



AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (AJCEM) ISSN 1595-689X

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1822 http://www.ajol.info/journals/ajcem COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.1

AFR. J. CLN. EXPER. MICROBIOL. 19 (3): 159-164

ANTIMICROBIAL RESISTANCE PATTERN OF CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA AND ESCHERICHIA COLI ON CARBAPENEMS

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ABSTRACT

Background: Carbapenems are the most effective and important therapeutic options to serious infections caused by *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates. However, Carbepenems resistant isolates of *Enterobacteriaceae* and *Pseudomonas aeroginosa* are increasing worldwide. This study, therefore, was carried out to determine the resistance pattern of clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* to Carbapenems.

Methods: Fifty (50) *E. coli* and forty seven (47) *Pseudomonas aeruginosa* isolates were studied. Antibiotic Susceptibility test was performed as recommended by the CLSI. The antibiotics used were Ertapenem, Imipenem, Colistin Sulphate, Levofloxacin, and Piperacillin/Tazobactam.

Results: Out of 97 clinical isolates subjected to drug susceptibilities test, *Pseudomonas aeruginosa* showed resistance to Ertapenem (87.2%); followed by Levofloxacin (19.1%), Colistin sulphate (12.8%), Piperacillin/tazobactan (4.3%) and Imipenem (2.1%) while *E.coli* displayed resistance to Ertapenem (30%), Levofloxacin (20%) and Colistin sulphate (4%). Interestingly, *E coli* was susceptible to Imipenem (0%) and Piperacillin/tazobactan (0%). A significant effect of Ertapenem on *Pseudomonas aeruginosa* was recorded. Also a significant effect of Piperacillin/Tazobactam was recorded on *E coli*. No significant effect was recorded among the other antibiotics on *P aeruginosa* or *E coli*.

Conclusion: There is a high level of Carbapenems resistance among the clinical isolates of *Pseudomonas aeruginosa* compared to *Escherichia coli* in this study. Considering the therapeutic value of Carbapenems as one of the last options for the treatment of *Enterobacteriaceae* and *Pseudomonas aeruginosa* infections, rational Carbapenems usage is essential to reduce selective pressure over *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates.

Keywords: Carbapenems, Antibiotics, Nosocomial, Susceptibility

PROFIL DE RÉSISTANCE AUX ANTIMICROBIENS DES ISOLATS CLINIQUES DE PSEUDOMONAS AERUGINOSA ET ESCHERICHIA COLI SUR CARBAPÉNÈMES

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RÉSUMÉ

Contexte : Carbapénèmes sont les plus efficaces et les options thérapeutiques importants d'infections graves causées par les entérobactéries et Pseudomonas aeruginosa isolats. Cependant, Carbepenems isolats résistants d'entérobactéries et Pseudomonas aeroginosa sont en augmentation dans le monde entier. En conclusion, cette étude a été réalisée pour déterminer le profil de résistance des isolats cliniques de Pseudomonas aeruginosa et Escherichia coli de carbapénèmes.

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Méthodes: Cinquante (50) E. coli et quarante sept (47) des isolats de Pseudomonas aeruginosa ont été étudiés. Test de sensibilité aux antibiotiques a été effectuée comme recommandé par le QIMV. Les antibiotiques utilisés étaient l'ertapénème, imipenem, sulfate de colistine, Lévofloxacine, et de l'association pipéracilline-tazobactam.

Résultats : Sur 97 isolats cliniques de médicaments soumis à des test de susceptibilité, Pseudomonas aeruginosa ont montré une résistance à l'ertapénème (87,2 %), suivie par la lévofloxacine (19,1 %), sulfate de colistine (12,8 %), l'association pipéracilline-tazobactam (4,3 %) et l'imipénème (2,1 %) tandis que les E. coli affiche une résistance à l'ertapénème (30 %), la lévofloxacine (20 %) et sulfate de colistine (4 %). Fait intéressant, E coli était sensible à l'imipénème (0 %) et de la pipéracilline/tazobactam (0 %). Un effet significatif de l'ertapénème sur Pseudomonas aeruginosa a été enregistrée. Aussi un effet significatif de l'association pipéracilline-tazobactam a été enregistré sur E coli. Aucun effet significatif n'a été enregistré parmi les autres antibiotiques sur *P aeruginosa* ou *E coli*.

Conclusion : Il y a un haut niveau de résistance aux carbapénèmes parmi les isolats cliniques de Pseudomonas aeruginosa par rapport à Escherichia coli dans cette étude. Compte tenu de la valeur thérapeutique des carbapénèmes comme l'une des dernières options pour le traitement d'entérobactéries et Pseudomonas aeruginosa les infections, l'utilisation rationnelle des carbapénèmes est essentielle pour réduire la pression sélective sur les entérobactéries et Pseudomonas aeruginosa isolats cliniques.

Mots-clés : Carbapénèmes, antibiotiques, infections nosocomiales, la sensibilité

INTRODUCTION

Antibiotic resistance of pathogenic organisms has become a worldwide problem with serious concern both in hospital and community settings posing threatening consequences on the treatment of infectious diseases. The increased use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon (1, 2). Pseudomonas aeruginosa is one of the most frequent (10-20%) pathogens associated to among nosocomial infections, especially patients immunocompromised (2) exhibiting notorious versatility and capacity to acquire resistance mechanisms to antimicrobial therapy(3). Within the hospital, P. aeruginosa finds numerous reservoirs in disinfectants, respiratory equipment, food, sinks, taps and mops. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by ingestion of contaminated foods and water (4).

Mechanisms of resistance to Carbapenems in *P. aeruginosa* are impermeability, including closure of porin channels in the bacterial cell wall; and extrusion of antibiotics by efflux pumps, which can lead to resistance to multiple classes of antibiotics. *E. coli*, on the other hand, produce extended-spectrum β lactamases (ESBLs) which may develop on the basis of a change in only one amino acid in the β lactamases normally produced (5). By contrast to plasmid-mediated production of ESBLs, AmpC β lactamases are chromosomally-mediated and occur in ICU pathogens such as *P. aeruginosa, Enterobacter spp*, and in recent years in *E. coli* (6).

Carbapenems are a class of potent β -lactams considered as the last resort option for treating serious nosocomial infections caused by a broad spectrum of Gram-negative bacteria. They are known

not to easily diffuse through the bacterial cell wall (7). They enter the Gram-negative bacteria through the outer membrane proteins (OMPs), after transversing the periplasmic space; Carbapenems 'permanently' acylate the penicillin-binding proteins (PBPs) which are enzymes that catalyze the formation of peptidoglycan in the cell wall of bacteria (8, 9, 10). Carbapenems act as mechanism-based inhibitors of the peptidase domain of PBPs which inhibits peptide cross-linking as well as other peptidase reactions. Carbapenems are prominent for their ability to bind to multiple different PBPs and eventually weakening the peptidoglycan ultimately leading to cell burst due to osmotic pressure (8, 11).

Carbapenems resistance is modulated by acquired carbapenemases in association with intrinsic mechanisms such as down-regulation or loss of OPrD porin, efflux pumps hyperextension, chromosomal AmpC β -lactamase production, and target alterations. However, since carbapenemases have the ability to hydrolyse Carbapenems, Gram-negative bacteria carrying a carbapenemase-encoding gene frequently exhibit resistance to virtually all β -lactams.

Majority of the non-fermenting Gram-negative bacteria (e.g., *pseudomonas* spp., *Acinetobacter* spp., and *Stenotrophomonas* spp.), as well as the *Enterobacteriaceae* (e.g., *Klebsiella* spp., *Escherichia coli*, and *Enterobacter* spp.) and Gram-positive bacteria (e.g., *Staphylococcus* spp., *Streptococcus* spp., *enterococcus* spp., *Nocardia* spp.), have shown resistance to most clinically available carbapenems and this is of grave public health concern (12).

Given the importance of Carbapenem for the treatment of infections caused by *P. aeruginosa* and *E. coli*, this present study was carried out to examine the antimicrobial resistance pattern of Carbapenems on the clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* in order to determine its efficacy.

MATERIAL AND METHODS Collection of samples

Ninety seven (97) isolates were obtained from the University College Hospital, comprising of fifty (50) *E. coli* and forty seven (47) *Pseudomonas aeruginosa* isolates. They were sculptured onto sterile slant bottles. They were transferred to the Microbiology Research Laboratory of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, South-Western part of Nigeria for further analysis.

Processing of samples

Inoculum preparation was done under asepsis by picking isolates from the slant bottles into universal bottles containing 5ml of normal saline to obtain a suspension equivalent to the turbidity of 0.5 McFarland standards.

Antibiotic susceptibility testing

Susceptibility test was determined using the disc diffusion method on Mueller-Hinton agar plates and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014) as described by (13). Five antibiotics were tested, including Ertapenem ($10\mu g$), Imipenem ($10\mu g$), Colistin Sulphate ($25\mu g$), Levofloxacin ($5\mu g$), and Piperacillin/Tazobactam ($110\mu g$).

Statistical analysis

Statistical analysis was carried out with the Statistical Package for Social Sciences (SPSS) version 20.0; ANOVA was done at p-value < 0.05 and at a confidence interval of 95%.

RESULTS

Table 1 showed the antibiotics susceptibility profiles of the clinical isolates cum variation using diskdiffusion methods. *Pseudomonas aeruginosa* had the highest resistance rates to Ertapenem (87.2%), followed by Levofloxacin (19.1%), Colistin sulphate (12.8%), Piperacillin/Tazobactam (4.3%) with the least resistance observed in Imipenem (2.1%).

TABLE 1: THE ANTIBIOTICS SUSCEPTIBILIT	Y PROFILES OF P. AERUG	GINOSA AND E. COLI

Organisms (is	solates)							Antib	iotics						
	Ertap	sener	m	In	nipen	em	(Colis	sin sulp	hate	Levo	floxacin	Piper	acillin	/tazobactan	n
	(10	μg)			(10µ	(g)			(25µg)			(5µg)			(110µg)	
	S	Ī	R	\boldsymbol{S}	Ι	R	S	Ι	R	S	Ι	R	S	Ι	R	
P. aeruginosa	2(%)4.3	8.5	87.2	4.3	93.6	2.1	6.4	80.9	12.8	80.9	0	19.1	89.4	6.4	4.3	
E. coli	56% 1	4%	30%	100%	0 %	0%	90 %	6 6%	6 4%	80%	0%	20%	96%	4%	0%	
					K	ey: S	= Sus	scept	tible, I=	Interm	ediat	e and R= R	esistant	;		

E. coli had the highest resistance rates to Ertapenem (30%) followed by Levofloxacin (20%). The least was recorded in Colistin sulphate (4%). No resistance was recorded in Imipenem and Piperacillin/Tazobactam, respectively. *P. aeruginosa* isolates had highest susceptibility to Piperacillin/Tazobactam (89.4%) followed by Levofloxacin (80.9%), Colistin sulphate (6.4%), with the least recorded in Imipenem and Ertapenem (4.3% each), respectively. *E. coli* had the highest susceptibility to Imipenem (100%) followed by Piperacillin/Tazobactam (96%), Colistin sulphate (90%), Levofloxacin (80%). The least was recorded in

These results suggest that Ertapenem (56%). Ertapenem is least effective against E. coli and P. *aeruginosa* since about 57.7% of these clinical specimen isolates resistant are to it, while Piperacillin/Tazobactam is most effective because about 92.8% of these organisms are susceptible to it. The result of ANOVA revealed a significant effect of Ertapenem on Pseudomonas aeruginosa. Also a significant effect of Piperacillin/Tazobactam was recorded on *E coli*. No significant effect was recorded among the other antibiotics on *P* aeruginosa and *E* coli (Table 2).

Organisms	Antibiotics								
	Ertapenem	Imipenem	Colisin sulphate	Levoflox	Piperacillin/tazobactam				
P. aeruginosa	1.27 <u>+</u> 0.07 ^b	1.37 <u>+</u> 0.02 ^a	1.95 <u>+</u> 0.03ª	1.48 <u>+</u> 0.02 ^a	0.88 <u>+</u> 0.00 ^a				
E. coli	1.24 <u>+</u> 0.40 ^a	1.84 <u>+</u> 0.04ª	1.32 <u>+</u> 0.01 ^a	1.74 <u>+</u> 0.02 ^a	2.32 ± 0.05^{ab}				

TABLE 2: ANOVA FOR MEAN EFFECT OF ANTIBIOTICS ON ISOLATES

Key: Values are mean scores \pm standard error. a, b = Mean values followed by the same superscript in the columns are not significantly different by Duncan's Multiple Range test (P \leq 0.05).

DISCUSSION

Antibiotic resistance determinants have been circulating within the microbial genome for millennia, largely predating the manufacture and use of antibiotics by human beings (14). Antibiotic resistance correlates well with the frequency of drug use and in a country like Nigeria where drugs are easily available over-the-counter, bacterial resistance to antibiotics grows rapidly, putting our health care system in a dilemma. Drug resistance facilitates growth and increases prevalent of persistent pathogens which become difficult to exterminate (15).

P aeruginosa is the most common non-fermenting bacterium isolated from clinical samples posing a serious therapeutic threat for the treatment of both community-acquired and nosocomial infections. Identification and selection of appropriate antibiotic to initiate therapy is essential to optimizing clinical outcome. *E. coli*, on the other hand, produce extended-spectrum β -lactamases (ESBLs) which may develop on the basis of a change in only one amino acid in the β -lactamases normally produced (6). By contrast to plasmid-mediated production of ESBLs, AmpC β -lactamases are chromosomally-mediated and occur in ICU pathogens such as *P. aeruginosa*, *Enterobacter spp*, and in recent years in *E. coli* (14).

From this study, *P aeruginosa* had more resistance to the antibiotics used as compared with *E coli*. The study showed majority (87.2%) of *P aeruginosa* isolates were resistant to ertapenem, followed by levofloxacin (19.1%) and colistin sulphate (12.8%). Similar resistant pattern against the isolate was reported in different studies conducted by ¹⁶, ¹⁷ and ¹⁸. Imipenem and piperacillin/tazobactam were most effective drug observed in this study showing resistant rates of 2.1% and 4.3% respectively. This pattern is in accordance with the reports of (19, 20, 21).

On the other hand, *E coli* showed a major (30%) resistance to ertapenem followed by levofloxacin (20%) and Colistin sulphate (4%). This is in accordance with the findings of (22, 23, 24). Interestingly, the isolates were 100% susceptible to imipenem and piperacillin/Tazobactam, respectively. This is in accordance and in disagreement to the report of 26 who recorded 100% sensitivity for imipenem and nearly 40% sensitivity to piperacillin/Tazobactam.

In Nigeria, the sensitivity of the isolates to imipenem and piperacillin/tazobactam is in accordance with the reports of (27, 28, 29). The high susceptibility pattern of these drugs could be associated to less drug abuse by the population due to their cost preventing patient's self-medication. However, the pattern disagrees with the findings of ³⁰, ³¹ where a high resistant was recorded, thus demonstrating the evolution of imipenem-resistant strains of *P. aeruginosa* to imipenem.

One of the reasons for resistance might be due to misuse and overuse of antibiotics, that is, not adhering to the prescription of antibiotics. Also, it can be transferred horizontally between bacteria. P. aeruginosa resistance to Carbapenems may be due to a result of complex interactions of several mechanisms production of carbapenemase, including overproduction of efflux system and loss of outer membrane porins (21). P aeruginosa resistance to imipenem and piperacillin/Tazobactam might be as a result of movement of various types of patients being referred to UCH, a tertiary hospital, both locally and internationally for management or continuation of therapy, thus the selective pressure of use, misuse and overuse of antibiotics cannot be farfetched. Also, there is the likelihood of transfer of resistance genes from other clinics around the world.

Resistance in *E coli* might be as a result of transfer of plasmids between commensal organisms and potential pathogens through inappropriate or overprescribing of antibiotics and difficulty in establishing bacterial etiology at the time of prescription. Therefore, there is need for public awareness on the prudent use of antibiotics and strict adherence to minimize the misuse of effective drugs. Overall, the data obtained indicate that imipenem and piperacillin/Tazobactam are the most effective for the treatment of *P. aeruginosa* and *E. coli* infections. Though effective, proper monitoring of resistance to these antibiotics is necessary.

Conclusion: This study revealed that most of the isolates were susceptible to imipenem and piperacillin/tazobactam. To avoid resistance, illicit use of antibiotics is advised. Continued monitoring of antimicrobial resistance patterns in hospitals and community settings is essential to guide effective empirical therapy. Furthermore, piperacillin/tazobactam may be considered as reserve drug for treatment of *P. aeruginosa* infections.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1823 http://www.ajol.info/journals/ajcem COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.2

AFR. J. CLN. EXPER. MICROBIOL. 19 (3): 165-170

PUBLIC HEALTH IMPLICATIONS OF SALMONELLA SPECIES CONTAMINATION OF NAIRA NOTES OBTAINED FROM BUTCHERS IN ABAKALIKI MEAT MARKET, EBONYI STATE

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ABSTRACT

Daily transactions have made paper currencies to pass through many hands, and pathogens become imposed on them before they are finally deposited in banks. This study evaluates the public health implications of *Salmonella* species contamination of naira notes obtained from butchers in Abakaliki meat market, Ebonyi State. A total of 95 samples of naira notes in different denominations (N5 to N1000) were randomly collected from butchers within Abakaliki metropolis between the months of March and May, 2016. All the naira note samples were analysed using standard microbiological procedures. A total of nine (9.5%) *Salmonella* spp isolates were obtained from the 95 naira note samples. All the *Salmonella* spp isolates were completely resistant (100%) to ceftazidime. The highest susceptibility frequency was observed for ciprofloxacin, gentamicin, meropenem, imipenem, and ofloxacin with values ranging from 88.9% - 77.8%. There was a statistically significant difference in the mean percentage resistance and susceptibility of the *Salmonella* spp isolates of antibiotics. The average multiple antibiotic resistance indices (MARI) of the *Salmonella* spp isolates was 0.35. This study shows that ciprofloxacin, gentamicin, meropenem, imipenem, and ofloxacin are still effective in the treatment of bacterial infections caused by *Salmonella* spp. Proper and hygienic handling of paper currencies is of public health importance so as to avert health challenges associated with microbial contamination of bank notes especially *Salmonella* spp.

Keywords: Salmonella, Naira notes, butchers, antibiotics, public health

CONSÉQUENCES SUR LA SANTÉ PUBLIQUE DE L'ESPÈCE SALMONELLA CONTAMINATION DE NAIRA NOTE OBTENUE À PARTIR DE LA VIANDE DE BOUCHERIE À ABAKALIKI, MARCHÉ DE L'ETAT D'EBONYI

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Résumé

Transactions quotidiennes ont fait des monnaies papier à passer entre de nombreuses mains, et d'agents pathogènes deviennent imposées sur eux avant qu'ils ne sont finalement déposés dans des banques. Cette étude évalue les conséquences sur la santé publique de l'espèce Salmonella contamination de naira note obtenue à partir de la viande de boucherie à Abakaliki marché, l'état d'Ebonyi. Un total de 95 échantillons de naira notes dans différentes dénominations (N5 à N1000) ont été prélevés au hasard à partir de bouchers au sein de Metropolis Abakaliki entre les mois de mars et mai 2016. Tous les échantillons ont été analysés note naira en utilisant les procédures microbiologiques standard. Un total de neuf (9,5 %) des isolats de Salmonella spp ont été obtenues à partir de la naira 95 remarque d'échantillons. Tous les isolats de Salmonella spp, étaient totalement résistants (100 %) de la ceftazidime. La plus haute fréquence de sensibilité a été observée pour la ciprofloxacine, gentamicine, méropénem, imipenem, et de l'ofloxacine avec des valeurs allant de 88,9 % - 77,8 %. Il y avait une différence statistiquement significative dans le pourcentage moyen de résistance et de sensibilité des isolats de Salmonella spp, le < (P 0,05). La plupart des isolats de Salmonella spp, présentait des caractéristiques multi-résistante car ils étaient résistants à au moins deux classes différentes d'antibiotiques.

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La moyenne des indices de résistance multiple aux antibiotiques (MARI) de la isolats de Salmonella spp, était de 0,35. Cette étude montre que la ciprofloxacine, gentamicine, méropénem, imipenem, et l'ofloxacine sont toujours efficaces pour le traitement des infections bactériennes causées par Salmonella spp. Bon et la manipulation hygiénique des monnaies de papier est de l'importance de la santé publique, afin d'éviter les défis de santé liés à la contamination microbienne des billets de banque en particulier Salmonella spp.

Mots-clés: Salmonella, Naira notes, des bouchers, des antibiotiques, de la santé publique

INTRODUCTION

Paper currency notes could be one of the most potential vehicles of transmitting diseases amongst the people. Daily transactions have made the paper currency to pass through many hands, and pathogens become imposed on them before they are finally deposited in banks. Modern scientific studies have confirmed the presence of various pathogenic bacteria on paper currencies. In most day-to-day cash transactions, money, in form of notes and coins, pass through the hands of many people; as against exchange dependent on double coincidence of wants [1]. Various denomination of the naira notes have been minted by the Central Bank of Nigeria (CBN). They are released to the public, through the commercial banks. Currently, there are eight denominations of the naira notes: N5, N10, N20, N50, N100, N200, N500 and N1000 notes. The N5, N10, N20, N50, N100 and N200 naira notes are the most common and are more involved in daily cash transactions. They are common especially among the populace while the N500 and N1000 notes are commonly used among the wealthy and in corporate transactions [2]. Individuals handling the notes shed some of their body flora on the notes; leading to the spread of the microorganisms among the handlers. This has been implicated in serious health hazard such as impairment of lungs function [3]. The contamination of the notes can be traced to dust, soil, water, microflora of the body of handlers (hand, skin, etc.), and the saliva often used when counting the notes and wounds. Some money handling habits such as: keeping naira notes in brassiere, socks, pockets, under the carpet or rugs, and squeezing in the hand frequently introduce microbes to the notes. Citrobacter spp, Salmonella spp, Shigella spp, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa have been isolated from naira notes [4]. Most of them are normal flora of the human skin; however, some examples are opportunistic pathogens. This suggests that the notes could serve as fomites for some infectious agents [2]. Salmonella is one of the most frequent pathogen associated with food borne disease outbreaks. Cooked food products and raw milk were most commonly contaminated with food borne pathogens and many of them were resistant to different antibiotics. Meat and milk products are often contaminated with bacterial pathogens. It is currently not possible to effectively and consistently exclude

such multi-drug resistant strains from the human food chain, which means that they continue to pose a significant clinical threat to consumers and concomitant economic threats to food production and processing industry. The Presence of enterotoxigenic and antimicrobial resistant strains of *Salmonella* has become remarkably widespread in foods. This requires a better control of food contamination sources and distribution of antimicrobial-resistance organisms [5]. This study evaluates the public health implications of *Salmonella* species contamination of Nigerian naira notes obtained from butchers in Abakaliki meat market, Ebonyi State.

MATERIALS AND METHODS

Study Area: Ebonyi State is popularly known as the 'Salt of the Nation,' apparently because of the large deposits of salt water in the state. The State capital is Abakaliki. It lies approximately within longitude 7^o 30' and 8^o 30' E and 5^o 40' and 6^o 45'N. According to data from the 2006 Population and Housing Census, Ebonyi State has an estimated population of 2.3 million and a land mass of 5,935 km².

Collection of Samples

A total of 95 samples of naira notes in different denominations ($\underline{N5} - \underline{N}$ 1000) were randomly collected from butchers within Abakaliki metropolis between the months of March and May, 2016. The naira notes were collected with hands covered with sterile gloves. The notes were then immediately placed inside sterile polythene bags and labeled accordingly. In some instances, the naira notes were obtained during exchange for bigger denominations; others were obtained after buying items with bigger denominations. The naira note samples were then immediately transported to the department of Applied Microbiology laboratory, Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis. The currencies were observed to have been in circulation for about 2 - 4 years (2011-2014). Mint paper currencies were used as controls.

Culturing, isolation, phenotypic characterization and identification of the isolates

A sterile swab stick moistened with sterile physiological saline (0.85 % NaCl) was used to swab

both sides of each note. Each of the swabs was then aseptically transferred into test tubes containing 5 ml of peptone water and incubated overnight at 37 °C. After incubation, the swabs were then streaked onto already prepared Salmonella-Shigella (SS) agar (Oxoid, UK). The inoculated plates were then incubated at 37 °C for 24 hours. Colonies displaying typical Salmonella characteristics (smooth, small and colourless colonies with dark centre) were picked and sub-cultured on freshly prepared Salmonella-Shigella agar plates so as to obtain pure colonies. Pure colonies of Salmonella isolates were picked from the Salmonella-Shigella agar plates and inoculated on prepared nutrient agar slants and preserved in the refrigerator at 4 °C for further tests. The suspected Salmonella isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test, and other biochemical tests which include oxidase test, indole test, citrate utilization test, H₂S production test, Voges-Proskauer test, methyl red test, urease test, nitrate reduction test, and sugar fermentation test (Cheesbrough, 2004).

Antibiotic susceptibility test

Antibiotic susceptibility of the Salmonella isolates was done using the Kirby Bauer disc diffusion method and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2007) formerly known as National Committee for Clinical Laboratory Standards (NCCLS). Mueller-Hinton agar was prepared according to the manufacturer's instructions. The medium was cooled to 45-50 °C and poured into plates. Plates were allowed to set on a level surface to a depth of approximately 4 mm. When the agar has solidified, plates were allowed to dry before use. An 18-24 hour old broth culture of the Salmonella isolate was standardized by diluting to 0.5 Mcfarland's standard. A sterile swab stick was inserted into the standardized Salmonella inoculum, drained to remove excess inoculum load and inoculated by spreading on the surface of prepared Mueller-Hinton agar plate. After this, the inoculated Mueller-Hinton agar plate was allowed to dry for a few minutes at room temperature with the lid closed. After the agar surface has dried for few minutes, antibiotic impregnated discs (Oxoid, UK) of known concentrations; gentamycin (30 µg), aztreonam (30 μ g), ofloxacin (5 μ g), ceftazidime (30 μ g), cefepime (30 μ g), meropenem (10 μ g), ciprofloxacin (5 μ g), and imipenem (10 µg) were carefully applied on the inoculated Mueller-Hinton agar plates using sterile forceps. The plates were then incubated at 37 °C for 18-24 hours. After incubation, the diameters of the zones of inhibition were measured with a ruler to the nearest millimeter and recorded. The results were recorded as resistant, intermediate and susceptible according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2007).

Determination of Multiple Antibiotics Resistance Index (MARI)

Multiple antibiotic resistance indices (MARI) of the bacterial isolates were calculated using the technique described by Christopher *et al.* (2013) and Subramani *et al.* (2012). This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).

STATISTICAL ANALYSIS

Statistical analysis was performed using T-test and Pearson correlation index at P < 0.05 with statistical software SPSS Data Editor Version 16.

RESULTS

TABLE 1: CURRENCY DESCRIPTION POSITIVE SALMONELLA SPP

		5/11	MONL		
S/N	Currency	Smooth	and	Rough and	Salmonella
	Type(N)	Neat		Dirty	spp
					growth
					after 24
					hours
1	500	+		-	+
2	50	-		+	+
3	200	-		+	+
4	50	-		+	+
5	100	-		+	+
ť	1000	-		+	+
5	10	-		+	+
٤	20	-		+	+
ç	5	-		+	+
1	Control	-		-	-
	(mint)				
	Kor	r + = Posit	ivo: _ =	Nogativo	

Key: + = Positive; - =Negative

All the naira notes were rough and dirty except one (\mathbb{N} 500) which was smooth and neat. All the naira notes were positive for *Salmonella* species except the control (mint)

TABLE 2: PERCENTAGE OCCURRENCE OF RESPONSES OF THE SALMONELLA ISOLATES TO DIFFERENT ANTIBIOTICS

Antibiotics Used	Resistance (%)	Susceptible (%)
Ciprofloxacin	1 (11.1 %)	8 (88.9 %)
Cefepime	4 (44.4 %)	5 (55.6 %)
Gentamicin	1 (11.1 %)	8 (88.9 %)
Aztreonem	4 (44.4 %)	5 (55.6 %)
Meropenem	1 (11.1 %)	8 (88.9 %)
Imipenem	2 (22.2 %)	7 (77.8 %)
Ofloxacin	2 (22.2 %)	7 (77.8 %)
Ceftazidime	9 (100 %)	0 (0 %)

All the *Salmonella* spp isolates were completely resistance (100 %) to ceftazidime. This was closely followed by aztreonam (44.4 %) and cefepime (44.4 %). The highest susceptibility frequency was observed for ciprofloxacin, gentamicin, meropenem with values of 88.9 % each. This was closely followed by imipenem and ofloxacin with values of 77.8 % each.

TABLE 3: ANTIBIOTIC RESISTANCE PATTERNS OF THE SALMONELLA ISOLATES

S/N	Isolate Code	Resistance Patterns
1	Sal 14	CAZ ^R
2	Sal 16	FEP ^R MEM ^R IPM ^R CAZ ^R
3	Sal 18	FEP ^R ATM ^R IPM ^R OFX ^R CAZ ^R
4	Sal 19	CAZ ^R
5	Sal 20	FEPR ATMR CAZR
6	Sal 27	CAZ ^R
7	Sal 38	ATM ^R CAZ ^R
8	Sal 17	CAZ ^R
9	Sal 46	CIP ^R FEP ^R CN ^R ATM ^R OFX ^R CAZ ^R

Key: R= Resistant, CIP = Ciprofloxacin, FEP = Cefepime, CN = Gentamincin, ATM = Aztreonam, MEM = Meropenem, IPM = Imipenem, OFX = Ofloxacin, CAZ = Ceftazidime

Five (Sal 2, Sal 3, Sal 5, Sal 7, and Sal 9) out of the nine (9) isolates were multi-drug resistant as they were resistant to at least two different classes of antibiotics.

TABLE 4: MULTIPLE ANTIBIOTIC RESISTANCE INDEX (MARI) OF THE SALMONALLA ISOLATES

Isolates	Sal 14	Sal 16	Sal 18	Sal 19	Sal 20	Sal 37	Sal 38	Sal 17	Sal 46	Total	Average
MARI value	0.13	0.50	0.63	0.13	0.38	0.13	0.3	0.13	0.8	3.13	0.35

The multiple antibiotic resistance indices (MARI) of the *Salmonella* spp isolates ranged from 0.13 to 0.8. This high MARI might be an indication of abuse and misuse of antibiotics.

DISCUSSION

Paper currencies have been used for transaction purposes both within Nigeria and beyond. However, this essential item have often times been predisposed to microbial contamination through unhygienic practices by traders and pedestrians alike. *Salmonella*, an enteric Gram-negative rod-shaped bacterium has been frequently implicated in paper currency contamination.

This study evaluates the role of *Salmonella* spp. in Nigeria's paper currency (Naira notes) contamination and their antibiotic susceptibility patterns. A total of ninety five (95) naira notes ranging from N 5 to N1000 were collected from butchers in Abakaliki meat market while a naira note in mint condition obtained from a commercial bank was used as a control. All the 95 naira notes obtained from butchers at meat market, Abakaliki showed bacterial growth after 24 hours.

Most of the naira notes that had bacterial growths were rough and dirty in appearance. The control (mint) obtained from a commercial bank in the same study area did not show any bacterial growth. Out of the 95 naira notes obtained for this study, 9 were positive for *Salmonella* spp. growth after 24 hours of incubation at 37 °C (Table 1). This result is in agreement with the work of Ayandele and Adeniyi [10] but differs from a separate report by Omar and Bassan [11] that isolated only one (1) *Salmonella* species from paper currency in Saudi Arabia.

All the *Salmonella* spp. isolates were completely resistant (100 %) to ceftazidime. This was closely followed by aztreonam (44.4 %), and cefepime (44.4 %) (Table 2). This observation is similar to report by Alemu [12] in Ethiopia where he recorded 87.5 % resistance among *Salmonella* isolates recovered from paper currency. The highest susceptibility frequency was observed for ciprofloxacin, gentamicin, and meropenem with susceptibility frequency value of 88.9 % each. This was closely followed by imipenem and ofloxacin with susceptibility frequency value of 77.8 % each (Table 2). The results of the one sample t-

test conducted showed that there was a statistically significant difference in the mean percentage resistance of the Salmonella isolates (mean = 33.3125, SD = 30.28495), t = 3.111, P = 0.017 (P < 0.05). One sample t-test also showed that there was a statistically significant difference in the mean percentage susceptibilities of the Salmonella isolates (mean = 66.6875, SD = 30.28495), t = 6.228, P = 0.000 (P < 0.05). Results of the independent samples t-test conducted to compare the percentage resistances and percentage susceptibilities of the Salmonella isolates to different classes of antibiotics showed that there was a statistically significant difference in the mean percentage resistance (mean = 33.3125, SD = 30.28495) and mean percentage susceptibility (mean = 66.687, SD = 30.28495); t = -2.204, P = 0.045 (P < 0.05). The result of this work shows that ciprofloxacin, gentamicin, meropenem, imipenem and ofloxacin are still active against Salmonella spp. isolates in Abakaliki, Ebonyi state. Results also showed that ceftazidime, a third generation cephalosporin, is no longer effective in treating bacterial infections caused by Salmonella spp. as all the 9 Salmonella isolates obtained in this study were resistant to this particular antibiotic. Four (44.4 %) out of the 9 Salmonella isolates did not exhibit multi-drug resistance traits as they were resistant to only one class of antibiotic; ceftazidime, a third generation cephalosporin (Table 3). However, the remaining 5 (55.6 %) Salmonella spp. isolates were multi-drug resistant as they exhibited resistance to at least two classes of antibiotics (Table 3). The average multiple antibiotic resistance index (MAR1) value of the Salmonella spp. isolates was 0.35 and this signifies their high antibiotic resistance frequency values to commonly used antibiotics for treating bacterial infections (Table 4). The presence of microorganisms on paper currencies suggests that it

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can be a favourable environment for their growth. The presence of Salmonella in paper currencies could possibly arise from faecal contamination. It is a common practice among traders (butchers and meat sellers inclusive) to handle paper currencies improperly without maintaining good handling hygienic practices especially washing of hands properly after butchering of animals or displaying meats for sale on dirty tables in open places where flies are always perching on the meat being sold. These unhygienic practices predisposes the traders to Salmonella infections especially Salmonella food poisoning, typhoid fever, paratyphoid fever among others. Consequently, proper handling of bank notes is of public health importance considering the fact that some communicable diseases can spread by means of fomites including money.

CONCLUSION

Salmonella contamination of paper currencies could be a potential source of community-acquired infection. This research has shown the presence of Salmonella species on Nigeria's paper currencies collected from butchers in Abakaliki meat market, Ebonyi State. The study also revealed that Salmonella isolates were susceptible to most of the antibiotics except ceftazidime. This study has also revealed that ciprofloxacin, gentamicin, meropenem, imipenem and ofloxacin are still effective in treating bacterial infections caused by Salmonella spp. Salmonella species can cause serious community acquired infections. Hence, appropriate hygienic measures should be adopted while handling naira notes.

CONFLICT OF INTEREST STATEMENT: The authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1824 COPYRIGHT 2018 <u>https://dx.doi.org/10.4314/ajcem.v19i3.3</u> AFR. J. CLN. EXPER.MICROBIOL. 19 (3): 171-176

A SURVEY ON URINARY TRACT INFECTION ASSOCIATED WITH TWO MOST COMMON UROPATHOGENIC BACTERIA

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ABSTRACT

The aim of this study was to determine the prevalence of urinary tract infection (UTI) caused by the uropathogens – *Escherichia coli* and *Klebsiella pneumoniae* among outpatients attending a hospital in Benin and the antibiotic susceptibility pattern of both isolates. Mid-stream urine samples were collected from 96 patients, analysed microscopically and streaked on the surface of prepared plates of blood and MacConkey Agar. Isolates resulting from incubated plates were identified by standard microbiological techniques. Susceptibility testing was done using the disk diffusion technique on Mueller Hinton Agar. The prevalence of UTI was 22.9%. It was significantly more prevalent in females (30.6%) than males (8.8%) (p < 0.05) and in age group 31 - 40 years (45.5%). *Escherichia coli* was the most commonly isolated and more prevalent (14.6%) followed by *Klebsiella pneumoniae* (5.2%). High level resistance (100%) to amoxycillin/clavulanic acid, ampicillin and ceftazidime was observed among the isolates. The active drugs against the bacteria were nitrofurantoin, ofloxacin and imipenem. Routine diagnostic procedure/screening is necessary to avoid complications.

Keywords: Infection, prevalence, resistance, uropathogens.

UNE ENQUÊTE SUR L'INFECTION DES VOIES URINAIRES ASSOCIÉS À DEUX PRINCIPALES BACTÉRIES UROPATHOGÈNES

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Résumé

Le but de cetteétudeétait de déterminer la prévalence de l'infectiond'appareilurinaire (UTI) causéeparl'*Escherichia coli* uropathogènes - et *Klebsiellapneumoniae* chez les patients fréquentant un hôpital au Bénin et le modèle de sensibilité aux antibiotiques des isolats des deux. Les échantillonsd'urine mi-jet ontétérecueilliesauprès de 96 patients, analysées au microscope et de rayuressur la surface des plaques préparées du sang et de la géloseMacConkey. Les plaques d'isolatsrésultantd'incubésontétéidentifiés par des techniques microbiologiques standard. De sensibilitéaétéréalisé en utilisant la technique de diffusion surgélose Mueller Hinton. La prévalence de l'UTIétait de 22,9 %. Il a étésignificativement plus élevée chez les femmes (30,6 %) que chez les hommes (8,8 %) (p < 0,05) et dans le grouped'âge 31 - 40 ans (45,5 %). Escherichia coli a été le plus souventisolés et plus fréquente (14,6 %) suivi par *Klebsiellapneumoniae* (5,2 %). La résistance de haut niveau (100 %) à l'amoxycilline-acideclavulanique, à l'ampicilline et la ceftazidime a étéobservéeparmi les isolats. Les médicamentsactifscontre les bactériesontéténitrofurantoin, l'ofloxacineetl'imipénème. Procédure de diagnostic/dépistagesystématiqueestnécessaire pour éviter les complications.

Mots clés : Infection, prévalence, résistance, uropathogènes.

INTRODUCTION

Bacteria are the commonest cause of both community and hospital-acquired infections globally. Infection of the urinary tract is the second most prevalent infection (after respiratory tract infection), but the most hospital-acquired infection accounting for 39 – 40% of total number of reported cases by acute health care hospitals (1, 2, 3). Urinary tract infection (UTI) refers to the presence, growth and multiplication of microbial pathogens within any organ that make up the urinary system, including the two kidneys, two ureters, single bladder and urethra (4).

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The system is concerned with the filtering out of excess fluid and other substances from the blood stream in the form of urine (which is a good growth medium for many species of bacteria though normally sterile) (5, 6).

Infections of the kidney (pyelonephritis), bladder (cystitis) and urethra (urethritis) are among the commonly reported infections (7, 8), often leading to the presence of bacteria in the urine and could be asymptomatic or symptomatic depending on the host immune status and virulence of the invading pathogen. UTIs caused by bacteria are mostly common while that by fungi or viruses are rare (6), but related to abnormalities of the urinary system or its instrumentation (catherisation) (9, 10). Esherichia coli is the cause of 80 - 85% of community acquired and 50% of hospital acquired UTIs (11, 12). Klebsiella and Proteus species account for 5 - 10%, and the Gram positive species such as Staphylococcus saprophyticus, accounts for another 5 – 10% of UTIs in young women (5). Several studies conducted in Nigeria and other parts of the world have reported Escherichia coli as the major organism isolated in significant numbers from urine followed by Klebsiella pneumoniae (in some studies) (13, 14, 15, 16). The frequency of these pathogen depends on age, sex, hospitalization and urethral catheterisation and thus are predisposing factors (17) as they could affect the prevalence of urinary tract infections (UTIs) (18).

Strains that cause UTIs often originate from the gut/intestinal flora of the patient, with infection occuring in an ascending manner. Uropathogenic *Escherichia coli* adheres to uroepithelial cells by means of adhesins, pili, type 1 and P fimbriae – all of which induce cytokine production and chemotaxis of neutrophils, initiating an inflammatory response (19, 20). Other known virulence factors produced by Gram negative uropathogens include haemolysins, capsule and proteases which contribute to damaging host cells and evading the immune system (21, 22).

Some strains that cause UTIs are resistant to the drugs used for treatment. This creates the need for their detection and susceptibility profile on a regular basis as it would enhance therapeutic decision. Thus, the aim of this study was to determine the prevalence of UTI due to *Escherichia coli* and *Klebsiella pneumoniae* among outpatients attending the Central Hospital, Benin, and the susceptibility pattern of both isolates.

Subjects and Method

A total of 96 outpatients attending the General Outpatients Department of the Central Hospital, Benin were included in this study after obtaining relevant ethical clearances from the Hospital Management Board and the Central Hospital's management. Subjects who were on antibiotic therapy were excluded. Data of subjects including age and sex were obtained from laboratory record/request form.

Specimen collection and bacterial isolation

Voided mid-stream urine specimens were collected in sterile universal containers. Samples were examined microscopically and loopful (0.001ml) streaked onto the surface of dried plates of blood and MacConkey Agar (Lab M, UK). Culture plates with bacteria counts greater than or equal to 10⁵cfu/ml of urine were considered as positive for UTI according to Cheesbrough (7). Isolates were identified by standard microbiological techniques including morphological (Gram staining) and biochemical (catalase, coagulase, indole, voges-proskauer citrate, and sugar fermentation) tests.

The prevalence/isolation rate of *Escherichia coli* or *Klebsiella pneumoniae* was determined by the formula: $= \frac{Number of isolates recovered}{Total number of samples screened} \times \frac{100}{1}$

Susceptibility testing

Sensitivity of the isolates to 16 antibiotics was performed using the disk diffusion technique on Mueller Hinton Agar (Lab M, UK). Zones of inhibition diameter measured in millimeter (mm) were interpreted using the interpretive chart published by the Clinical and Laboratory Standards institute (CLSI, 2012) and reported as sensitive (S), intermediate (I) and resistant (R).

Statistical analysis

Data were analysed using SPSS 16.0. Chi-square test was used to test for significance.

RESULTS

A total of 96 urine samples were studied. The patients included 34 (35.4%) males and 62 (64.6%) females giving a male to female ratio of 1: 1.82. Their age range was from 21 – 80 years with a mean age of 33.7 years. A total of 22 patients had UTI with majority of the isolates recovered from age groups 31 – 40 years (45.4%) and 21 – 30 years (31.8%) but was most prevalent in the former age group (45.4%) (Table1). The prevalence was also higher in females (30.6%) compared to males (8.8%) (Table2). The total number of *Escherichia coli* and *Klebsiella pneumoniae* recovered were 14 (63.7%) and 5 (22.7%) respectively with *Escherichia coli* being most prevalent (14.6%) (Table3). No mixed culture was encountered.

Age	NI- of anotimen	Number pos	itive	- Total positive(%)	Prevalence rate (%)	
8-	collected	Male	Female	F (///	(//)	
21 - 30	54	1	6	7 (31.8)	12.9	
31 - 40	22	2	8	10 (45.5)	45.4	
41 - 50	10	0	3	3 (13.6)	30	
51 - 60	5	0	1	1 (4.55)	20	
61 – 70	3	0	1	1 (4.55)	33.3	
71 - 80	2	0	0	0 (0)	0 (0)	
Total	96	3	19	22 (100)	22.9	

TABLE 1: PREVALENCE OF UTI IN RELATION TO AGE AND GENDER

TABLE 2: DISTRIBUTION OF BACTERIAL ISOLATESBY GENDER OF PATIENTS

Organisms	Gender	- Total (%)	
	Male (n = 34)	Female (n = 62)	
Escherichia coli	2 (14.3)	12 (85.7)	14 (100)
Klebsiella pneumoniae	0 (0)	5 (100)	5 (100)
Staphylococcus aureus	1 (50)	1 (50)	2 (100)
Proteus mirabilis	0 (0)	1 (100)	1 (100)
Total recovered (%)	3 (13.6)	19 (86.4)	22 (100)
Prevalence rate (%)	8.8	30.6	22.9

TABLE 3: FREQUENCY OF BACTERIAL ISOLATES IN URINE (N = 96 SAMPLES)

Organisms	Number isolated (%)	Prevalence rate (%)
Escherichia coli	14 (63.6)	14.6
Klebsiella pneumoniae	5 (22.7)	5.2
Staphylococcus aureus	2 (9)	2.1
Proteus mirabilis	1 (4.55)	1
Total	22 (100)	22.9

The comparative antibiotic susceptibility pattern of isolates revealed a high level resistance to amoxicillin/clavulanic acid (100%), ampicillin (100%) and ceftazidime (100%) by both groups of organisms (Table 4). *Escherichia coli* isolates were more resistant to cefpodoxime (71.4%), cefotaxime (64.2%) and

ceftriazone (71.4%) than *Klebsiella pneumoniae* isolates with 20% resistant to each drug respectively. Nitrofurantoin, ofloxacin and imipenem were the active drugs against the isolates as indicated by the sum of percentage sensitive and intermediate.

Antibiotic (ug)	Escherichia col	<i>i</i> (n = 14)		Klebsiella pne	umoniae (n=5)	
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Amoxicillin/clavulanate (20/10)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	5 (100)
Ampicillin (10)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	5 (100)
Cefuroxime (30)	0 (0)	(0)	14 (100)	0 (0)	0 (0)	5 (100)
Cefpodoxime (10)	3 (21.4)	1 (7.1)	10 (71.4)	4 (80)	0 (0)	1 (20)
Cefotaxime (30)	4 (28.5)	1 (7.1)	9 (64.2)	4 (80)	0 (0)	1 (20)
Cefoxitin (30)	6 (42.8)	0 (0)	8 (57.1)	2 (40)	1 (20)	2 (40)
Ceftazidime (30)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	5 (100)
Ceftriaxone (30)	2 (14.2)	2 (14.2)	10 (71.4)	0 (0)	4 (80)	1 (20)
Ciprofloxacin (5)	1 (7.1)	5 (35.7)	8 (57.1)	1 (20)	1 (20)	3 (60)
Cloxacillin (1)	0 (0)	0 (0)	14 (100)	0 (0)	0 (0)	5 (100)
Gentamicin (10)	8 (57.1)	0 (0)	6 (42.8)	1 (20)	0 (0)	4 (80)
Nitrofurantoin (300)	9 (64.2)	2 (14.2)	3 (21.4)	3 (60)	2 (40)	0 (0)
Ofloxacin (5)	9 (64.2)	4 (28.5)	1 (7.1)	3 (60)	1 (20)	1 (20)
Piperacillin (100)	3 (21.4)	4 (28.5)	7 (50)	1 (20)	1 (20)	3 (60)
Piperacillin/tazobactam (100/10)	8 (57.1)	4 (28.5)	2 (14.2)	1 (20)	2 (40)	2 (40)
Imipenem (10)	5 (35.7)	8 (57.1)	1 (7.1)	3 (60)	1 (20)	1 (20)

TABLE 4: COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF Escherichia coli AND Klebsiella pneumoniae ISOLATES

Key: S =sensitive, I = intermediate, R = resistant, µg = microgram

DISCUSSION

The genitourinary system is one of the portals of entry for pathogens and infection can occur anywhere along the tract. Urinary tract infections (UTIs) due to *Escherichia coli* and *Klebsiella pneumoniae* are among the widely encountered in clinics worldwide as both organisms have displayed marked predominance in the invasion of the tract. Infections due to the former organism are more common compared to the latter which is often devastating in immunocompromised patients (23).

In this study, the overall prevalence of UTI was 22.9%. This is higher than 2% (Abakaliki), 9.2% (Ethiopia) and 13% (Abuja) (16, 4, 24). On the other hand, the rate recorded is comparatively lower than 25% (Bangladesh), 39.69% (Okada), 43.1% (Jordan) and 48% (Rivers) (25, 14, 26, 27). The reason for the slightly low prevalence observed in this study may be due to the fact that only outpatients were studied. Outpatients are at lower risk of infection than inpatients who may have been predisposed by urethral catheters (28, 29) or other debilitating diseases. This is

among outpatients (11%) (24). In a related development, the prevalence/isolation rate of *Escherichia coli* and *Klebsiella pneumoniae* as

well buttressed by a study where the prevalence of

UTI among inpatients (36%) to be three times that

rate of *Escherichia coli* and *Klebsiella pneumoniae* as observed in this study was 14.6% and 5.2% respectively. Thus, *E. coli* is the most prevalent and predominant cause of UTI in our environment (p < 0.05) – a situation that is linked to its myriad of virulence factors. This trend has also been respectively observed in similar studies: 22% and 13.7% (24); 16.5% and 1.7% (14); 15.4% and 5.7% (26). Slight deviation from this pattern was recorded with *K. pneumoniae* being the most prevalent (17.3%) compared to *E. coli* (12.7%) (27). *Staphylococcus aureus* was also reported to be the most prevalent (28%) in a different study in Benin (30).

Also in this study, majority of the isolates were recovered from age group 31 - 40 (45.5%) followed by 21 - 30 (31.8%) but the highest prevalence was seen in the former age group (45.4%). This is partly in line

with a similar study where UTI was most prevalent in age group 38 - 42 (66.7%) (26). This finding is however contrary to two seperate studies that reported highest prevalence in age groups 21 - 30(44.67%) (14) and 18 - 29 (46%) (27), though there is a level of closeness in these age groups. Nevertheless, the high prevalence recorded in the above age groups is related to the indiscriminate sexual activity and high pregnancy rate (among females) that characterize these groups – all which are predisposing factors.

With respect to gender, UTI was significantly more prevalent among females (30.6%, 1 in 3) compared to males (8.8%, 1 in 11) (p< 0.05). Females were thus 3.4 times more likely to develop UTI than males. This finding has been observed in other studies (11, 13, 30, 27). Higher prevalence of UTI in females has been attributed to physiological and anatomical differences in both sexes. UTIs occur more frequently in women than men because the shorter, wider, female urethra appears to be less effective in preventing access of bacteria to the bladder (8). Different studies have also demonstrated that women who are prone to UTIs possess epithelial cells with significantly more receptors for uropathogenic bacteria than healthy controls (31, 32). The similarities, disparities and variations in prevalence rates recorded in different studies may be partly due to population difference and criteria (inclusion/exclusion) used by different researchers (14).

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The comparative antibiotic susceptibility pattern revealed a high level resistance by Escherichia coli and *Klebsiella pneumoniae* isolates to amoxicillin/clavulanic acid, ampicillin, cefuroxime and ceftazidime. Suffice to add, these isolates were multidrug resistant which might have been due to the expression of several resistance mechanisms (including production of multiple beta-lactamases) arising from selective pressure (33, 34). This makes the choice of drug for empirical treatment a challenging task. Similar observations have also been reported in other studies (4, 14, 35). However, the active drugs were nitrofurantoin, ofloxacin and imipenem. Low resistance to the last drug (imipenem) is related to its high cost and difficulty in accessibility which have often discouraged its abuse. Nevertheless, organisms causing UTI in different patient population differ in their pathogenic potentials and susceptibility to antimicrobials (22).

Conclusion

The human urinary tract is normally sterile except for the distal end of the urethra closest to the external environment. Microbial invasion of this tract is quite common leading to a wide range of clinical conditions in millions of people worldwide. This study has implicated *Escherichia coli* as the major cause of UTI especially in females of reproductive age. Some of the aetiologic agents were also found to be multidrug resistant to the penicillins and cephalosporins. Regular fluid intake and routine diagnostic procedure/screening are necessary to avoid complications.

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ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1825 https://dx.doi.org/10.4314/ajcem.v19i3.4 AFR. J. CLN. EXPER. MICROBIOL. 19 (3): 177-185

PREVALENCE OF HELICOBACTER PYLORI IGG AND STOOL ANTIGEN DETECTION FROM DYSPEPTIC PATIENTS IN JOS, NIGERIA

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ABSTRACT

Helicobacter pylori is a helical shaped gram negative microaerophilic bacterium, that can inhabit various areas of the stomach. The study was carried out to determine the prevalence of *Helicobacter pylori* infection among dyspeptic patients attending Endoscopy unit, Jos University Teaching Hospital (JUTH). The prevalence of H *pylori*; antibodies in plasma and antigen in stool samples of 80 patients examined was studied using ELISA (DIA PRO, Italy) and ICA (BIOTEST China) respectively. Socio-demographic and clinical information was obtained through the assistance of attending gastroenterology staff. Blood grouping was also performed by slide agglutination test for all patients. *Helicobacter pylori* IgG antibodies were detected in plasma of 28 (35%) patients of which 27 patients out of the sero-positive cases were antigen positive while *Helicobacter pylori* antigen was detected in the stool of 31 (38.8%) patients of which 27 patients out of the sero-positive is easy sere antigen positive cases were also seropositive. No significant association was found between *Helicobacter pylori* and age, sex, ABO blood groups was found to be 32.6%, 46.7%, 33.3% and 33.3% in blood groups O, B, A and AB respectively, while *Helicobacter pylori* stool antigen test positive results was found to be 33.3%, 36% and 39.5% in blood groups O, B, A and AB respectively. Hence, no statistical association was significantly associated with *Helicobacter pylori* antibody test (P<0.05). There is need for government to encourage people about the *Helicobacter pylori* screening test since it is one of the etiologic agent of ulcer.

Keywords: Helicobacter pylori, Jos, Peptic ulcer, Prevalence.

PREVALENCE D'HELICOBACTER PYLORI IGG ET DETECTION D'ANTIGENE STOOL CHEZ DES PATIENTS DYSPEPTIQUES A JOS, NIGERIA

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ABSTRAIT

Helicobacter pylori est une bactérie microaérophile gram négatif de forme hélicoïdale, qui peut habiter diverses zones de l'estomac. L'étude a été réalisée pour déterminer la prévalence de l'infection à Helicobacter pylori chez les patients dyspeptiques participant à l'unité d'endoscopie, Hôpital universitaire de Jos (JUTH). La prévalence de H pylori; Les anticorps dans le plasma et l'antigène dans des échantillons de selles de 80 patients examinés ont été étudiés en utilisant ELISA (DIA PRO, Italie) et ICA (BIOTEST Chine) respectivement. L'information socio-démographique et clinique a été obtenue grâce à l'assistance du personnel de gastro-entérologie. Le groupage sanguin a également été réalisé par un test d'agglutination sur lame pour tous les patients.

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Des anticorps IgG d'Helicobacter pylori ont été détectés dans le plasma de 28 patients (35%) dont 27 patients séropositifs étaient antigènes positifs tandis que l'antigène Helicobacter pylori a été détecté dans les selles de 31 patients (38,8%) dont 27 patients des cas positifs d'antigène étaient également séropositifs. Aucune association significative n'a été trouvée entre Helicobacter pylori et l'âge, le sexe, le groupe sanguin ABO, le statut économique, la source d'eau et la consommation d'alcool. Ainsi, la séropositivité à Helicobacter pylori vis-à-vis des groupes sanguins était respectivement de 32,6%, 46,7%, 33,3% et 33,3% dans les groupes sanguins O, B, A et AB, tandis que les résultats positifs au test antigénique Helicobacter pylori 33,3 %, 33,3%, 46% et 39,5% dans les groupes sanguins O, B, A et AB respectivement. Par conséquent, aucune association statistique n'a été trouvée entre l'infection à Helicobacter pylori et les groupes sanguins des patients (P> 0,05). Cependant, l'état matrimonial était significativement associé au test d'anticorps Helicobacter pylori (P <0,05). Il est nécessaire que le gouvernement encourage les gens au sujet du test de dépistage de l'Helicobacter pylori puisqu'il est l'un des agents étiologiques de l'ulcère.

Mots-clés: Helicobacter pylori, Jos, ulcère peptique, prévalence.

INTRODUCTION

*Helicobacter pylori*is a helical shaped, gram negative, microaerophilic bacterium that can inhabit various areas of the stomach particularly the antrum. Transmission probably occurs mostly by the faecaloral routes and through contaminated food, water and unclean hands (1). Oral-oral transmission has been identified in the case of African women who pre-masticate foods given to their infants (2). It causes a chronic low level inflammation of the stomach lining and is strongly linked to the development of duodenal, gastric and stomach ulcer (3).

Helicobacter pylori infection is wide spread among human populations and is considered to play a major role in the pathogenesis of several gastroduodenal diseases including gastric ulcer, duodenal ulcer, peptic ulcer, gastric mucosa associated lymphoid tissue (MALT) Lymphoma. Previous seroepidemiologic studies indicated that about 50% of adults in developed countries and nearly 90% in developing countries are positive for serum antibodies against Helicobacter pylori (4, 5). There are puzzles in defining the exact role of Helicobacter pylori infection in humans. The bacterium, which is associated with human disease of the upper gastrointestinal tract, may otherwise exist as a commensal with probable symbiotic association in some human hosts (6).

Despite significant advances in the understanding of the biology of the bacterium, the factors that determine the outcome of infection are poorly Epidemiological studies understood. have demonstrated high frequencies of the O blood group among most peptic ulcer patients. Although, the host factors might be important with regard to the outcome of infection by this organism, bacterial factors seem to influence the inflammatory response and the development of a more severe pathology. Cytotoxin associated antigen A (Cag A) is thought to be the major virulence factor involved in the pathogenesis of Helicobacter pylori diseases (7). Thus, presently, its role has been established in chronic antral gastritis, duodenal ulcer, chronic gastric ulcer, dyspepsia, gastric cancer and gastric lymphoma. World Health Organization added *Helicobacter pylori* to its list of known carcinogens (8).

The diagnosis of Helicobacter pylori gastritis can be made through many laboratory tests. The techniques are divided into two groups the invasive and noninvasive tests (9). Stool antigen tests as a non-invasive test have recently been welcomed with great expectations as they are convenient to the patients and can be easily performed even in small laboratories (10). However, the accuracy of stool antigen tests in different clinical situations is a matter of concern (11). Whereas, serological tests are reported to be unreliable for the diagnosis of Helicobacter pylori since they may return false negative results up to 60 days after infection and remain positive for a considerable time after eradication, but serology for IgG, against *Helicobacter pylori* may play an important role in decreasing the need for endoscopy provided the cut-off values must be determined for easy assay based on the prevalence of antibodies in the population (12). However, stool antigen test and serology test methods for H pylori are suitable for epidemiological studies, since their performance characteristics has been compared with endoscope-based methods, demonstrating an overall accuracy of 98% and 78% respectively.

In Nigeria, there are considerable controversies over the management of *Helicobacter pylori* infection. There is no national or regional consensus guidelines and very few documented pathological role of the bacteria (13). The updates in Knowledge of the prevalence of *Helicobacter pylori* infection and its associated risks factors is necessary for intervention programs that will reduce the morbidity as well as mortality caused by the bacteria.

The present study was aimed at comparative evaluation of stool antigen test and blood antibody test methods for diagnosis of *Helicobacter pylori* infection in cases of dyspepsia and some risk factors from patients attending the endoscopy clinic at JUTH.

MATERIALS AND METHODS

Study area: The study area of this research work was Jos University Teaching Hospital. Jos is the Capital City of Plateau State. Plateau State is the twelfth largest State of Nigeria, and is roughly located in the center of the country. With an area of 26,899 square kilometers, the State has a population of 3,178,712 people according to 2006 census. It is located between latitude 80°24'N and longitude 80°32' and 100°38' east.

Study Population and Sample size determination:

A total of 80 patients were recruited for the study due to the few amount of dyspeptic patients that attended the endoscopy clinic within the period of this study. The attending physician interviewed each volunteer who completed a detailed questionnaire. The questionnaire was designed to obtain demographic data, socioeconomic status, and other risk factors.

The sample size was determined using the formula described by IFAD 2003. Thus: $n = t^2 \times p (1-p)/m^2$ Where: n=required sample size; t=confidence level at 95%=1.96; p = established local prevalence for serum antibodies = 80% (14) and established local prevalence for stool antigen = 80% (15); m = margin of error at 7.5 = 0.075; n = 109

10% attrition rate = 10.9; Total sample size: 109+10.9 = 119.9.

Ethical approval: Ethical clearance was sought and obtained from the Ethical and Research Committee of Jos University Teaching Hospital (JUTH).

Blood Sample Collection and Procession: The study was carried out between September and November 2016. The clinics were visited between 8am and 9am on Mondays and Wednesday during the week days. Physicians (Gastroenterologist) determined the diagnostic relevance of each volunteer who completed a detailed questionnaire. All the patients had dyspepsia with ulcer which was determined by Endoscopy. After an informed consent was obtained and appropriate pretest counseling conducted, about 5ml of a venous blood was collected in vacutainer tubes. A tourniquet was applied to the upper arm of the patient (to enable the Median cubital veins to be seen and felt) and the patient was asked to make a tight fist (Thus, making the veins prominent). The punctured site was cleaned with sterile alcohol and with the thumb of the left hand held down the skin below the selected punctures site, a vein was punctured with the level of the needle directed upwards in the line of the vein. When sufficient blood is collected the tourniquet is released and the patient was instructed to open his or her fist. The blood was then transported to the laboratory for analysis.

ABO Blood grouping and Rhesus Status

Blood grouping was conducted before plasma was separated, by slide agglutination test using monoclonal anti; A, anti-B anti-D antibodies. A drop of blood was placed on a clean white tile in three places in a vertical row. A drop of anti-A, anti-B, and anti-D was added respectively. Each blood sample was mixed using a glass rod. Blood group was determined on the basis of agglutination of test plasma by the respective antiserum.

Detection of *Helicobacter pylori* IgG Antibodies in Plasma

Plasma was recovered from the blood samples by centrifugation at 2,000rpm for 5 minutes. Serological status of Helicobacter pylori was determined using a commercially available Helicobacter pylori IgG ELISA Kit (Diagnostic Bioprobes-Italy) according to the manufacturers instruction a value of >1.1µml was taken as positive. The ELISA kit was for the quantitative/qualitative determination of IgG antibodies to Helicobacter pylori in human serum and plasma. Samples were diluted at 1:100 into properly defined dilution tubes. The micro wells were placed in the microwell holder, microwell A1 and B1 were left empty for the operation of Blanking. 100µl of calibrators and $100\mu/g$ control serum was dispensed in duplicates. Then 100µl of diluted samples were dispensed in each properly identified well and sealed properly. The microplates were incubated for 60minutes at +37°C. Microplates were washed with an ELISA microplate washer 5 times per cycle. 100µl enzyme conjugate was pipette into each well except A1 + B1 blanking wells, and they were covered with sealer. I checked that the red coloured component has been dispensed in all the wells except A1 + B1. The microplates were incubated for 60minutes at 37°C. Microwells were washed using ELISA microplate washer.100µl of chromogen/substrate mixture was pipetted into each well, the blank wells A1 and B1 included. The microplates were then incubated at room temperature 24ºC for 20 minutes. 100µl of sulphuric acid was pipette to stop the enzymatic reaction into all the wells the addition of the acid turned the positive calibrators the control serum and positive samples from blue to yellow. The colour intensity of the solution in each well was measured by ELISA reader at 450 nm with the help of the gen5 software.

Detection of *Helicobacter pylori* using Stool Antigen Test (SAT)

Stool samples were collected in clean sample containers from patients whose blood samples was collected and a qualitative immunochromatographic assay was done to determine bacteria antigen according to the detail information provided within the available commercial kit (Biotest Biotech, china): Fecal specimens were collected in sufficient quantity (2g) in a clean dry specimen collection container so as to obtain maximum antigens (if present). The cap of the specimen collection tube was unscrewed, and then the specimen was randomly stabbed with the collection applicator in three different sites so as to collect approximately 50 mg of feces into the extraction buffer. For liquid specimens a dropper was held vertically so as to aspirate fecal specimens, and then two drops approximately 80µl of the specimen was dispensed into the collection tube containing the extraction buffer. The cap of the collection tube was tightened, and then the specimen collection tube was shaken vigorously to mix. The specimen and the extraction buffer in the tube were left alone for two minutes. The specimen collection tube was held upright and the cap of the collection tube was opened, the tube was inverted and two full drops of the extracted specimen (approximately 80μ l) was transferred to the specimen well of the test cassette, and the timer was started. Trapping the specimen well(s). Result was read at ten minutes after the specimen was dispensed.

Interpretation of Results: Positive-Two lines appeared, one red line was in the control line region (c) and another apparent red line was in the test line region (T). **Negative-** One red line appeared in the control line region (c) and no line appeared in the test line region (T).

Statistical analysis: Chi-square was used to compare the categorical data. $P \le 0.05$ was considered statistically significant.

RESULTS

.The result of the eighty (80) patients that participated in the study which comprised 44 (55%) males and 36 (45%) females shows that 28 (35.0%) were positive for anti-*H. pylori* antibodies, while 31 (38.8%) were positive for *Helicobacter pylori* stool antigen test (SAT).(Table 1). Blood group showed that 79 (98.8%) of the total patients were rhesus positive and 1(1.1%) of the patients was rhesus negative(Table not shown)

TABLE 1: RELATIONSHIP BETWEEN HELICOBACTER PYLORI SEROPREVALENCE AND STOOL ANTIGEN TEST IN DYSPEPTIC PATIENTS

No examined	Seroprevalence(%)	Stool antigentest (%)	No. Positive for Abs/Ag
80	28(35.0)	31(38.8)	27
Total	80	28(35.0)	31(38.8)

X²=0.837df=1

The values for ELISA positive samples ranged from $\geq 1.1 \mu/ml$ to $2.8 \mu/ml$ while values for negative samples ranged from $\leq 1.0 \mu/ml$ to $0.1 \mu/ml$. Among the participants blood group O was the most common blood group 43 (53.8%) followed by blood group B 15 (18.8%), A 12 (15%) and AB 9 (11.3%) (Table 2).

Helicobacter pylori seropositivity with respect to blood groups was found to be 32.6%, 46.7%, 33.3% and 33.3% in blood groups O, B, A and AB respectively (Table 2). While, *Helicobacter pylori* stool antigen test

positive results was 33.3%, 33.3%, 46% and 39.5% in blood groups O, B, A and AB respectively. Hence, no statistical association was found between *Helicobacter pylori* infection and blood groups of the patients (P>0.05) (Table 2)..

Table 3 shows the relationship between *Helicobacter pylori* infection and age of the patients. Majority of the patients were young adults aged 20 to 40 years. There was no significant association between *Helicobacter pylori* seropositivity and age of patients since (p>0.05).

Blood group	No examined	Seroprevalence (%)	Stool antigen test(%)	p value
0+	43	14(32.6)	17(33.3)	0.837
A+	12	4(33.3)	4(46.7)	
B+	15	7(46.7)	7(33.3)	
AB	9	3(33.3)	3(39.5)	
O-	1	0(0.0)	0(0.0)	
Total	80	28(35.0)	31(38.8)	

TABLE 2: RELATIONSHIP BETWEEN BLOOD GROUPS ABO WITH HELICOBACTER PYLORI SEROPREVALENCE AND STOOL ANTIGEN TEST AMONG DYSPEPTIC PATIENTS

X²=0.837df=1

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The sex specific prevalence was 43.2% in males and 25.0% in females. There was no statistically significant association between *Helicobacter pylori* seropositivity and sex of the patients since (P>0.05) (Table 3). With respect to marital status 24.4% of the married patients

were positive, while 48.6% single unmarried patients were positive. There was a statistical significance association between *Helicobacter pylori* seropositivity and marital status of the patients since (P<0.05)(Table 3).

TABLE 3: RELATIONSHIPS BETWEEN SEROPREVALENCE OF H. PYLORI AND GENERAL INFORMATION AMONG DYSPEPTIC PATIENTS.

Factors	No.Examined	No. Positive (%)	Total no. of subjects p=80
Age (years)			
<20	5	3(60.0)	0.700
20-29	29	12(41.4)	
30-39	23	6(26.1)	
40-49	11	3(27.3)	
50-59	9	3(33.3)	
60-69	3	1(33.3)	
Sex			
Male	44	19(43.2)	0.090
female	36	9(25.0)	
Marital status			
Single	35	17(48.6)	0.025
married	45	11(24.4)	

X2=0.700, df=1, X2=0.090, df=1, X2=0.025, df=1

The result of the relationships between seroprevalence of H. *pylori* infection and risk factors among dyspeptic patients with respect to the economic status of the patients shows that 24.4% were considered average and seropositive, 25.0% were very good and seropositive, while 78.6% were poor and seropositive. However, there was no statistical significance between the patients' economic status and *Helicobacter pylori* seropositivity at P>0.05(Table 4). Seropositivity of anti-*Helicobacter pylori* antibodies was not significantly associated with the patients source of water since (P>0.05). It was also observed that seropositivity of anti-*Helicobacter pylori* antibodies was not significantly associated with alcohol consumption (P>0.05) (Table 4).

TABLE 4: RELATIONSHIPS BETWEEN SEROPREVALENCE OF H. PYLORI INFECTION AND RISK FACTORS AMONG
DYSPEPTIC PATIENTS

Risk factors	No. examined	No. positive (%)total no. of subjec	ts) P -value
Economic status			
Average	41	10(24.4)	0.081
Very good	4	1(25.0)	
Poor	35	17(48.6)	
Source of water			
Тар	2	0(0.0)	0.293
Sachet	75	26(34.7)	
Well	3	2(66.7)	
Alcoholism			
Yes	2	0(0.0)	0.293
No	78	28(35.0)	

X2 =0.081 df=2, X2=0.293 df=1, X2=0.293 df=1

Table 5 shows the relationship between *Helicobacter pylori* infection and age of the patients. From the result it was observed that there was no association between *Helicobacter pylori* stool antigen and age of the patients since (P>0.05) (Table 5). The sex specific prevalence was 45.5% in males and 30.6% in females. There was no statistically significant association between *Helicobacter pylori* stool antigen and sex of the patients since (P>0.05) (Table 5).

Regarding marital status 31.1% of the patients were positive and married, while 48.6% were positive and single. There was no statistical significance association between *Helicobacter pylori* stool antigen and marital status of patients since (P>0.05) (Table 5).

With respect to economic status of the patients 31.7% were considered average and positive, 25.0% were very good and positive and 48.6% were poor and positive for stool antigen test. There was no statistical association between the patients economic status and *Helicobacter pylori* stool antigen test since (P>0.05) (Table 6).

It was observed that *Helicobacter pylori* stool antigen was not statistically associated with the patients source of drinking water (P>0.05) (Table 6). *Helicobacter pylori* stool antigen was not significantly associated with patients' alcohol consumption. (P>0.05) (Table 6).

Factors	No. Examined	No. Positive (%(Total no. of subjects) N=80	p value	
Age				
<20	5	3(60.0)	0.716	
20-29	29	12(41.4)		
30-39	23	8(34.8)		
40-49	11	3(27.3)		
50-59	9	3(33.3)		
60-69	3	2(66.7)		
Sex				
Male	44	20(45.5)	0.174	
Female	36	11(30.6)		
Marital statu	s			
Single	35	17(48.6)	0.112	
Married	45	14(31.1)		

TABLE 5: RELATIONSHIPS BETWEEN STOOL ANTIGEN OF H. *PYLORI* INFECTION AND GENERAL INFORMATION AMONG DYSPEPTIC PATIENTS

X² =0.716 df=5, X²=0.174 df=1, X²=0.112 df=1

DISCUSSION

Detection of *Helicobacter pylori* infection with noninvasive methods such as serological tests and stool antigen test are useful and widely available (16). The result of this study revealed that the prevalence of *Helicobacter pylori* among patients attending endoscopy unit in Jos University Teaching Hospital (JUTH) was 35% for seroprevelance and 38.8% for stool antigen test respectively. This finding is lower than the previous report from Keffi showing a prevalence of 56.3% (17). It is well lower than another previous report from a developing country showing a prevalence of 53.5% for *Helicobacter pylori* (18).

Epidemiological studies have demonstrated a higher frequency of the blood group O among patients suffering from peptic ulcer. These findings of this study supports the epidemiological view of the greater sensitivity of those with blood group O to infection by *Helicobacter pylori* which is apparently in line with the conclusion of (18) who demonstrated that the H-antigen in blood group O represents an important receptor expressed in the gastroduodenal mucosa cells to which Helicobacter pylori adheres which also enables colonization of the bacterium. Blood group A and AB patients in this study were less prone to *Helicobacter pylori* infection than other blood groups which is not consistent with earlier studies. The present study does not demonstrate any significant difference in Helicobacter pylori infection of patients with varying blood groups which is consistent with similar studies from other countries (19).

This result revealed that there was no statistically significant association between *Helicobacter pylori* infection and age of the patients (P>0.05). Therefore,

this work is not consistent with previous reports which indicated high frequencies in the elderly (20).

TABLE 6: RELATIONSHIPS BETWEEN STOOL ANTIGEN OF *H.PYLORI* INFECTION AND RISK FACTORS AMONG DYSPEPTIC PATIENTS

Risk Factors	No. Examined	No. Positive (%)Total no. subjects N=80) p value
Economic status			
Average	40	13(31.7)	0.273
Very good	4	1(25.0)	
Poor	35	17(48.6)	
Source of water			
Тар	2	0(0.0)	0.325
Sachet	75	29(38.7)	
Well	3	2(66.7)	
Alcoholism			
Yes	2	0(0.0)	0.255
No	78	31(39.7)	

X2=0.273 df= 2, X2=0.325 df=2, X2 =0.255 df=1

The result of this present study showed that *Helicobacter pylori* infection was higher in males 43.2%, 45.5% than females 25.0%, 30.6% respectively. It was not statistically significant (P>0.05). This is consistent with some studies that have reported higher prevalence of the infection in males which may be attributed to higher exposure of males to potential environment sources of infection (21). It was also observed that *Helicobacter pylori* infection was high among patients whose source of drinking water was sachet water because about 94% of the population depends on sachet water as their source of drinking water. However there was no statistically significant since (P>0.05).

There was no relationship between *Helicobacter pylori* infection and alcohol consumption in this study (P>0.05). This is in line with studies from other

countries that have reported no significant association between *Helicobacter pylori* and alcohol consumption. However, history of alcohol (Local alcohol like burukutu) consumption has been shown to be a risk factor for *Helicobacter pylori* infection (17). The reason for this difference may be due to the difference in the type of alcohol beverages consumed or the source of water used in its production. n conclusionthe prevalence of *Helicobacter pylori* among the study group was 35% for blood antibody and 38.8% for stool antigen. This study employed ELISA and ICA to determine the presence or absence of *Helicobacter pylori* infection among the study population. Hence, there is need to employ advance techniques like PCR to identify this organism.

It is necessary for government to encourage people about the *Helicobacter pylori* screening test since it is one of the etiologic agent of ulcer. Also there is need for adopting proper hygienic practices such as avoidance of eating food prepared in an unhygienic settings, washing hands when visiting hospitals and when leaving the hospital should be a common practice and ensuring that water are obtained from

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reputable sources and finally, mothers should stop the habit of pre-masticating food before giving their children.

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ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2018 ISBN 1595-689X VOL19 No.1 AJCEM/1826 http://www.ajol.info/journals/ajcem COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.5

AFR. J. CLN. EXPER. MICROBIOL. 19 (1): 186-194

ASSESSMENT OF MICROBIOLOGICAL AND CHEMICAL QUALITIES OF SELECTED COCOA, TEA AND COFFEE BRANDS IN NIGERIAN MARKETS

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ABSTRACT

Background: Cocoa, Tea, and Coffee products are consumed worldwide; they are rich in nutrient and, thus, prone to microbial contaminations that could cause food infections and intoxication. Objective: the objectives of the paper are: To evaluate the microbial and chemical qualities of some popular brands of cocoa, coffee and tea products in Nigerian market and benchmark it with standard specifications, to determine the products safety for human consumption and proffer solutions on ways to prevent possible food borne hazards associated with these products. Materials and Methods: This study examined the microbiological and chemical qualities of some brands of these products at the consumer level using standard analytical methods. Results: Five (50%) of the cocoa products had coliform counts (cfu/g) significantly (p<0.05) higher than acceptable limits and range from 2.6×10¹±0.01 to 4.6×10¹±0.01. The mean total aerobic plate count and fungal counts (cfu/g) cocoa, coffee, and tea were not significantly (p<0.05) different from standard specifications. The microbial isolates include species of Bacillus (59.2%), Staphylococcus(12.0%), Enterobacter(1.6%), Aspergillus(20.0%), Penicillium(14.4%) and Saccharomyces (12.0%). Moisture contents higher than 6% and 3% standard specifications in tea and cocoa products was detected in 7(70%) of tea and 2(20%) of cocoa products respectively. The samples are rich in phenol contents (mg/ml) and contain varying concentrations of manganese, calcium, iron, and copper. Free Radical Scavenging (DPPH) activity of 6.2±0.01 to 16.3±0.02µg/ml was detected in the samples. Conclusion: Some cocoa products contain unacceptable levels of coliforms, the high moisture contents above 3 and 6% standard specifications in some cocoa and tea products could encourage the proliferation of mycotoxigenic moulds and pathogenic bacteria to hazardous levels. The use of good raw materials, compliance to good manufacturing practices (GMP) and apt storage are advocated.

Keywords: Chemical qualities, Cocoa, Coffee, Microbial qualities, Mycotoxigenic moulds, Tea

ÉVALUATION DES QUALITÉS MICROBIOLOGIQUES ET CHIMIQUES DE CACAO, THÉ ET CAFÉ RÉPUTÉES DANS LES MARCHÉS NIGÉRIANS

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RÉSUMÉ

Contexte: Cacao, thé, café et produits sont consommés dans le monde et ils sont riches en nutriments et, par conséquent, sujettes aux contaminations microbiennes qui pourraient causer des infections et intoxications alimentaires. Objectif : les objectifs de l'étude sont : d'évaluer les qualités microbiologiques et chimiques de certaines marques populaires de cacao, café et thé produits en marché nigérian et comparaison avec les spécifications standard, pour déterminer l'innocuité des produits pour la consommation humaine et de proposer des solutions sur les moyens de prévenir les risques d'origine alimentaire associées à ces produits. Matériels et méthodes : Cette étude a examiné les qualités microbiologiques et chimiques de certaines marques de ces produits au niveau de la consommation à l'aide de méthodes analytiques.

Résultats: cinq (50 %) de la produits de cacao avait des coliformes fécaux (ufc/g) de manière significative (p < 0,05) plus élevée que les limites acceptables et vont de 2,6×101±0,01 à 4,6×101±0.01. La moyenne d'une numération totale sur plaque et le nombre de cellules fongiques (CFU/g) de cacao, café, thé et n'ont pas été significativement (p < 0,05) de différentes spécifications standard. Les isolats microbiens comprennent des espèces de Bacillus(59,2 %), Staphylococcus(12,0 %), Enterobacter(1,6 %), Aspergillus (20,0 %), Penicillium(14,4 %) et Saccharomyces(12,0 %). Contenu d'humidité plus élevé que 6 % et 3 % dans les spécifications standard de plateau et de produits de cacao a été détectée dans 7(70 %) de plateau et 2(20 %) de produits de cacao, respectivement. Les échantillons sont riches en matières de phénol (mg/ml) et contiennent différentes concentrations de manganèse, calcium, fer, et cuivre. De radicaux libres (DPPH) activité de 6,2 ± 0,01 à 16,3±0,02µg/ml a été détecté dans les échantillons.

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Conclusion: Certains produits de cacao contiennent des niveaux inacceptables de coliformes, le contenu d'humidité élevée au-dessus de 3 et 6 % des spécifications standard dans certains produits de cacao et de thé pourrait encourager la prolifération des moisissures et bactéries pathogènes mycotoxigenic à des niveaux dangereux. L'utilisation de bonnes matières premières, de la conformité aux bonnes pratiques de fabrication (BPF) et de stockage sont préconisées. apt Mots-clés: qualités chimiques, cacao, café, qualités microbiennes, moules, thé Mycotoxigenic

INTRODUCTION Cocoa, Tea, and Coffee have received much attention due to their significant human roles in the diet. the beverage/confectionery industries and high polyphenols contents. Products of cocoa, tea and coffee present varied microbial contamination levels and chemical contents and possess different levels of antioxidant potentials (1, 2, 3). To reduce contamination of coffee, tea and cocoa products, factors such as use of quality raw materials, correct storage conditions, application of hazard analysis, critical control points (HACCP) and other quality systems in good manufacturing practices (GMP), hygienic processing environment, training and education of food handlers should be well implemented. Cocoa, coffee, and tea contribute significantly to the economy of Nigeria and some African countries; they are a major non-oil foreign exchange earner for the countries: Cote d'Ivoire, Ghana, Cameroon, Uganda, Togo, Sierra Leone, Ethiopia, and Kenya and contribute substantially to the rural economy (4, 5, 6). There have been recorded outbreaks of food infections and intoxication, as a result of consumption of tea, coffee and cocoa products due to poor manufacturing practices or poor storage facilities (7, 8). The high level of unemployment consequent on poor economy/recession has lead to the emergence of large numbers of small scale home made products, packaged under substandard conditions and brought into the market without quality control/assessments to ascertain their health benefits and implications. Cocoa and coffee beans and tea can be contaminated with toxigenic fungal species and deleterious fungal toxins especially ochratoxin A and aflatoxins (7, 9, 10, 11, 12, 13, 14). Toxic elements of modern day environmental pollution fluoride, lead, and aluminum have also been found in tea (15, 17). Knowledge of nutritional, microbiological, chemical and biochemical compositions of food is important to health, well-being, and safety of the consumers (18) and to the manufacturers in understanding the importance of various nutritional contents so that a number of essential nutrients may be maintained or improved during and after processing. Beverages (coffee, tea, and cocoa) are nutritionally rich to support the proliferation of microbial spp (19) and contain polyphenols and minerals that have diverse beneficial biochemical, antioxidant and antimicrobial effects (20, 21, 22), This study is therefore set to assess the microbiological and chemical qualities of some cocoa, coffee and tea products in Nigerian markets.

METHODS

Sample collection

One hundred and twenty-five (125) samples made up of five each of ten different brands of cocoa and tea products and 5 different brands of coffee products were purchased from supermarkets in Ota (Ogun state) and Lagos (Lagos State) Nigeria. Ota in Ogun state is located at 6° 41' 00" N 3° 41' 00" E while Lagos is situated at 6° 27' 14.65" N 3° 23' 40.81" E. All the samples were collected within the months of February to July 2016 and all samples purchased are within expiry date. The samples were transported to the laboratory for analyses. The microbiological and phytochemicals analyses were carried out at the Microbiology and Biochemistry laboratories of Covenant University while the proximate and mineral analyses were assayed at the Research Laboratory of Bells University of Science and Technology, Ota, Ogun State. Prior to analyses, the manufacture/expiry dates, batch number and manufacturers address were documented.

Sample analysis

Microbiological analysis

Standard methods for microbiological analysis (23, 24)was adopted for Total Aerobic Plate Count (TAPC), Total Coliform Count (TCC), Total Fungal count (TFC), Staphylococcus aureus count (SAC), and isolation of some organisms of such concerns as Salmonellae, Shigella, Cronobacter, and moulds. Cultural characteristics and Biochemical tests and employing the API kits Biomerieux® sa, were used in the identification of the bacterial isolates. Fungal isolates were identified on the basis of their Macroscopic and Microscopic characteristic (25).

Chemical analysis

The method of the Association of Official Analytical Chemists (26) was adopted for determination of proximate compositions (moisture, ash, crude fibre, protein, fat, and carbohydrate). The mineral contents (elements) of the samples: calcium (Ca), magnesium (Mg), iron (Fe) and copper (Cu) were determined using the atomic absorption spectrophotometer (G105 UV-VIS, Thermo Fisher Scientific, GeneSys, Madison, USA) as described in the methods of the Association of Official Analytical Chemists (26). The AlCl₃ method as described by Oranusi *et al* (27) was used for the determination of the total flavonoid content of the sample extracts. Total phenolics were determined using Folin-Clocalteus method following the description of Oranusi *et al* (27). The antioxidants scavenging activity of the samples on the stable free radical DPPH was measured by the method as described by Brand-Williams *et al*, (28).

Statistics

Results for microbial count parameters were presented as a mean and standard deviation, chemical compositions and microbial isolates were presented as percentages of occurrence. One way analysis of variance was employed to compare mean microbial loads and correlation analysis and test of significance for the proximate, mineral and microbial compositions for the three products (p=0.05).

RESULTS

Microbiological evaluation

The mean microbial counts (total aerobic plate count, S. aureus count, fungal count and coliform count) for cocoa, tea and coffee products are presented in Table1. It shows that all the samples have significantly (p < 0.05) low TAPC of the order 10¹ to 10². The cocoa and tea products were however relatively more contaminated (not significant at p=0.05) when compared to coffee samples, counts ranging from 1.7×10² - 2.9×10² cfu/g were obtained from cocoa products. Tea products had counts ranging from 1.7×10² -4.5×10² cfu/g, while coffee products gave counts that ranged from $2.2 \times 10^1 - 4.5 \times 10^1$ cfu/g. Mean S. aureus count of the samples reveals that most of the samples had significantly (p < 0.05) low counts of the order 101 to 102. The cocoa and tea products, however, had more counts when compared to coffee samples. Counts ranging from <10 - 7.6×101 cfu/g were obtained from cocoa products. Tea products gave counts of < 10 -4.7×10¹ cfu/g. Coffee products had no S. aureus count.

TABLE 1: MEAN TOTAL MICROBIAL COUNTS	(cfu/g) FOR COCOA, TEA AND COFFEE SAMPLES
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Сосоа					Tea				Coffee		
SC	TAPC	TFC	SAC	TCC	SC	TAPC	TFC	SAC	SC	TAPC	TFC
C1	$1.2 \times 10^{1} \pm 0.00$	2.9×10 ¹ ±0.00	< 10	-	T1	3.3×10 ² ±0.03	3.6×10 ² ±0.01	3.4×10 ¹ ±0.00	CF1	4.5×10 ¹ ±0.00	2.2×10 ¹ ±0.00
C2	2.7×10 ² ±0.02	3.0×10 ² ±0.02	7.6×10¹±0.00	3.2×10 ¹ ±0.00	T2	3.0×10 ² ±0.01	4.0×10 ² ±0.01	-	CF2	5.5×10 ¹ ±0.00	2.7×10 ¹ ±0.00
C3	1.7×101±0.02	2.8×10 ² ±0.01	< 10	4.2×10 ¹ ±0.00	Т3	4.1×10 ² ±0.01	3.5×10 ² ±0.01	-	CF3	2.2×10 ¹ ±0.00	2.8×10 ¹ ±0.00
C4	1.3×10 ¹ ±0.00	2.9×10 ¹ ±0.00	< 10	-	T4	4.5×10 ² ±0.02	4.6×10 ² ±0.02	4.7×10 ¹ ±0.00	CF4	1.0×10 ¹ ±0.00	2.7×10 ¹ ±0.00
C5	2.1×10 ² ±0.01	2.2×10 ¹ ±0.00	< 10	-	T5	3.5×10 ² ±0.01	4.5×10 ² ±0.01	-	CF5	2.2×10 ¹ ±0.00	2.9×10 ¹ ±0.00
C6	1.0×10 ¹ ±0.00	3.4×10 ² ±0.02	< 10	-	Т6	2.8×10 ² ±0.00	2.9×10 ² ±0.01	-			
C7	2.9×10 ² ±0.01	4.4×10 ² ±0.03	1.1×10 ² ±0.00	4.6×10 ¹ ±0.01	T7	1.7×10 ¹ ±0.00	2.8×10 ¹ ±0.00	-			
C8	1.7×10²±0.00	3.6×10 ² ±0.02	2.9×10 ¹ ±0.01	2.6×10 ¹ ±0.00	Т8	2.9×10 ² ±0.01	3.1×10 ² ±0.00	-			
С9	2.6×10 ¹ ±0.00	2.3×10 ¹ ±0.00	-	-	Т9	2.2×10 ² ±0.01	2.6×10 ¹ ±0.00	-			
C10	1.7×10 ¹ ±0.00	3.8×10 ² ±0.03	-	3.8×10 ² ±0.01	T1 0	3.7×10 ¹ ±0.00	2.7×10 ¹ ±0.00	< 10			

Key: SC= Sample code; TAPC= Total aerobic plate count; SAC= *S. aureus* count; TCC= Coliform count; TFC= Fungal count; C= Cocoa products; T= Tea products; CF= Coffee products; — = No Bacterial Growth

The table also reveals the presence of coliforms in some cocoa samples with counts ranging from 2.6×10^1 cfu/g in C8 to 4.6×10^1 cfu/g in C7. Tea and coffee samples were however not contaminated by coliforms. All the samples had fungal counts of the order 10^1 to 10^2 . The cocoa

and tea products, however, had more fungal contaminants when compared to the coffee samples though not significant p= 0.05. Microbial isolates documented were mainly species of *Bacillus* (59.2%), *Aspergillus* (20.0%) *and Penicillium* (14.4%) and pockets of *Saccharomyces cerevisae*

(12.0%), *Staphylococcus* (12.0%) and *Enterobacter* (1.6%).

Chemical evaluation

Tables 2, 3 and 4 present the proximate and mineral compositions of the samples. Cocoa samples (C7 and C10) had higher proximate parameters with the exception to carbohydrates. Tea and coffee products had low lipids (Ether extracts) except, however, for coffee sample CF4 with 3.67 % lipid. The tea samples have high moisture contents but for T7, T9 and T10. There is

a weak positive correlation among the proximate and mineral values of cocoa, coffee and tea with significant values of 0.333, 0.117 and 0.054 respectively. Similarly, the result shows that proximate values and microbial counts are positively correlated with 0.329 and a significant relationship with 0.001 p-values. The association was stronger for TAPC and TFC to protein, ash and Mg components of cocoa products but not with tea and coffee All the samples contained phytochemicals (phenolics and flavonoids) and with antioxidant property (see Table 5).

TABLE 2: PERCENTAGE (%) PROXIMATE AND MINERAL (Mg/Kg) COMPOSITIONS FOR COCOA PRODUCTS

Sample	Moisture	Protein	Ether extract	Ash	Crude fiber	Carbo hydrate	Mg	Ca	Fe	Cu
C1	2.31±0.01	5.934±0.89	3.559±0.07	1.808±0.01	0.281±0.04	86.108±0.03	210.9±3.08	0.606±0.01	90.438±1.80	0.01±0.00
C2	1.72±0.01	7.601±0.12	5.227±0.02	1.481±0.01	0.805±0.06	83.176±0.05	208.5±1.79	0.330±0.02	4.292±1.60	0.37±0.00
C3	1.44±0.13	8.935±0.07	0.790±0.08	5.380±0.13	0.122±0.06	83.333±0.09	213.3±2.27	1.466±0.07	33.044±1.19	0.06±0.00
C4	2.22±0.07	15.385±0.04	5.841±0.02	4.583±0.04	0.123±0.04	71.848±0.42	216.4±3.66	0.876±0.08	6.9723±1.76	0.26±0.01
C5	2.34±0.75	16.827±2.90	4.385±0.07	4.461±0.21	0.750±0.01	71.237±0.79	211.9±2.85	0.204±0.07	3.529±1.21	0.24±0.02
C6	0.88±0.03	8.847±0.06	1.572±0.05	4.056±0.08	0.770±0.01	83.875±0.08	221.3±2.08	0.373±0.05	36.729±1.61	0.01±0.00
C7	6.89±0.04	25.115±0.11	8.527±0.09	9.025±0.04	0.152±0.05	50.291±0.60	230.4±1.60	1.118±0.05	865.94±4.82	15.01±0.14
C8	1.43±0.02	11.485±0.01	8.580±0.08	2.646±0.22	0.215±0.08	75.644±0.09	259.3±2.03	0.266±0.01	46.044±1.85	0.08±0.00
С9	2.25±0.11	9.026±0.01	2.497±0.05	3.884±0.30	0.206±0.07	82.137±0.12	213.1±4.31	0.178±0.04	11.492±1.81	0.27±0.01
C10	4.04±0.02	25.135±0.01	20.062±0.10	6.845±1.71	0.391±0.02	43.527±0.18	230.7±3.27	0.425±0.02	148.52±3.20	40.5±0.40
Sample	Moisture	Protein	Ether extract	Ash	Crude fiber	Carbo hydrate	Mg	Ca	Fe	Cu
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T1	7.27±0.04	2.802±0.10	0.896±0.02	5.357±0.23	9.329±0.12	74.346±0.12	217.3±4.02	0.364±0.02	27.770±0.08	0.16±0.00
T2	8.11±0.07	2.747±0.05	0.684±0.05	4.772±0.05	11.013±0.42	72.674±0.13	199.5±2.37	0.400 ± 0.01	4.360±0.06	0.18±0.01
T3	7.11±0.02	2.853±0.07	0.437±0.01	4.453±0.09	9.461±0.28	75.686±0.09	207.7±3.82	0.599±0.03	5.329±0.24	0.12±0.00
T4	8.13±0.06	2.641±0.08	0.868±0.06	2.909±0.22	8.049±0.16	77.403±0.12	205.6±3.47	0.480±0.00	12.677±0.29	0.44±0.02
T5	6.73±0.96	2.542±0.08	0.448±0.00	3.670±0.05	6.084±0.01	80.526±0.22	205.2±3.14	0.580±0.01	3.622±0.01	0.28±0.01
T6	10.00±0.0	2.488±0.02	0.499±0.01	3.848±0.14	12.260±0.33	70.905±0.11	207.2±3.06	0.678±0.03	33.885±0.09	0.60±0.02
	5									
T7	0.73+0.01	1.700+0.07	0.554+0.02	0.139+0.04	0.037+0.01	96.840+0.02	178.9+2.88	0.099+0.02	6.124+0.05	0.11+0.00
Т8	7 77+0 01	2 952+0 01	0 585+0 01	4 221+0 04	9 420+0 08	75 011+0 30	208 5+3 11	0 340+0 01	10 529+0 93	0 02+0 00
10	7.7720.01	2.90220.01	0.00020.01	1.22120.01	5.12020.00	75.01120.50	200.020.11	0.01020.01	10.02920.90	0.0210.00
Т9	0 54+0 10	0 981+0 03	0 173+0 00	3 020+0 03	0 001+0 00	95 285+0 42	70 6+1 80	0 120+0 01	00 000+0 00	0 16+0 01
17	0.0120.10	0.90120.00	0.17.510.00	0.01010.000	0.00120.00	JO.20010.42	,0.011.00	0.12020.01	00.000±0.00	0.1010.01
T10	1 50+0 01	1 015+0 14	0 239+0 01	2 014+0 02	0.005+0.00	95 200+0 04	81 9+2 10	0 240+0 04	0 065+0 01	0.01+0.00
110	1.50±0.01	1.013±0.14	0.209±0.01	2.017±0.02	0.003±0.00	JJ.200±0.04	01.7±4.10	0.440±0.04	0.005±0.01	0.01±0.00

TABLE 3: PERCENTAGE (%) PROXIMATE AND MINERAL (Mg/Kg) COMPOSITIONS FOR TEA PRODUCTS

TABLE 4: PERCENTAGE (%) PROXIMATE AND MINERAL (Mg/Kg) COMPOSITIONS FOR COFFEE PRODUCTS

Sample	Moisture	Protein	Ether extract	Ash	Crude fiber	Carbo hydrate	Mg	Ca	Fe	Cu
CF1	3.71±0.03	2.567±0.08	0.090±0.00	6.272±0.43	0.795±0.02	86.566±0.38	216.4±3.77	0.259±0.03	1.524±0.10	0.02±0.00
CF2	2.70±0.02	2.308±0.08	0.507±0.01	4.745±0.24	2.077±0.03	87.663±0.36	227.8±4.09	0.486±0.12	36.216±2.00	3.81±0.13
CF3	3.57±0.17	2.711±0.01	0.139±0.01	4.110±0.13	0.047±0.00	89.423±0.32	208.6±3.78	0.231±0.01	0.980±0.01	0.01±0.00
CF4	2.45±0.09	2.459±0.02	3.669±0.33	4.285±0.02	0.235±0.01	86.902±0.50	216.4±2.76	0.300±0.04	0.880±0.02	0.65±0.02
CF5	3.40±0.06	2.242±0.01	0.177±0.01	3.454±0.01	1.528±0.05	89.199±0.14	218.7±5.54	0.260±0.03	1.699±0.10	0.00±0.00

DISCUSSION

MicrobiologicalanalysisAll the products analyzed had TPC and fungalcounts not significantly (p<0.05) different from</td>

the standard requirement as indicated in (29, 30, 31) these standards specify a TAPC of 10^2 - 10^4 cfu/g and fungal counts of 10^2 - 10^3 cfu/g sample.

TAPC (heterotrophic contaminants) if made up of mere environmental contaminants of no health concern, will pose no threat to consumers but for food spoilage. However, the presence of organisms of public health concern makes them unacceptable. Fungi are spore bearers that are known to be common environmental and food contaminants, their presence in these samples, therefore, corroborate earlier reports (32). There is abounding evidence, however, that although fungi are common food contaminants, some species of *Aspergillus, Penicillium, Fusarium* can produce deleterious toxins ¹², and have been implicated in life threatening food borne diseases (32).

The level of *S. aureus* count in the samples was low; however, it is advisable that *Staphylococcus aureus* should not be present in a refined food product. The presence of *Staphylococci* could be attributed to the human beings involved in the processing. *Staphylococci* are normal flora of man and have been reported as contaminants in foods and also implicated in foodborne illnesses (33, 34).

The FAM(29), TRISL(30), and FDA(31) specify total *Coliform* counts of not more than 10^1 cfu/g (1.8- 10^1 cfu/g) for cocoa, coffee powder products and tea, some products analyzed in this work (C2, C3, C7, and C8) had significantly higher counts 2.6×10^1 cfu/g – 4.6×10^1 cfu/g. The coffee and tea products samples were negative for total coliform. Coliforms were indicator organisms; their presence in some products above acceptable limits connotes lapses in the quality control processes of manufacturing practices (35). This also portends a potential health hazard to consumers because these products are ready-to-eat.

The presence of bacillus spp as the major contaminants of products analyzed is in tandem with reports that bacilli are spore formers that are ubiquitously distributed. Some species *B. cereus, B. anthraces* have been implicated in foodborne diseases (36, 37), the presence of bacillus, therefore, must be closely monitored and not just be treated as mere contaminants.

All the products analyzed met the required microbiological standard, with the exception of some cocoa samples containing sub-standard coliform counts; this could indicate that most of the products were of good quality assuming all other organisms/metabolites of concern not assayed for were absent. The positive correlation established for proximate values and microbial counts of products corroborate reports that have shown cocoa powders and powdered cocoa beverages as good culture media for bacteria and fungi despite their low moisture content and powdery or granular nature (38, 19). Cocoa, tea and coffee products just like other food products can be contaminated by the food handlers and the environment with a wide varietv of microorganisms during manufacturing and packaging processes. Thus if these products are not properly handled and stored in a hygienic environment, the shelf life could be reduced and the hygroscopic nature of these products could support the growth and proliferation of microbial contaminants to hazardous levels.

Chemical analysis

The proximate compositions of the samples were within standard specifications except for percentage moisture which is high in some cocoa and tea products and low ash contents in some tea samples. The high moisture of C7 and some tea products could account for its higher microbial load compared to other samples (39). The quality of initial raw materials, processing conditions, and handling practices have been reported to reflect product quality (40, 41), This could explain why some cocoa products (C2, C3, and C8) with low moisture contents have relatively high contaminants level while some tea products (T2, T3, T5, T6, T8) with high moisture contents tend to have low microbial loads. Although all the products except for the once earlier mentioned, had low moisture level that might not constitute suitable medium for the growth of microbes, poor storage conditions, and increase in moisture has been identified as factors that could affect products shelf life, favour microbial growth, proliferation and subsequent production of toxic microbial metabolites such as mycotoxins (39).

The mineral compositions as reported for cocoa samples are in consonance with the presentations of Jayeola and Oluwadun(19). All the samples had copper compositions within standard required limit of 30 mg/kg, except for sample C10 with a copper level of 40.5 mg/kg which is within the SLTB maximum acceptable limit of 100 mg/kg. Copper is a heavy metal and copper toxicity is allied with stomach upset, queasiness, and diarrhea and can front tissue injury and disease (42). At high concentrations, copper is known to produce oxidative damage to biological systems, including peroxidation of lipids or other macromolecules (43, 44). Copper, however, is an essential trace mineral for man, it plays an important role in diseases in which oxidant stress is elevated. Deficiency of copper has been observed to alter the role of other cellular constituents involved in antioxidant activities, such as iron, selenium, and glutathione (45, 46, 47).

The samples all contained some levels of phytochemicals; beverages (coffee, tea, and cocoa) have been reported to contain polyphenols and minerals that have diverse beneficial biochemical, antioxidant and antimicrobial effects (48, 49, 21). Tea contributes to approximately 63 % of dietary flavonoid in the diet, and 69-85 % of the flavonoid content is soluble in hot water brewing within the first 3-5 min. (50,51) opined that the phenols and phytochemicals content in beverages are a function of the starting materials, roasting levels, and brewing method. Free radical scavenging assay is used to investigate the antioxidant capacity/ activity of foods (52, 53). The products analyzed exhibited different levels of antioxidant activity. Many studies have considered fruits, vegetables, and teas as the major sources of dietary antioxidative phenolics, this report observed cocoa product CI exhibited high scavenging activity (high antioxidant level with lowest IC₅₀) against DPPH free radicals. Arts et al,(54) reported that the antioxidant catechin content of chocolate (cocoa product) is four times that of tea.

In conclusion, some of the cocoa and tea products failed the required standard for coliform and moisture contents; this could be due to poor manufacturing practice and storage, it was observed in this study that some tea and cocoa products which did not meet the required

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standards are products produced and consumed locally in Nigeria. Use of quality raw materials, efficient and good manufacturing practice (GMP), proper storage conditions, application of hazard analysis critical control points (HACCP) should be well implemented and enforced by regulatory agencies. Quality products with good antioxidant activity should be encouraged for the health benefits.

Conflict of interest: Both authors declare no conflict of interest and have approved the submission of this article to this journal

Significance Statement: This study discovers that Cocoa, Coffee & Tea beverages at consumer level do contain microbes including coliforms that can foodborne infection/intoxication. cause Α positive relationship was established for microbial load, proximate compositions and mineral values in addition to the antioxidant property of these beverages. This study will help the researcher to uncover the critical area of beverages in functional foods, as a carrier medium for probiotics and fortification for better antioxidant activity. The need for strict adherence to good manufacturing practices and hazard analysis critical control point in beverage production is emphasized.

Authors Contributions: Author Oranusi, S. designed, supervised and wrote the manuscript Author Anosike S. O. Performed the laboratory analysis and wrote the draft

Acknowledgement: Authors appreciate the Covenant University management for the platform created for this research.

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ORIGINAL ARTILE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2018 ISBN 1595-689X VOL19 No.2 AJCEM/1827 COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.6 AFR. J. CLN. EXPER. MICROBIOL. 19 (2): 195-201

RADIATION SENSITIVITY OF WATER- BORNE MULTI DRUG RESISTANT ESCHERICHIA COLI

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ABSTRACT

The spread of antibiotic-resistant microorganisms in the environment is recognized widely as an important public health issue, with concerns about future ability to treat infectious diseases. The main risk to public health is that the resistance genes are transferred from environmental bacteria to human pathogens. Safe water is one of the most important needs in public health in the twenty first century. Radiation sensitivity (D_{10}) is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold. The aim of this paper is to determine the association between multiple antibiotic resistance and radiation sensitivity (D_{10}).Four hundred and sixty four (464) water samples were collected for assessment. *E. coli* isolation and identification were done using API 20E, and a PCR based DNA STRIP technology that allows simultaneous detection of virulence genes and confirmation of *E. coli* isolates. Antibiotic susceptibility testing was also conducted using the Kirby-bauer method. Radiation sensitivity was done using a cobalt 60 source. Sixty-three percent (63%) of the multidrug resistant *E. coli* isolates. L *coli* isolates is 0.33 ± 0.11 kGy. The study confirmed a high prevalence of multiple antibiotic resistant indexes and radiation sensitivity (D_{10}) of the *E. coli* isolates. Lastly, there is no association between multiple antibiotic resistant indexes and radiation sensitivity.

SENSIBILITÉ AU RAYONNEMENT DE L'EAU D'ESCHERICHIA COLI RÉSISTANTES AUX MÉDICAMENTS MULTIPLES

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Résumé

La propagation des micro-organismes résistants aux antibiotiques dans l'environnement est largement reconnue comme un important problème de santé publique, avec des préoccupations au sujet de la capacité à traiter les maladies infectieuses. Le principal risque pour la santé publique est que les gènes de résistance sont transférés de bactéries environnementales aux pathogènes humains. La salubrité de l'eau est un des principaux besoins en matière de santé publique au xxie siècle. Sensibilité au rayonnement (D10) est définie comme la dose de rayonnement (kGy) nécessaires pour réduire le nombre de micro-organisme par un facteur 10. Le but de cet article est de déterminer l'association entre la résistance multiple aux antibiotiques et sensibilité au rayonnement (D10).Quatre cent soixante quatre (464) des échantillons d'eau ont été prélevés à des fins d'évaluation. L'isolement et l'identification de E. coli ont été réalisés à l'aide de l'API 20E, et une bande d'ADN PCR Technologie qui permet la détection simultanée des gènes de virulence et la confirmation de l'E. coli. L'antibiogramme a également été effectuée à l'aide de la méthode Kirby-Bauer. Sensibilité au rayonnement a été fait à l'aide d'une source de cobalt 60. Soixante-trois pour cent (63 %) de la multirésistance des souches E. coli a enregistré une résistance multiple aux antibiotiques (MAR) index de >0,2.

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La moyenne sensibilité au rayonnement (D10) des isolats d'E. coli le est de 0,33 0,11 kGy. L'étude a confirmé une forte prévalence de résistance multiple aux antibiotiques des isolats de *E. coli*. Enfin, il n'y a pas d'association entre plusieurs index et résistantes aux antibiotiques sensibilité au rayonnement (D10) de *E. coli* résistantes aux antibiotiques.

Mots clés: antibiotiques, Escherichia coli, d'origine, multi-résistante, la sensibilité.

INTRODUCTION

Radiation sensitivity D₁₀ is defined as the radiation dose (kGy) required to reduce the number of that microorganism by 10-fold (one log cycle), or required to kill 90% of the total When a suspension of a number [1] microorganism is irradiated at incremental doses, the number of surviving cell forming colonies after each incremental dose may be used to construct a dose survival curve (figure 1). The relative sensitivity of different microorganisms to ionizing radiation is based on their respective D₁₀ values (which is the dose required to reduce the population by 90%). Lower D_{10} values indicating greater sensitivity of the organism in question may be explained by multiple targets and/or certain repair processes being operative at low doses. Microbial cells, whether pathogenic or comprising the normal microflora of foods, exhibit differences in their responses to ionizing radiation. As ionizing radiation is a suitable method to control pathogenic bacteria in food, a large number of D₁₀ values have yet been published [2]

In Ghana today very few published research are available radiation sensitivity on of microorganism. Radiation has been used to improve the safety of food substances as well as in studies involving the use of pure isolates of bacteria. Mahami et al., [3] sought to investigate the relative susceptibilities of planktonic versus biofilm cells of *Listeria monocytogenes* on glass to gamma radiation. Result from their study demonstrated that ionizing radiation effectively reduced the populations of both planktonic and biofilm-associated L. monocytogenes. The study further showed that, in contrast to chemical antimicrobial treatments, the antimicrobial efficacy of ionizing radiation is preserved or enhanced when treating biofilm associated bacteria.

In a similar study Adu Gyamfi *et al.*, [4] they determined the D_{10} value (decimal reduction dose) of *Escherichia coli* in refrigerated and frozen retailed chicken. This study was done alongside the investigation of the microbiological quality of chicken, at different retail outlets. Their study observed low D_{10} values of *E. coli* especially

under refrigerated conditions. This suggests susceptibility to low dose irradiation and the possibility of controlling spoilage and pathogenic microflora of fresh poultry.

However, Radiation sensitivity (D₁₀) is known to be a virulence factor. For example, the radiation sensitivity of three strains of Escherichia coli O157:H7 were found to increase after being induced to the antibiotic nalidixic acid [5]. However previous work, studied the radiation sensitivity only in relation to decimal reduction of bacteria but not to antibiotic resistance. Generally the effect of radiation on the antibiotic resistance of bacteria has not been extensively researched worldwide. This is particularly important when antibiotic resistance of inoculated bacteria is used as selective marker. For example, bacteria resistant to the antibiotic nalidixic acid (NalR) have recently been validated for use as a marker in studies of chemical interventions and for use in food [5]. Furthermore, the radiation sensitivity of *E. coli* isolates from water has not been determined. This is critical to further our understanding of radiation doses that will be required to eliminate water borne E. coli from water for safe public consumption. In addition, we do not know the relationship between multi antibiotic resistant E. coli and radiation sensitivity. This information is needed to provide baseline knowledge for future research in the application of radiation sensitivity to the control of multi antibiotic resistant *E. coli*. The aim of this paper is to determine the radiation sensitivity of water bourne multi-drug resistant E. coli

METHODOLOGY AND METHODS Sample collection sites

After several preliminary visits to various communities in the districts, 57 sampling sites comprising six different water sources that include dams, boreholes, stream sources, rivers, canals and hand-dug wells in 27 communities were selected. Samples were taken from locations that were representative of the water sources and/or distribution networks at which water is delivered to the inhabitants and/or points of use based primarily on factors such as population and extent of usage or level of patronage of water from these sources. Most of the communities are dominated by farmers. Each community selected had at least a borehole or a stream as the principal sources of water for the inhabitants.

Site Observation Details

Prior to water sampling, important observations were made of sanitary conditions and possible sources of contamination, both anthropogenic and natural that occur in the proximity of water bodies and/or are likely to influence water quality from all the sources sampled.

Field records for the following environmental factors were also recorded: Water clarity/turbidity (visual clarity in the water i.e. leaves, debris, algae] weather conditions (temperature, wind, rainfall) presence of animals (birds/ducks). Other comments (e.g. system problems i.e. disinfection/filtration equipment, faecal accidents)

Sample size and sampling frequency

Total of one hundred and twenty two water samples were collected for assessment. The sample collection period spanned over the two seasons in Ghana i.e. the dry and raining seasons. All water sampling and preservation procedures were performed according to Standard Methods for the examination of water and wastewater [6,7] and WHO guidelines for drinking water quality [8,9]. Sampling for bacteriological analysis was done aseptically with care, ensuring no external contamination of samples. All samples were transported to the laboratory within 2 hours.

Bacteria isolation and identification

All gram-positive organisms were identified by conventional methods, such as Gram reaction, positive catalase, Tube coagulase and Deoxyribonucleases (DNAse) test etc, whiles an API 20E kit was used to identify the gram negative organism. *E. coli* strain 25922 was used as the positive control for the E. *coli* isolates.

Anti-bacteria susceptibility testing of E. coli

Each of the isolates [*E. coli*] were subjected to antibiotic susceptibility testing using the Kirby bauer-method that has been standardized and evaluated by the methods of national committee for clinical laboratory standards. Isolates grown overnight on Nutrient Agar were suspended in sterile normal Saline (0.9% w/v NaCl) using a sterile wire loop until the turbidity was equivalent to 0.5 Mcfarland standards. A sterile non toxic cotton swabs dipped into the standardized innocula were used to streak the entire surface of Mueller Hinton Agar plates. The *E. coli* isolates were then tested against fourteen The data was analyzed and graphs were drawn. The inverse of the slope of each graph was taken (14)antibiotics as follows: ampicillin($10\mu g$), Pipemidic acid (20ug), Chloramphenicol ($30\mu g$), Ciprofloxacin (5 μg), Co-trimoxazole ($25 \ \mu g$), Erythromycin ($15 \ \mu g$), Nitrofurantoin ($300 \ \mu g$), penicillin ($10 \ IU$), Cefuroxime ($30 \ \mu g$), Cefotaxime ($30 \ \mu g$), Nalidixic acid ($30 \ \mu g$), Amikacin ($30 \ \mu g$), Tetracycline ($30 \ \mu g$), and Gentamicin ($10 \ \mu g$), Antibiotics disks were aseptically placed using a sterile forceps, and all plates incubated (Gallenkamp England model IH-150) at 37oC for 24hrs (Mills, R., et. al. 2003). The result was interpreted using NCCLS [10].

Determination of radiation sensitivity (D₁₀) of *E. coli*

The Radiation sensitivity (D_{10}) of *E. coli* was carried out on 29 (60%) of the multidrug resistant *E. coli* isolates. Stored isolates of *E. coli* were revived by culturing them on EMB and nutrient agar media overnight at 37°C in an incubator. Colonies from the overnight cultures were taken into a 100ml of peptone water. The peptone water was allowed to stand for a while for the organism to reach the stationary phase. The broth was dispensed 10ml into 7 McCarty bottles and labeled 0, 500, 600, 700, 800, 900, 1000 Gray for irradiation using the (⁶⁰Co source).

The exposure to the various doses of radiation was controlled from a radiation-controlled system. After irradiation with the doses mentioned above, the samples were aseptically dispensed into conical flacks containing 90 ml of peptone water. The samples were placed on a mechanical shaker for 5 minutes. The 100 ml peptone water was serially diluted into 8 other McCarty bottles containing 9ml of peptone water.

The dilutions were immediately plated in triplicates on EMB and incubated at 37°C to estimate survival rates. The samples were incubated at 38°C. The average of the surviving population of each dose was determined after 24 hours of incubation. This selection protocol was repeated independently three more times. Each replicate was initiated using an isolated colony derived from frozen stock of the founder.

The formula $-\log [N/N_o]$ was used to calculate the survival curve and the dose resistance of the *E. coli* organism where N is the number of surviving *E. coli* on a plate after each dose administered and N_o is the inoculums concentration of the samples sent for irradiation. as the dose to reduce the population of the *E. coli* organism by 1log cycle (D_{10}).

RESULTS

Table 1 shows the seasonal distribution of Multiple resistant *E. coli* isolates in the water. The number of multiple resistant *E. coli* isolated

ranged between 1 and 8 in the dry season. The highest number (8) of multidrug resistant isolates in the dry reason was obtained from stream water sources, whilst the least was from river water sources (1).

TABLE 1: A SEASONAL DISTRIBUTION OF MULTIPLE RESISTANT E. COLI ISOLATES

	No. of Multiple resistance isolates				
Water source	Dry Season	Rainy Season			
Borehole	4	6			
Canal	3	1			
Dam	8	6			
Hand-dug well	5	4			
River	1	0			
Streams	7	3			
Ν	6	6			
Mean	4.67	3.33			
Std Dev	2.58	2.50			
S. E	1.05	1.02			

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 2 presents the paired t test analysis for season distribution of multiple resistances in *E. coli*. The total number of multi resistance isolates (n=6) averaged 4.67 ± 2.58 and (n=6) averaged

 3.33 ± 2.50 in the dry and rainy seasons respectively. There was a significance difference (t-test, and d.f. = 5, *P*<0.05) between the number of multiple antibiotic resistance *E. coli* isolated in the dry and rainy seasons.

TABLE 2: T TEST FOR THE SEASONAL DISTRIBUTION OF MULTIPLE RESISTANT E. COLI ISOLATES

Season	Mean <u>±</u> SD	Min	Max	d.f	P value	
Dry	4.66 ±2.58	1	8	5	0.1576	
Rainy	3.33 ±2.50	1	6	5		
CD_ standard deviction of device of the day Mine minimum Mane manimum						

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 3 shows the distribution of the antibiotic resistant *E. coli* isolates and the number of antibiotic to which they were observed to be resistant. The highest number (16) of antibiotics to which *E. coli* was resistance in a single water source was observed in stream water sources and the least (1) was from borehole and river water

sources. Two *E. coli* isolates were observed to be resistance to 10 different antibiotics. This was recorded in dams and stream waters sources. A summary of the resistant profiles and the kind of antibiotics to which the *E. coli* showed resistant are presented in tables 4.24 and 4.25

					50	UNCLU						
Antibio	tic resistan	t profile								Number isolates	of	resist
PEN	СХМ	ERY	TET	CHL	PA	AMP	СОТ	CIP	NIT	2		
PEN	CXM	ERY	TET	CHL	PA	AMP	NAL	GEN		2		
PEN	CXM	ERY	TET	CHL	PA	AMK	COT			2		
PEN	CXM	ERY	TET	CHL	PA	CTX				3		
PEN	CXM	ERY	TET	CHL	PA					2		
PEN	CXM	ERY	TET	CHL						5		
PEN	CXM	ERY	TET							13		
PEN	CXM	ERY								5		
PEN	CXM									14		
PEN										49		

TABLE 3: ANTIBIOTIC RESISTANCE PROFILE (ANTIBIOGRAM) OF E. COLI FROM VARIOUS WATER SOURCES

CHL= Chloramphenicol; COT= Co-trimoxazole; ERY= Erythromycin; NIT= Nitrofurantoin; AMP= Ampicillin; PEN= Penicillin; PA= Pipemidic acid; CIP= Ciprofloxacin; NAL= Nalidixic acid; TET = Tetracycline; CXM = Cefuroxime

Figure 2 Shows the seasonal occurrence of *E. coli* isolates with MAR index>2. The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected. It can be observed from the graph that sixteen (16) *E. coli* isolates with MAR index>2 representing 53% were obtained in the dry reason. Fourteen (14) isolates *E. coli* isolates with MAR index>2 representing 47 % were obtained in the rainy season.



Seasonal occurrence of *E. coli* isolates with MAR

index > 2



FIGURE 2: THE SEASONAL OCCURRENCE OF E. COLI ISOLATES WITH MAR INDEX >2

Table 4 shows the multiple antibiotic resistant indexes of *E. coli* isolates at various water sources. Borehole water sources recorded the highest MARp values of 0.9. This was followed by canal water source with a value of 0.7. The least was obtained from stream water sources and hand-dug well water sources, both recorded values of 0.4

The general statistical summary is presented first in table 5. A scatter plot of radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E. coli* are also provided. Finally, a simple regression analysis of the association between D_{10} and multiple antibiotic resistances index of *E. coli* is presented.

Sampling site	Total numbers of test (isolates)	No of resistant test (resistant isolates)	MAR p
Borehole	11	10	0.9
Canal	6	4	0.7
Dam	28	14	0.5
Hand-dug well	24	9	0.4
River	2	1	0.5
Streams	26	10	0.4

TABLE 4: MULTIPLE ANTIBIOTIC RESISTANT INDEXES OF *E COLI* ISOLATES AT VARIOUS WATER SOURCES

MAR p = MAR index per sampling source.

TABLE 5: STATISTICAL SUMMARY OF THE RADIATION SENSITIVITY (D₁₀) OF THE MULTI-RESISTANT E.

Parameter	Parameter Mean±SD Min Max d.f 95.0% Confidence Interval				al	
					Lower Boundary	Upper Boundary
D_{10}	0.33±0.11	0.05	0.5	28	-0.133	0.79

SD= standard deviation, d.f= degree of freedom, Min= minimum, Max= maximum

Table 6 shows the descriptive statistical summary of the measures of radiation sensitivity (D_{10}) obtained from the multi-resistant *E. coli* isolates.

The radiation sensitivity (D_{10}) for 29 multidrug resistant isolates was measured. The mean

radiation sensitivity (D_{10}) is 0.33±0.11 kGy. This means that average radiation doses of 0.33±0.11 kGy will be required to reduce the number of *E. coli* by 10-fold (one log cycle) or required to kill 90% of the total number [1].

TABLE 6: A SIMPLE REGRESSION ANALYSIS CORRELATION BETWEEN D10 AND	MULTIPLE ANTIBIOTIC

	RESISTANCES OF E. COLI INDEX						
	Coefficient	Standard	Standardized	t	Р		
		error	coefficient				
Intercept	0.270	0.043	0	6.237	0.000		
Slope	0.223	0.153	0.270	1.459	.0.156		
Correlation coefficient (r)	=0.270, r ² =0.039						
Source	df	MS	F	Р			
Regression	1	0.025	2.12	0.156			
Residual	27	0.012					
Regression Residual	1 27	0.025 0.012	2.12	0.156			

Significant at 0.05

Table 6 presents a simple regression analysis of the test of association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) index of E. coli. The correlation coefficient (r) value of 0.27 indicates that there is no linear relationship between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) index of E. coli. The corresponding significance level of 0.156 also implies that there is no relationship or association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of E. coli. The larger insignificant P value (0.156) also suggests that changes in the predicator (multiple resistance indexes of multiple resistant E. coli) are not associated with the changes in the response) radiation sensitivity (D_{10})

DISCUSSION

Is there any association between antibiotic resistance and radiation sensitivity (D_{10}) ? The results obtained from this research have answered this question, providing new data on radiation sensitivity values for multidrug resistant E. coli. Ionizing radiation is a suitable method to control pathogenic bacteria in food and water, a large number of D₁₀ values have been published [2,11]. Antibiotic resistance of bacteria is a commonly used selective marker. Bacteria resistance to antibiotics is believed to have an increased sensitivity to irradiation. The data obtain from the current study indicates that the multidrug resistant *E. coli* had low D₁₀ values with a mean of 0.33 ± 0.11 KGy. Second, test of association between D₁₀ and antibiotic resistance, was P>0.05, an indication of no association between the two parameters.

The range of *E. coli* D₁₀ values obtained from the current study is consistent with previously published D_{10} values for *E. coli* [12,13]. The primary mode of action of ionizing radiation is via hydrogen and hydroxyl radical molecules resulting from the ionization of water molecules within the target organism. These radicals can disrupt membranes and interfere with the functioning of proteins, but the most significant target within the cell is DNA, where radicals are responsible for strand breakage [13]. NalR strains of E. coli O157:H7 and Salmonella have been recently shown to be more sensitive to irradiation than the NalS parent strains from which they comprehensive were derived [5]. А understanding of why a given isolate may be more or less sensitive to irradiation than related isolates of the same pathogen is yet to be formulated [12,13].

This current study did not find any association between the radiation sensitivity (D_{10}) and the multiple antibiotic resistances (MAR) of *E coli*. Furthermore, the results showed that antibioticresistant bacteria were preferentially associated with low D_{10} values. Thus, the study has demonstrated that ionizing radiation effectively reduces the populations of both antibiotic resistant *E. coli*. However, it is worth noting the likelihood that a survey of a larger number of isolates would result in a more linear progression of D_{10} values, that can bridge the gap seen among the statistical clusters observed among the isolates evaluated in this current study.

The implications and uses of radiation sensitivity (D_{10}) are of enough benefit to the society. First, in countries such as Austria, Czech Republic irradiation is used for drinking water disinfection [14]. Safe drinking water should not present any significant risk to health over a lifetime of consumption, including different sensitivities

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that may occur between life stages [15] (WHO, 2006). However *E. coli* in waters is a major cause of water borne diseases particularly in developing countries, where the chunk of WHO's estimated 30 000 deaths daily from water related diseases occurs [16] (Dauda, 2010). Irradiation is one of the best means of water disinfection. However, radiation sensitivity of bacteria depends on several factors [17,18]. This requires that the radiation sensitivities be evaluated for each and every organism. This study has proven that a radiation doses of 0.33±0.11 kGy could be used to disinfect E. coli including resistant isolates of the same in drinking water. Sachet water and bottle water producers in Ghana may have their water products disinfected for public consumption by the uses of the established recommended doses from this current study.

Finally, analysis of association between antibiotic resistance and radiation sensitivity (D_{10}) showed that that antibiotic-resistant bacteria were preferentially associated with low D_{10} values. Also a radiation dose of (0.33 ± 0.11 KGY) can be used to disinfect water contaminated with multidrug resistant *E. coli*, for safe human consumption. Furthermore, ionizing radiation effectively reduces the populations of antibiotic resistant *E. coli*.

The analysis of the findings further suggests that inactivation kinetics for controlling pathogen inactivation in food and water systems have to be estimated on the basis of specific microorganisms in food or water matrices of concern and should include further extrinsic factors. Lastly, this study has provided a model for which further research on the association between bacteria antibiotics resistances and their corresponding radiation sensitivities could be investigated.

CONFLICT OF INTEREST: Authors reports of no conflict of interest.

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ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1828 https://www.ajol.info/journals/ajcem COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.7 AFR. J. CLN. EXPER. MICROBIOL. 19 (3): 202-206

PREVALENCE STUDIES OF HUMAN T-LYMPHOTROPHIC VIRUS TYPE 1 AMONG PULMONARY TUBERCULOSIS PATIENTS IN DUTSE METROPOLIS, NORTH-WESTERN NIGERIA

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ABSTRACT

Human T-lymphotrophic virus type 1 (HTLV-1) is a causative agent of tropic spastic paraparesis and adult Tcell leukaemia, Information regarding the involvement of HTLV-1 in presentation of some subclinical immune suppression that may results in increased rate of HIV and TB infections has long been documented. Sixty (60) Confirmed Pulmonary TB subjects consisting of 41 males and 19 females were recruited in this study, Tuberculosis was confirmed by collecting their sputum samples and analysed using GeneXpert. The prevalence of HTLV-1 IgG antibodies among TB subjects was 6.6%, while that of IgM was 1.6%. Accordingly, sexually active couple group has the highest prevalence of 2.3% when compared to single and widow categories, age group 15-24 has the highest percentage of 3.3% for HTLV-1 IgG antibodies.

Key words: HTLV-1, Tuberculosis, IgG, IgM.

DES ÉTUDES DE PRÉVALENCE DU VIRUS HUMAIN T-LYMPHOTROPE HUMAIN DE TYPE 1 CHEZ LES PATIENTS ATTEINTS DE TUBERCULOSE PULMONAIRE EN MÉTROPOLE DUTSE, NORD-OUEST LE NIGERIA

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RÉSUMÉ

Les T-lymphotrope humain de type 1 (HTLV-1) est un agent causal de la paraparésie spastique tropicale et des profils à cellules T, de l'information au sujet de l'implication de l'HTLV-1 dans la présentation de certains suppression immunitaire subclinique qui peuvent se traduit par une augmentation des taux de VIH et tuberculose. Soixante (60) a confirmé la tuberculose pulmonaire (composé de 41 sujets de sexe masculin et 19 de sexe féminin ont été recrutés dans cette étude, la tuberculose a été confirmé par la collecte de leurs échantillons d'expectoration et analysées à l'aide d'GeneXpert. La prévalence des anticorps IgG HTLV-1 chez les sujets CT était de 6,6 %, tandis que celle d'IgM a été de 1,6 %. En conséquence, groupe couple sexuellement actifs a les taux de prévalence de 2,3 % par rapport à une seule et veuve, catégories d'âge 15-24 ans a le plus fort pourcentage de 3,3 % pour l'HTLV-1 anticorps IgG.

Mots	clés:	HTLV-1,	la	tuberculose,	IgG,	IgM

INTRODUCTION

The human T-lymphotropic virus, or human T-cell leukaemia-lymphoma virus (HTLV) belongs to the family of viruses that are known to cause a type of cancer called adult T-cell leukaemia/lymphoma and a diseases called HTLV- I associated myelopathy/tropical spastic paraparesis (HAM/TSP). The HTLV-1 genome is diploid, composed of two copies of a singlestranded RNA virus whose genome is copied into a double-stranded DNA form that integrates into the host cell genome, at which point the virus is referred to as provirus (1).

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Human T-cell lymphotropic virus type 1 (HTLV-1) is a human retrovirus that causes persistent infection in the host. While most infected persons remain asymptomatic carriers (ACs), 3-5% develop a T-cell malignancy termed adult T-cell Luekemia (ATL), and another 0.25-3% develop a chronic progressive inflammatory neurologic disease known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (2). An increased prevalence of the virus infection among individuals with active TB and higher mortality in co-infected individuals are described by several authors (3). However, contradictory results are reported in other studies (4,5). Furthermore, high frequencies of HTLV-1 infection among TB patients have been reported in Japan, Nigeria and Brazil (6,7,8). On the other hand, in studies conducted in Senegal and Papua New Guinea, few TB patients were HTLV-1 infected (9,10). Fewer studies started with HTLV-1infected individuals and looked at the occurrence of TB. In one Japanese report, TB history was more frequent among HTLV-1-infected than among seronegative men (11) In another Japanese study, chest X-ray findings compatible with old pulmonary TB were more frequent among HTLV-1 carriers than among non-carriers (12). The present research aimed at reporting prevalence of HTLV-1 antibodies among patients infected with pulmonary Tuberculosis (PTB)

MATERIALS AND METHODS

Study Design

The present study is a cross- sectional study. **Study Population**

Sixty (60) individuals infected PTB with no history of other chronic diseases were recruited in this study.

Specimen Collection

About 5mls of whole blood was collected aseptically from each subject using standard venepuncture. Samples were dispensed into appropriately labelled screw – capped containers and was left at room temperature for about an hour, after which it was spun at 3,000 rpm for 10minites to separate serum from blood clot. Serum were dispensed into corresponding labelled plain containers and was stored at – 20% until needed for assay

Determination of Tuberculosis

Tubercule bacilli was confirmed using GeneXpert as follows; 2 volumes of sample reagent was mixed with one part of sample (Ratio 2:1),

It was mixed vigorously 20 times, incubation at room temperature for 10 minutes and mixed again until sample liquefies. 2mls of sample was transferred into the open port of Xpert MTB/RIF Cartridge and lid closed, after 30 minutes, cartridge Barcode was scanned using barcode reader of the machine. Instrument module door was opened with the blinking green light and load cartridge. And the result was recorded.

ELISA technique for HTLV-1 Determination

Fifty (50µl) of negative and positive controls were added to the negative and positive control wells respectively. In sample wells, 40µl Sample dilution buffer and 10µl sample were added. Samples were loaded onto the bottom without touching the well wall and mix well with gentle shaking. After 30 minutes of incubation at room temperature and sealed with closure plate membrane, diluted with buffer 30 times for 96T and washed 5 times. 50µl HRP-Conjugate reagent was added to each well except the blank control well, it was incubated for 30 minutes and washed. 50µl Chromogen Solution A and B were added to each well, mixed with gentle shaking and incubated at 37 °C for 15 minutes, light was avoided during colouring. Lastly, 50µl of stop solution was added to each well to terminate the reaction and colour in the well changed from blue to yellow. Absorbance was Read at 450nm using a Microtiter Plate Reader and OD value of the blank control well is set as zero.

Ethical Clearance

An ethical approval was obtained from Jigawa State Ministry of health ethical committee before the commencement of the study, and the consent/accent of the subjects was sought for during this study. The subject was giving an opportunity to accept or reject enrolment into the study.

Statistical Analysis

The results were entered in Microsoft Excel and later transferred in statistical software SPSS Version 20 and OpenEpi version 2.3 for analysis.

RESULTS

Table 1 shows the prevalence of HTLV-1 IgG and IgM antibodies among the Pulmonary Tuberculosis patients. Of the sixty screened patients 6.6% that is 4 tested positive to HTLV-1 IgG antibodies while 1.6% that is 1 patient tested positive to IgM antibodies. The statistical analysis shows that there is no significant difference between IgG and IgM antibodies among Tuberculosis patients enrolled in this study with p-value >0.005.

Table 2 shows relationship between HTLV-1 IgG and IgM antibodies and marital status of the Tuberculosis patients. Of the sixty study participants 3.2% and 1.6% of the married couples tested positive to HTLV-1 IgG and IgM antibodies respectively, single couples have 1.6% for IgG and 0% for IgM antibodies. However, all the participants in Divorce category have 1.6% for IgG antibodies

and 0% for IgM. Widow Categories tested negative to both IgG and IgM antibodies.

Table 3 shows age distribution with respect to HTLV-1 IgG and IgM antibodies among Pulmonary tuberculosis patients. Age group 5-14 and 55-64 all tested negative to both IgG and IgM antibodies.

Age group 15-234 has highest percentage prevalence of IgG antibodies with 3.3%, age group 25-34 and 45-54 have both 1.6%, while 55-64 have 0% prevalence. Age group 45-54 is the only group that have participant who tested positive to HTLV-1 IgM antibodies

TABLE 1: PREVALENCE OF HTLV -1 IG	GG AND IGM ANTIBODIES	AMONG STUDY POPULAT	ION
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Sex	No. Screened	IgG (%)	IgM (%)
Male	41	04(6.6)	00
Female	19	00	01(1.6)
Total	60	04(6.6)	01(1.6)
$\chi^2 = 0.7013$			P – valve = 0.4017

TABLE 2: MARITAL STATUS AND HTLV -1 IGG AND IGM ANTIBODIES AMONG STUDY POPULATION

Marital Status	No Screened	IgG (%)	IgM (%)
Married	32	02(3.2)	01(1.6)
Single	21	01(1.6)	0(0.0)
Divorce	05	01(1.6)	00(0)
Widow	02	00(0)	00(0)
Total	60	04(6.6%)	01(1.6%)
$\chi^2 = 1.068$			P - valve = 0.381

TABLE 3: AGE DISTRIBUTION AND HTLV -1 IGG AND IGM ANTIBODIES AMONG PULMONARY TUBERCULOSIS PATIENTS

Age Range	No Screened	IgG (%)	IgM (%)	
5 - 14	02	00(0)	00(0)	
15 - 24	08	02(3.3)	00(0)	
25 - 34	21	01(1.6)	00(0)	
35 - 44	18	01(1.6)	00(0)	
45 - 54	06	00(0)	00(1.6)	
55 - 64	05	00(0)	00(0)	
Total	60	04(6.6%)	01(1.6%)	

DISCUSSION

According to the results obtained from the present study, a percentage prevalence of 6.6% for IgG and 1.6% IgM antibodies among TB patients were recorded, this is in accordance with work of Olaleye et al. 1994 (13) who reported a prevalence of 3.6%. Degree of subclinical immunosuppression caused by HTLV-1 infection has been documented by Goon et al., 2004 (14) resulting to other maladies such as crusted scabies, strongyloidiasis and Tuberculosis (15,8). In this study the percentage prevalence of participants who are Tuberculosis and HTLV-1 coinfected was 6.6% for IgG antibodies. A study conducted in Salvador a city with highest prevalence of HTLV-1 in Brazil, found that 11% of patients hospitalised with Tuberculosis were infected with HTLV-1(16). Similarly, it was

documented that in Guinea Bissau a West African country infection with HTLV-1 alone is not sufficient enough to increase the risk of Tuberculosis, however HTLV-1 increase the risk of Tuberculosis among HIV patients (5). Many studies suggested an increase prevalence of HTLV-1 infection with active TB (17,1,3), this is in contrast to the findings of Norrgren *et al.*, 2008 (5). However, in Nigeria there is little or no information that showing the prevalence of HTLV-1 infection among Tuberculosis patients. Based on the results obtained from this study, PTB/HTLV-1 co-infection is higher among sexually active groups with 2(3.3%) compared to widow category which have zero percent prevalence

CONCLUSION

Conclusively, the present research revealed a 6.6% and 1.3% prevalence of HTLV-1 IgG and IgM antibodies respectively among patients with PTB. Also, a higher prevalence of 2.3% has been found among married couples unlike widow group which

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ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1829 COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.8 AFR. J. CLN. EXPER. MICROBIOL. 19 (3): 207-213

DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS AMONG INDIVIDUALS PRESENTING WITH FEBRILE ILLNESS IN LAGOS, NIGERIA

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RUNNING TITLE: DETECTION OF HIV IN FEBRILE INDIVIDUALS

ABSTRACT

Introduction: The Human Immunodeficiency Virus (HIV) is the aetiological agent of Acquired Immunodeficiency Syndrome (AIDS), which is a chronic and potentially life-threatening condition. Fever is mostly associated with the early stage of virus replication known as acute HIV infection or syndrome; as such, determination of HIV status during this critical period is a good means of improving clinical outcome in those infected. Thus, this study aimed to determine the prevalence of HIV among febrile individuals in Lagos, Nigeria.

Materials and Methods: A cross sectional study of 250 febrile individuals attending General Hospitals at Isolo, Mushin and Surulere, Lagos, Nigeria. Analysis was carried out at the Virology Research Laboratory, Central Research Laboratory, College of Medicine of the University of Lagos from July to October 2017. Sample analysis was done according to the Nigerian National Testing Algorithm to determine HIV status using Enzyme Immunoassay (EIA) and data analyzed using Statistical Package for Social Sciences (SPSS) version 20.

Results: Out of the 250 febrile participants, 8 were positive for HIV, with an overall prevalence of 3.2%. Further analysis however showed that 90% of the HIV positive participants had four or more episodes of fever in a month. HIV infection was still majorly among the ages 15-24 and 25-34 for male and ages 25-34, 35-44 and 45-54 for females.

Conclusion: This findings showed that different cohorts are significantly at risk of HIV infection. Hence, policies and all efforts to reduce the burden of HIV are paramount for a HIV free future for Nigeria.

Keywords: Acute Retroviral Syndrome (ARS), Fever, Asymptomatic and Enzyme-Linked Immunosorbent Assay (ELISA).

La DÉTECTION DES VIRUS DE L'IMMUNODÉFICIENCE HUMAINE CHEZ LES PERSONNES PRÉSENTANT UNE MALADIE FÉBRILE À LAGOS, NIGERIA

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TITRE : DÉTECTION DU VIH DANS LES INDIVIDUS FÉBRILES

Résumé

Introduction : Le virus de l'immunodéficience humaine (VIH) est l'agent causal du syndrome d'immunodéficience acquise (SIDA), qui est une maladie chronique et potentiellement mortel. La fièvre est le plus souvent associé avec le début de la réplication des virus connus sous le nom de l'infection à VIH aiguë ou un syndrome ; à ce titre, la détermination du statut VIH durant cette période critique est un bon moyen d'améliorer les résultats cliniques dans les personnes infectées. Ainsi, cette étude visait à déterminer la prévalence du VIH parmi les personnes fébriles à Lagos, Nigeria.

Matériels et méthodes : Une étude transversale de 250 individus fébriles qui fréquentent les hôpitaux généraux à Dubuque, Mushin et Surulere, Lagos, Nigeria.

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L'analyse a été effectuée à la virologie, Laboratoire de recherche Laboratoire Central de recherche, Collège de médecine de l'Université de Lagos, de juillet à octobre 2017. L'analyse des échantillons a été faite selon le test national nigérian algorithme pour déterminer le statut sérologique à l'aide de dosage immunoenzymatique (EIA) et les données analysées en utilisant le progiciel de statistiques pour les sciences sociales (SPSS), version 20.

Résultats : Sur les 250 participants fébriles, 8 étaient positifs pour le VIH, avec une prévalence globale de 3,2 %. Cependant d'autres analyses ont montré que 90 % des séropositifs les participants avaient quatre épisodes ou plus d'une fièvre dans un mois. L'infection à VIH est encore plus parmi les 15-24 ans et 25-34 ans pour les hommes et de l'âge 25-34, 35-44 et 45-54 pour les femmes.

Conclusion : Cette conclusions ont montré que différentes cohortes sont grandement à risque d'infection à VIH. Par conséquent, les politiques et tous les efforts visant à réduire le fardeau du VIH sont primordiales pour un avenir sans VIH pour le Nigeria.

Mots-clés : syndrome rétroviral aigu (EI), de la fièvre, asymptomatiques et de dosage immuno-enzymatique (ELISA).

INTRODUCTION

The Human Immunodeficiency Virus (HIV) is a known retrovirus to cause human immunodeficiency infection, with sequel advancement to Acquired Immunodeficiency Syndrome (AIDS) if proper management is not instituted[1] Since the initial recognition of AIDS as a distinct syndrome with associated severe and life-threatening clinical conditions, HIV/AIDS remains one of the world's most significant public health challenge^[2],^[3]. The pandemic proportion of HIV is further reflected in an estimated 36.7 million people living with HIV-1 infection with 1.0 million deaths from HIV-related causes globally in 2016[3]. Furthermore, current estimates demonstrate very heterogeneous spread of HIV-1, but with Sub-Saharan Africa more affected, as 70% of the global total of new HIV infections has been reported from the region.[4] An estimated 60% of new HIV infections in Western and Central Africa in 2015 occurred in Nigeria, which is designated to have the second largest HIV epidemic in the world. Although HIV prevalence among adults is remarkably low (3.1%) compared to other Sub-Saharan African countries such as South Africa (19.2%) and Zambia (12.9%), the size of Nigeria's population means 3.5 million people were living with HIV in 2015[4]. The sustained pandemicity of HIV is due to the multi route of transmission of the virus, with its chronicity perpetuated by the gradual depletion of primary cells of the immune defence; which among others include monocytes, macrophages and dendritic cells[5]. The sequel undermined immune response from these cells play a significant role in HIV pathogenesis, as there is a direct killing and increased apoptosis of these infected cells, which progresses to a total decline of CD4+ T cells if there is no appropriate treatment intervention[6]

During the first few weeks following HIV-1 acquisition, many people develop an acute retroviral syndrome (ARS), which is a set of nonspecific symptoms and signs that includes fever, body pain, fatigue among other symptoms[7]. There is a steep rise in the HIV-1 viral load during this period that coincides with a spurt of inflammatory cytokines which is elicited as a febrile response in the individual[8]. Febrile illness or fever is a term for elevated body temperature, which is due to the body's natural reaction to invasion by an infectious pathogen. It is clinically established when an individual has a temperature ≥ 38 °C and is one of the leading causes of visit to the hospital[9]. In Nigeria, major agents of febrile illnesses include *Plasmodium falciparum* responsible for *malaria, Salmonella typhi* (typhoid fever) plus a number of parasitic and viral infections[10]. While a number of persons can be positive for HIV but remain asymptomatic, fever is a common feature in HIV infected adults and may be a presenting sign of acute HIV-1 infection or indeed HIV infection at any stage[10].

The association of fever with ARS has been shown by studies reporting up to 75% of individuals experiencing an acute 'malaria-like' illness approximately 2 weeks after infection[11],[12] Furthermore, Bebell et al. reported in 2010, that 1-3% of adults who sought care for suspected malaria in Uganda actually had acute or early HIV infection[13] Considering the surge in HIV-1 replication and viral load in individuals with ARS, who are highly contagious and the fact that such persons may not be aware of their status. Coupled with the fact that their febrile illness is often thought to be caused by malaria and typhoid fever in Nigeria; HIV status determination among these cohorts is clinically important for referral for early treatment, which promotes good clinical outcome for persons positive to HIV. Early detection is also significant for deterring spread of the virus by identifying persons at risk of HIV spread. Hence, the objective of this study was to detect HIV infection among febrile individuals presenting to the hospital for treatment, so as to determine the prevalence of HIV among febrile individuals, while promptly referring positive persons to a HIV treatment centre.

SUBJECTS AND METHODS

Study Centre/Site: A cross sectional study of febrile individuals at selected General Hospitals in Lagos State, Nigeria. Laboratory analysis was carried out at the Virology Research Laboratory, Central Research Laboratory, College of Medicine of the University of Lagos.

Study Population: Study participants were 250 individuals \geq 15 years of age who sort health care

for febrile illness at General Hospitals (Isolo, Mushin, and Surulere) in Lagos State. Study population was calculated by the minimum sample size (N), determined using the equation as described by Pourhoseingholi *et al* in 2013[14]: $n=Z^2P(1-P)/d^2n=Z^2P(1-P)/d^2$

Where n= sample size, Z=statistics for a level of 95% confidence interval=1.96, d= precision (allowable error) =5%=0.05. P=prevalence rate (3.17%) as reported by NACA[15]. The population of Isolo, Mushin, and Surulere are reported as 837,300, 841,100 and 669,400 persons respectively[16]. Meanwhile, a minimum of 48 participants were calculated for the study, but was marked up to 250 for improved quality of data.

Ethical Consideration: Ethical approval with reference number CMUL/HREC/06/17/188 was obtained from the Health Research Ethics Committee (HREC), College of the Medicine of the University of Lagos. Duly informed consent was obtained from the participants that met criteria for the study.

Inclusion criteria: Febrile individuals who do not have previous history of chronic illness and do not know their HIV status as at the time of presentation at the hospitals.

Exclusion criteria: Acutely ill febrile individuals, those who declined participation and individuals less than 15 years of age were excluded from the study.

Data/ Sample Collection and Storage: Sociodemographic data was obtained by Health Research Ethics Committee validated structured interviewer administered questionaires. Participants designated as febrile after seeing the Clinician proceeded to sample collection for laboratory testing. Three to five mililitres (ml) of whole blood samples was collected into an EDTA bottle labelled with unique code for confidentiality and transported in cold chain using triple level packaging system to the laboratory. Samples were spun in the centrifuge (Eppendorf, Germany) at 300 rpm for 10 minutes and plasma separated into labelled cryovials and stored at in a freezer at -20 °C until analysis.

Sample Analysis using Enzyme Immunoassay (EIA): Analysis of all samples to determine HIV status were done according to the Nigerian National Testing Algorithm (serial testing)^[17] and according to the manufacturer's instructions (Alere Determine HIV-1/2: Catalog number 7D2342/7D2343; Trinity Biotech Uni-Gold: Catalog number 1206502; SD-Bioline HIV 1/2 3.0: Catalogue number: 03FK10CE. The test kits were rapid test based on the principle of Enzyme Immunoassay (EIA)[17] All kits were checked before use to ascertain that they were not expired or damaged and all procedures were carried out using universal safety precautions.

Statistical Analysis: Analysis was done using Statistical Package for Social Sciences (SPSS) version 20 to compute descriptive data and prevalences, which were recorded as simple percentages. Associations between non-parametric variables were tested using Fishers's exact test. The level of significance was set at 0.05.

RESULTS

Two hundred and fifty (250) febrile individuals were recruited for this study. There were 80 (32%) males and 170 (68%) females, giving a male/female ratio of 1:2.1. The mean age of the participants was 36.5 years, with a range of \geq 15 - 84 years. The demographic features of the febrile individuals are as shown in table 1, with no significant associations found among the demographic variables collated from the participants.

Out of the two hundred and fifty (250) samples analysed, 8 samples were positive for HIV, with an overall prevalence of 3.2% in these febrile individuals. Off these, 5 (62.5%) and 3 (37.5%) were females and males respectively without any statistical significant difference (p = 0.32) as shown in table 2.

VARIABLES	CHARACTER	NUMBER OF <i>p-values</i> PARTICIPANTS (%)
Marital Status	Single	140 (56.0) 0.76
	Married	103 (41.2)
	Divorced	5 (2.0)
	Widow	10 (0.0)
Religion Belief	Christianity	110 (44.0) 0.83
	Islam	125 (50.0)
	Traditional	10 (4.0)
	Others	5 (2.0)
Tribe	Yoruba	168 (67.2) 0.09
	Igbo	48 (19.2)
	Hausa	23 (9.2)
	Others	11 (4.4)
Educational Level	Primary	82 (32.8) 0.63
	Secondary	102 (40.8)
	Tertiary	61 (24.4)
	None	5 (2.0)
Occupation:	Civil Servant	35 (14.0) 0.74
	Business	41 (16.4)
	Artisan	123 (49.2)
	Farmer	4 (1.6)
	Unemployed	47 (18.8)
Fever Episodes in a Month	Once	49 (19.6) 0.81
	Twice	95 (38.0)
	Thrice	85 (34.0)
	≥Four Times	21 (8.4)

TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

TABLE 2: PREVALENCE OF HIV AMONG FEBRILE INDIVIDUALS

SEX DISTRIBUTION	NUMBER (%)	NUMBER POSITIVE (%)	NUMBER NEGATIVE (%)	p-values
Male	80 (32)	3 (37.5)	77 (96.2)	0.32
Female	170 (68)	5 (62.5)	165 (97.1)	
Grand Total	250 (100)	8 (3.2)	242 (96.8)	
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Further analysis however showed that 90% of the positive participants had four or more episodes of fever in a month and HIV positive participants fell

into the 15 to 54 years age range. Distribution and comparison of HIV positivity among the three general hospitals in Isolo, Mushin and Surulere

used for this study showed no statistical significant difference. HIV-1 detection among the 8 positive individuals from the study sites (Isolo, Mushin and Surulere) taking into consideration the numbers of samples collected from the sites were 2.5%, 4.1% and 3.6% respectively (figure 1).



FIGURE 1: THE PERCENTAGE DISTRIBUTION OF POSITIVE SAMPLES FROM THE STUDY SITE

DISCUSSION

Febrile illness or fever is a common reason for presenting and seeking healthcare in low and middle-income countries. Among patients with febrile illness requiring admission, case fatality ratios are high, sometimes exceeding 20%[18]. The many etiologies of fever are difficult to distinguish clinically and laboratory services may be limited or absent in such settings. Consequently, presumptive significantly affects the proper treatment management of febrile illnesses and HIV positive persons[18],[19]. These positive febrile individuals may probably be at the acute HIV-1 infection stage, remain asymptomatic, highly contagious and account for a large number of new HIV-1 infections. Thus, diagnosis and prompt initiation of Antiretroviral drugs (ARTs) for these and other cases of HIV infection should be identified as a 'top priority' for HIV prevention, particularly in Nigeria.

The prevalence of 3.2% of HIV-1 among febrile individuals in this study falls within the overall range prevalence of 3.17% in Nigeria[15] This finding shows that the burden of HIV infection in Nigeria is still a cause for concern particularly among febrile individuals seeking healthcare at various hospitals. Our finding among these febrile participants was similar to the study by Bebell et al.[13] who reported a similar prevalence of HIV among adults who sought care for suspected malaria in Uganda. Likewise, and also in 2010, Serna-Bolea et al.[20] reported that 3.3% of patients who sought care for fever at a district hospital in Southern Mozambique had acute HIV infection. However, the prevalence detected from this study was lower than respective 4.1%, 9%, 4.88% and

11.4% reported from Oyo,[21] six geopolitical zones,[22] Plateau,^[23] and Ogun States in Nigeria.^[24]

Our study also shows that positive samples were detected in the three locations, which are densely populated and are representative of most areas in Lagos. Corresponding variations in the prevalence's in several reports from different parts of the country is majorly attributed to the different population samples, geographic locations, testing platforms and other socio-economic factors related to the population and site studied.

In this study, more females presented at the hospitals seeking healthcare for febrile illnesses and were found to be more infected than males. Although, the percentage of positivity among males and females was not statistically significant. This findings was similar to the study of Omoniyi *et al.*^[22] who also reported a higher prevalence in females than in males. The larger area of exposure in females (vagina/ comapared to the penis) has been known to put females at greater risk of HIV infection.

A high prevalence of HIV infection was more prominent in age group < 54 years, also similar to the study by Omoniyi *et al.*[22] who reported high HIV prevalence within same age range. This is clearly indicative of the association of HIV with a more sexually active age group in our society; although, all gender and age groups are still at risk of HIV infection.

While febrile patients should be encouraged for HIV testing, a provider-initiated HIV-1 testing and counseling (PITC) programme should be instituted for all patients visiting health facilities. Unfortunately the uptake of PITC has been low in sub-Saharan Africa[25]. Frequently mentioned reasons for the low uptake documented in literature include patient burden, absence of test kits, patients' perceived HIV-1-negative status following a previous HIV-1 test, added costs for patients accessing care at private facilities and weak health systems in general[26] However, focusing PITC efforts on patients with signs of acute infection (e.g. fever, sexually transmitted diseases, and diarrhea) may greatly reduce the number of patients requiring testing, while still allowing the identification of a large number of patients with undiagnosed prevalent HIV-1. Early detection through screening at the point of presentation for patients with febrile illness should be advocated for as a policy towards management. This would be significant for improved clinical care and reduction in HIV transmission in our environment.

CONCLUSION

The findings in this study shows that HIV was detected in individuals with febrile illnesses and was detected more in the sexually active age. It is important to put up a policy that will promote identification and monitoring of patients presenting in Nigerian clinics with febrile conditions and engage them in voluntary HIV counselling and testing. Thus, the government at all levelsl should further support HIV prevention programmes in population at risk, particularly as identified in this study.

FINANCIAL SUPPORT AND SPONSORSHIP: None.

CONFLICTS OF INTEREST: There are no conflicts of interest.

ACKNOWLEDGMENT

The authors would like to acknowledge and thank all patients and staffs who contributed to the collection and analysis of samples in the laboratory,

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especially the medical laboratory scientists of the Virology Unit Laboratory, College of Medicine of the University of Lagos.

AUTHORS' CONTRIBUTIONS

S.O.B., N.F.O. and B.T.E. conceptualized the study and were responsible for the experimental and project design, analysis of data and writing the manuscript. O.B.O. and J.A.B. made conceptual contributions, performed experimental analysis and assisted in preparing the manuscript. While O.S.A was the laboratory director, team lead of the Virology Research Group and was responsible for the experimental and project design, analysis of data and writing the manuscript. All authors read and approved the manuscript.

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AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 VOL19 No.3 AJCEM/1830 http://www.ajol.info/journals/ajcem COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.9 AFR. J. CLN. EXPER.MICROBIOL. 19 (3): 214-220

PREVALENCE OF MALARIA INFECTION AMONG PATIENTS ATTENDING MURTALA MUHAMMED SPECIALIST HOSPITAL KANO, NIGERIA

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ABSTRACT

Malaria is one of the most common diseased conditions in Nigeria and across most developing countries triggered by one of four species of *Plasmodium*. The objective of this study was to screen and detect for the presence of *Plasmodium* species via microscopic analysis on Malaria patients attending a healthcare facility Northern Nigeria and present the epidemiological data of malaria.Finger prick blood samples, Thick and Thin Giemsa-stained blood smears, were collected from 350 malaria-suspected individuals representing all age groups. The Giemsa-stained blood smears were examined microscopically. Demographic information on rural and urban dwellings, use of insecticides and mosquito nets were collected using structured questionnaires. Malaria cases were detected in in 227 (64.9%) of the participants with a higher infection rate amongst the males (147) than the females (80). The predominant specie found was *Plasmodium falciparum*. All age groups in this study were vulnerable in the order of 61-above>31-40>51-60>41-50>1-10>21-30> 11-20 years of age.A large number of participants dwelling in urban area (219) were tested positive for malaria in contrast to eight(8) from the rural area. Not using insecticides and mosquito treated nets were significantly associated with the prevalence of malaria as 59.4% of participants who were tested positive for *Plasmodium falciparum* infection utilized insecticides, while 66.9% of those who did not were also tested positive. Individuals residing close to gutters and bushes were the most susceptible (85.6%) to *Plasmodium* infection.

Keywords: Prevalence, Malaria, Plasmodium falciparum, Kano.

PRÉVALENCE DE L'INFECTION PARMI LES PATIENTS FRÉQUENTANT L'HÔPITAL SPÉCIALISTE MURTALA MUHAMMED KANO, NIGÉRIA

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Résumé

Le paludismeestl'une des plus fréquentes conditions malades au Nigéria et dans la plupart des pays en développement, déclenchéparl'une des quatreespèces de Plasmodium. L'objectif de cetteétudeétaitd'écran et de détecter la présenced'espèces de Plasmodium par analysemicroscopiquesur le paludisme les patients fréquentantunétablissement de santé dans le nord du Nigéria et présente les donnéesépidémiologiques du paludisme. Des échantillons de sang par piqûre au doigt, épaisseset minces colorées au Giemsa, frottis de sang ontétérecueilliesauprès de 350 personnessoupçonnées de paludismereprésentanttous les groupesd'âge. Les frottissanguinscolorés au Giemsa-ontétéexaminées au microscope.L'informationdémographiquesur les logementsrurauxeturbains, l'utilisationd'insecticides et de moustiquairesontétérecueillies à l'aide de questionnaires structurés. Les cas de paludismeontétédétectésdans la région de 227 (64,9 %) des participants avec un tauxd'infection plus élevéparmi les hommes (147) que les femelles (80). La principaleespècetrouvéeétait Plasmodium falciparum.

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Tous les groupesd'âgedanscetteétudeontété exposés dansl'ordre de 61-au-dessus de >31-40 >51-60 >>1-10 41-50 21-30 >>11-20 ans. Un grand nombre de participants en zone urbained'habitation (219) ontététestéspositifs pour le paludisme en revanche à 8 de la régionrurale. N'utilisez pas d'insecticides et de moustiquairesimprégnéesontétésignificativementassociée à la prévalence du paludismecomme 59,4 % des participants qui ontététestéspositifs pour l'infection à Plasmodium falciparum insecticides utilisésalorsque 66,9 % de ceux qui n'ont pas ontégalementététestéspositifs. Les individusrésidant à proximité de gouttières et les buissonsétaient les plus susceptibles (85,6 %) infection à Plasmodium. Mots-clés : la prévalence, le paludisme, Plasmodium falciparum, Kano.

1. INTRODUCTION

The scourge of malaria which has a high incidence across most of the developing world, particularly the tropical and subtropical regions of the world is caused by the bite of the female anopheles mosquito, thereby transmitting one of four protozoan parasites of the *Plasmodium family; Plasmodium falciparum, Plasmodium*

vivax, Plasmodium ovale and Plasmodium malariae(1, 2). Of these, *Plasmodium falciparum* is the most common and deadly (3).

Recent data indicated that an estimated 1 million lives globally has been claimed by malaria infection of which Africa bore the highest rate of mortality (90%), particularly among victims under the age of 5 years old across the sub-Saharan African continent (4,5).

Studies reported that in Nigeria, malaria infected patients represent approximately 60% of outpatient hospital visits in Nigeria, 30% of hospitalizations, 30% of under-five mortalities, 25% of infant mortalities and 11% of maternal mortalities (6). In the last decade, the fight against malaria and its propagating agents in Nigeria and across Africa has not been as effective due to the emergence of resistant species of the parasites, coupled with the advent of vectors that appear resistant to commercially available insecticides (7). This has caused a paradigm shift in behavioral pattern of the malaria vectors in which they are now just as devastating when encountered outdoors as well as indoors. Its rate of transmission is characteristically distinct between the rainy and dry seasons (8). Data revealed that the countries microclimate, topography, population densities, cultural practices, etc also contribute towards the spread of the disease (9). These include residence and leisure activities within close proximity to stagnant bodies of water, public exposure to rural lavatories, general neglect for routine check-up or screening, monthly rainfall, etc (10, 11, 12, 13).

It has long been established that the emergence and spread of drug resistance to commonly used chemotherapeutics are major factors contributing to this increasing burden and most of the mortality and morbidity are borne by children and pregnant women. Pregnant women and their infants are susceptible to common and preventable infectious diseases including malaria but are woefully left unscreened and untreated. According to an estimate, approximately 125 million pregnant women worldwide are exposed to the risks of malaria in pregnancy (MIP) each year, resulting in 200,000 infant deaths (14).

In Nigeria, the federal ministry of health in conjunction with the then millennium development goals (MDG) had proposed the goal of reducing the malaria associated rate of mortality and morbidity by half as of 2013 (8). With the inability to achieve the set target at the end of the set project date, it is necessary to conduct community-wide malaria surveys as a means of monitoring the impact and effectiveness of malaria control measures and programs at different levels. Data obtained from such studies would aid in defining up-to-date malaria burden as well as develop suitable measures of intervention whilst attempting to address adequate control measures for the disease throughout the country.

This study was carried to assess prevalence of malaria in patience attending the Murtala Mohammed Specialist hospital in Kano State, Northern Nigeria. It would also attempt to establish the prevalence of *P. falciparum* infection among different age groups of consenting participants in order to provide information useful to relevant government agencies involved in the control of malaria towards the development of suitable policies for maximum efficiency.

2. MATERIALS AND METHODS 2.1. Study Area

The study location is Kano metropolis, Nigeria situated between latitudes 11° 25′ N to 12°47′ N and longitude 8° 22′ E to 8°39′ E east and 472m above sea level and a population of over 3 million (16). Kano metropolis is bordered by Madobi and Tofa Local Government Areas (LGAs) to the South West, Gezawa LGA to the East, Dawakin Kudu LGA to the South East, and Minjibir LGA on the North East.

2.2. Study Population and Ethical Permission

The study population was made up of 211 male and 139 female patients attending the Murtala specialist hospital Kano state, Nigeria who all gave their consent to participate in the study.

2.3. Ethical Permission

Approval to conduct the study was obtained from the Ethical Clearance committee, Kano State Ministry of Health prior to sample collection. Subjects used in this study were those who's informed consent or that of their guardian were sought. They were patients within the all age groups examined for malaria parasite infection at the general outpatient department (GOPD) of Murtala Mohammed specialist hospital Kano state, Nigeria.

2.4. Blood Collection and Screening

Patients attending the hospital, who were feverish with asymptotic signs of malaria were screened for Plasmodium infection. Finger prick blood samples were collected to prepare thick blood films (in duplicate) were also prepared, stained with 10% Giemsa stain for 20 minutes and examined for malaria parasites by microscopy. Each of the films was assessed and the mean value was recorded. The parasite density was estimated by counting the number of asexual parasites against a minimum of 200 white blood cells (WBCs) (15).

2.5. Statistical Analysis

Statistical analysis was done using SPSS Software.

3. RESULT

This result in (Table 1, Figure 1) revealed that the male patients had the highest cases of malaria infection (69.7%) positive for *Plasmodium falciparum*

while the female patients recorded (57.6%) positive cases of malaria infection for Plasmodium falciparum. More so, (30.3%) and (42.4%) of the respondents (male and female respectively) were found to be negative for all the Plasmodium species out of 350 samples tested in the study. There was also no significant association between the prevalence of Plasmodium species that causes malaria infection in relation to gender status of respondents that participated in this study (P < 0.05.The prevalence rate of malaria infection caused by *Plasmodium falciparum* in relation to mode of settlement pattern of the respondents with respect of those living in the urban settlement is having the highest cases of malaria infection of (65.6%) cases while those living in the rural settlement recorded the least prevalence rate of (50%) cases (Table 2). There was no significant association between prevalence of *Plasmodium* species that causes malaria infection in relation to gender status of respondents (P < 0.05). Results of the about prevention and control of respondents or guardians obtained in this study shown in (Table 3). Statistical analysis of the findings from the results above, revealed that

there were significant associations (p >0.05) the prevalence of *Plasmodium* species that causes malaria infection in relation to knowledge about prevention and control of malaria infection by the respondents or guardians that participated in this study. Finally, all the 350 respondents that participated in this study were found to be negative for *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale* under this category.

TABLE 1: DISTRIBUTION OF PLASMODIUM SPECIES IN RELATION TO AGE OF RESPONDENTS

Age/years	Number examined	Male		Female	
		Positive	Negative	Positive	Negative
1 - 10	19	5	4	7	3
11 - 20	77	30	16	15	16
21 - 30	118	43	22	26	27
31 - 40	65	32	10	18	5
41 - 50	28	13	6	5	4
51 - 60	28	14	4	7	3
61 and above	15	10	2	2	1
Total	350	147	64	80	59
Total Percentage (%)		69.66	30.34	57.55	42.45

P < 0.05.



TABLE 2: DISTRIBUTION OF PLASMODIUM SPECIES IN RELATION TO AGE OF RESPONDENTS				
	No. examined	Malaria Positive	Malaria Negative	
Age Group (years)				
1 - 10	19	12	7	
11 - 20	77	45	22	
21 - 30	118	69	49	
31 - 40	65	50	15	
41 - 50	28	18	10	
51 - 60	28	21	7	
61 and above	15	12	3	
Sex				
Male	211	147	64	
Female	139	80	59	
Residence				
Urban	334	219 (65.6%)	115 (34.4%)	
Rural	16	8 (50%)	8 (50%)	

Method	No. examined	P. falciparum	P. malarae	P. vivax	P. ovale	% positive
Use of insecticide						
Yes	96	57	-	-	-	59.4
No	254	170	-	-	-	66.9
Bushes and gutters						
Yes	139	119	-	-	-	85.6
No	211	108	-	-	-	51.2
Use of mosquito nets						
Yes	43	22	-	-	-	51.2
No	307	207	-	-	-	67.4

TABLE 3: DISTRIBUTION OF MALARIA IN RELATION TO PREVENTION AND CONTROL

4. DISCUSSION

The frequency of malaria infections across all age groups in Nigeria and Africa as a whole is worrisome particularly for community health establishments, primary and secondary schools as well public or private businesses. It is of major concern in the 21st century as it is one of the most prevalent causes of human mortality in developing countries as a direct result of either the increase in regular over-the-counter anti-malaria agents or a high level of parasitemia, the latter which if detected early would fast track the treatment and recovery process. Apart from death, malaria infection greatly diminishes the overall effectiveness of humans thereby impacting negatively on commerce, economy and tourism in the affected location. The spread of malaria is high all across Nigeria, making the country one of those in the area among the malariaendemic regions of the world. In this study, data obtained revealed that a large number of the studied population (227 of 350) were infected with Plasmodium falciparum (Table 1-3, Figure 1) as assayed via the gold standard of malaria detection (microscopy). Among the infected individuals, data revealed that the males were far more susceptible to infection (147 out of 211 male patients) in comparison to 80 out of 139 female participants (Table 1). Data obtained revealed that the most vulnerable age group in the study population for each sex was 21-30. The result suggests that if the retirement age within the study location is set at 60 years, then at any given time, over 50% (58.4% for ages 21-30, 76.9% for ages 31-40, 64.3% for ages 41-50 and 75% for ages 51-60) of the active workforce suffers from malaria infection, thereby diminishing their efficiency in the workplace or in institutions of higher learning (Table 1, Figure 1). The finding of the current study revealed that malaria infection in the study participants was 64.85%, representing almost double the figure obtained in other recent studies that revealed a 36.6-39.5% rate of infection in northern Nigeria (16, 9). This variation may be attributed to climate change which could affect the reproductive rate of Plasmodium species, dwindling or ineffective control measures in the target location as well as the level of infrastructural development pertaining to the conditions of the participants dwelling over the last decade (17).

Majority of the participants in this study (334 out of 350) resided within the peri-urban environment of Kano metropolis of which 219 were tested positive for malaria (Table 2). 16 of the 350 participants resided in the rural area of Kano state of which 8 (50%) were tested positive for malaria. These results were in stark contrast to that obtained for studies in other countries of Africa where it is generally expected that the more urban the environment, the lower the chances of malaria infection (17). It is generally perceived that in homes made of mud, local bricks and sticks, the prevalence of mosquitos would be higher as such materials used in building the homes provide the preferred porous surfaces for propagation of the mosquito vectors (12). Industrial activities coupled with the need for more stringent environmental practices in the urban areas may contribute for increased numbers of stagnant water bodies which provides the avenue for increased mosquito growth, thereby accounting for the higher incidence of malaria amongst residence in the urban areas. Between the rural and urban dwellers of the study location, the immediate exposure to not only stagnant water bodies but bushes and gutters as well as the use of control measures like mosquito nets and insecticides would also aid in the reduction of malaria incidence, thus was also accessed (Table 3). In this study, it was discovered that out of 307 out of 350 participants who did not utilized WHO approved mosquito nets, 207 representing 67.4% were tested positive for Plasmodium falciparum infection. By contrast, 22 out of 43 patients who slept in mosquito nets were infected by P. falciparum (Table 3). These findings suggest that other environmental factors are responsible for the propagation of malaria in the study region which should be responsible for the change in breeding and transmission pattern of the malaria vectors to account for the high incidence of P. falciparum infection detection (8, 9). Assaying the use of

insecticides to prevent malaria as another control measure revealed that 59.4% of the population who apply this measure were still tested positive for P. falciparum infection (Table 3). The significance of this discovery was further exacerbated by the findings that 66.9% of participants who did not use insecticides were found to be infected further confirms that an overwhelming environmental factor is responsible for the failing antimalarial regimes employed by the study population. To this end, the proximity of bushes and gutters to the dwellings of the studied populace was accessed for which it was discovered that the highest recorded incidence of infection was among participants (119 out of 139, 85.6%) who reside close to such (Table 3). Almost half the populations of participants' living far away from bushes and gutters by at least 1 kilometer away from their places of residence were at risk (Table 3). The frequency of infection recorded among the high risk groups pertaining to individuals residing close to gutters and bushes is similar to data obtained in various studies across Nigeria, Africa and major developing countries (18, 19, 17, 20).

The major limitation of this study was that since it was carried out at a certain interval in time, it does not represent annual seasonal pattern of the disease. It is therefore conceivable that seasonal variations could and may have affected the findings in this study. Furthermore, the data obtained in this study does not embody that of the entire metropolis as the findings were obtained from only willing participants that attended the Murtala Mohammed Specialist hospital whilst the study was conducted.

5. CONCLUSION As part of the Sustainable Development Goals mandate

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geared towards the global control and eradication of malaria, Plasmodium falciparum is the overarching dominant Plasmodium specie in Nigeria which impacts negatively on commerce, economy and tourism in the affected locale (16, 9). Findings from this study revealed a high prevalence of malaria among the males (69.66%) than the females(57.55%). The prevalence of malaria was highest for participants between age group 31-40 (76.9%) of the workforce age but highest overall for participants age 61 and above. The most significant observation was the high prevalence of P. falciparum infection amongst participants residing in urban areas (Table 2). The culmination of these two results buttresses the need for early, routine screening and treatment of malaria among the males in the study location. Additionally, in light of the use of WHO approved mosquito netting, only 43 participants' use the nets for which 51.2% were tested positive for P. falciparum infection. Also, in the face of a malaria endemic in Northern Nigeria, 66.9% of the study population who did not use insecticides were diagnosed with malaria. As environmental factors are generally considered to be the medium by which mosquito breed and spread, the number of participants diagnosed with malaria was highest amongst patients living in close proximity to gutters and bushes (Table 3).

In view of the sizable population at risk in this malaria endemic region of Nigeria, it is suggested that effective environmental sanitation, geared towards the clearing of bushes and gutters could substantially reduce the spread of mosquitos, hence reduce the incidence of malaria in endemic regions. The findings of this study would assist in decision making towards improved malaria prevention and control.

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ORIGINAL ARTILE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY JULY 2018 ISBN 1595-689X VOL19 No.3 AJCEM/1831 COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.10 AFR. J. CLN. EXPER. MICROBIOL. 19 (3): -221-228

ANTIFUNGAL PROPERTIES OF METHANOLIC EXTRACTS OF SOME MEDICAL PLANTS IN ENUGU, SOUTH EAST NIGERIA

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ABSTRACT

Objective: The current study evaluated the anti-fungal activities of methanolic extracts of the leaves and fruits of some medicinal plants of health value in Enugu, southeast Nigeria.

Materials and methods: Volumes of the different extracts into molten Sabouraud Dextrose Agar to make up for concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. Concentrations of 125 µg/ml, 250 µg/ml and 500 µg/ml of Miconazole were incorporated to serve as a positive control while 0.5 ml of DMSO served as a negative control. The plants extracts employed were from Azadirachta indica (Neem), Anacardium occidentale (Cashew), Euphorbia hirta (Asthma weed), Jatropha curcas (Physic nut), Acantus montanus (Mountain thistle) and Picralima nitida (òsúigwe in Igbo). A 2 mm agar disc cut out from the margin of actively growing cultures of each dermatophyte (*Trichophyton soudanense*, *T.mentagrophytes*, *Cladosporium* sp, *T.rubrum* and *Fusarium* sp) were inoculated on the agar plates containing varying at 28 °C.

Results: All the extracts exhibited antifungal activities of varying degrees with radial growth inhibitions (RGIs) ranging from 0-0.45 mm radius. Fifty percent (50 %) of the plants extracts comprising of *Picralima nitida, Euphorbia hirta* and *Acantus montanus* exhibited complete inhibition at 100 mg/ml against all the clinical isolates under investigation (p < 0.05) . *Picralima nitida* seed was the only plant extract with complete inhibition at 25 mg/ml against *T.soudanense* and *T.rubrum*. *Picralima nitida* was the closest in activity to miconazole. *Azadirachta indica* (Neem) did not exhibit complete inhibition at 35 mg/ml yet exhibited a significantly lower RGI at the lowest concentration of 25 mg/ml better than others.

Conclusion: Antifungal activities showed that leaves extract of fruits plants against the clinical isolates are good source of medicinal applications

Key words: Antifungal agents, medical plants, Enugu, Nigeria.

PROPRIÉTÉS ANTIFONGIQUES D'EXTRAITS MÉTHANOLIQUES DE CERTAINES PLANTES MÉDICALES À ENUGU, AU SUD-EST DE NIGERIA

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RÉSUMÉ

Objectif: La présente étude a évalué les activités anti-fongiques des extraits méthanoliques des feuilles et fruits de certaines plantes médicinales de valeur santé à Enugu, au sud-est du Nigeria.

Matériel et méthodes: Volumes des différents extraits dans molten Sabouraud Dextrose Agar pour compenser pour les concentrations de 25 mg/ml, 50 mg/ml et 100 mg/ml respectivement. Les concentrations de 125 µg/ml, 250 µg/ml et 500 µg/ml de miconazole ont été intégrés pour servir comme contrôle positif alors que 0,5 ml de DMSO a servi de témoin négatif. Les extraits de plantes utilisés étaient d'Azadirachta indica (neem), noix de cajou (Anacardium occidentale), Euphorbia hirta (asthme), Jatropha curcas (pourghère),Acantus montanus (chardon de montagne) et de Picralima nitida (òsúigwe en Igbo). Un disque de 2 mm d'agar coupé à partir de la marge de croissance active, les cultures de chaque (dermatophytes Trichophyton soudanense, T. mentagrophytes, Cladosporium sp, T.rubrum et Fusarium sp) ont été inoculés sur les géloses contenant des concentrations variables des différents extraits de plantes, la norme agent antifongique et contrôle négatif en triplicats et incubées à 28 °C.

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Résultats: Tous les extraits de l'exposé des activités antifongiques avec divers degrés d'inhibitions de la croissance radiale (RGIs) allant de 0-0,45 mm de rayon.

Cinquante pour cent (50 %) des extraits de plantes comprenant de Picralima nitida, Euphorbia hirta et Acantus montanus présentaient une inhibition complète à 100 mg/ml contre tous les isolats cliniques soumis à l'enquête (p < 0.05).

Picralima nitida semence a été le seul extrait de plantes avec une inhibition complète à 25 mg/ml contre T.soudanense et T.rubrum. Picralima nitida était la plus proche de l'activité pour le miconazole. Azadirachta indica (neem) n'ont pas d'inhibition complète à l'égard d'aucun des isolats cliniques à 100 mg/ml mais montrent un souci nettement inférieur à la plus faible concentration de 25 mg/ml mieux que d'autres.

Conclusion: Activités antifongique a montré que l'extrait de feuilles de fruits plantes contre les isolats cliniques sont une bonne source d'applications médicinales

Mots clés: antifongiques, plantes médicinales, Enugu, Nigéria.

INTRODUCTION

The increasing incidence of growing resistance to antifungal agents despite the intensive use of antifungal drugs in the treatment of fungal infection [1], has become a great health challenge. However, there has been some claims by the traditional healers that some medicinal plants are more efficient in the treatment of infectious diseases than synthetic antibiotics. Medicinal plants have been practiced for centuries as remedies for human diseases as they incorporate elements of therapeutic value. At that place are numerous plant natural products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally [2]. Medicinal plants are considered to be an important source of new chemical substances with possible therapeutic effects [3]. The World Health Organization (WHO) estimates that up to 85% of people still rely primarily on traditional remedies such as herbaceous plants for their medicine [4]. Traditional healing plays an integral role in black African culture as it provides health care needs for a large majority of the society [5]. Presently, there is growing awareness of scientific and medicinal plants in the health care system of many developing countries [6]. In Nigeria, there is a rich tradition in the use of herbal plant products for the treatment of several ailments. Plants such as Euphorbia hirta (Asthma weed), Anacardium occidentale (Cashew), Picralima nitida (Òsúigwe), Jatropha cucas (Physic nut), Azadirachta indica (Neem) and Acantus montanus (Mountain thistle) have been used by the people within the Enugu metropolis for the local treatment of skin diseases because of their effectiveness, availability and cost effectiveness.

Furthermore, nature has bestowed a very rich botanical wealth of vegetation, and a large number of diverse types of plants grow in different parts of the country. They constitute the richest source of drugs for traditional systems of medicine, modern medicines, food supplements [7]. The increasing prevalence of serious mycoses coupled with the frequent use of the available antifungal drugs has resulted in rising resistance of fungal pathogens to antifungal agents. However, these topical drugs are generally ineffective against fungal infections of the nails due to their inability to penetrate the entire nail unit and eradicate the infection. The increasing prevalence of serious mycoses coupled with the frequent use of antifungal drugs currently available has resulted in rising resistance of fungal pathogens

to antifungal agents. There is, therefore, a need for new broad-spectrum antifungal agents that can be used empirically in immune compromised patients, organ transplant patient, and other challenging situations. Use of herbal medicine in the treatment of infection with microorganisms predates the introduction of antibiotics [8]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented too, as well as representing a rich source of antimicrobial agents [9], with many of them readily available in rural areas at a relatively cheaper price. Medicinal plants extracts are promising as alternative or complementary control means because of their antimicrobial activity, non-phytotoxicity, as well as biodegradability properties [10].

Considering the vast potential of plants materials in the health care and the challenges in the management of fungal infections, this current study was designed to evaluate the antifungal properties of methanolic extracts of some traditional medicinal plants in Enugu, South East, Nigeria and their phytochemistry properties in order to reformulate the existing antifungals that are essential for improving patient management.

MATERIALS AND METHODS Preparation of Plant Materials

Fresh leaves of the plants, *Azadirachta indica* (Neem), *Anacardium occidentale* (cashew). *Euphorbia hirta* (Asthma weed), *Jatropha curcas* (Physic nut), *Acantus montanus* (Mountain thistle) and the *Picralima nitda* (Òsúigwe) Seeds & Rind were collected within the Enugu metropolis and authenticated in the Botany Department of the University of Nigeria, Nsukka. They were washed under running tap water and air dried in room for 5 days (for the leaves) and 12 days (for the seeds and rind). The materials were ground into fine powder and stored in labelled air tight containers.

Preparation of Crude Extracts

100 g of each of the powdered form of the plant materials was exhaustively extracted by Soxhlet extraction method [11], using absolute methanol at 30 °C.

Preparation of fungal isolates

Microscopically identified isolates of *Trichophyton* soudanense, *T. mentagrophytes*, *T. rubrum*, *Cladosporum* sp and *Fusarium* sp, were obtained from the Mycology Laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla,Enugu. The isolates were subcultured in Sabouraud Dextrose Agar (SDA) to get pure cultures. Pure cultures were prepared in slanted cultures, stored in MacCartney bottles and kept at 2-8 °C for further experimental purposes.

Determination of Antifungal Activities Reconstitution of plant extracts

A stock concentration was reconstituted by weighing 2 g of the individual plant residue by adding 1 ml of DMSO to it in a test tube to make a concentration of 2 g/ml (2000 mg/ml).

Preparation of molten SDA plates containing the extract

The agar plate method was adopted as described [12].

Culture Process

The Culture medium of the different concentrations was inoculated with 2 mm agar disc cut out from the margin of the actively growing culture of the dermatophyte. This was carried out in triplicates and incubated at 28 °C, with controls run concurrently. The radial growth was measured daily for 4 days and the average reading taken for the test, positive and negative controls.

Percentage radial growth inhibition was calculated by employing the following formula:

Percent inhibition = C-T÷ C x 100 [13]. Where, C = Radial growth of negative control; T = Radial growth of the test

Column Chromatography

This was done using n-haxane, chloroform and ethyl acetate based on increasing polarity [14].

Phytochemical Analysis

The phytochemical analysis of fractionated *Picralima nitida* seed extract was carried out using standard methods [15].

Other test analysis for alkaloids, flavonoids, steroids and terpenoids, saponins, tannins carbohydrate, glycosides, resins (Precipitation test), proteins, fats and oil were carried using standard methods.

Data analysis

All generated data were subjected to statistical analysis using a one-way analysis of variance (ANOVA), followed by Dunnetts test (multiple comparison post-test) at p < 0.05.

RESULTS

Antifungal activities of the methanolic extracts of six medicinal plants were determined against five clinical isolates of fungi species. Miconazole nitrate was used as the standard for comparing the plant's extract. In Table 1, Miconazole nitrate at 500μ m/ml had the highest antifungal effect on the growth of

Cladosporium sp by inhibiting it completely with a percentage radial growth inhibition (PRGI) of 100 %. Picralima nitida seed extract exhibited the highest antifungal effect with all the three concentrations (Tables 2 and 3).

TABLE 1: PERCENTAGE RADIAL GROWTH INHIBITION OF POSITIVE CONTROL ANTIFUNGAL AGENT (MICONAZOLE) AGAINST FUNGAL ISOLATES

Fungal isolates	Concentr nitrate	ation of	Miconazole
	125 μg/ml	250 μg/ml	500 μg/ml
T.soudanense	89.2 %	90 %	93.8 %
T.mentagrophytes	75 %	78 %	85 %
Cladosporium sp	86.7 %	95 %	100 %
T.rubrum	94 %	95 %	98 %
Fusarium sp	82.9 %	75.3 %	95.3 %

TABLE 2: PERCENTAGE RADIAL GROWTH INHIBITION OF *PICRALIMA NITIDA* SEED EXTRACT AGAINST FUNGAL ISOLATES

Fungal Isolates	Concentrations of extracts				
	25 mg/ml	50 mg/ml	100 mg/ml		
	Radial Gro	owth inhibitio	n		
T.soudanense	100 %	100 %	100 %		
T.mentagrophytes	90 %	100 %	100 %		
Cladosporium sp	91.7 %	95 %	100 %		
T.rubrum	100 %	100 %	100 %		
Fusarium sp	94.1 %	100 %	100 %		

TABLE 3: PERCENTAGE RADIAL GROWTH INHIBITION OF *PICRALIMA NITIDA* SEED FRACTIONS AGAINST FUNGAL ISOLATES

Fungal Isolates	Hexane	Chloroform	Ethylether
	fraction	fraction	fraction
	(100	(100 mg/ml)	(100
	mg/ml)		mg/ml)
	Radial growth	Inhibition	
T.soudanense	55 %	77.1 %	26.7 %
T.mentagrophytes	-33.3 %	25 %	-66.7 %
Cladosporium sp	25 %	50 %	-50 %
T.rubrum	35.7 %	88.6 %	57.1 %
Fusarium sp	-14.3 %	83.3 %	0%

At 100 mg/ml the extract inhibited completely all the investigated isolates with PRGI of 100 %. At 50 mg/ml, there was complete inhibition against all isolates except against Cladosporium spp. and at 25 mg/ml it inhibited completely T.soudanense and T.rubrum. The inhibitions exhibited bv T.mentagrophytes, Cladosporium spp. and significant. Fusarium spp. were, however,

T.soudanense and T.rubrum appeared to be the most sensitive of the fungi under investigation. This was followed by T.mentagrophytes and Fusarium spp. with a PRGI of 90 – 100 %. Cladosporium spp. with a PRGI of 91.7 -100 % appeared to be the least sensitive. The inhibition range of Picralima nitida seed extract for all the isolates was 90 -100 %. There was no inhibition observed in the negative control. The PRGIs for the antifungal activities of Picralima nitida seed extract at 25 mg/ml, 50 mg/ml and 100 mg/ml, respectively were significant (P<0.05) compared with the standard.

The phytochemistry of the chloroform fraction of Picralima nitida seed revealed the presence of flavonoids, alkaloids, and terpenoids (Table 3). The highest antifungal effect was exhibited by Chloroform fraction with a PRGI of 88.6 % against T.rubrum. All the fungi under investigation appeared sensitive to the chloroform extract. Ethyl ether fraction had a lesser antifungal effect on the experimental isolates. There was a significant difference of (p < 0.05) in the treatment of chloroform fraction against control.

From Table 4, Picralima nitida rind extract had its highest antifungal effect at 100 mg/ml by inhibiting completely all the investigated isolates with a PRGI of 100 %. RGs of 0.03±0.03 mm to 0.1±0.05 mm at 25 mg/ml and 0.03±0.03 mm to 0.05±0.05 mm at 50 mg/ml were observed for all the isolates. The activities of Picralima nitida rind at these concentrations show a significant difference (p <0.05) compared to the activities of the standard antifungal agent. Table 5 shows that Azadirachta indica (Neem) seed extract did not inhibit any of the fungi completely at its highest concentration of 100 mg/ml. The inhibition range of Neem seed extract for all the isolates was 54.2-95.4 %. Neem seed was statistically significant (p < 0.05) only at 100mg/ml when compared with Miconazole nitrate.

INHIBITION OF PICRALIMA NITIDA RIND EXTRACT AGAINST FUNGAL ISOLATES Fungal isolates Concentration of Extracts

TABLE 4: PERCENTAGE RADIAL GROWTH

91.7 %. T.rubrum had a PRGI of 88-100 % and appeared to be the most sensitive followed by T.soudanense with a PRGI of 76.9-100 %. The percentage inhibition range of A.occidentale extract for all the isolates was between 23.5-100 % giving a significant difference (p < 0.05) on all the concentrations with respect to the standard control (Table 6),

TABLE 5: PERCENTAGE RADIAL GROWTH
INHIBITION OF AZADIRACHTA INDICA SEED
EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial growth	inhibition	
T.soudanense	86.2 %	95.4 %	89.2 %
T.mentagrophytes	64 %	94 %	94 %
Cladosporium sp	54.2 %	66.7 %	91.7 %
T.rubrum	94 %	94 %	95 %
Fusarium sp	88.2 %	79.4 %	89.4 %

TABLE 6: PERCENTAGE RADIAL GROWTH INHIBITION OF ANACARDIUM OCCIDENTALE LEAF (CASHEW) SEED EXTRACT AGAINST FUNGAL ISOLATES

	100211120		
Fungal isolates	Concentration of Extracts		
	25 mg/ml	50 mg/ml	100 mg/ml
	Radial grow	th inhibition	
T.soudanense	76.9 %	84.6 %	100 %
T.mentagrophytes	68 %	82 %	100 %
Cladosporium sp	43.3 %	80 %	91.7 %
T.rubrum	88 %	92 %	100 %
Fusarium sp	23.5 %	5 2.9 %	100 %

— TABLE 7: PERCENTAGE RADIAL GROWTH INHIBITION OF EUPHORBIA HIRTA LEAF EXTRACT AGAINST FUNGAL ISOLATES

		50 mg/ml 1		AGAINST FUNGAL ISOLATES			
	25 mg/ml		100 mg/ml	Fungal isolates	Concentrat	ion of Extra	cts
Tanulaunaa	Radial grov	wth inhibition	100.0/		25 mg/ml	50 mg/ml	100 mg/ml
T.mentagrophytes	92.3 % 80 %	95.4 % 94 %	100 % 100 %	Tooudananca	Radial grov	vth inhibiti	on
Cladosporium sp	86.7 %	91.7 %	100 %	T.souunense T.mentaaronhutes	30.8 %	40.2 %	100 %
T.rubrum	94 %	94 %	100 %	Cladosnorium sp	0%	33 3 %	100 %
Fusarium sp	90.6 %	94.1 %	100 %	T.rubrum	0%	60 %	100 %
				Fusarium sp	-5.9 %	35.3 %	100 %

Anacardium occidentale (cashew) leaf extract as shown in Table 5 had its highest antifungal effect of 100 % RGI at 100 mg/ml. All investigated isolates were completely inhibited at 100 mg/ml by the extract except for Cladosporium sp with a PRGI of

In table 7, Euphorbia hirta exhibited its highest antifungal effect at 100 mg/ml by completely inhibiting all the fungal isolates. No inhibition was observed in most of the isolates under investigation at 25 mg/ml. T.rubrum appeared to be the most sensitive fungi with a PRGI of 60 % at 50 mg/ml and 100 % at 100 mg/ml. However, the concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml had a significant difference of (p < 0.05) when compared with the Standard. In Table 8, Jatropha curcas exhibited its highest antifungal effect of 100 % at 100 mg/ml by inhibiting all tested isolates except for Fusarium sp. Mild inhibition of 58.3 % was observed at 25 mg/ml and 50 mg/ml for Cladosporium sp. Others appeared to be resistant at these concentrations.

TABLE 8: PERCENTAGE RADIAL GROWTH INHIBITION OF JATROPHA CURCAS LEAF EXTRACT AGAINST FUNGAL ISOLATES

Fungal isolates	Concentration of Extracts			
	25 mg/ml	50 mg/ml	100 mg/ml	
	Radial Growth Inhibition			
T.soudanense	38.5 %	53.8 %	100 %	
T.mentagrophytes	10 %	20 %	100 %	
Cladosporium sp	58.3 %	58.3 %	100 %	
T.rubrum	30 %	40 %	100 %	
Fusarium sp	29.4 %	47.1 %	97.1 %	

TABLE 9: PERCENTAGE RADIAL GROWTH INHIBITION OF ACANTUS MONTANUS LEAVES EXTRACT AGAINST FUNGAL ISOLATES

Fungal Isolates Concentration of extracts

25 mg/ml	50 mg/ml	100 mg/ml
Radial Growth inhibition		
84.6 %	92.3 %	100 %
52 %	64 %	100 %
50 %	66.7 %	100 %
-40 %	0 %	100 %
41.2 %	52.9 %	100 %
	25 mg/ml Radial Grov 84.6 % 52 % 50 % -40 % 41.2 %	25 mg/ml 50 mg/ml Radial Growth inhibiti 84.6 % 92.3 % 52 % 64 % 50 % 66.7 % -40 % 0 % 41.2 % 52.9 %

Cladosporium sp appeared to be the most sensitive fungi with a PRGI of 58.3-100 %. The extract was significantly higher (p < 0.05) at the three concentrations with respect to the standard. In Table 9 shows that Acanthus montanus at 100 mg/ml exhibited its highest antifungal effect by completely inhibiting all the tested isolates at a100 % RGI. T.rubrum and Fusarium sp were not inhibited at 25 mg/ml and 50 mg/ml of extract. T.soudanense appeared to be the most sensitive with a PRGI of 84.6-100 % followed by T.mentagrophytes with PRGI of 52-100 %. However, there was a significant difference (p < 0.05) in activities of the plant extract to the standard. The phytochemical studies of these plants revealed the presence of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Table 10).

TABLE 10: PHYTOCHEMICAL CONSTITUENTS OF
CHLOROFORM EXTRACT

S/No	Phytoconstituent	CE
1	Alkaloids	+
2	Carbohydrates	_
3	Flavonoids	+
4	Glycosides	_
5	Fats and oil	_
6	Tannins	-
7	Saponins	_
8	Resins	_
9	Proteins	_
10	Terpenoids	+
11	Steroids	_

Key: + = Present, - = Absent, CE = Chloroform extract

DISCUSSION AND CONCLUSION

The methanolic extracts of six different plant species were assayed for antifungal activities and compared with Miconazole nitrate using the agar plate method. Miconazole belong to the azole group of antifungal agents used clinically against fungal infections. They owe their antifungal activity by inhibiting the enzyme lanosterol 1,4-α-demethylase; the enzyme necessary to convert lanosterol to ergosterol, which is the predominant sterol in fungal cell membranes responsible for maintaining cell integrity, viability, function and normal growth [16]. Results from this study showed that the methanolic extract of Picralima nitida (seed and rind), Azadirachta indica (Neem), Anacardium occidentale (Cashew), Euphorbia hirta, Acantus montanus and Jathropha curcas exhibited antifungal activity against T. soudanense, T.mentagrophyte, T. rubrum, Cladosporium sp and Fusarium sp. These plants were considered to possess biochemically related substances with similar inhibitory properties with miconazole with the antifungal activities varying differently at the same concentrations of each of the extracts against the
respective fungal isolate. It generally increases with increase in concentration of the extracts. This finding agrees with other study [17], which showed higher concentrations of antimicrobial substances led to appreciable growth inhibition.

The phytochemical studies of these plants also reveal the presence of secondary metabolites such as tannins, terpernoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Table 10). The antifungal activities of these plants may probably be due to the presence of these rich secondary metabolites in plants which is tandem with other studies [18, 19, 20]. The patterns of radial growth inhibition of these plant extracts in this study were similar to those of miconazole nitrate. This could suggest the presence of similarly active ingredients present in Miconazole nitrate (the control antifungal agent) which is used predominantly as a drug of choice against superficial fungal infection especially Trichophyton spp, Epidermophyton spp and Micosporium spp. The activities of Picralima nitida seed extract appeared to be better than the other plant extracts investigated. This could be based on the concentration of active antifungal ingredients such as the phenolics (Tannins and flavonoids) present in that part of the plant material. The activities of Picralima nitida (seed and rind) in this study justifies its use in the treatment of skin conditions including of Tinea corporis (ringworm of the skin), Tinea capitis (ringworm of the head) as reported by other works [21, 22]. Azadirachta indica (Neem) seed oil has been used in the treatment of various skin infections by alternative system of medicine [23]. Its activities in this study are in agreement with the work done [24], where neem oil was shown to have different inhibitory effects on different fungi including T. rubrum, T. mentagrophytes and Fusarium sp. However, though there was no complete inhibition observed in this study with any of the fungi, which could be due to neem oil from different localities may possess different rates of the antifungal activities with significant effect on all the fungal species tested. This variation could be due to the difference in the quality of the active ingredients in the oil sample. The leaves of Anacardium occidentale (cashew) in this study exhibited poor inhibitions at lower concentrations, though Rajesh et al [25] reported that, the nuts have proven antifungal properties of more than 94 % inhibition against Aspergillus fumigatus, A niger, Curvalaria sp and Fusarium sp. This is an indication of uneven distribution of the active ingredients of the plant. Euphorbia hirta exhibited resistance at lower

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concentrations on some of the isolates, but complete inhibition at a higher concentration. This finding agrees with the other findings [9], as sensitivity increases with increase in concentration. Jatropha curcas in this study showed a poor inhibition of 10 % and 30 % at 25 mg/ml for T. mentagrophytes and T.rubrum respectively. However, Adejumo et al. 2009 who worked on Fusarium sp observed it to be the most resistant. The difference in their susceptibilities could be attributed to an inherent resistance factor of the test organisms among other factors, though other studies [26, 27] observed that, the leaves of Jatropha curcas had no inhibitory effect against T. mentagrophytes rather it was observed in the seed extract. This discrepancy is likely due to the fact that no two plants of the same species may have experienced the same environmental challenges considering the fact that plants phytoanticipins are uniformly distributed within the plants while phytoalexins are restricted to the tissue colonized by the fungus and the cells surrounding the infection site [28]. Some antifungal compounds may be present constitutively in one part of a plant, but induced as phytoalexins in other organs. Acantus montanus had not really been widely used in folk medicine for the treatment of skin diseases, rather for pain, inflammation and other ailments [29, 30]. Its antifungal activities in this study support its usage in folklore treatment. T.rubrum and T.soudanense appear to be the most sensitive fungi at lower concentrations of the extracts. This could be due to the difference in the concentrations of the bioactive components in the sample or synergistic reactions of the various phytochemicals in the extract.

Euphorbia hirta (Asthma weed), Anacardium occidentale L (Cashew), Picralima nitida (Akuamma plant), Jatropha curcas (Barbados nut), Azadirachita indica A (Neem plant), and Acantus montanus (Mountain thistle) have antifungal activities. Their antifungal activities increase with increase in concentrations. Crude extract of Picralima nitida seed possesses the highest antifungal activity. The phytochemistry of its chloroform fraction reveals the presence of flavonoids, alkaloids and terpenoids. Trichophyton rubrum was the most sensitive fungal under investigation. Further investigation of the purified components of the seed extracts of Picralima nitida to determine the metabolites responsible for their activities will make it serve as a good base for consideration in the pharmaceutical industries for the production and packaging of antifungal products.

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ORIGINAL ARTILE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY ISBN 1595-689X JULY 2018 Vol.19 No.3 AJCEM/1832 http://www.ajol.info/journals/ajcem COPYRIGHT 2018 https://dx.doi.org/10.4314/ajcem.v19i3.11 http://dx.doi.org/10.4314/ajcem.v17i4.1 AFR. J. CLN. EXPER. MICROBIO. 19 (3): -229-237

INDENTIFYING AND MODELING THE DISTRIBUTION OF CRYPTIC RESERVOIRS OF EBOLA VIRUS USING ARTIFICIAL INTELLIGENCE

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Running title: Distribution of Cryptic Reservoirs of Ebola Virus

ABSTRACT

Fruit bats (Megachiroptera) have been found to be the principal reservoirs of Ebola virus (EBOV) to humans. However, bats do not appear to be the primary reservoir in the environment and between outbreaks. The cryptic reservoir species of EBOV and its distribution have not been identified. The purpose of the study was to identify the most likely cryptic reservoir species of EBOV and the probable distribution of cryptic reservoir species where EBOV could be maintained in Sierra Leone. The Bioagent Transport and Environmental Modeling System (BioTEMS) was used to analyze mammals, arthropods, plants and protists in order to identify the most likely species to be the cryptic reservoir for EBOV. ArcGIS and BioTEMS were used to determine the probable distribution of cryptic reservoir species. BioTEMS identified free-living pathogenic amoebae (FLPA) as the probable cryptic reservoir species (Test Performance = 93.3). Diptera in the order Chrysops were also identified as possible secondary reservoirs and mechanical vectors of EBOV. Distribution of likely hot spots for FLPA and phytotelmata/tree-holes were identified in several regions of Sierra Leone, primarily in the southeast and are similar to those predicted by other authors, but at a much higher resolution (15 m for BioTEMS verses up to 5 km in other studies). Water-filled cavities (phytotelmata), specifically tree-holes, were identified as the most likely sites for the cycle of transmission to occur among FLPA and susceptible secondary reservoirs. Free-living pathogenic amoebae are not only pathogenic to humans and animals but they serve as reservoirs and Trojan horses of infection as well. Identifying what and where cryptic reservoirs of EBOV persist between outbreaks provides an opportunity for the first time to conduct environmental epidemiologic surveillance to mitigate outbreaks and to test anti-microbial delivery systems such as the ProVector® to reduce EBOV and FLPA.

Keywords: Filovirus, Amoeba, Epidemiology, Machine Learning, Vector, Disaster Management

IDENTIFICATION ET MODÉLISATION DE LA DISTRIBUTION DES RÉSERVOIRS DU VIRUS EBOLA CRYPTIQUE EN UTILISANT L'INTELLIGENCE ARTIFICIELLE

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Runimg Email Titre : Distribution de réservoirs cryptique du virus Ebola

Résumé

Des chauves souris (Megachiroptera) ont été trouvés à être les principaux réservoirs du virus Ebola (EBOV) à l'homme. Cependant, les chauves-souris ne semblent pas être le réservoir principal de l'environnement et entre les poussées. Les espèces réservoirs cryptique du virus Ebola et de sa distribution n'ont pas été identifiés. Le but de l'étude était d'identifier les plus susceptibles d'espèces réservoirs cryptique EBOV et la distribution probable des espèces réservoirs cryptique où EBOV pourrait être maintenu en Sierra Leone. La modélisation des transports et de l'environnement Bioagent Système (BioTEMS) a été utilisé pour analyser les mammifères, les arthropodes, les plantes et les protistes afin d'identifier les espèces les plus susceptibles d'être le réservoir pour EBOV cryptique. ArcGIS et BioTEMS ont été utilisés pour déterminer la distribution de déterminer l'évolution probable des espèces réservoirs.

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BioTEMS a identifié des amibes pathogènes (FLPA) comme le probable espèces réservoirs cryptiques (Test Performance = 93,3). Dans l'ordre des Diptères Chrysops ont également été identifiées comme vecteurs mécaniques secondaires possibles de EBOV. La distribution des points chauds susceptibles de FLPA et phytotelmata/arbre-trous ont été identifiés dans plusieurs régions de la Sierra Leone, principalement dans le sud-est et sont similaires à ceux prévus par d'autres auteurs, mais à une beaucoup plus haute résolution (15 m pour BioTEMS les versets jusqu'à 5 km dans d'autres études). Cavités remplies d'eau (phytotelmata), en particulier les trous d'arbres, ont été identifiés comme sites les plus probables pour le cycle de transmission de se produire entre FLPA et sensible des réservoirs secondaires. Des amibes pathogènes ne sont pas seulement des pathogènes pour l'homme et les animaux, mais ils servent de réservoirs et les chevaux de Troie de l'infection. Identifier ce qui et où les réservoirs de EBOV cryptique persistent entre les poussées est l'occasion pour la première fois d'effectuer la surveillance épidémiologique de l'environnement pour atténuer les épidémies et de tester les systèmes de prestation anti-microbiens tels que le ProVector® pour réduire EBOV et FLPA.

Mots-clés: Filovirus, amibes, l'épidémiologie, de l'apprentissage machine, scénario, la gestion des catastrophes

INTRODUCTION

The first cases of Ebola virus (EBOV), family Filoviridae, appeared in 1976, causing acute and often fatal illness. There have been five species described, with the Zaire EBOV having the highest rate of fatality, 88% (1). Since its discovery, there have been over 31,000 cases and nearly 13,000 deaths attributed to EBOV, most of which occurred in the latest outbreak in 2014-2016 (2). The index case for the 2014-2016 outbreak was traced to a boy living in Meliandou, Guinea who was most likely playing near a large harboring bat (2,3). From Guinea it spread to Sierra Leone, Nigeria, Liberia, Nigeria, Senegal, Spain, the United States and Mali, with Sierra Leone experiencing the most cases and the second highest number of fatalities. In a retrospective epidemiologic study, EBOV was introduced into Sierra Leone by a woman who had visited the home of the index case in Meliandou (4). Sierra Leone is within the area modeled where the mammalian reservoir species, e.g. fruit bats, and environmental conditions are suitable to sustain EBOV transmission but no zoonotic transmission has been recorded.5 However, the identity of the cryptic reservoirs of EBOV and where these infected reservoirs are located have not been modeled in Sierra Leone or in other areas where EBOV may persist.

Great progress has been made in understanding the pathogenesis and molecular biology of EBOV; however, the maintenance and transmission of EBOV in the environment remains unclear (6,7). In a review of animal sampling, suggested four strategies which should be undertaken for the purposes of identifying reservoir sources and to isolate suspected virus in an animal outbreak; 1) surveillance of free-ranging nonhuman primate mortality and morbidity,2) investigation of every wildlife morbidity or mortality event as this may hold the most promise for locating virus or viral genome sequences, 3) surveillance of suspected bat species to detect evidence of exposure, and 4) prioritization of dogs and pigs for testing, including morbidity, mortality, and serology to detect virus or previous exposure (8). Pourrut noted that human outbreaks and animal mortality do not appear to be reliable indicators of the presence of the virus in a region, and suggested antibody detection may serve as

a better epidemiological indicator of the presence of EBOV in an area (9).

The three bat species suspected of serving as the primary reservoirs of EBOV are in the suborder (Megachiroptera); Hypsignathus monstrosus, Epomops franqueti, and Myonycteris torquata; other mammals may become infected when eating fallen fruit contaminated with saliva or feces from infected bats (10). Insectivorus bats (Microchiroptera) may also serve as reservoirs of EBOV in West Africa (1). The source of EBOV infection in bats has not been determined. The conditions of EBOV transmission from bats to great apes or even to humans are also unknown; environmental factors, animal demography and viral factors may all contribute to the outbreak of EBOV, e.g. during dry seasons when great ape mortality increases from EBOV, two of the three primary bat species give birth, and when apes and bats compete for scarce fruit.9 Species positive for EBOV and/or EBOV antibodies, polymerase chain reaction (PCR) and isolation have been identified in several mammal orders: Primates, Chiroptera, Artiodactyla, Carnivora and Rodentia (5,8,11).

Species other than mammals have been hypothesized as possible reservoirs for EBOV. Monath suggested possible non-mammalian reservoirs of EBOV could include plants, blood-feeding and other arthropods, and that step wise mutation may be an alternative to transmission through a cryptic reservoir (12). Despite a relatively long search for the origin of Ebola viruses, their reservoirs remain elusive; including origin and ecology of Ebola viruses. In a study of Marburg virus, over 2,000 insects were tested by PCR using a Filovirus specific primer set; all were negative (13). Leendertz recommended biologically plausible hypotheses of persistence and transmission be developed that may not align with current dogma; suggesting EBOV emerges in mammals when the precursor virus jumps from mayflies or other riverine insects to insectivorous bats, made possible by the thermodynamics of the digestive system of insectivorous bats (14). In a thermodynamic model, to support the possibility emphemeral insects may serve as reservoirs Gale proposed the following; 1)

the evidence that arthropods are refractory is not definitive, 2) a combination of filovirus filament length and the high temperature of the insect virus once ingested by a flying bat, in combination with the large number of insects eaten by bats (e.g. during an ephemeral mass emergence of mayflies), facilitated jumping the species barrier, 3) phospholipid phosphatidylserine in the virus envelope promotes filament formation through fusion of single glycoprotein (Gc) particles within the ingested insect within the high temperature of the bat, increasing their infectivity to bats, and 4) increase the affinity of the filaments for bats, while having no effect on the binding affinity of the single Gc virions (15).

Despite decades of research and thousands of animals tested and over 40 years of research, monitoring of EBOV is limited or impossible due to the paucity of knowledge about its ecology e.g. when, where, and how EBOV circulates in the environment (1,16). The exact reservoir species that harbors the virus is not determined, although most wildlife epidemiologic efforts have been focused on fruit bats(17). Future sampling campaigns, in-depth serological studies, and modeling efforts should take into account the possibility that fruit bats may not always be the ultimate source of EBOV outbreaks.1 Where EBOV survives between outbreaks and replicates is unknown; this unknown species is defined as the cryptic reservoir of EBOV. There are two opposing hypotheses for the emergence of EBOV, the first is long-term local persistence in a cryptic and infrequently contacted reservoir; the second is a recent introduction of EBOV with spreading through susceptible populations (6). Here we present a hypothesis that free-living pathogenic amoeba (FLPA) serve as the cryptic reservoir of EBOV, with susceptible mammalian species and possibly invertebrate reservoirs becoming infected through contact with FLPA in the environment, most likely via phytotelmata. Also presented is the probable distribution cryptic reservoirs where EBOV could be introduced and maintained in Sierra Leone.

MATERIAL AND METHODS

Biotic and abiotic factors were analyzed in order to determine the location of the probable cryptic reservoir species. Niche analysis of protists, mammals, arthropods and plants was conducted to identify the most likely cryptic reservoirs of EBOV. ArcGIS geospatial analysis software, Statistica statistical software, and the Bioagent Transport and Environmental Modeling System (BioTEMS) were used to analyze geographic information and conduct data analysis. BioTEMS utilizes up to several hundred abiotic and biotic factors to produce risk and vulnerability

assessments for biological agents and infectious diseases. Examples of biotic and abiotic factors include pathogen strain, nucleic acid sequences, vector/host relationship, vectorial capacity, host/vector physiology, colonization ability, population dynamics of hosts and vectors, plants species, soil, and weather conditions, such as wind, temperature, precipitation, and shade. Analytical methods within BioTEMS include artificial intelligence, fuzzy logic, niche analysis, Baysian and general additive regression. The BioTEMS consists of a set of algorithms and models that have been used as a stand-alone system for risk assessments of bioagents, infectious diseases, vectors, and to supplement HPAC. BioTEMS has been used to analyze risk of selected bioagents and/or to optimize placement of Biological Integrated Detection Systems (BIDS) units at military installations within the US and overseas, during national and international training exercises, national political conventions and to assist the Defense Threat Reduction Agency during a presidential inauguration. The BioTEMS risk assessment maps (RAMS) have also been developed for several infectious and zoonotic diseases and distribution of vectors e.g., Zika, West Nile and Eastern Equine Encephalitis viruses, Lyme disease, Rocky Mountain spotted fever, avian influenza, plague, Shigella, tularemia, mosquito, tick and mite species. The BioTEMS RAMS have been produced for several countries, including Bangladesh, Brazil, Cameroon, China, India, Iran, Libya, Georgia, Turkey, United Arab Emirates, Sierra Leone, and several cities in the US (Kollars in press). BioTEMS was used in the present study to analyze abiotic and biotic factors to identify the location of sites of hot spots for cryptic reservoirs of EBOV and to conduct niche analysis to determine likely reservoir species of mammals, arthropods, plants and microbes. BioTEMS and ArcGIS were used to identify locations of hot spots and validate the model using geographic coordinates of published EBOV cases, isolations and other published models of EBOV.

RESULTS AND DISCUSSION

BioTEMS identified phytotelmata/ tree holes as the principal microhabitat for cryptic reservoir species of EBOV, and where long term survival of EBOV between outbreaks and the cycle of EBOV transmission occurs. BioTEMS identified free-living amoeba, specifically freeliving pathogenic amoeba (FLPA) species of Acanthamoeba, Naegleria and Balmuthia, as the most likely cryptic species of EBOV; other FLA such as Hartmanella and Dictyostelium may also serve as reservoirs. Transmission in tree holes is predicted to occur among FLA and secondary reservoirs, principally fruit bats, secondarily in primates, and possibly arthropods (Test Performance = 93.3). Distribution of likely hot spots for FLPA and phytotelmata/tree-holes are shown in Figure 1.



FIGURE 1 BIOTEMS RISK ASSESSMENT MAP OF EBOV AND FLPA IN SIERRA LEONE (RED POLYGON- HIGHEST RISK OF ENVIRONMENTAL SURVIVAL OF EBOV Orange polygon- moderate risk of EBOV, Yellow polygon-lower risk of EBOV; blue circles identify recommended sites for EBOV

surveillance and environmental sampling.

The predicted sites for the cryptic species are similar to those predicted by other authors, but at much higher resolution than most (15 m for BioTEMS verses up to 5 km in previously published models.5, 18,19 BioTEMS identified species within the genus Chrysops as a secondary reservoir and mechanical vector of EBOV. It is unclear whether EBOV can replicate within arthropods. One laboratory study demonstrated replication of Marburg virus, another Filovirus, in the mosquito Aedes aegypti; other studies failed to demonstrate EBOV replication in Aedes aegypti (20,21). A lesion from a fly or spider bite coincided with a patient with Marburg virus (22). Whether arthropods can mechanically transport EBOV has also not been demonstrated. Laboratory trials indicated low potential for mechanical transmission of EBOV by house flies under the conditions tested, but repeated expsosure from EBOV/fly exposures could reach the 100 plaque forming units/ml threshold necessary to result in mucosal transmission (23).

In addition to vertebrates (proven) and invertebrates (hypothesized) as reservoirs or mechanical vectors for EBOV, we suggest free-living pathogenic amoeba (FLPA) are the most likely cryptic and maintenance reservoirs of EBOV. That BioTEMS accurately predicted FLPA as the cryptic reservoir is supported by biological and environmental evidence; 1) FLPA act as suitable hosts for invasion and replication for several pathogens (bacteria and viruses), 2) FLPA can vector pathogens into hosts, 3) FLPA infect vertebrates and invertebrates 4) FLPA protect pathogens from harsh environmental conditions, 5) FLPA are effective air-borne colonizers of micro-habitats. 6) FLPA can survive harsh environmental conditions for decades, 7) FLPA undergo

blooms when environmental conditions are right, and 8) some species of pathogens become more pathogenic when passaged with FLPA. Figure 2 demonstrates the life cycle during wet and dry periods. During suitable environmental conditions, some FLPA species such as *Acanthamoeba* are in the amoeba form, other species, e.g. Naegleria can exist as an amoeba or a flagellate form. During harsh environmental conditions the FLPA can encyst in order to survive the inhospitable conditions of their microhabitat.

Ebola viruses (EBOV) are enveloped RNA viruses that infect cells through a pH-dependent process mediated by viral glycoproteins involving endocytosis of virions and their routing into acidic endosomes (24,25). Host cell factors used by EBOV to invade macrophages include Mer, integrin, and NPC1 are required for efficient GP-mediated transduction and EBOV infection of macrophages (26). Perhaps these same mechanisms enable EBOV to infect free-living amoebae (FLA). Freeliving amoebae are virtually free-living macrophages living in the environment. FLPA may be more suitable reservoirs than closely related non-pathogenic species/strains due to cell membrane composition. For example, Naegleria fowleri demonstrated a higher concentration of integrin-like proteins than N. lovaniensis (27). Perhaps, the integrin-like protein in N. fowleri enables binding and entry of EBOV. Several bacteria and virus pathogens are able to invade and replicate within FLPA and during replication they are protected from harsh environmental conditions and even anti-microbials when the FLPA encyst The(28,29). cyst of FLPA can stay viable for at least 20 years, (30) and we suggest, serve as a maintenance reservoir for EBOV. Although often temporal, tree holes can serve as

exceptional aquatic micro-ecosystems for several microbial species and are hotspots for microfungi (31). Protists feed upon micro-fungi and bacteria and have been found in phytotelmata ecosystems, on tree limbs and llianas (32,33). Larval mosquito species decrease several forms of protists in tree holes, such as ciliates; however, cyst stages become more abundant (34). Cyst forming amoebae appear to be very effective colonizers of aquatic habitats, and have even been isolated in the atmosphere.³⁵ Naegleria fowleri, the etiologic agent of primary amoebic meningo-encephalitis is found in soil, water, wastewater, and biofilms, even on rough top tanks (36). Temperature and bacterial density are positively correlated with the population density of N. fowleri biofilms (37). In addition to air-current transport of infected FLA cysts, mechanical transport of infected cysts or secretions from infected reservoirs, could deposit material in tree-holes, thereby infecting FLPA inhabiting the tree hole and secondary hosts utilizing resources in the treehole.

Among the FLA, only species within four genera, Acanthamoeba, Naegleria, Balamuthia and Sappinia, are responsible for opportunistic and non-opportunistic infections in mammals (38,39). Infected animals can potentially contribute spreading in pathogenic Acanthamoeba in homes by contaminating the domestic environment; bedding, fur, cornea and tissue may be infected with FLPA (40). Free-living pathogenic amoebae are also able to infect insects, such as locusts, mosquitoes, and biting flies. Larvae of syrphid fly larvae, Eristalis tenax, may have been infected with Marseillevirus either by direct ingestion or through the ingestion of an infected free-living pathogenic amoeba (41). Acanthamoeba can invade and cause disseminated infection within insects likely providing a vehicle for bacterial and viral pathogens to be disseminated as well (42). Mycobacterium species, a pathogen that replicates in several free-living amoeba species, can be mechanically transmitted by Syrphid flies to pigs (43). Mycobacterium ulcerans, the etiologic agent of Burndi ulcer, is acquired from the environment, but the exact mode of transmission remains a mystery. Mycobacterium ulcerans presents an example of a pathogen that may be transmitted by free-living pathogenic amoeba or through an insect vector. Laboratory trials demonstrated ICR mice were infected by M. ulcerans through passive infection through skin abrasions, and co-culturing of M. ulcerans-with AP enhances pathogenesis (44).

In Australia, *M. ulcerans* was detected by PCR in several species of mosquitoes during peak transmission time, and the hypothesis was proffered that transmission by mosquitoes offers a partial explanation for outbreaks in southeastern Australia (45). Viruses and bacteria are often isolated from the same soil and water environments as are amoebae, and experimental models using *Acanthamoeba spp.* and *Dictyostelium discoideum* and other FLA have demonstrated these organisms are resistant to digestion by free living amoeba, are able to replicate and the FLA are a training ground for pathogens (46,47).

Several studies have demonstrated the ability of FLPA to infect mammals. Contact with Naegleria species by wild mammals was demonstrated for the first time with the discovery of antibodies to FLA in several mammalian species with variation among species and age groups (48). Several species of primates have been infected by Balmuthia; the mandrill (Papio sphinx), whitecheeked gibbon (Hylobates concolor), a western lowland gorilla (Gorilla gorilla), Kikuyu colobus monkey (Colobus guereza) (49). In zoos in America, infection with B. mandrillaris has been reported to account for 2.8% of captive gorilla deaths in North America over the past 19 years (50). In Africa, Balmuthia and Acanthamoeba have been found in humans living in the Tai Forest area of Côte d'Ivoire, near the Liberian border; interestingly the prevalence of Balmuthia was correlated with hunting activity and age in this area where hunters may have become exposed to Balmuthia through handling bloody meat or exposure to the environment through cuts and Balmuthia and Acanthamoeba are abrassions (51). capable of infecting through abrasions in the skin (39). Acanthamoeba, N. fowleri, and B. mandrillaris have been isolated from water bodies from Guinea-Bissau, and this is the first report of the isolation of B.mandrillaris from environmental sources in Africa (52). Naegleria and Acanthamoeba species have also been isolated from Benin (53). Acanthamoeba and Naegleria species have been isolated from the Queen Elizabeth Park in the same area of western Uganda, where the first outbreak of Bundibugyo EBOV occurred in western Uganda.54,55 This does not mean that there is causation, however, it does demonstrate the wide dispersion of FLPA in areas where EBOV is present at least temporarily. There would seem to be an opportunity for EBOV to interact with FLPA in the environment and within infected mammals (Figure 2).



FIGURE 2 PHYTOTELMATA (SUCH AS TREE HOLES) SERVE AS SITES FOR INTERACTION AMONG CRYPTIC RESERVOIRS (FREE-LIVING PAGHOGENIC AMOEBAE), SECONDARY RESERVOIRS (SUCH AS BATS, PRIMATES, RODENTS) AND POSSIBLY MECHANICAL VECTORS (CHRYSOPS SPECIES), ALLOWING EBOLA VIRUS TO SURVIVE BETWEEN OUTBREAKS, REPLICATE AND INFECT SECONDARY RESERVOIR (Illustrated by Kathryn E. Kollars)

Reperant, suggested the application of the theory of island biogeography, developed by MacArthur and Wilson, to emerging pathogen epidemiology to identify: 1) interactions among recipient host species, reservoirs and vectors, 2) intraspecific interactions of reservoir interactions, and 3) mechanism driving disease emergence within host species (56,57). Application of the theory of island biogeography to emerging pathogens may assist in predicting from which animal species future zoonotic and vector-borne pathogens are to emerge most likelv to and identify arthropod/parasite/host interactions and distribution Habitat islands, created by human activity, (56,58). have a major impact on biodiversity (59). Forest fragmentation creates habitat islands associated with EBOV. Spillover from wildlife reservoirs of EBOV to humans occurred mainly in hotspots of fragmented forest and reduction of closed forests (60,61).

Tree holes can be described as islands in the forest; the diversity of microbial communities utilizing tree holes can vary depending on size, and tree holes in fact serve as island reservoirs for species, protecting them from a hostile environment (62,63). Tree holes are often created when trees are damaged, and logging often damages adjacent trees. Free-living amoebae thrive in islands of fertility, were water penetrates and remains in gaps (64). Fruit bats and other bat species will use tree holes as roosting places in West Africa (65,66). Monkeys and apes use tree holes as a source for drinking water, during habitat-specific and seasonal water shortages (.67)

Host and spatial heterogeneity are linked and management strategies can be developed to quantitatively assess the contribution of habitat patches, pathogens, and reservoirs to cycles of transmission (68). Niche models are also used to identify patterns of pathogen distribution. Peterson stated that limitation using GARP niche models may occur due to current small sample sizes and ecologic dimensions of the model that do not capture other dimensions that may be more important, such as climate variability.¹⁸ There is host specificity in the filovirus host-parasite system with patterns of combulerery and codistribution and they survey for EBOV; 1) African EBOV reservoirs would be primarily be found in evergreen broadleaf forest, 2) the main focus of the geographic distribution of the reservoir(s) would be in the Congo Basin; 3) a disjunct distributional area would be present in West Africa; 4) a related taxon in eastern Africa would range in more arid habitats; 5) the reservoir would belong to a clade more broadly distributed across Africa and Southeast Asia(18). BioTEMS identified the probable cryptic reservoirs of EBOV and was used to map its potential distribution between and during outbreaks.

Conclusion

BioTEMS has been used to accurately predict the presence and distribution of infectious diseases, vectors and to develop risk assessment maps for bioagents at military installations (69). Several members of the FLPA fit the characteristics of the prioritized EBOV reservoir; 1) protists are found in high density in phytotelmata, 2) Chiroptera may play a critical role in distribution of infected FLPA as well as infecting FLPA in the Congo Basin, when roosting or drinking from tree-holes, 3) phytotelmata are microhabitat islands within the fragmented forest islands that would serve as abundant and optimal habitats for the cycle of transmission between FLPA and secondary reservoirs, and 4) FLPA are found at higher densities in habitat islands in arid habitats, and 5) there is increased evidence of the broad distribution of FLPA in tropical environments. There is evidence that FLPA serve as the cryptic reservoir for EBOV; however this needs to be tested across western Africa by field sampling and in laboratory trials. Antimicrobial technologies, auto-disseminated through devices such as the ProVector®, may be utilized to reduce EBOV in FLPA in microhabitats where hotspots have been identified and to keep EBOV below the epidemiologic threshold, thereby reducing the risk of an EBOV outbreak. Additional studies should be conducted to determine the environmental epidmiologic threshold required for an EBOV outbreak in order to assist medical and public health officials in developing medical prevention and emergency response plans to reduce morbidity and mortality due to EBOV

ACKNOWLEDGMENTS: We wish to acknowledge the efforts and sacrifices of the health care workers who paid with their lives during the concluded EBOV outbreak in Sierra Leone and other countries in West Africa. Among these colleagues is our former Afro European Medical and Research Network Country representative and Medical Coordinator in Sierra Leone, Dr. Sheik Umar Khan MB. Ch.B, MWACP, Physician Specialist, In-Charge Lassa Fever Program, Kenema Government Hospital (KGH) and In-Charge, Internal Medicine Department, KGH, Ministry of Health and

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