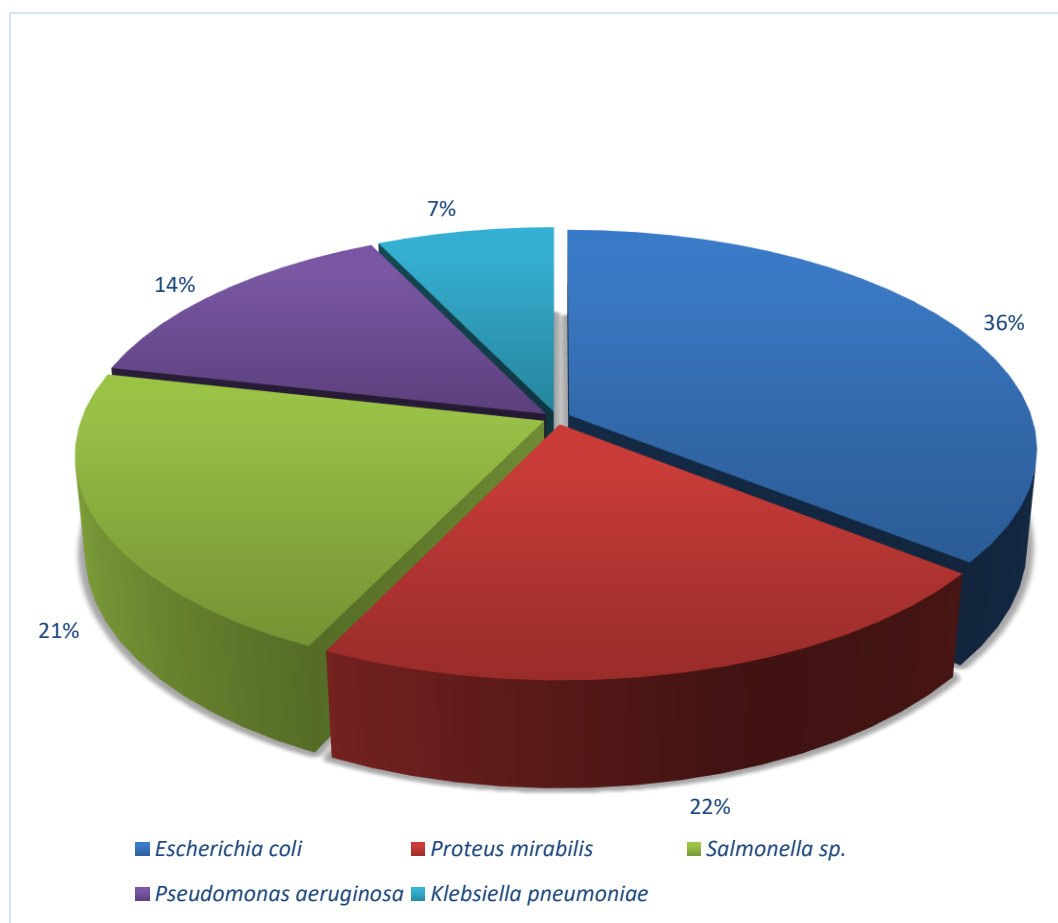


Fig. 1: Frequency of isolation of bacteria from wastewater samples

Table 2: Phytochemical composition of *Mangifera indica* leaves and stem barks

Test	Reagent used	Observation	Leaf extract					Stem bark extract				
			Ls	Aq.	Eth	Meth	Hex	Bs	Aq.	Eth	Meth	Hex
Tannins	FeCl ₃	Blue black coloration	+	+	+	+	+	+	+	+	+	+
Alkaloids	Marquis reagent	Dark orange or purple	+	+	+	+	+	+	+	+	+	+
Saponins	Distilled water	Stable foams	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	Glacial acetic acid and FeCl ₃	Brownish green ring	+	+	+	+	+	+	+	+	+	+
Flavonoids	NH ₃ + H ₂ SO ₄	Yellow coloration	+	—	+	+	+	+	—	—	—	+
Phenols	Aqueous FeCl ₃	Blue coloration	—	—	+	—	+	—	—	+	—	+
Protein	Conc. nitric acid	Yellow coloration	—	—	—	—	+	—	—	—	—	+
Phytosterol	Acetic anhydride + H ₂ SO ₄	Brown ring	+	+	+	+	+	+	+	+	+	+

Ls = leaf sample; Aq.= aqueous; Eth = ethanolic; Meth = methanolic; Hex = hexane; Bs = bark sample; - = negative result; + = positive result

The results for qualitative phytochemical screening of *M. indica* leaf and bark are presented on Table 2. Alkaloids, saponins, tannins, phytoesters and glycosides were present in all the methanolic, aqueous, ethanol and hexane leaf and bark extracts. Protein was present in the hexane extract but absent in aqueous, ethanol and methanol extracts of both leaf and bark samples. Flavonoids were present in the ethanolic, methanolic and hexane extracts of the leaf, but absent in the aqueous, ethanol and methanol extracts of the bark. Phenol was present in both the ethanol

and hexane extracts of the leaf and bark, but absent in the aqueous and methanolic extracts of the plant samples.

The spectrum of *M. indica* was recorded in wavelength 4000-600 cm^{-1} . The result of the FT-IR analysis confirmed the presence of functional groups such as O-H, C=C, C-H, C-O and CH_3 (Tables 3 and 4). The highest (strong instance) peak appears at a wavelength of 3321.1 cm^{-1} and a transmittance of 63.892%, which indicates the presence of O-H stretching and H-bonded of alcohols and phenols.

Table 3: FT-IR interpretation of compounds of methanol leaf extract of *Mangifera indica*

Wavenumber cm^{-1} (Test Sample)	% Transmittance	Functional Group assignment	Expected Phyto compounds identified
3321.1	63.892	H- bonded, O-H stretch	Hydroxyl compound, alcohols, phenols
2942.7	74.583	Asymmetric stretching of -CH (CH_2) vibration	Saturated aliphatic compounds
2834.6	77.550	Symmetric stretching of -CH (CH_2) vibration	Fatty acids, lipids, protein
1612.1	84.397	C=O stretching	Carbonyl compound
1448.1	76.760	C=C-C, aromatic ring	Aromatic compound
1110.7	79.321	O-H bend, alcoholic group	Phenol, tertiary alcohol
1017.6	24.291	PO_3 stretch	Phosphate ion

Table 4: FT-IR Interpretation of compounds of methanol stem bark extract of *Mangifera indica*

Wavenumber cm^{-1} (Test Sample)	% Transmittance	Functional Group assignment	Expected Phyto compounds identified
3304.3	64.450	H- bonded, O-H stretch	Hydroxyl compound, alcohols, phenols
2946.5	76.168	Asymmetric stretching of -CH (CH_2) vibration	Saturated aliphatic compounds
2834.6	79.684	Symmetric stretching of -CH (CH_2) vibration	Fatty acids, lipids, protein
1619.5	84.578	C=O stretching	Carbonyl compound
1488.1	78.893	C=C-C, aromatic ring	Aromatic compound
1112.6	80.859	O-H bend, alcoholic group	Phenol, tertiary alcohol
1015.7	28.059	PO_3 stretch	Phosphate ion

Fig 2 shows the combined FI-TR spectra of *M. indica* leaf and bark extracts. In Fig 3, the FT-IR spectra peak at $3,321.1\text{ cm}^{-1}$ for *M. indica* stem bark extract revealed the presence of alcohols and phenols (O-H stretch, H-bonded), the peaks at 2942.7 and 2834.6 cm^{-1} showed the presence of alkanes (C-H stretch), the peak at 1619.5 cm^{-1} revealed the presence of carboxylic acid group (C=O stretch), the peak at 1488.1 cm^{-1} showed the presence of aromatic compounds (C-N stretch), and the peaks at 1112.6 and 1015.7 cm^{-1}

indicate presence of alcohols, esters, ethers, and phosphate (O-H bend).

Fig 4 shows the FT-IR spectra of *M. indica* leaf extracts, with the peaks at $3,304.3$, 2946.5 , 2834.6 , 1619.5 , 1488.1 , 1112.6 and 1015.7 cm^{-1} revealing the presence of alcohols, (O-H stretch, H-bonded), fatty acids and lipids (H-C=O, C-H stretch), nitriles (C=O stretch), carbonyls (C=O stretch), aromatics (C=C-C), phenols (O-H bend), and phosphate (O-H bend).

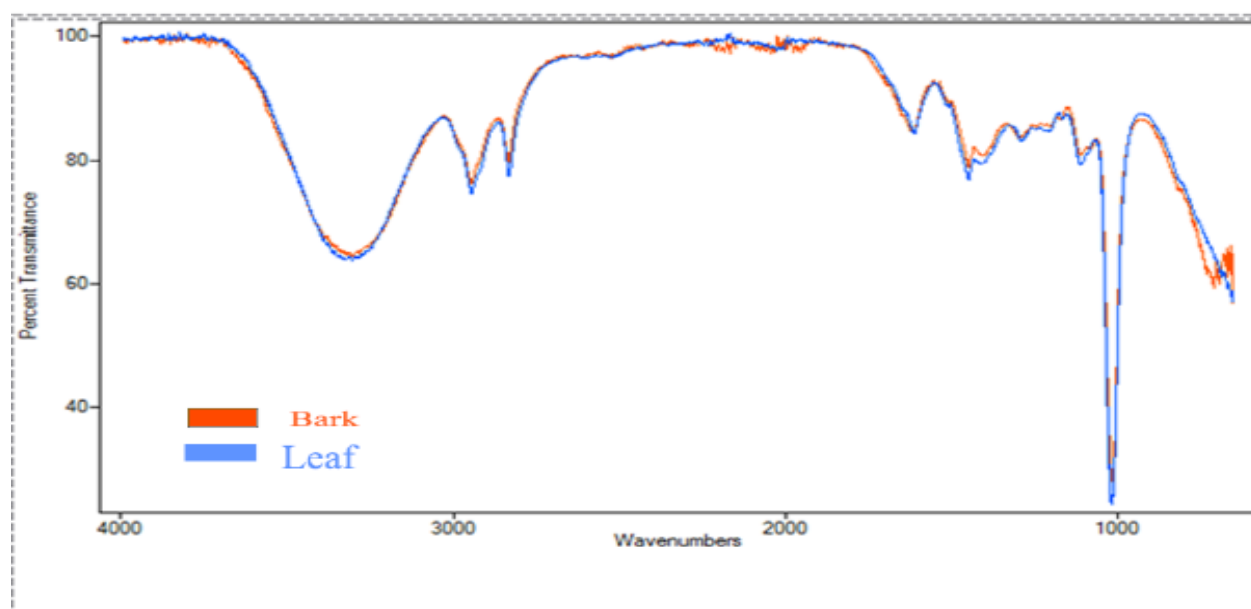


Fig. 2: FTIR Spectra analysis of methanolic stem bark and leaf extract of *Mangifera indica*

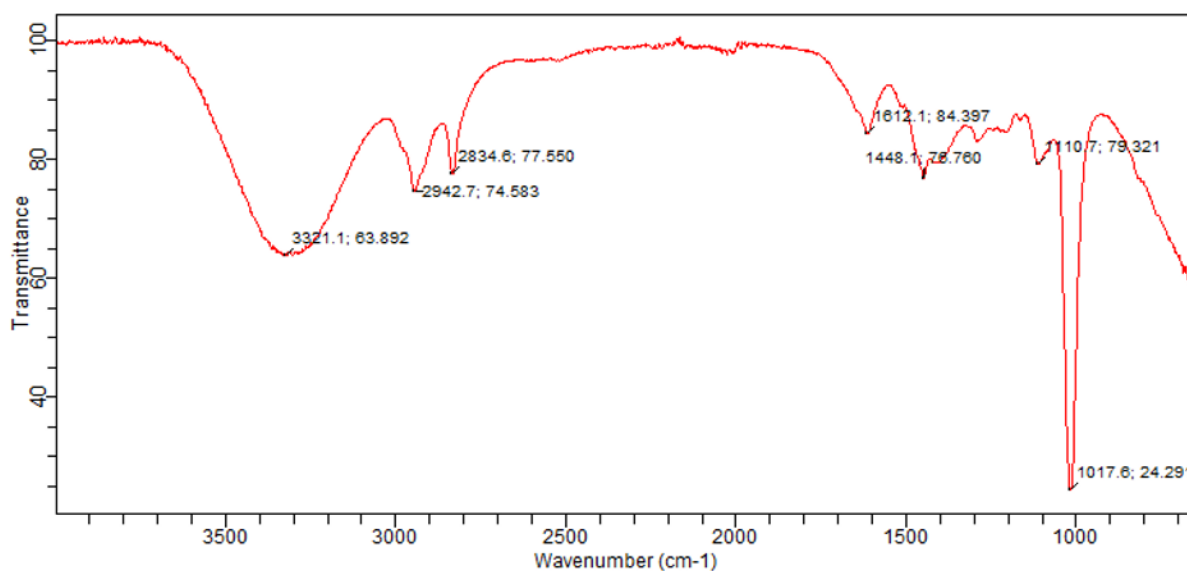
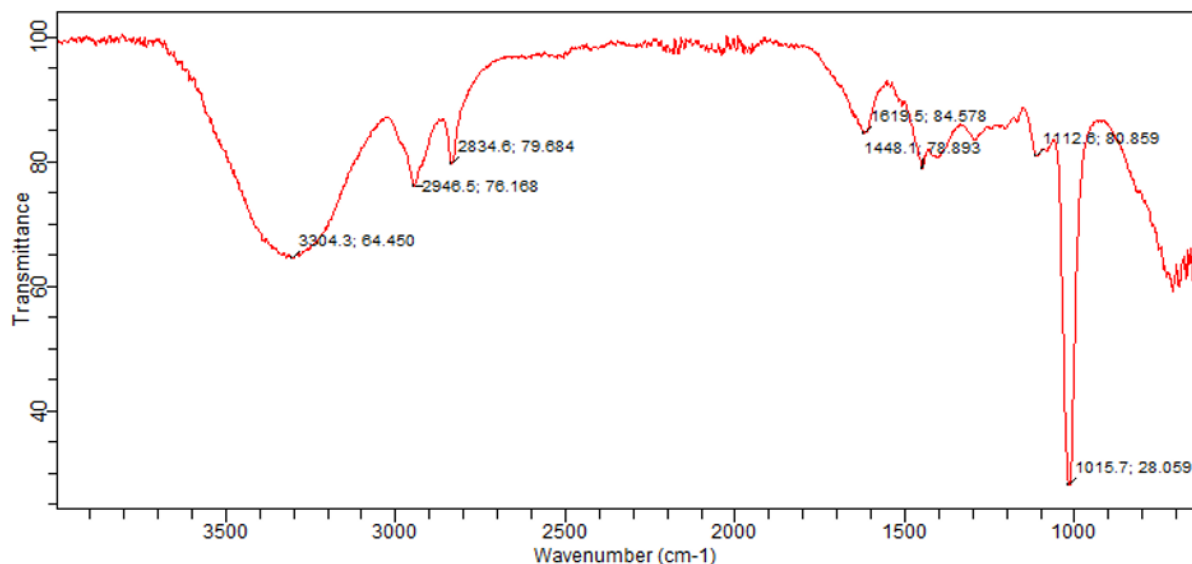


Fig. 3: FTIR spectrum analysis of methanol stem bark extract of *Mangifera indica*

Fig. 4: FT-IR spectrum analysis of methanol leaf extract of *Mangifera indica*Table 5: Antibacterial activity of methanolic extracts of *Mangifera indica* on selected bacterial isolates

Bacteria isolate	Zone diameter of inhibition (mm)		
	Mango leaf (30 µg)	Mango bark (30 µg)	Tetracycline (30 µg)
<i>Pseudomonas aeruginosa</i>	12.5	13	0
<i>Staphylococcus aureus</i>	8.5	0	5.5
<i>Klebsiella pneumoniae</i>	10.5	0	13.5
<i>Salmonella</i> spp	15	12	18
<i>Escherichia coli</i>	11	8	13
<i>Proteus mirabilis</i>	10	0	10

The result of the antimicrobial assay in Table 5 shows the diameters of inhibition zone (in millimetres) of the isolates to the plant extracts. Leaf extracts of *M. indica* (30µg) exerted highest antimicrobial activity against *Salmonella* spp (inhibition zone diameter of 15 mm) and *P. aeruginosa* (inhibition zone diameter of 12.5mm), and least activity against *S. aureus* (inhibition zone diameter of 8.5 mm).

The extract of *M. indica* bark (30µg) exerted highest antimicrobial activity against *P. aeruginosa* (inhibition zone diameter of 13mm) and *Salmonella* spp (inhibition zone diameter of 12mm) and no activity against *S. aureus*, *K. pneumoniae* and *P. mirabilis* (inhibition zone diameter of 0mm each). Comparatively, tetracycline (30µg) exerted highest antimicrobial activity against *Salmonella* spp (inhibition zone diameter of 18 mm) but no activity against *P. aeruginosa* (inhibition zone diameter of 0 mm).

Discussion:

Water is such a common everyday substance that its significance in biological systems may tend to be underestimated. Various microorganisms may be commonly found in wastewater, many of which are pathogenic. Gram negative bacteria belonging to five genera were isolated from the wastewater samples; *E. coli*, *Proteus mirabilis*, *Salmonella* spp, *P. aeruginosa* and *K. pneumoniae*. These Gram-negative bacteria are usually implicated in a number of important water-borne diseases and thus their presence in the sampled wastewater indicates such water requires adequate treatment before discharge into the environment, particularly through run-off into surface water bodies. In a similar study (15) evaluating presence of pathogenic organisms in wastewater samples, bacteria such as *P. aeruginosa*, *K. pneumoniae*,

E. coli, *Bacillus cereus* and *Streptococcus pneumoniae* were isolated from wastewater. Ghai et al., (16) also isolated *E. coli*, *B. cereus*, *Enterobacter* sp. and *S. aureus* from standing pond water, with the highest frequency of occurrence of *E. coli* (36%) with *K. pneumoniae* the least frequency of occurrence (7%). This indicates that faecal matters may be a major pollutant of the wastewater being generated from the point sources of their collection. In another study by Shivani et al., (17), *Bacillus* spp had the highest frequency of occurrence (38.86%) while *P. aeruginosa* had the lowest frequency (7.69%). Though these organisms are opportunistic pathogens, their presence in these wastewater samples could prove lethal when such waters find their way into surface or groundwater consumed by residents in the surrounding community.

The Mango extracts exerted high *in vitro* antimicrobial activity against *Salmonella* spp and *P. aeruginosa* isolates, with inhibition zone diameters of 15mm and 12.5mm for the leaf extract, and 12mm and 13mm for the bark extracts respectively. Although, the antimicrobial activity of the standard antibiotic (tetracycline) used as control against *Salmonella* spp (inhibition zone diameter of 18mm) was higher than the *M. indica* leaf and bark extracts, tetracycline did not exert any antimicrobial effect on *P. aeruginosa* (inhibition zone diameter of 0mm). Plants have long been identified as very important sources of potentially useful bioactive principles for the development of new chemotherapeutic agents (18). The FT-IR analysis of the Mango leaf and bark extracts in this study showed high presence of hydroxyl groups, phenols, alkanes, aromatic amines, carboxylic acids, esters, and ether compounds. Rajiv et al., (19) reported the presence of phenols, alkanes, aromatic amines, carboxylic acids, esters and ethers when an FT-IR spectrum analysis was conducted on methanolic extracts of *Myristica dactyloids* fruits.

Previous studies on Mango leaf extracts have been reported by many workers to show the presence of active functional groups such as xanthenes (mangiferin), flavonols (quercetin), benzophenones and terpenoids (20). The presence of these compounds in Mango stem bark and leaf may have contributed to the antimicrobial activity of the extracts against the bacteria isolates in our study. The implication of this relatively abundant composition of active principles within the Mango leaf and bark extracts confirms their wide application in local treatment of infectious diseases.

The physico-chemical parameters of the wastewater samples indicated that the pH

values obtained (6.5-8.0) favored the growth of the bacteria, which grew well within this pH range. The sulphate and phosphate contents of the wastewater samples in this study were well within permissible range when compared with maximum recommended levels of the sulphate and phosphate index values in domestic drinking water (21,22,23). This fact notwithstanding, it is emphasized that release of non-treated domestic wastewater directly into water bodies will cause nutrient enrichment of such waters, which may lead to eutrophication, that results in ecosystem imbalance through disruption of natural food chain and web relationships and other adverse environmental reactions.

Plants are generally known to contain several phytochemicals such as alkaloids, flavonoids, saponins, tannins and other phenolic compounds that have antimicrobial activities (24). Phytochemical screening of Mango leaf and bark extracts have shown the presence of important phytochemical constituents such as tannins, alkaloids, saponins, cardiac glycosides, flavonoids, phenols, proteins and phytosterols. In our study, alkaloids, tannins, saponins, and cardiac glycosides were present in the extracts, while phytosterols and flavonoids were absent in the aqueous and the ethanolic extracts of the Mango leaf. Variation in phytochemical components of the various extracts may result from differences in the chemistry of the extracting solvents, which may selectively affect extraction of various bio-active metabolites.

The natural tendency of saponins to wall off microbes makes them good candidates for treating bacterial, fungal and yeast infections, boost effectiveness of certain vaccines, and destroy some types of tumors, particularly lung and blood cancers. Therefore, the presence of saponins in the leaf and bark extracts of *M. indica*, as found in our study, indicates their bioactivity against bacterial pathogens in wastewaters. The phytochemical analysis in this study confirmed the presence of active functional components in the leaf and bark extracts of *M. indica* which be responsible for the antibacterial properties of the plant materials. A potential promising candidate therefore for biological water treatment and other environmentally remediated functions would be the Mango stem bark and leaf plant parts.

Conclusion:

In conclusion, the results of this study indicated the presence of potentially pathogenic Gram-negative bacteria in wastewater. These bacteria could pose serious health risks

to man and aquatic habitats thereby killing economically important aquatic lives. The physico-chemical parameters of the wastewater samples used in this study underscores the importance of treating wastewater adequately before release into natural water bodies, to prevent adverse environmental reactions. The release of untreated wastewater directly into water bodies may lead to eutrophication, which is the nutrient enrichment of these water bodies that negatively impacts aquatic life and may lead to ecosystem imbalance, and disruption of natural food chain and web relationships in such environments.

The phytochemical screening of the leaf and bark extracts confirmed Mango as a potential source of antibacterial agents and purifying agents for green and ecofriendly water treatment technology development that can reduce microbial loads in wastewaters to acceptable levels without causing harm to the environment from contaminated effluents.

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**Short Communication****Open Access****Prevalence and risk factors of acute gastroenteritis caused by Rotavirus among children in tertiary hospitals, southeastern Nigeria**

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Abstract:

Background: Diarrhea is a worldwide problem and rotavirus is the commonest viral etiologic agent. In Nigeria, diarrhea causes more than 315,000 deaths of preschool-age children annually. In Ebonyi State, it is among the leading causes of pediatric emergency visits and one of the major causes of infant morbidity and mortality. This study was aimed at determining the prevalence and associated risk factors of acute gastroenteritis due to rotavirus infection among under-five children in Abakaliki, Ebonyi state.

Methodology: This was a cross-sectional study of 275 children under 5 years of age hospitalized for acute watery diarrhea, who were consecutively recruited into the study. Stool samples were collected from each child for rotavirus antigen detection using an enzyme-linked immunosorbent assay (ELISA). Socio-demographic information of each child and selected risk factors were collected using structured questionnaire. Data analysis was done on SPSS software version 20.0, and association of demographic characteristics and risk factors with rotavirus diarrhoea was measured using Chi-square test, odds ratio (and 95% confidence interval). Significant value was set at $p < 0.05$.

Results: The prevalence of rotavirus diarrhea among children under 5 years of age in this study was 26.5% (73/275). Aside from educational level of the mothers, there was no significant association between any of the socio-demographic characteristics and prevalence of rotavirus diarrhoea ($p > 0.05$). Although the prevalence of diarrhoea in the children was lower with the use of maize gruel (pap) as weaning feed (26.3%, 71/270) compared to the use of other complementary feeding such as 'Cerelac' and 'NAN' (40%, 2/5), this association did not reach a significant level (OR=0.5352, 95% CI=0.0875-0.3270, $p=0.6110$), probably due to the small number of children weaned using other complementary feeds.

Conclusion: The relatively high prevalence of rotavirus diarrhea in children under 5 years of age in this study is an indication of the need for the parents/guardians of these children to improve child feeding hygiene.

Keywords: Acute gastroenteritis; Rotavirus; ELISA; antigen test; children; Abakaliki; Nigeria

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Prévalence et facteurs de risque de gastro-entérite aiguë causée par le rotavirus chez les enfants des hôpitaux tertiaires, sud-est du Nigéria

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Résumé:

Contexte: La diarrhée est un problème mondial et le rotavirus est l'agent étiologique viral le plus courant. Au Nigeria, la diarrhée provoque chaque année plus de 315 000 décès d'enfants d'âge préscolaire. Dans l'État d'Ebonyi, c'est l'une des principales causes de visites aux urgences pédiatriques et l'une des principales causes de morbidité et de mortalité infantiles. Cette étude visait à déterminer la prévalence et les facteurs de risque associés de gastro-entérite aiguë due à une infection à rotavirus chez les enfants de moins de cinq ans à Abakaliki, dans l'État d'Ebonyi.

Méthodologie: Il s'agissait d'une étude transversale portant sur 275 enfants de moins de 5 ans hospitalisés pour diarrhée aqueuse aiguë, qui ont été recrutés consécutivement dans l'étude. Des échantillons de selles ont été prélevés sur chaque enfant pour la détection de l'antigène du rotavirus à l'aide d'un dosage immuno-enzymatique (ELISA). Les informations sociodémographiques de chaque enfant et les facteurs de risque sélectionnés ont été collectés à l'aide d'un questionnaire structuré. L'analyse des données a été effectuée sur le logiciel SPSS version 20.0, et l'association des caractéristiques démographiques et des facteurs de risque avec la diarrhée à rotavirus a été mesurée à l'aide du test du chi carré, du rapport de cotes (et de l'intervalle de confiance à 95%). La valeur significative a été fixée à $p < 0,05$.

Résultats: La prévalence de la diarrhée à rotavirus chez les enfants de moins de 5 ans dans cette étude était de 26,5% (73/275). Mis à part le niveau d'éducation des mères, il n'y avait pas d'association significative entre les caractéristiques sociodémographiques et la prévalence de la diarrhée à rotavirus ($p > 0,05$). Bien que la prévalence de la diarrhée chez les enfants ait été plus faible avec l'utilisation de gruaux de maïs (pap) comme aliment de sevrage (26,3%, 71/270) par rapport à l'utilisation d'autres aliments complémentaires tels que «Cerelac» et «NAN» (40%, 2/5), cette association n'a pas atteint un niveau significatif (OR=0,5352, IC 95%=0,0875-0,3270, $p=0,6110$), probablement en raison du faible nombre d'enfants sevrés utilisant d'autres aliments complémentaires.

Conclusion: La prévalence relativement élevée de la diarrhée à rotavirus chez les enfants de moins de 5 ans dans cette étude est une indication de la nécessité pour les parents/tuteurs de ces enfants d'améliorer l'hygiène alimentaire des enfants.

Mots-clés: Gastro-entérite aiguë; Rotavirus; ELISA; test d'antigène; enfants; Abakaliki; Nigeria

Introduction:

Diarrhea is a worldwide problem, and despite the global public health intervention in water supply, diarrhea remains the second leading cause of death, after pneumonia, in children under 5 years of age (1). Globally, an estimate of more than 110 million diarrheal cases and 2 million hospitalizations, resulting in more than 17% of childhood deaths, are reported annually (2). In developing countries, most of the diarrhea cases are usually severe enough to require hospitalization, with 1.87 million deaths in children before the age of 5 years (2). Again, in surviving children, severe diarrhea presents with some complications such as malabsorption syndrome, malnutrition (conditions often associated with poor cognitive function), failure to thrive in early childhood, and increased susceptibility to other infections (3).

In children, rotavirus is the commonest viral etiologic agent of diarrhea globally, and rotavirus group A is the most frequent (4). Also, a previous study confirmed that rotavirus accounted for 37.1% of acute diarrheal hospitalizations (5). The virus is detected more in diarrhea stools (87%) than in normal stools (30.6%) and younger children are more vulnerable than the older ones with the peak incidence between 6 and 24 months (6).

In Nigeria, reports have shown that rotavirus is a very common etiologic agent of diarrhea in children under 5 years of age and causes more than 315,000 deaths of preschool-

age children annually (5,7-14). In Ebonyi State of Nigeria, diarrhea is among the leading causes of paediatric emergency visits and one of the major causes of infant morbidity and mortality (15). The aim of this study, therefore, was to determine the prevalence of and risk factors for rotavirus diarrhea among hospitalized children under 5 years of age in two tertiary hospitals in southeastern Nigeria.

Materials and method:

Study setting

The study was conducted in Abakaliki, the capital city of Ebonyi State, Nigeria. The State borders with Enugu State in the west, Cross River State in the east, Benue State in the north, and Abia State in the south. There are two distinct seasons; wet and dry seasons occurring between April to October and November to March respectively. The State has poor access to adequate potable water, poor sanitation, and hygiene, with a consequent increase in diarrhea cases, especially in children. It has a population of about two million people out of which 42.7% are children, with 11.7% below 4 years of age (16).

The study area is traversed by several rivers which constitute sources of water supply especially to the suburbs and rural communities. This may serve for drinking, washing and bathing, and irrigation purposes for medium and small-scale farming which is a major occupation of the people.

Study design

This was a descriptive cross-sectional study of children below 5 years of age conducted in the two major hospitals; Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA), and Mile 4 Hospital, Abakaliki, Ebonyi State, from Jan to April 2019.

Sample size and study eligibility

Sample size for the study was calculated using the formula; $N = z^2pq/d^2$ (17), where N is the desired sample size, 'z' is the standard normal deviation usually set at 1.96 corresponding to 95% confidence interval, 'p' is the prevalence of rotavirus which was 19.2% from a previous study (10), 'q' is 1-p, and 'd' is the standard error (margin of error) set at 0.05. This gave a sample size of 238 that was increased after considering 15% attrition to 275 participants.

Eligibility criteria for the study were children below 5 years of age who presented with acute watery stools (passage of 3 or more loose stools per day) with or without blood or fever at the participating health facilities, and informed consents of each parent or guardian. Children with chronic or persistent diarrhea (diarrhea that has lasted for more than 14 days) were excluded.

Ethical approval

Ethical approval was obtained from the Research and Ethics Committee of the two hospitals. Written informed consent, explained in the local language where necessary, was also obtained from each parent/guardian.

Sampling technique and data collection

Eligible children at each of the health-care facility was consecutively recruited into the study until the sample size was attained (this was over a period of 4 months). A structured questionnaire was used to collect socio-demographic information and some selected risk factors such as age, gender, weight, place of residence, educational level of parent/caregivers, occupation of fathers, rotavirus immunization and feeding history.

Sample collection and laboratory analysis

Stool samples from eligible participants were collected into a clean plastic bottle and transported in a Giostyle cool box containing some icepacks to Virology Laboratory in the Alex Ekwueme Federal University Teaching Hospital where they were stored in a deep freezer at -70°C. The samples were then transported in a triple layer-packaging with icepacks to the Central Research Laboratory, College of Medicine, University of Lagos, for analysis.

The AccuDiag™ Rotavirus (Fecal) ELISA kits (Diagnostic Automation Incorporation, DAI, USA) were used to detect rotavirus antigens present in the stool samples, according to the standard operating procedure of the manufacturer's instruction.

First, all reagents were brought to room temperature (15-30°C) and all frozen samples were thawed completely before use. Ninety-six test wells were placed in a strip holder. One hundred micro liter (100 µl) each of the negative control, positive control, and stool supernatant were pipetted to the appropriate test wells. This was incubated at room temperature for 30 minutes, then washed three times. After the last wash, the wells were slapped out on a clean absorbent towel to remove excess wash buffer. Two drops of Reagent 1 (blue solution) were added to each well, incubated at room temperature for 5 minutes, and then washed. After the last wash, the wells were slapped out on a clean absorbent towel to remove excess wash buffer. Two drops of Reagent 2 (red solution) were added to each well, incubated at room temperature for 5 minutes, then washed 3 times. After the last wash, wells were slapped out on a clean absorbent towel to remove excess wash buffer. Two drops of Chromogen were then added to each well followed by incubation at room temperature for 5 minutes. Finally, 2 drops of Stop Solution were added to each well and mixed thoroughly by gently tapping the side of the strip holder with the index finger.

Washings at each stage were done by vigorously filling each well to overflowing and decanting contents 3 separate times. Results were read using the ELISA plate reader. All the wells were read using bichromatic reading with fiber at 450nm and 620-650 nm. The cut-off mark was at 0.15 optic density (OD) with an absorbance reading of 0.15 OD and above taken to be positive while reading less than 0.15 OD was taken to be negative.

Statistical analysis of data

All data were analyzed using SPSS statistical software for Windows® version 20, 2012 (IBM Inc., Chicago Illinois, USA). Categorical and discrete variables were summarized using frequency and percentage. Association of variables with prevalence of rotavirus diarrhoea was measured using Chi-square test, odd ratio (and 95% confidence interval). Significant value was set at $p < 0.05$.

Results:

Sociodemographic characteristics of children

As shown in Table 1, of the total of 275 children recruited into this study, 168 (61.1%)

were males while 107 (38.9%) were females. Most of the children (n=152, 55.1%) were within the ages of 1–11 months while 123 (44.7%) were between the ages of 12 and 59 months, 120 (43.6%) lived in rural areas while 155 (56.4%) lived in urban settings.

Of the 275 children, 73 tested positive for rotavirus antigen, giving a prevalence of 26.5%. The prevalence of rotavirus diarrhea in male children was 25% (42/168), which was not significantly different from the prevalence of 29% (31/107) in female children (OR=0.8172, 95% CI 0.4741-1.409, $p=0.481$). The rotavirus prevalence of 25.7% (39/152) in children between 1-11 months of age was also not significantly different from the rotavirus prevalence of 27.6% (34/123) in children 12-59 months of age (OR=0.9034, 95% CI=0.5279 - 1.546, $p=0.7838$). However, the prevalence of rotavirus was significantly higher in children whose mothers had secondary level of education (33.8%, 49/145) than those whose mothers had higher level (University and Polytechnic) of education (17.3%, 13/75) and those whose mothers had no education or only primary school leaving certificate level of education

(20.0%, 11/55) ($\chi^2=13.680$, $p=0.0084$). There was no significant difference ($p>0.05$) in the prevalence of rotavirus diarrhoea among the children in relation to other sociodemographic factors such as family type, care giver, and occupation of the fathers (Table 1).

Risk factors for rotavirus diarrhea

Table 2 depicts the investigated risk factors in relation to the prevalence of rotavirus diarrhea, which showed that none of the factors was significantly associated with prevalence of rotavirus diarrhoea ($p>0.05$). Although the prevalence of diarrhoea in the children was lower with the use of maize gruel (pap) as weaning feed (26.3%, 71/270) compared to the use of other complementary feeding such as 'Cerelac' and 'NAN', (40%, 2/5), this association did not reach a significant level (OR=0.5352, 95% CI= 0.0875-0.3270, $p=0.6110$).

The prevalence of rotavirus diarrhea was higher in those who were not exclusively breastfed (29.2%, 28/96) than in those who were exclusively breast fed (25.1%, 45/179) but this also did not reach a significant level (OR= 0.8156, 95%CI=0.4683-1.420, $p=0.4772$).

Table 1: Prevalence of rotavirus diarrhoea in relation to socio-demographic characteristics of children in tertiary hospitals, southeastern Nigeria

Socio-demographic variables	Rotavirus status by ELISA antigen results			OR (95% CI)	p value
	No positive (%) n=73 (26.5)	No negative (%) n=202 (73.5)	Total n=275		
Age group (in months)					
1-11	39 (25.7)	113 (74.3)	152	0.9034 (0.5279 - 1.546)	0.7838
12-59	34 (27.6)	89 (72.4)	123		
Gender					
Male	42 (25.0)	126 (75.0)	168	0.8172 (0.4741 - 1.409)	0.4861
Female	31 (29.0)	76 (71.0)	107		
Care giver					
Mother	70 (27.2)	187 (72.8)	257	1.872 (0.5256 - 6.664)	0.4167
Nanny	3 (16.7)	5 (83.3)	23		
Family type					
Monogamous	67 (26.6)	185 (73.4)	252	1.026 (0.3883-2.712)	1.0000
Polygamous	6 (26.1)	17 (73.9)	23		
Educational level of mother					
University/Polytechnic	13 (17.3)	62 (82.7)	75	13.680 ⁺	0.0084 [*]
Secondary school	49 (33.8)	96 (66.2)	145		
FSLC/No education	11 (20.0)	44 (80.0)	55		
Occupation of father					
Professional/civil servant	20 (24.4)	62 (75.6)	82	3.174 ⁺	0.5292
Politician	3 (23.1)	10 (76.9)	13		
Businessman	38 (25.3)	112 (74.7)	150		
Skilled artisan	6 (40.0)	9 (60.0)	15		
Unskilled worker	6 (40.0)	9 (60.0)	15		

⁺=Chi square value; ^{*}=statistically significant ($p>0.05$); OR=Odds Ratio; CI=Confidence Interval; ELISA=Enzyme-linked immunosorbent assay; FSLC=First school leaving certificate

Table 2: Prevalence of rotavirus diarrhea in relation to selected risk factors among children in tertiary hospitals, southeastern Nigeria

Risk factors	Rotavirus status by ELISA test results			OR (95% CI)	p value
	No positive (%) n=73 (26.5)	No negative (%) n=202 (73.5)	Total n=275		
Exclusive breastfeeding					
Yes	45 (25.1)	134 (74.9)	179	0.8156 (0.4683 - 1.420)	0.4772
No	28 (29.2)	68 (70.8)	96		
Complementary feeding					
Maize gruel	71 (26.3)	199 (73.7)	270	0.5352 (0.0875 - 0.3270)	0.6110
Others	2 (40.0)	3 (60.0)	5		
Preparation of baby's food by;					
Mother	73 (27.0)	197 (73.0)	270	4.094 (0.2234 - 75.007)	0.3295
Nanny	0	5 (100)	5		
Source of drinking water					
Processed water (bottle/sachet)	38 (23.6)	123 (76.4)	161	3.095 ⁺	0.3772
Rain	2 (18.2)	9 (81.8)	11		
Borehole/Well	28 (33.3)	56 (66.7)	84		
Stream	5 (26.3)	14 (73.7)	19		
Method of fecal waste disposal					
Water closet	43 (24.7)	131 (75.3)	174	3.397 ⁺	0.3344
Pit latrine	11 (28.2)	28 (71.8)	39		
Bush	18 (29.5)	43 (70.5)	61		
Stream	1 (100.0)	0	1		

⁺ = Chi square value; OR = Odds Ratio; CI = Confidence Interval; ELISA = Enzyme-linked immunosorbent assay

Discussion:

This study showed the burden of rotavirus diarrhea among under 5 years of age in Abakaliki, with a high prevalence of rotavirus diarrhea of 26.5% being reported. This is comparable to similar studies in Abuja with a prevalence of 25% (18), Lagos with 25.8% (19) and Sokoto with 25.5% (20). However, the rate in our study is lower than the prevalence of 31.5% in Enugu (13), 37.1% in Asaba (5), 38.1% in Maiduguri (12), and 32.2% in Kaduna (21). The observed similarities and differences in prevalence with these studies and our study may be attributed to differences in methodology, study design, time and season of sample collection, sample storage, geographical location, or changes in environmental or socio-economic factors. Again, it might as well reflect changing trends of rotavirus infection (22).

Our study revealed that there was no statistically significant difference in the prevalence of rotavirus infection in relation to age groups (1-11 months and 12-59 months) and gender. This finding is consistent with a previous study in Jos (7). Although the detection rate of rotavirus antigen was slightly higher in female (29%, 42/168) than in male children (25%, 31/107), this difference was not significantly different ($p=0.4861$). Similar observations were made in studies from Abuja, Jos and Katsina (7,18,23). However, it is in contrast with the studies from Benin and Asaba where significantly higher percentages in females than males

were reported (5,10). The only socio-demographic factor significantly associated with prevalence of rotavirus diarrhoea in our study was educational level of the mothers, which showed that the prevalence was higher in mothers with secondary level of education than those with higher education (University and Polytechnic), and those with no education or school leaving certificate education level ($p=0.0086$).

None of the selected risk factors such as the history of exclusive breastfeeding, preparation of children meal, sources of drinking water, and methods of fecal disposal was significantly associated with prevalence of rotavirus diarrhoea. Although the use of maize gruel (pap) as weaning meal was associated with a lower prevalence of rotavirus diarrhoea (26.3%) compared to use of other complementary feeding such as 'Cerelac' and 'NAN' (40.0%), this association did not reach statistically significant level ($p=0.6110$), probably due to the small number of children weaned with complementary feeds ($n=5$). Nevertheless, the higher prevalence of rotavirus diarrhea in these children may be explained by the fact that preparation of maize gruel (pap) requires the use of boiling water (100°C) as opposed to complementary feeds that do not require such procedure in their preparation. Rotaviruses are not likely to survive the high temperature of boiling.

Our study findings agree largely with a similar study in Asaba (5) where apart from younger age group, no other risk factors including complementary feeding type, was asso-

ciated with prevalence of rotavirus diarrhoea. Again, the findings in our study of exclusive breastfeeding and sources of drinking water not significantly associated with prevalence of rotavirus diarrhea were similar to those reported in the study conducted in Benin (10).

Conclusion:

The relatively high prevalence of rotavirus diarrhea in children under 5 years of age in Abakaliki, reported in our study is an indication of the need for the parents/guardians of these children to improve child feeding hygiene.

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CONFERENCE ABSTRACTS/RÉSUMÉS DE LA CONFÉRENCE

Lassa virus persistence in body fluids after recovery from acute Lassa fever: a 2-year interim analysis of a prospective longitudinal cohort study

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Background: There is anecdotal evidence for Lassa virus persistence in body fluids. We investigated various body fluids after recovery from acute Lassa fever and describe the dynamics of Lassa virus RNA load in seminal fluid. The primary objective of this study was to quantitatively describe virus persistence and clearance and assess the infectivity of seminal fluid.

Methodology: In this prospective, longitudinal, cohort study, we collected plasma, urine, saliva, lacrimal, vaginal and seminal fluids from Lassa fever survivors at Irrua Specialist Teaching Hospital in Edo State, Nigeria. Inclusion criteria for participants were RT-PCR-confirmed Lassa fever diagnosis and age 18 years and above. Samples were taken at discharge from hospital (month 0) and at months 0.5, 1, 3, 6, 9, 12, 18, and 24 after discharge. Lassa virus RNA was detected using real-time RT-PCR. Infectivity was tested in cell culture and immunosuppressed mice. We used a linear mixed-effect model to analyse the dynamics of virus persistence in seminal fluid over time.

Results: Between Jan 31, 2018, and Dec 11, 2019, 165 participants were enrolled in the study, of whom 159 were eligible for analysis (49 women and 110 men). Low amounts of Lassa virus RNA were detected at month 0 in plasma (45%, n=49/110), urine (34%, 37/110), saliva (5%, 5/110), lacrimal fluid (9%, 10/110), and vaginal fluid (21%, n=7/33 female participants). Virus RNA was cleared from these body fluids by month 3. However, 35 (80%) of 44 male participants had viral RNA in seminal fluid at month 0 with a median cycle threshold of 26.5. Lassa virus RNA remained detectable up to month 12 in seminal fluid. Biostatistical modelling estimated a clearance rate of 1.19 log₁₀ viral RNA copies per month and predicted that 50% of male survivors remain Lassa virus RNA-positive in seminal fluid for 83 days after hospital discharge, and 10% remain positive in seminal fluid for 193 days after discharge. Viral RNA persistence in seminal fluid for 3 months or more was associated with higher viraemia ($p=0.006$), more severe disease ($p=0.0075$), and longer hospitalisation during the acute phase of Lassa fever ($p=0.0014$). Infectious virus was isolated from 48 (52%) of 93 virus RNA-positive seminal fluid samples collected between month 0 and 12.

Conclusion: Lassa virus RNA is shed in various body fluids after recovery from acute disease. The persistence of infectious virus in seminal fluid implies a risk of sexual transmission of Lassa fever.

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Persistence du virus de Lassa dans les fluides corporels après la guérison de la fièvre de Lassa aiguë: une analyse intermédiaire de 2 ans d'une étude de cohorte longitudinale prospective

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Contexte: Il existe des preuves anecdotiques de la persistance du virus Lassa dans les fluides corporels. Nous avons étudié divers fluides corporels après la guérison de la fièvre de Lassa aiguë et décrit la dynamique de la

charge d'ARN du virus de Lassa dans le liquide séminal. L'objectif principal de cette étude était de décrire quantitativement la persistance et la clairance du virus et d'évaluer l'infectiosité du liquide séminal.

Méthodologie: Dans cette étude de cohorte prospective, longitudinale, nous avons collecté du plasma, de l'urine, de la salive, des fluides lacrymal, vaginal et séminal de survivants de la fièvre de Lassa à l'hôpital universitaire spécialisé d'Irrua dans l'État d'Edo, au Nigéria. Les critères d'inclusion pour les participants étaient le diagnostic de fièvre de Lassa confirmé par RT-PCR et l'âge de 18 ans et plus. Des échantillons ont été prélevés à la sortie de l'hôpital (mois 0) et aux mois 0,5, 1, 3, 6, 9, 12, 18 et 24 après la sortie. L'ARN du virus Lassa a été détecté par RT-PCR en temps réel. L'infectiosité a été testée en culture cellulaire et sur des souris immunodéprimées. Nous avons utilisé un modèle linéaire à effets mixtes pour analyser la dynamique de la persistance du virus dans le liquide séminal au fil du temps.

Résultats: Entre le 31 janvier 2018 et le 11 décembre 2019, 165 participants ont été inscrits à l'étude, dont 159 étaient éligibles pour l'analyse (49 femmes et 110 hommes). De faibles quantités d'ARN du virus de Lassa ont été détectées au mois 0 dans le plasma (45%, $n=49/110$), l'urine (34%, $37/110$), la salive (5%, $5/110$), le liquide lacrymal (9%, $10/110$) et les sécrétions vaginales (21%, $n=7/33$ participantes). L'ARN du virus a été éliminé de ces fluides corporels au mois 3. Cependant, 35 (80%) des 44 participants masculins avaient de l'ARN viral dans le liquide séminal au mois 0 avec un seuil de cycle médian de 26,5. L'ARN du virus de Lassa est resté détectable jusqu'au 12e mois dans le liquide séminal. La modélisation biostatistique a estimé un taux de clairance de $1,19 \log_{10}$ de copies d'ARN viral par mois et a prédit que 50% des hommes survivants restent positifs pour l'ARN du virus de Lassa dans le liquide séminal pendant 83 jours après la sortie de l'hôpital, et 10% restent positifs dans le liquide séminal pendant 193 jours. La persistance de l'ARN viral dans le liquide séminal pendant 3 mois ou plus était associée à une virémie plus élevée ($p=0,006$), à une maladie plus grave ($p=0,0075$) et à une hospitalisation plus longue pendant la phase aiguë de la fièvre de Lassa ($p=0,0014$). Le virus infectieux a été isolé de 48 (52%) des 93 échantillons de liquide séminal positif à l'ARN du virus prélevés entre le mois 0 et le mois 12.

Conclusion: L'ARN du virus Lassa est excrété dans divers fluides corporels après guérison d'une maladie aiguë. La persistance du virus infectieux dans le liquide séminal implique un risque de transmission sexuelle de la fièvre de Lassa.

Prevalence of thyroid dysfunction in pregnancy

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Background: Thyroid dysfunction is frequent in pregnant women and is often associated with an increased risk of adverse maternal and fetal outcome. Many women attending the antenatal clinic with thyroid dysfunction may go unnoticed until the long-term sequelae is seen in the child. Therefore, thyroid assessment is important for identifying, treating, and preventing complication of thyroid dysfunctions.

Methodology: This was a multi-center hospital based descriptive cross-sectional study with 501 apparently healthy pregnant women enrolled. Blood specimen from participants were collected and analysed for serum thyroid stimulating hormone (TSH), free thyroxine (free T_4), free triiodothyronine (free T_3), thyroid peroxidase antibody (anti-TPO) by Enzyme-Linked Immunosorbent Assay (ELISA). Statistical analysis of data was done using SPSS.

Results: The prevalence of thyroid dysfunction in pregnant women using thyroid function tests was 12.4% ($n=62$), with 9.6% ($n=48$) hypothyroid, 2.0% ($n=10$) hyperthyroid, and 2.3% ($n=12$) autoimmune thyroid disorder.

Conclusions: The prevalence of thyroid dysfunction in pregnancy of 12.4% from this study, is relatively high in thyroid dysfunction assessment, therefore routine screening of women attending antenatal clinic should be incorporated.

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Prévalence du dysfonctionnement thyroïdien pendant la grossesse

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Contexte: La dysfonction thyroïdienne est fréquente chez les femmes enceintes et est souvent associée à un risque accru d'issues maternelles et fœtales indésirables. De nombreuses femmes fréquentant la clinique prénatale avec un dysfonctionnement thyroïdien peuvent passer inaperçues jusqu'à ce que les séquelles à long terme soient observées chez l'enfant. Par conséquent, l'évaluation de la thyroïde est importante pour identifier, traiter et prévenir les complications des dysfonctionnements thyroïdiens.

Méthodologie: Il s'agissait d'une étude transversale descriptive multicentrique hospitalière avec 501 femmes enceintes apparemment en bonne santé inscrites. Des échantillons de sang des participants ont été collectés et analysés pour la thyroïdostimuline sérique (TSH), la thyroxine libre (T_4) libre, la triiodothyronine libre (T_3) libre,

les anticorps anti-peroxydase thyroïdienne (anti-TPO) par dosage immuno-enzymatique (ELISA). L'analyse statistique des données a été effectuée à l'aide de SPSS.

Résultats: La prévalence du dysfonctionnement thyroïdien chez les femmes enceintes utilisant les tests de la fonction thyroïdienne était de 12,4% (n=62), avec 9,6% (n=48) d'hypothyroïdie, 2,0% (n=10) d'hyperthyroïdie et 2,3% (n=12) trouble thyroïdien auto-immun.

Conclusion: La prévalence du dysfonctionnement thyroïdien pendant la grossesse de 12,4% de cette étude, est relativement élevée dans l'évaluation du dysfonctionnement thyroïdien, par conséquent, le dépistage systématique des femmes fréquentant la clinique prénatale devrait être intégré.

Low lying giant chorangioma with myometrial attachment: report of a rare histopathological finding

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Chorangioma is an expansile nodular lesion composed of capillary vascular channels, intervening stroma cells and surrounding trophoblast. Placental chorangioma is the most common neoplasm of the placenta with an incidence of approximately 1% of all pregnancies. It presents as solitary nodule or less frequently, as multiple nodules in the foetal surface of the placenta or within the placental parenchyma. The clinical significance of chorangioma is size dependent. Small chorangiomas (< 5cm in diameter) are usually asymptomatic and are either not diagnosed or incidentally found at a histological examination or screening obstetric ultrasound examinations. Giant chorangiomas (> 5cm in diameter), are rarely seen in obstetric practice, occurring in approximately 1 in 10000 pregnancies and may be associated with hydramnios, haemorrhage, premature delivery, premature placental separation and placenta praevia. Foetal complications arising from giant chorangioma may include non-immune foetal hydrops, heart failure, anaemia, thrombocytopenia, weight loss and death. From the review of literature, there is no reported association of giant chorangioma with placenta accreta or an incidental finding of giant chorangioma in a hysterectomy specimen. We present the first report of an incidental low lying giant chorangioma partly invading the myometrium, in a hysterectomy specimen that followed an elective caesarean section and subsequent hysterectomy for intractable Postpartum Haemorrhage. The new born baby was in good medical condition.

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Chorangiome géant de basse altitude avec attache myométriale: rapport d'une constatation histopathologique rare

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Le chorangiome est une lésion nodulaire expansible composée de canaux vasculaires capillaires, de cellules stromales intermédiaires et de trophoblastes environnants. Le chorangiome placentaire est la tumeur la plus fréquente du placenta avec une incidence d'environ 1 % de toutes les grossesses. Il se présente sous la forme de nodules solitaires ou, moins fréquemment, sous forme de nodules multiples à la surface fœtale du placenta ou à l'intérieur du parenchyme placentaire. La signification clinique du chorangiome dépend de la taille. Les petits chorangiomes (< 5cm de diamètre) sont généralement asymptomatiques et sont soit non diagnostiqués, soit découverts fortuitement lors d'un examen histologique ou d'une échographie obstétricale de dépistage. Les chorangiomes géants (> 5cm de diamètre) sont rarement observés en pratique obstétricale, survenant dans environ 1 grossesse sur 10 000 et peuvent être associés à un hydramnios, une hémorragie, un accouchement prématuré, une séparation placentaire prématurée et un placenta praevia. Les complications fœtales résultant d'un chorangiome géant peuvent inclure l'anasarque fœtal non immunitaire, l'insuffisance cardiaque, l'anémie, la thrombocytopénie, la perte de poids et la mort. D'après la revue de la littérature, il n'y a pas d'association rapportée de chorangiome géant avec placenta accreta ou de découverte fortuite de chorangiome géant dans une pièce d'hystérectomie. Nous présentons le premier rapport d'un chorangiome géant de basse altitude envahissant en partie le myomètre, dans une pièce d'hystérectomie qui a suivi une césarienne électorale et une hystérectomie subséquente pour une hémorragie du post-partum réfractaire. Le nouveau-né était en bon état de santé.

Arboviral infections in pregnancy: the Ibadan experience

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Background: Arboviruses are a threat to global health as they are major cause of human and animal diseases. They typically present as febrile illnesses which may be misdiagnosed as malaria especially in malaria holo-endemic regions such as Ibadan, Nigeria. Hence, the burden of arboviral infections is underestimated due to misdiagnosis, under-reporting and lack of surveillance. The study was carried out to determine the seroprevalence of some arboviruses among pregnant women in Ibadan, Nigeria

Methodology: Sera of 362 pregnant women attending antenatal clinic in Ibadan were screened for anti-Zika virus immunoglobulin M (IgM) and immunoglobulin G (IgG) by enzyme-linked immunosorbent assay. A subset of 36 anti-Zika virus IgM and/or IgG positive sera were tested for antibodies to Zika, Yellow fever, Dengue-1 & -2, Spondweni, West Nile, and Chikungunya viruses using haemagglutination inhibition.

Results: Overall, 22 (61.1%) of the 36 sera tested had antibodies to the tested arboviruses. All the sera which tested positive, had antibodies to at least two or more viruses, 4 (11.1%) tested positive to 6 of the viruses, 4 (11.1%) tested positive to 5 of the viruses, 1 (2.8%) to 4 of the viruses, 8 (22.2%) to 3 of the viruses, and 5 (13.9%) to 2 of the viruses. Antibody to Dengue-1 was observed in all positive sera. Antibodies to Zika, Dengue-2 and Chikungunya were observed in 55.6%, 38.9%, and 25%, respectively while antibodies to Spondweni, West Nile and Yellow fever were detected in 22.2%, 13.9% and 11.1% respectively. About 47% of participants had antibodies to Zika and 2 other arboviruses, and 25% to Zika virus and 3 or more arboviruses. A low (11.1%) prevalence of antibodies to Yellow fever virus was observed.

Conclusion: This study showed high seroprevalence to arboviruses in pregnant women who have an increased risk of severe illness and vertical transmission to the foetus. It also demonstrated a very low herd immunity to Yellow fever despite Nigeria being in the category of countries recommended for vaccination in the Yellow Fever belt. Therefore, a large proportion of the community is at risk of Yellow fever. These results emphasize the importance of seroprevalence studies as an important tool in the assessment of epidemiology of arboviral infections as well as herd immunity.

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Infections à arbovirus pendant la grossesse: l'expérience d'Ibadan

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Contexte: Les arbovirus constituent une menace pour la santé mondiale car ils sont la principale cause de maladies humaines et animales. Ils se présentent généralement comme des maladies fébriles qui peuvent être diagnostiquées à tort comme du paludisme, en particulier dans les régions holo-endémiques du paludisme telles qu'Ibadan, au Nigeria. Par conséquent, le fardeau des infections à arbovirus est sous-estimé en raison d'un diagnostic erroné, d'une sous-déclaration et d'un manque de surveillance. L'étude a été menée pour déterminer la séroprévalence de certains arbovirus chez les femmes enceintes à Ibadan, au Nigeria

Méthodologie: Les sérums de 362 femmes enceintes fréquentant une clinique prénatale à Ibadan ont été testés pour l'immunoglobuline M (IgM) anti-virus Zika et l'immunoglobuline G (IgG) par dosage immuno-enzymatique. Un sous-ensemble de 36 sérums anti-virus IgM et/ou IgG positifs a été testé pour les anticorps contre les virus Zika, fièvre jaune, Dengue-1 et -2, Spondweni, West Nile et Chikungunya en utilisant l'inhibition de l'hémagglutination.

Résultats: Au total, 22 (61,1%) des 36 sérums testés présentaient des anticorps contre les arbovirus testés. Tous les sérums testés positifs présentaient des anticorps contre au moins deux virus ou plus, 4 (11,1%) testés positifs contre 6 des virus, 4 (11,1%) testés positifs contre 5 des virus, 1 (2,8%) contre 4 des virus, 8 (22,2%)

à 3 des virus, et 5 (13,9%) à 2 des virus. Des anticorps contre la Dengue-1 ont été observés dans tous les sérums positifs. Des anticorps contre Zika, Dengue-2 et Chikungunya ont été observés chez 55,6%, 38,9% et 25%, respectivement, tandis que des anticorps contre Spondweni, West Nile et la fièvre jaune ont été détectés dans 22,2%, 13,9% et 11,1% respectivement. Environ 47% des participants avaient des anticorps contre Zika et 2 autres arbovirus, et 25% contre le virus Zika et 3 arbovirus ou plus. Une faible prévalence (11,1%) d'anticorps dirigés contre le virus de la fièvre jaune a été observée.

Conclusion: Cette étude a montré une séroprévalence élevée aux arbovirus chez les femmes enceintes qui présentent un risque accru de maladie grave et de transmission verticale au fœtus. Il a également démontré une très faible immunité collective contre la fièvre jaune bien que le Nigeria soit dans la catégorie des pays recommandés pour la vaccination dans la ceinture de la fièvre jaune. Par conséquent, une grande partie de la communauté est à risque de fièvre jaune. Ces résultats soulignent l'importance des études de séroprévalence en tant qu'outil important dans l'évaluation de l'épidémiologie des infections à arbovirus ainsi que de l'immunité collective.

Prescription pattern of antibiotics in private healthcare facilities in Port Harcourt metropolis, Rivers State, Nigeria

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Background: The widespread use of antibiotics plays a major role in the development and spread of antibiotic resistance globally, the consequences of which are grave with mortality and morbidity continually on the rise. Knowledge backed by data will help improve antimicrobial stewardship and curb the growing challenge of antimicrobial resistance locally and globally. Therefore, an assessment of antibiotic prescription practices of medical personnel in private health facilities in Rivers State, Nigeria, was carried out.

Methodology: A structured questionnaire was used to collect information on medical training and antibiotic prescription practices among 102 medical doctors. Data were analyzed at 95% confidence interval using the SPSS v25 software and a *p*-value less than 0.05 was considered statistically significant.

Results: Demographic characteristics showed that 32.4% of the respondents were female and 67.6% were male practitioners. About 60.8% had MBBS degree only, 21.6% had a postgraduate medical fellowship and 17.6% had postgraduate degrees (PGD/MSc/PhD). The data showed that 35.3% of the respondents had practiced for at least 15 years, 33.3% had practiced for 1-5 years and 23.5% had practiced for 6-10 years. About 43% of the respondents commonly prescribed cephalosporins, followed by amoxicillin 20.6%, ceftriaxone 16.7%, and ampicillin 2.9%. Only 30.4% indicated that laboratory investigations were the basis of prescriptions while 69.6% indicated that empirical assessment was the basis for prescription of antibiotics. Analysis based on academic qualifications showed that empirical (clinical judgment) prescription was most frequent among persons with postgraduate medical fellowship (86.4%) and least frequent among individuals with MBBS degrees only (13.6%). Empirical prescription was also most frequent among individuals with 6-10 years practicing experience (75%). However, there was no statistically significant association between the basis of prescriptions with academic qualifications or years of practice.

Conclusion: Findings of the study indicate a high preference for prescription of cephalosporins especially based on empirical assessment and not laboratory investigations among doctors in private medical facilities in Port Harcourt metropolis. Urgent steps are recommended to improve antibiotic prescription patterns among private practitioners in a bid to control the growing rate of antimicrobial resistance locally.

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Modèle de prescription d'antibiotiques dans les établissements de santé privés de la métropole de Port Harcourt, État de Rivers, Nigéria

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Contexte: L'utilisation généralisée des antibiotiques joue un rôle majeur dans le développement et la propagation de la résistance aux antibiotiques à l'échelle mondiale, dont les conséquences sont graves avec une mortalité et une morbidité en constante augmentation. Les connaissances étayées par des données aideront à améliorer la gestion des antimicrobiens et à freiner le défi croissant de la résistance aux antimicrobiens aux niveaux local et mondial. Par conséquent, une évaluation des pratiques de prescription d'antibiotiques du personnel médical dans les établissements de santé privés de l'État de Rivers, au Nigéria, a été réalisée.

Méthodologie: Un questionnaire structuré a été utilisé pour collecter des informations sur la formation médicale

et les pratiques de prescription d'antibiotiques auprès de 102 médecins. Les données ont été analysées à un intervalle de confiance de 95% à l'aide du logiciel SPSS v25 et une valeur p inférieure à 0,05 a été considérée comme statistiquement significative.

Résultats: Les caractéristiques démographiques ont montré que 32,4% des répondants étaient des femmes et 67,6% étaient des hommes pratiquants. Environ 60,8% n'avaient qu'un diplôme MBBS, 21,6% avaient un doctorat en médecine et 17,6% avaient un diplôme de troisième cycle (PGD/MSc/PhD). Les données ont montré que 35,3% des répondants avaient pratiqué pendant au moins 15 ans, 33,3% avaient pratiqué pendant 1 à 5 ans et 23,5% avaient pratiqué pendant 6 à 10 ans. Environ 43% des répondants prescrivaient couramment des céphalosporines, suivies de l'amoxicilline 20,6%, de la ceftriaxone 16,7% et de l'ampicilline 2,9%. Seulement 30,4% ont indiqué que les analyses de laboratoire étaient à la base des prescriptions tandis que 69,6% ont indiqué que l'évaluation empirique était la base de la prescription d'antibiotiques. Une analyse basée sur les diplômes universitaires a montré que la prescription empirique (jugement clinique) était la plus fréquente chez les personnes titulaires d'une bourse médicale postdoctorale (86,4%) et la moins fréquente chez les personnes titulaires d'un diplôme MBBS seulement (13,6%). La prescription empirique était également la plus fréquente chez les personnes ayant 6 à 10 ans d'expérience dans la pratique (75%). Cependant, il n'y avait pas d'association statistiquement significative entre la base des prescriptions avec des diplômes universitaires ou des années de pratique.

Conclusion: Les résultats de l'étude indiquent une forte préférence pour la prescription de céphalosporines en particulier sur la base d'une évaluation empirique et non d'enquêtes de laboratoire chez les médecins des établissements médicaux privés de la métropole de Port Harcourt. Des mesures urgentes sont recommandées pour améliorer les schémas de prescription d'antibiotiques chez les praticiens privés dans le but de contrôler le taux croissant de résistance aux antimicrobiens localement.

Leveraging the potentials of companion diagnostics for personalized cancer care in Nigeria: prospects and challenges for pathologists and policy proponents

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To achieve effective cancer care for all, an important component is the adoption of personalized medicine as the most logical approach to cancer management to improve outcomes and reduce side effects of treatment protocols. This implies personalized diagnostic services including the use of companion diagnostics which employs modern methods of molecular medicine for a more targeted therapy. Unfortunately, the uncomfortable truth is that personalized medicine is undeniably expensive and often requires new levels of expertise beyond what is traditionally offered in developing countries like Nigeria. The objectives of this study are to highlight the importance of personalized care in oncology and to advocate for improved investment of resources towards the provision of facilities for companion diagnostics as a means of achieving our overarching objective of cancer care for all. Companion diagnostics (CDs) is defined by FDA as a device that provides information that is essential for the safe and effective use of a corresponding drug or biological product. Currently there are more than two CDs for oncology care, some of them include; PATHVYSION HER-DNA Probe Kit and HERCEPT test for breast cancer, DAKO C-Kit PharmDx for leukemia, and COBAS K-Ras mutation test for colorectal cancer. The importance of companion diagnostics for personalized medicine and targeted oncology care cannot be overemphasized. Unfortunately, there remains considerable challenges militating against its widespread adoption in Nigeria. We argue that concerted advocacy at various levels and concentrated investment strategies are very much needed to meet these challenges.

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Tirer parti du potentiel des diagnostics compagnons pour les soins personnalisés contre le cancer au Nigeria: perspectives et défis pour les pathologistes et les partisans des politiques

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Pour parvenir à des soins efficaces contre le cancer pour tous, un élément important est l'adoption de la médecine personnalisée comme approche la plus logique de la gestion du cancer pour améliorer les résultats et réduire les effets secondaires des protocoles de traitement. Cela implique des services de diagnostic personnalisés, y compris l'utilisation de diagnostics compagnons qui utilisent des méthodes modernes de médecine moléculaire pour une

thérapie plus ciblée. Malheureusement, la vérité inconfortable est que la médecine personnalisée est indéniablement chère et nécessite souvent de nouveaux niveaux d'expertise au-delà de ce qui est traditionnellement offert dans les pays en développement comme le Nigeria. Les objectifs de cette étude sont de souligner l'importance des soins personnalisés en oncologie et de plaider en faveur d'un meilleur investissement des ressources dans la fourniture d'installations pour le diagnostic compagnon comme moyen d'atteindre notre objectif primordial de soins contre le cancer pour tous. Le diagnostic compagnon (CD) est défini par la FDA comme un dispositif qui fournit des informations essentielles à l'utilisation sûre et efficace d'un médicament ou d'un produit biologique correspondant. Actuellement, il existe plus de deux CD pour les soins en oncologie, certains d'entre eux incluent; Kit de sonde PATHVYSION HER-DNA et test HERCEPT pour le cancer du sein, DAKO C-Kit PharmDx pour la leucémie et test de mutation COBAS K-Ras pour le cancer colorectal. On ne saurait trop insister sur l'importance des diagnostics compagnons pour la médecine personnalisée et les soins oncologiques ciblés. Malheureusement, il reste des défis considérables qui militent contre son adoption généralisée au Nigeria. Nous soutenons qu'un plaidoyer concerté à différents niveaux et des stratégies d'investissement concentrées sont indispensables pour relever ces défis.

Effect of COVID-19 pandemic and its mitigating actions on blood transfusion services in Lagos State, Nigeria

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Background: The Lagos State Blood Transfusion Service (LSBTS) is responsible for the provision of safe blood in the State. To provide this service, the LSBTS coordinates activities between the government, partnering organizations and potential voluntary blood donors. This study describes the effect of the coronavirus disease 2019 (COVID-19) pandemic on blood transfusion services in Lagos State, Nigeria, and proffered solutions.

Methodology: This study formed part of a quality improvement exercise. A qualitative design through conducting ten key-informant interviews and meeting sessions with staff of the LSBTS, partnering organizations and the public was used for the study. Themes were identified following interviews.

Results: The COVID-19 pandemic and its lockdown measures had three main effects on blood transfusion services in Lagos State; (i) cancellation of all advocacy meetings and large blood donation drives; (ii) limitation of free movement and security fears by voluntary blood donors and staff leading to a reduced blood supply, and (iii) a rebound increase in the emergency request for blood and blood products. Actions taken by the LSBTS include; (i) organization of small unit donor drives and production of blood components from all units donated to stock up the blood banks before the full COVID-19 lockdown and community spread to maintain blood supply; (ii) use of social media to sensitize the general public on LSBTS activities, to give reminders on donation appointments, information on car pick up options, and notification of community blood collection; and (iii) online education of health workers on the appropriate clinical use of blood, alternatives to transfusion, and the strengthening of patient blood management guidelines. The effect of these solutions resulted in three times increase in walk-in blood donation six months into the COVID-19 pandemic and in the efficient request and usage of blood.

Conclusion: The measures taken by the LSBTS were effective in mitigating the effect of the pandemic on blood transfusion services in Lagos State. Hence, this approach can form a framework for interventions in future disease outbreaks in Nigeria and similar settings.

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Effet de la pandémie de COVID-19 et de ses mesures d'atténuation sur les services de transfusion sanguine dans l'État de Lagos, au Nigéria

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Contexte: Le service de transfusion sanguine de l'État de Lagos (LSBTS) est responsable de la fourniture de sang sûr dans l'État. Pour fournir ce service, le LSBTS coordonne les activités entre le gouvernement, les

organisations partenaires et les donneurs de sang volontaires potentiels. Cette étude décrit l'effet de la pandémie de la maladie à coronavirus 2019 (COVID-19) sur les services de transfusion sanguine dans l'État de Lagos, au Nigéria, et propose des solutions.

Méthodologie: Cette étude faisait partie d'un exercice d'amélioration de la qualité. Une conception qualitative par le biais de la conduite de dix entretiens avec des informateurs clés et de séances de réunion avec le personnel de la LSBTS, des organisations partenaires et le public a été utilisée pour l'étude. Les thèmes ont été identifiés à la suite d'entretiens.

Résultats: La pandémie de COVID-19 et ses mesures de confinement ont eu trois effets principaux sur les services de transfusion sanguine dans l'État de Lagos; (i) l'annulation de toutes les réunions de plaidoyer et des grandes collectes de dons de sang; (ii) la limitation de la libre circulation et les craintes sécuritaires des donneurs de sang volontaires et du personnel entraînant une diminution de l'approvisionnement en sang, et (iii) un rebond de la demande d'urgence de sang et de produits sanguins. Les actions entreprises par le LSBTS comprennent : (i) l'organisation de petites collectes de donneurs et la production de composants sanguins à partir de toutes les unités données pour approvisionner les banques de sang avant le verrouillage complet du COVID-19 et la propagation communautaire pour maintenir l'approvisionnement en sang; (ii) l'utilisation des médias sociaux pour sensibiliser le grand public aux activités du LSBTS, pour rappeler les rendez-vous pour les dons, les informations sur les options de prise en charge en voiture et la notification de la collecte de sang communautaire ; et (iii) l'éducation en ligne des agents de santé sur l'utilisation clinique appropriée du sang, les alternatives à la transfusion et le renforcement des directives de gestion du sang des patients. L'effet de ces solutions a entraîné une multiplication par trois des dons de sang sans rendez-vous six mois après le début de la pandémie de COVID-19 et une demande et une utilisation efficaces du sang.

Conclusion: Les mesures prises par le LSBTS ont été efficaces pour atténuer l'effet de la pandémie sur les services de transfusion sanguine dans l'État de Lagos. Par conséquent, cette approche peut former un cadre pour les interventions lors de futures flambées de maladies au Nigéria et dans des contextes similaires.

Heavy metal contents of sachet water in Gombe, Nigeria

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Background: Provision of adequate clean drinking water is a basic human need in every society. In Nigeria, like many developing countries, the provision of clean drinking water is not adequate. The introduction of sachet water is seen by many as a solution to the problem. This is because it is affordable where more than a liter is sold at twenty Naira or even less. The availability and affordability of sachet water have made it a preferred choice for most people despite questions being raised about the purity and potential toxicity. Therefore, this study set out to evaluate the heavy metal contents of sachet water in Gombe, Nigeria

Methodology: Forty samples of sachets water were randomly purchased from the streets of Gombe and analyzed for heavy metals content (lead, cadmium, mercury, arsenic and nickel) using Atomic Absorption Spectrophotometer (AAS).

Results: None of the sachet water samples had date of manufacture or expiry date while about 23% (n=9) had no NAFDAC registration numbers. All the samples had lead levels above the WHO safety limits of 0.01mg/L. Mercury, cadmium and arsenic levels were higher than the safety limits in 23% (n=9), 15% (n=6) and 5% (n=2) of the samples respectively. No nickel was detected in all the samples.

Conclusion: Majority of the sachet water in Gombe are contaminated with heavy metals such as lead, mercury and cadmium in concentrations above the WHO safety limits. Processes of water purification should be put in place to reduce the heavy metal contents of sachet water.

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Teneur en métaux lourds d'un sachet d'eau à Gombe, au Nigeria

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Contexte: La fourniture d'eau potable adéquate est un besoin humain fondamental dans toute société. Au Nigeria, comme dans de nombreux pays en développement, l'approvisionnement en eau potable n'est pas suffisant. L'introduction de l'eau en sachet est considérée par beaucoup comme une solution au problème. C'est parce qu'il est abordable où plus d'un litre est vendu à vingt nairas ou même moins. La disponibilité et le prix abordable de l'eau en sachet en ont fait un choix privilégié pour la plupart des gens, malgré les questions soulevées sur la pureté et la toxicité potentielle. Par conséquent, cette étude visait à évaluer la teneur en métaux lourds de l'eau en sachet à Gombe, au Nigeria

Méthodologie: Quarante échantillons d'eau en sachet ont été achetés au hasard dans les rues de Gombe et analysés pour la teneur en métaux lourds (plomb, cadmium, mercure, arsenic et nickel) à l'aide du spectrophotomètre d'absorption atomique (AAS).

Résultats: Aucun des échantillons d'eau en sachet n'avait de date de fabrication ou de date de péremption, tandis qu'environ 23% (n=9) n'avaient pas de numéro d'enregistrement NAFDAC. Tous les échantillons avaient des niveaux de plomb supérieurs aux limites de sécurité de l'OMS de 0,01 mg/L. Les niveaux de mercure, de cadmium et d'arsenic étaient supérieurs aux limites de sécurité dans 23% (n=9), 15% (n=6) et 5% (n=2) des échantillons respectivement. Aucun nickel n'a été détecté dans tous les échantillons.

Conclusion: La majorité de l'eau en sachet à Gombe est contaminée par des métaux lourds tels que le plomb, le mercure et le cadmium à des concentrations supérieures aux limites de sécurité de l'OMS. Des processus de purification de l'eau doivent être mis en place pour réduire la teneur en métaux lourds de l'eau en sachet.

Molecular subtyping of breast cancers in Port Harcourt, Nigeria

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Background: Expressed biomarkers in breast cancer enable attainment of targeted therapy and molecular sub-classification. The aim of this study is to assess the immunohistochemical receptor status and molecular sub-classification of breast cancer patients in Port Harcourt, Nigeria

Methodology: This was a retrospective review and molecular sub-classification of immunohistochemistry tests for Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor-2 (HER-2) on tissues of breast cancer patients, processed and histologically evaluated in Anatomical Pathology laboratory, University of Port Harcourt Teaching Hospital (UPTH) and AimPath laboratory in Port Harcourt, Nigeria, between 2016 and 2021. ER and/or PR positivity is Hormone Receptor (HR) positive, HR+/HER-2- is Luminal A, HR+/HER-2+ is Luminal B, HER-2+/HR- is HER-2 enriched, and HR-/HER-2- is Triple Negative.

Results: Of the 280 cases, 4 (1.4%) were men while 276 (98.6%) were women. The mean age of the patients was 46.3±11.2 years, age range 21-90 years, with a peak at 41-50 years. Triple Negative constituted 55.4% (n=155), followed by Luminal A with 30% (n=84), HER2 enriched with 7.5% (n=21) and Luminal B with 7.1% (n=20). Of the 84 (30%) HR+ cases, 72 (25.7%) were positive for both ER and PR while 6 (2.1%) cases were positive for ER and PR only. Of the 20 Luminal B, 19 were ER+/HER-2+, while 1 was PR+/HER-2-. All male patients were luminal A. The relationship between patients age and biomarker expression was not statistically significant ($p>0.05$).

Conclusion: Although Triple Negative breast cancer is the most common molecular type in Port Harcourt, Luminal A is relatively common while Luminal B and HER-2 enriched are uncommon.

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Sous-typage moléculaire des cancers du sein à Port Harcourt, Nigéria

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Contexte: Les biomarqueurs exprimés dans le cancer du sein permettent d'atteindre une thérapie ciblée et une sous-classification moléculaire. Le but de cette étude est d'évaluer le statut des récepteurs immunohistochimiques et la sous-classification moléculaire des patientes atteintes d'un cancer du sein à Port Harcourt, au Nigeria.

Méthodologie: Il s'agissait d'une revue rétrospective et d'une sous-classification moléculaire des tests d'immunohistochimie pour le récepteur des œstrogènes (ER), le récepteur de la progestérone (PR) et le récepteur du facteur de croissance épidermique humain-2 (HER-2) sur les tissus de patientes atteintes d'un cancer du sein, traitées et histologiquement. évalué dans le laboratoire d'anatomie pathologique de l'hôpital universitaire de Port Harcourt (UPTH) et le laboratoire AimPath de Port Harcourt, au Nigéria, entre 2016 et 2021. La positivité ER et/ou PR est positive pour les récepteurs hormonaux (HR), HR+/HER-2- est Luminal A, HR+/HER-2+ est Luminal B, HER-2+/HR- est enrichi en HER-2 et HR-/HER-2- est triple négatif

Résultats: Sur les 280 cas, 4 (1,4%) étaient des hommes tandis que 276 (98,6%) étaient des femmes. L'âge

moyen des patients était de $46,3 \pm 11,2$ ans, la tranche d'âge 21-90 ans, avec un pic à 41-50 ans. Le triple négatif constituait 55,4% (n=155), suivi du Luminal A avec 30% (n=84), HER2 enrichi avec 7,5% (n=21) et Luminal B avec 7,1% (n=20). Sur les 84 (30%) cas HR+, 72 (25,7%) étaient positifs à la fois pour ER et PR tandis que 6 (2,1%) cas chacun étaient positifs pour ER et PR uniquement. Sur les 20 Luminal B, 19 étaient ER+/HER-2+, tandis que 1 était PR+/HER-2-. Tous les patients masculins étaient luminal A. La relation entre l'âge des patients et l'expression du biomarqueur n'était pas statistiquement significative ($p > 0,05$).

Conclusion: Bien que le cancer du sein triple négatif soit le type moléculaire le plus courant à Port Harcourt, Luminal A est relativement courant tandis que Luminal B et HER-2 enrichi sont rares.

Treatment outcome of COVID-19 in patients with haematological malignancies: case series from three Nigerian tertiary teaching hospitals

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Background: SARS CoV-2, the cause of the recent and ongoing COVID-19 pandemic is a cause of significant morbidity and mortality. Treatment outcome in patients with haematological malignancies in Nigeria has not been adequately reported. We describe the demographics, clinical characteristics, and treatment outcome of patients with haematological malignancies who had COVID-19 in three tertiary healthcare facilities in Nigeria.

Methodology: This is a case series of 9 patients with haematological malignancies who had COVID-19. The study centres include University of Benin Teaching hospital (UBTH), Rivers State University Teaching Hospital (RSUTH), and Federal Teaching Hospital Gombe. Data of the patients were retrieved from the various treatment centres and analysed using SPSS 23.0

Results: The 9 patients included 7 (77.8%) males and 2 (22.2%) females, with mean age of 64 ± 9.0 years and age range of 52-77 years. The underlying malignancies were CLL (n=5, 55.6%), multiple myeloma (n=2, 22.2%), NHL (n=1, 11.1%) and myelofibrosis (n=1, 11.1%). Two (22.2%) were newly diagnosed, 2 (22.2%) were in remission, 4 (44.4%) were in relapse, and 1 (11.1%) was in stable disease state. Most common COVID-19 related symptoms were cough (n=9, 100%), breathlessness (n=9, 100%), fever (n=7, 77.8%) and fatigue (n=7, 77.8%). Five (55.6%) had severe disease, 3 (33.3%) moderate and 1 (11.1%) critical at presentation. They were treated with steroids (n=4, 44.4%), azithromycin (n=3, 33.3%), ivermectin (n=3, 33.3%) and heparin (n=5, 55.6%) amongst other medications. The length of hospital stays following diagnosis ranged from 1-10 days with a mean of 5.6 ± 3.1 days. Mortality rate was 55.6% (n=5).

Conclusion: CLL patients were the most common haematological malignancy infected with SARS-CoV-2. COVID-19 was associated with high mortality rate in patients with haematological malignancies.

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Résultats du traitement du COVID-19 chez les patients atteints de malignités hématologiques: séries de cas de trois Hôpitaux Universitaires Nigériens

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Contexte: Le SRAS CoV-2, la cause de la pandémie récente et en cours de COVID-19, est une cause de morbidité et de mortalité importantes. Les résultats du traitement chez les patients atteints d'hétopathies malignes au Nigeria n'ont pas été adéquatement rapportés. Nous décrivons les caractéristiques démographiques, les caractéristiques cliniques et les résultats du traitement des patients atteints de malignités hématologiques qui avaient COVID-19 dans trois établissements de soins de santé tertiaires au Nigeria.

Méthodologie: Il s'agit d'une série de cas de 9 patients atteints d'hétopathies malignes qui avaient COVID-19. Les centres d'études comprennent l'hôpital universitaire de l'Université du Bénin (UBTH), l'hôpital universitaire de l'État de Rivers (RSUTH) et l'hôpital universitaire fédéral de Gombe. Les données des patients ont été récupérées dans les différents centres de traitement et analysées à l'aide de SPSS 23.0

Résultats: Les 9 patients comprenaient 7 (77,8%) hommes et 2 (22,2%) femmes, avec un âge moyen de $64 \pm 9,0$ ans et une tranche d'âge de 52 à 77 ans. Les tumeurs malignes sous-jacentes étaient la LLC (n=5, 55,6%), le

myélome multiple (n=2, 22,2%), le LNH (n=1, 11,1%) et la myélofibrose (n=1, 11,1%). Deux (22,2 %) ont été nouvellement diagnostiqués, 2 (22,2 %) étaient en rémission, 4 (44,4 %) étaient en rechute et 1 (11,1 %) était dans un état de maladie stable. Les symptômes liés au COVID-19 les plus courants étaient la toux (n=9, 100 %), l'essoufflement (n=9, 100%), la fièvre (n=7, 77,8%) et la fatigue (n=7, 77,8%). Cinq (55,6%) avaient une maladie grave, 3 (33,3%) modérée et 1 (11,1%) critique à la présentation. Ils ont été traités avec des stéroïdes (n=4, 44,4%), de l'azithromycine (n=3, 33,3%), de l'ivermectine (n=3, 33,3%) et de l'héparine (n=5, 55,6%) parmi d'autres médicaments. La durée des séjours hospitaliers après le diagnostic variait de 1 à 10 jours avec une moyenne de $5,6 \pm 3,1$ jours. Le taux de mortalité était de 55,6% (n=5).

Conclusion: les patients atteints de LLC étaient la tumeur maligne hématologique la plus courante infectée par le SRAS-CoV-2. Le COVID-19 a été associé à un taux de mortalité élevé chez les patients atteints de malignités hématologiques.

A descriptive study of the use of bone marrow biopsies in University of Port Harcourt Teaching Hospital: a two-and-half year study

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Background: The effective management of patients with haematological and some non-haematological disorders depends on correctly interpreted bone marrow biopsy (BMB). The aim of this study is to evaluate indications for utility of diagnoses in patients who underwent BMB in University of Port Harcourt Teaching Hospital (UPTH) and determine the concordance rate between BMB and bone marrow aspirates (BMA) diagnoses of the patients.

Methodology: Histologically processed BMB at Anatomical Pathology laboratory of UPTH between 1st January 2017 and 30th June 2019 were identified and clinico-pathologically evaluated. Patients' pathology request forms, folders, and slides stained with H & E and appropriate special stains, were reviewed. Where necessary, fresh slides were prepared from paraffin embedded tissue blocks. Information on patients' demographics, indications for biopsy, BMB diagnoses, full blood count (FBC) and smear results as well as BMA findings were documented. The obtained data were analysed using Statistical Package for Social Science version 21.0 (SPSS Inc. Chicago, IL, USA).

Results: Ninety-six BMB of 46 females and 50 males were reviewed. Patients' age ranged between 1-90 years and peaked at 40-49 years. The commonest indications were unexplained anaemia 28.1% (39/96), suspected multiple myeloma 13.5% (13/96) and suspected lymphoproliferative disease 12.5% (12/96). Erythroid hyperplasia 20.8% (20/96), acute myeloid leukaemia 12.5% (12/96) and multiple myeloma 11.5% (11/96) were the most frequent BMB diagnoses. BMB was useful in detecting non-haematological cases that were not suspected clinically by the haematologists such as chronic granulomatous inflammation and metastatic bone marrow disease in 10.4% (10/96) of patients. The diagnostic utility BMB was 95.8% while the concordance rate between BMB and BMA diagnoses was 73.8%.

Conclusion: While unexplained anaemia is the commonest indication for BMB, erythroid hyperplasia is the commonest diagnosis, with good concordance between BMB and BMA, in UPTH. BMB widens the spectrum of diagnoses beyond clinically suspected disease conditions, therefore its utilization should be encouraged through creation of improved awareness among Clinicians.

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Une étude descriptive de l'utilisation des biopsies de moelle osseuse à l'hôpital Universitaire de l'Université de Port Harcourt: une étude de deux ans et demi

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Contexte: La prise en charge efficace des patients atteints de troubles hématologiques et de certains troubles non hématologiques dépend d'une biopsie de la moelle osseuse (BMB) correctement interprétée. Le but de cette étude est d'évaluer les indications d'utilité des diagnostics chez les patients qui ont subi un BMB à l'hôpital universitaire de Port Harcourt (UPTH) et de déterminer le taux de concordance entre les diagnostics de BMB et d'aspiration de moelle osseuse (BMA) des patients.

Méthodologie: Des BMB traités histologiquement au laboratoire d'Anatomopathologie de l'UPTH entre le 1er janvier 2017 et le 30 juin 2019 ont été identifiés et évalués clinico-pathologiquement. Les formulaires de demande de pathologie des patients, les dossiers et les lames colorées avec H & E et les colorations spéciales appropriées

ont été examinés. Si nécessaire, des lames fraîches ont été préparées à partir de blocs de tissus inclus en paraffine. Des informations sur les caractéristiques démographiques des patients, les indications de biopsie, les diagnostics de BMB, la numération formule sanguine (FBC) et les résultats des frottis ainsi que les résultats de BMA ont été documentés. Les données obtenues ont été analysées à l'aide de Statistical Package for Social Science version 21.0 (SPSS Inc. Chicago, IL, USA).

Résultats: Quatre-vingt-seize BMB de 46 femmes et 50 hommes ont été examinés. L'âge des patients variait entre 1 et 90 ans et culminait entre 40 et 49 ans. Les indications les plus fréquentes étaient l'anémie inexpliquée 28,1% (39/96), la suspicion de myélome multiple 13,5% (13/96) et la suspicion de maladie lymphoproliférative 12,5% (12/96). L'hyperplasie érythroïde 20,8% (20/96), la leucémie myéloïde aiguë 12,5% (12/96) et le myélome multiple 11,5% (11/96) étaient les diagnostics de BMB les plus fréquents. Le BMB a été utile pour détecter les cas non hématologiques qui n'étaient pas suspectés cliniquement par les hématologues, tels que l'inflammation granulomateuse chronique et la maladie métastatique de la moelle osseuse chez 10,4 % (10/96) des patients. L'utilité diagnostique BMB était de 95,8% tandis que le taux de concordance entre les diagnostics BMB et BMA était de 73,8%.

Conclusion: Alors que l'anémie inexpliquée est l'indication la plus fréquente de BMB, l'hyperplasie érythroïde est le diagnostic le plus fréquent, avec une bonne concordance entre BMB et BMA, dans l'UPTH. Le BMB élargit le spectre des diagnostics au-delà des états pathologiques cliniquement suspectés. Par conséquent, son utilisation doit être encouragée en sensibilisant davantage les cliniciens.

Ultrasound guided fine needle aspiration cytology of thyroid lesions: a pilot study on the spectrum of radiologic and histopathologic findings

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Background: Ultrasound guided fine needle aspiration cytology (FNAC) plays a pivotal role in the cytopathological management of thyroid disease by providing a précised evaluation of thyroid lesions. This interventional radiologic procedure is minimally invasive, readily available and affordable. This study is aimed at evaluating the spectrum of radiologic and cytologic findings of thyroid disorders using ultrasound guided fine needle aspiration cytology.

Methodology: One hundred and twenty-one patients referred for ultrasound guided FNAC of the thyroid gland at the interventional radiology unit of the Rivers State University Teaching Hospital, Port Harcourt from September 2020 to August 2021, participated in the study. After obtaining informed consent, age and anthropometric parameters were taken. Patients had standard exposure and position for the study. Using an ultrasound machine (Logic F6, General Electric, USA, 2017), fitted with a 7.5-MHz linear transducer, the thyroid gland was scanned to locate the thyroid lesion. With a 20ml syringe and a 23G by 1½ inch needle, cells were aspirated from the thyroid lesion. Dry and wet slide smears were immediately prepared and subsequently taken to the histopathology laboratory for analysis. The data obtained were analysed using the Statistical Package for Social Sciences version 21.0 (SPSS Inc. Chicago, IL, USA).

Results: Males and females constitute 23 (19.0%) and 98 (81.0%) respectively, with a mean age of 41.33±3.11years. Radiological evaluation revealed that 86.8% (n=105) of the thyroid glands were enlarged with radiologic diagnosis of thyroid malignancy (9.0%, n=11), thyroiditis (19.8%, n=24), thyroid nodule (38.8%, n=47) and thyroid nodules with cystic degeneration (32.2%, n=39). Cytological findings revealed non-diagnostic sample (thy1/c) category (1.7%, n=2), benign colloid nodule (43.0%, n=52), follicular neoplasm (5.8%, n=7), and thyroiditis (17.4%, n=21).

Conclusion: Image-guided FNAC is a valuable tool in the diagnosis of thyroid lesions with the majority of the thyroid lesions being benign.

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Cytologie par aspiration à l'aiguille fine guidée par échographie des lésions thyroïdiennes: une étude pilote sur le spectre des résultats radiologiques et histopathologiques

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Contexte: La cytologie par aspiration à l'aiguille fine guidée par ultrasons (FNAC) joue un rôle central dans la gestion cytopathologique de la maladie thyroïdienne en fournissant une évaluation précise des lésions thyroïdiennes. Cette procédure radiologique interventionnelle est peu invasive, facilement disponible et abordable. Cette étude vise à évaluer le spectre des résultats radiologiques et cytologiques des troubles thyroïdiens à l'aide de la cytologie par aspiration à l'aiguille fine guidée par ultrasons.

Méthodologie: Cent vingt et un patients adressés pour une FNAC guidée par échographie de la glande thyroïde à l'unité de radiologie interventionnelle du Rivers State University Teaching Hospital, Port Harcourt de septembre 2020 à août 2021, ont participé à l'étude. Après avoir obtenu le consentement éclairé, l'âge et les paramètres anthropométriques ont été relevés. Les patients avaient une exposition et une position standard pour l'étude. À l'aide d'un appareil à ultrasons (Logic F6, General Electric, USA, 2017), équipé d'un transducteur linéaire de 7,5 MHz, la glande thyroïde a été scannée pour localiser la lésion thyroïdienne. Avec une seringue de 20 ml et une aiguille de 23G sur 1½ pouce, les cellules ont été aspirées de la lésion thyroïdienne. Des frottis secs et humides sur lame ont été immédiatement préparés et ensuite emmenés au laboratoire d'histopathologie pour analyse. Les données obtenues ont été analysées à l'aide du Statistical Package for Social Sciences version 21.0 (SPSS Inc. Chicago, IL, USA).

Résultats: Les hommes et les femmes constituent respectivement 23 (19,0%) et 98 (81,0%), avec un âge moyen de 41,33±3,11 ans. L'évaluation radiologique a révélé que 86,8% (n=105) des glandes thyroïdes étaient hypertrophiées avec un diagnostic radiologique de malignité thyroïdienne (9,0 %, n=11), de thyroïdite (19,8%, n=24), de nodule thyroïdien (38,8 %, n=47) et nodules thyroïdiens avec dégénérescence kystique (32,2 %, n=39). Les résultats cytologiques ont révélé une catégorie d'échantillon non diagnostique (thy1/c) (1,7%, n=2), un nodule colloïde bénin (43,0%, n=52), une néoplasie folliculaire (5,8%, n=7) et une thyroïdite (17,4%, n=21).

Conclusion: La FNAC guidée par l'image est un outil précieux dans le diagnostic des lésions thyroïdiennes, la majorité des lésions thyroïdiennes étant bénignes.

Assessment of thyroid function in patients with mental disorders

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Background: Thyroid hormones are metabolic hormones secreted by the thyroid gland, and include the thyroxine (T4) and triiodothyronine (T3). These hormones act on virtually all the cells in the human body. Their functions include; regulation of basal metabolic rates, protein synthesis, growth of long bones, neural development. The present study aimed to determine thyroid function among mentally ill patients (schizophrenic spectrum and mood spectrum disorders) attending the Neuropsychiatric clinic of University of Port Harcourt Teaching Hospital, as this will both provide a clear picture of the incidence in our environment as well as, aiding the early diagnosis and management of thyroid dysfunction.

Methodology: This comparative case-control study was conducted in the University of Port Harcourt Teaching Hospital; a tertiary health facility serving treatment, teaching, health research and referral purposes for primary and secondary health care facilities within Rivers State and its adjoining states. The serum levels of TSH, Free T3 and Free T4 were quantitatively determined using commercially available CALBIOTECH ELISA kits on 173 mentally ill patients attending the Neuropsychiatric clinic of University of Port Harcourt Teaching Hospital and 173 apparently normal healthy individuals used as control.

Results: A total of 173 participants with a mean age of 35.25±12.17 years and 173 controls with a mean age of 35.35±8.76 years were recruited. Male and female participants in the mentally ill group were 68 (39.3%) and 105 (60.7%) respectively, while those in the control groups were 69 (39.9%) and 104 (60.1%). The study observed that patients with bipolar disorders, schizophrenia and acute psychosis had high values of FT3, which were higher than the values for the controls, while the controls had higher FT3 than patients with depression and schizo-affective disorders. Patients with acute psychosis had higher FT4 values than controls. In addition, there was a reduction in the level of FT4 in the mentally ill participants when compared to that of the healthy controls. Grouping the mentally ill patients into two broad areas, patients with schizophrenic spectrum disorders had lower TSH and higher FT3 than mood spectrum disorders.

Conclusion: From the finding of high incidence of thyroid abnormality in mentally ill patients, it is recommended that assessments of thyroid hormone should be included in the standing order test for all mentally ill patients for effectiveness in their management.

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Évaluation de la fonction thyroïdienne chez les patients souffrant de troubles mentaux

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Contexte: Les hormones thyroïdiennes sont des hormones métaboliques sécrétées par la glande thyroïde et comprennent la thyroxine (T4) et la triiodothyronine (T3). Ces hormones agissent sur pratiquement toutes les cellules du corps humain. Leurs fonctions comprennent; régulation des taux métaboliques basaux, synthèse des protéines, croissance des os longs, développement neural. La présente étude visait à déterminer la fonction thyroïdienne chez les patients atteints de maladie mentale (troubles du spectre schizophrénique et du spectre de l'humeur) fréquentant la clinique neuropsychiatrique de l'hôpital universitaire de Port Harcourt, car cela fournira à la fois une image claire de l'incidence dans notre environnement ainsi que, aider au diagnostic précoce et à la gestion du dysfonctionnement thyroïdien.

Méthodologie: Cette étude cas-témoins comparative a été menée à l'hôpital universitaire de l'Université de Port Harcourt; un établissement de santé tertiaire servant à des fins de traitement, d'enseignement, de recherche en santé et de référence pour les établissements de soins de santé primaires et secondaires dans l'État de Rivers et ses États voisins. Les taux sériques de TSH, de T3 libre et de T4 libre ont été déterminés quantitativement à l'aide de kits ELISA CALBIOTECH disponibles dans le commerce sur 173 patients atteints de troubles mentaux fréquentant la clinique neuropsychiatrique de l'hôpital universitaire de Port Harcourt et 173 individus sains apparemment normaux utilisés comme contrôle.

Résultats: Un total de 173 participants avec un âge moyen de 35,25±12,17 ans et 173 témoins avec un âge moyen de 35,35±8,76 ans ont été recrutés. Les participants masculins et féminins du groupe des malades mentaux étaient respectivement 68 (39,3%) et 105 (60,7%), tandis que ceux des groupes témoins étaient 69 (39,9%) et 104 (60,1%). L'étude a observé que les patients atteints de troubles bipolaires, de schizophrénie et de psychose aiguë avaient des valeurs élevées de FT3, qui étaient supérieures aux valeurs des témoins, tandis que les témoins avaient des FT3 plus élevés que les patients souffrant de dépression et de troubles schizo-affectifs. Les patients atteints de psychose aiguë avaient des valeurs de FT4 plus élevées que les témoins. De plus, il y avait une réduction du niveau de FT4 chez les participants atteints de maladie mentale par rapport à celui des témoins sains. En regroupant les patients atteints de maladie mentale en deux grands domaines, les patients atteints de troubles du spectre schizophrénique présentaient une TSH plus faible et un FT3 plus élevé que les troubles du spectre de l'humeur.

Conclusion: À partir de la découverte d'une incidence élevée d'anomalies thyroïdiennes chez les patients atteints de maladie mentale, il est recommandé d'inclure des évaluations des hormones thyroïdiennes dans le test d'ordre permanent pour tous les patients atteints de maladie mentale pour l'efficacité de leur prise en charge.

The epidemiology of haematological malignancies in Port Harcourt: a twenty-year retrospective study

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Background: Haematological malignancies (HM) include a large group of various myeloid and lymphoid malignancies associated with a high mortality and morbidity and constitute an economic burden to the affected families. The epidemiologic pattern of HM has not been described in our locality. The aim of this study is to describe the epidemiology of HM in our environment.

Methodology: This was a 20-year retrospective study, all cases diagnosed with HM between July 2001 to June 2020 were included. Data were retrieved from the cancer registry and from case notes of patients.

Results: A total of 404 HM were diagnosed and made up 7.7% of all malignancies from July 2010 to June 2021 in the cancer registry (319 of 4833 total cancers). Peak incidence occurred in the year 2013 (33 new cases) followed by the year 2020 (32 new cases). The annual incidence rate of HM ranged from 0.7 to 1 case per 100,000 with increasing incidence rates over the period and peak incidence in 2013 and 2020 (33 and 32 new cases respectively). The commonest HM were chronic myeloid leukaemia (CML) 20.8% (n=84), multiple myeloma (MM) 16.6% (n=67), acute myeloid leukaemia (AML) 15.3% (n=62), and chronic lymphocytic leukaemia (CLL) 13.9% (n=56), with idiopathic myelofibrosis and myelodysplastic syndromes being the least (1.0%, 4 cases each). From 2007 to 2012, CML had the highest incidence (peak in 2010 with 13 new cases), however from 2015 to 2019, AML had the highest incidence (peak in 2019 with 10 new cases). In 2020, MM had the highest number of new cases recorded. Males were generally more affected than females (M: F ratio overall of 1.3: 1) except in CLL and Hodgkin lymphoma (M: F ratio of 1: 1.5 and 1: 1.4 respectively). The median age of onset for HM was 45 years (range 7 months - 83 years). Acute lymphoblastic leukaemia had the youngest median age of onset 8 years (7 months - 44 years), while the oldest age of onset was seen in CLL and MM (55 years each, range 28 - 83 years and 24 - 83 years respectively).

Conclusion: The incidence of HM increased over time. Although CML was the most common HM, in the past 5 years AML had the highest incidence.

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L'épidémiologie des hémopathies malignes à Port Harcourt: une étude rétrospective sur vingt ans

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Contexte: Les hémopathies malignes (HM) comprennent un grand groupe de diverses malignités myéloïdes et lymphoïdes associées à une mortalité et une morbidité élevées et constituent un fardeau économique pour les familles touchées. Le schéma épidémiologique de l'HM n'a pas été décrit dans notre localité. Le but de cette étude est de décrire l'épidémiologie de la MH dans notre environnement.

Méthodologie: Il s'agissait d'une étude rétrospective de 20 ans, tous les cas diagnostiqués avec HM entre juillet 2001 et juin 2020 ont été inclus. Les données ont été extraites du registre du cancer et des notes de cas des patients.

Résultats: Un total de 404 HM ont été diagnostiqués et représentaient 7,7% de toutes les tumeurs malignes de juillet 2010 à juin 2021 dans le registre du cancer (319 sur 4833 cancers totaux). Le pic d'incidence s'est produit en 2013 (33 nouveaux cas) suivi de l'année 2020 (32 nouveaux cas). Le taux d'incidence annuel de HM variait de 0,7 à 1 cas pour 100 000 avec des taux d'incidence croissants au cours de la période et une incidence maximale en 2013 et 2020 (33 et 32 nouveaux cas respectivement). Les HM les plus courantes étaient la leucémie myéloïde chronique (LMC) 20,8 % (n=84), le myélome multiple (MM) 16,6 % (n=67), la leucémie myéloïde aiguë (LAM) 15,3 % (n=62) et la leucémie lymphoïde chronique (LLC) 13,9% (n=56), la myélobiose idiopathique et les syndromes myélodysplasiques étant les moins nombreux (1,0%, 4 cas chacun). De 2007 à 2012, la LMC avait l'incidence la plus élevée (pic en 2010 avec 13 nouveaux cas), mais de 2015 à 2019, la LMA avait l'incidence la plus élevée (pic en 2019 avec 10 nouveaux cas). En 2020, MM a enregistré le plus grand nombre de nouveaux cas. Les hommes étaient généralement plus touchés que les femmes (rapport M: F global de 1,3: 1), sauf dans la LLC et le lymphome hodgkinien (rapport M: F de 1: 1,5 et 1: 1,4 respectivement). L'âge médian d'apparition de l'HM était de 45 ans (intervalle de 7 mois à 83 ans). La leucémie lymphoblastique aiguë avait l'âge médian le plus jeune d'apparition de 8 ans (7 mois - 44 ans), tandis que l'âge d'apparition le plus élevé était observé dans la LLC et le MM (55 ans chacun, de 28 à 83 ans et de 24 à 83 ans respectivement).

Conclusion: L'incidence de l'HM a augmenté avec le temps. Bien que la LMC soit la HM la plus courante, au cours des 5 dernières années, la LMA avait l'incidence la plus élevée.

The role of neutrophil gelatinase-associated lipocalin and beta-trace protein in detection of diabetic nephropathy

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Background: Type 2 Diabetes Mellitus (T2DM) is a common metabolic medical problem worldwide that is associated with adverse multisystemic complications such as nephropathy, neuropathy, retinopathy amongst others. More importantly, a considerable proportion of T2DM patients have poor glycaemic control, which with time will lead to the onset of these complications. This study was carried out to investigate the predictive role of serum beta-trace protein (BTP) and neutrophil gelatinase-associated lipocalin (NGAL) in diabetic nephropathy in T2DM.

Methodology: A sequence generation randomisation method was used to select 240 age and gender-matched case (diabetic nephropathy patients) and control (apparently healthy persons) groups for the study. Serum BTP and NGAL were estimated using Elabscience ELISA kit, serum creatinine was estimated using Jaffe Method (kinetic), glycated haemoglobin was done using fluorescence immunoassay, fasting plasma glucose (FPG) was estimated using glucose oxidase method, microalbumin was estimated by using turbidimetry, and glomerular function rate (GFR) was calculated using the Modified Diet in Renal Disease Equation (MDRD).

Results: The mean serum concentration of NGAL was 70.2 ±19.9 and 113.2 ±21.5 in the control and case groups respectively. The mean serum BTP concentration was 3.95 ±0.72 in the control and 4.03 ±0.79 in the case group. The mean HBA1c concentration was 5.14 ±0.81 and 9.42 ±2.27 in the control and the case groups respectively. There was a positive correlation between FPG and NGAL and between FPG and BTP in the case group with diabetic nephropathy. The NGAL cut-off point was found to be 110.2 ng/mL, with a 94% sensitivity and 90% specificity. The BTP cut-off point was found to be 4.16 ng/mL with an 88.1% sensitivity and 81.0% specificity. Using the ROC curve, NGAL performed better than BTP as an indicator for diabetic nephropathy.

Conclusion: Both BTP and NGAL are useful as diagnostic markers for early detection of renal dysfunction in patients with diabetes and for populations with slight impairment in GFR (<90mL/min/1.73m²). NGAL has a higher sensitivity and specificity than BTP, therefore it is recommended as a biomarker for the detection of diabetic nephropathy in diabetic patients, and can show diabetic nephropathy earlier than serum creatinine.

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Le rôle de la lipocaline associée à la gélatinase neutrophile et de la protéine bêta-trace dans la détection de la néphropathie diabétique

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Contexte: Le diabète sucré de type 2 (DT2) est un problème médical métabolique courant dans le monde entier qui est associé à des complications multisystémiques indésirables telles que la néphropathie, la neuropathie, la rétinopathie, entre autres. Plus important encore, une proportion considérable de patients atteints de DT2 ont un mauvais contrôle glycémique, ce qui, avec le temps, conduira à l'apparition de ces complications. Cette étude a été réalisée pour étudier le rôle prédictif de la protéine bêta-trace sérique (BTP) et de la lipocaline associée à la gélatinase neutrophile (NGAL) dans la néphropathie diabétique dans le DT2.

Méthodologie: Une méthode de randomisation par génération de séquences a été utilisée pour sélectionner 240 groupes de cas appariés selon l'âge et le sexe (patients atteints de néphropathie diabétique) et témoins (personnes apparemment en bonne santé) pour l'étude. La BTP et la NGAL sériques ont été estimées à l'aide du kit ELISA Elabscience, la créatinine sérique a été estimée à l'aide de la méthode de Jaffe (cinétique), l'hémoglobine glyquée a été déterminée à l'aide d'un immunodosage par fluorescence, la glycémie à jeun (FPG) a été estimée à l'aide de la méthode de la glucose oxydase, la microalbumine a été estimée à l'aide de la turbidimétrie, et le taux de fonction glomérulaire (DFG) a été calculé à l'aide de la Régime alimentaire modifié dans l'équation de la maladie rénale (MDRD).

Résultats: La concentration sérique moyenne de NGAL était de $70,2 \pm 19,9$ et de $113,2 \pm 21,5$ dans les groupes témoins et cas respectivement. La concentration sérique moyenne de BTP était de $3,95 \pm 0,72$ dans le groupe témoin et de $4,03 \pm 0,79$ dans le groupe des cas. La concentration moyenne de HBA1c était de $5,14 \pm 0,81$ et $9,42 \pm 2,27$ dans les groupes témoins et cas respectivement. Il y avait une corrélation positive entre FPG et NGAL et entre FPG et BTP dans le groupe de cas avec néphropathie diabétique. Le point de coupure NGAL s'est avéré être $110,2$ ng/mL, avec une sensibilité de 94% et une spécificité de 90%. Le point de coupure BTP s'est avéré être $4,16$ ng/mL avec une sensibilité de 88,1% et une spécificité de 81,0%. En utilisant la courbe ROC, le NGAL a obtenu de meilleurs résultats que le BTP en tant qu'indicateur de néphropathie diabétique.

Conclusion: Le BTP et le NGAL sont tous deux utiles en tant que marqueurs diagnostiques pour la détection précoce de la dysfonction rénale chez les patients diabétiques et pour les populations présentant une légère altération du DFG (<90 ml/min/1,73 m²). Le NGAL a une sensibilité et une spécificité plus élevées que le BTP, il est donc recommandé comme biomarqueur pour la détection de la néphropathie diabétique chez les patients diabétiques, et peut montrer une néphropathie diabétique plus tôt que la créatinine sérique.



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