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# PERCEPTION OF THE EFFICACY OF ARTEMISININ-BASED COMBINATION THERAPY (ACT) AND CHLOROQUINE PRESCRIPTION PATTERN AND AMONG NURSES IN SOUTH-WEST NIGERIA

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**ABSTRACT**

**Background-Malaria remains a threat to millions of children despite the recent advances recorded in the fight against the disease which remain the 3rd largest killer of children below the age of 5 years in endemic regions. Drug resistant plasmodium species continues to limit the fight against malaria, while the spread of fake and substandard antimalarial drugs has been recognized as a major problem across Africa because of its association with drug resistant parasite. We aim to find out the prescription pattern of chloroquine among nurses in South-West Nigeria and perception of artemisinin-based combination therapy (ACT).**

**Design and methods-About 180 pre-tested questionnaires were administered to randomly selected nurses out of which 155 were sufficiently completed and suitable for analysis. Results-Majority (56.1%) still have confidence in the efficacy of CQ which was still being prescribed by 45.2% of the respondents. CQ was mostly prescribed by those who had previous ACT treatment failure experience (54.3%) with their patients, P=0.03; as well as those who believe that ACT resistance malaria is now in circulation (44.3%). Fifty (32.3%) of our respondents claimed that they had come across fake and substandard ACT, from which 40.0% now prescribe CQ.**

**Discussion-The high rate of CQ prescription in this study showed that many of the health workers were still resistant to the change in antimalarial treatment policy, which is related to unsatisfactory experience with ACT. Additional measures are urgently required to verify this experience so as to win the confidence of healthcare workers away from chloroquine.**

**Key words- Artemisinin-based combination therapy (ACT), chloroquine, substandard antimalarial**

# LA PERCEPTION DE L'EFFICACITE DE LA THERAPIE COMBINEE A BASE D'ARTEMISININE. (ACT) ET LA MODELE DE PRESCRIPTION DE CHLOROQUINE CHEZ LES INFIRMIERS AU SUD – OUEST DU NIGERIA.

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**RESUME :**

**Contexte : Le paludisme reste une menace aux millions d'enfants en dépit des progrèsrécentsenregistrés dans la lutte contre la maladie et ce qui reste la troisième cause de décès des enfants de moins de 5 ans dansles régionsendémiques. Les espèces de plasmodium résistants aux médicaments continuent à limiter la lutte contre le paludisme, alors que la dispersion des drogues anti paludismes contrefaits et non conformes a été reconnu comme un problème majeur à travers l'Afrique en raison son association avec parasite résistant aux médicaments. Nous nous efforçons de trouver le modèle de prescription de chloroquine parmi les infirmiers au Sud – Ouest du Nigeria et la perception de thérapiecombinée à base d'artemisinine (ACT).**

**Conception and Méthodes : Environ de 180 questionnairespré – testes ont été administrés aux infirmiers choisis au hasard dont 155 ont été suffisamment complétés et appropries pour analyser.**

**Résultats : La majorité (56,1%) ont encore confiance dans l'efficacité de CQ qui était encore prescrit par 45,2% des répondants. CQ était surtout prescrit par ceux qui ont eu une expérienceprécédente de l'échec du traitement de ACT (54,3%)avec leurs patients=0,03, et également ceux qui croient que la résistance du paludisme au ACT est maintenant en circulation (44,3%). Cinquante (32,3%) de notrerépondants ont maintenu qu'ils avaient rencontré par hasard un ACT contrefait et non confirmé, desquels 40,0% prescrivent maintenant CQ.**

**Discussion : Le taux élevé de prescription de CQ dans cette étude a montré que beaucoup de travailleurs de santéétaient encore résistants au changement de po ;itique de traitement antipaludique, qui est lié à l'expérience insatisfaisante avec ACT. Méthodessupplémentaires sont nécessaires de toute urgence pour vérifier cette expérience pour gagner la confiance des travailleurs de la sante de la chloroquine.**

**Mots – clés : La thérapie à base d'artemisinine ; (ACT), Chloroquine antipaludique non conformé.**

**INTRODUCTION**

Malaria is among the top 3 killers of children all over the world where it is estimated that more than 3 billion people are at risk of infection. About 198 million malaria cases with associated 584,000 deaths were estimated to have occurred all over the world in 2013 (1). Malaria burden is heaviest in Africa where about 90% of all the death occurs, with 78% of such deaths in children below the age of 5 years. Malaria is also the 3rd on the list of top ten causes of death in Nigeria, and the country is said to incur malaria death than any other country in the world (2). The current malaria control strategy include the use of long lasting insecticide treated nets (LLITN), use of indoor residual insecticide spray (IRS), prompt diagnoses and treatment of cases with artemisinin bases combination therapy (ACT). Malaria control has been hampered by the development of resistance by mosquitoes to commonly used insecticides as well as development of resistance by plasmodium species to every antimalarial drug that has ever been deployed in malaria chemotherapy.

Chloroquine (CQ) has been in the frontline of malaria treatment and control since it was first discovered in 1934. It is also said to be the most successful antimalarial drug of all time (3,4). Resistance against CQ was believed to have emerged from South America (5) and South East Asia (6) from where it spread to West Africa and other countries of the world (7,8). The reduced efficacy of CQ prompted the WHO to come up with the policy change in antimalarial chemotherapy whereby it was agreed that CQ should be substituted as the first line drug in countries where resistance is more that 25% threshold (9).

Nigeria formally adopted policy change from CQ to ACT in 2004, and since then, there were many challenges from health care workers as well as patients in terms of compliance to the new treatment guidelines (1).

The most recent WMR which showed that prevalence of malaria infection in Sub-Sahara Africa among the children aged 2-10 years fell from 26% in 2000 to 14% in 2013. And in Nigeria which has the largest number of global malaria death, under 5 mortality rate also reduced from 213 per 1,000 live birth in 1990 to 117 per 1,000 live birth in 2013 (10). Against the background of this positive development, only about 9-26% of children with malaria as at 2013 were treated with ACTs. Some of the reasons behind this was said to be due to the fact that many of the children did not seek treatment, while many of those who sort treatment were not given any antimalarial drug (1).

A study conducted in 2013 on the use of ACT in Nigeria showed that CQ was still being used by about 39% of the respondents while ACT was used by only about 13.6%. The same study also revealed that nurses were the second most important group of health care workers that prescribe antimalarial drugs following doctors (11). A similar study conducted in Kenya showed that insufficient supply of ACT, cost and availability of inappropriate antimalarial such as amodiaquine were some of the reasons why healthcare workers still don't comply with ACT treatment policy (12). Another possible reason why health care workers may still be having problem with the compliance to ACT treatment policy despite the fact that it has been in place for the past 10 years might be because many still have confidence in the older drugs. Presence of fake and substandard ACT in circulation may be associated with treatment failure which might cause health care workers to have diminished confidence and be tempted to fall back on the older drugs. A recent survey of fake antimalarial drugs in Nigeria showed that 6.8% of the ACT in circulation was substandard, while another 1.3% was already degraded. About 1.2% of the circulating ACT was also found to be falsified (13). Fake and substandard antimalarial drugs, especially ACT, mostly find their way to Africa from Asian countries. In a study by Newton *et al* that investigated the rate of counterfeit antimalarial drugs en route to Africa from

Asia, fake drugs were found in 8 of the 11 countries sampled. Some of the unwholesome antimalarial drugs sampled included counterfeit artesunate that contains chloroquine, counterfeit dihydroartemisinin containing just acetaminophen, and counterfeit artemether-lumefantrine containing pyrimethamine (14).

Several studies have shown that nurses are important part of the health care team involved in management of malaria in the community and are often involved in prescription of antimalarial drugs. They sometimes constitute the first point of contacts in malaria treatment at primary health care level (15,16,17). Despite the change in treatment policy, Onyeaso and Oluwole found out that Chloroquine was the most frequently prescribed antimalarial drug for malaria prophylaxis by primary healthcare providers in a study in Nigeria. The reason for this was said to include ease of availability, affordability and insufficient knowledge of healthcare providers regarding efficacy and resistance of antimalarial drugs (17). Study in Tanzania also showed that SP was not well received by the healthcare workers several years after it was substituted for CQ (18). Similar studies in Kenya and Ghana also showed that Health care workers were resistant to malaria treatment policy change in a similar way malaria seems to be resistant to the changed medication (19, 20). The study in Kenya even showed that qualified healthcare workers (Doctors and Trained Nurses) were more likely to be less compliant with the new treatment policy. Longitudinal study in Tanzanian where malaria treatment policy had been changed 2ce in a decade found out that healthcare workers compliance to the new antimalarial drugs worsens over time (21). It was suggested that this may be based on their routine experiences in the clinical management of patients whereby the healthcare workers may be the first to notice inadequate clinical response to treatment before the attention of other stakeholders is eventually drawn to it.

Ability of malaria to develop resistance to ACT has never been in doubt; there are reports of resistance to ACT in South –East Asia, the same area where resistance to CQ was believed to have originated (21, 22, 23). A recent publication from eastern part of Nigeria reported 3 cases of *Plasmodium falciparum* malaria that showed early treatment failure (ETF) to artemisinin-based combination therapy. All the 3 cases showed adequate clinical response when treated with quinine. The publishers suggested that the failure might as well be due to questionable quality of the ACT, but then other patients that were treated with the same batch of drug were said to have responded adequately (24). This study aimed at investigating the level of chloroquine prescription among nurses in South West in Nigeria as well the possible role their experience with the use of ACT might play.

**MATERIALS AND METHODS**

About 180 pre-tested questionnaires were administered to randomly selected nurses in Sagamu, South-West Nigeria between the month of March and May 2015 to access the level of confidence they still had in CQ. Nurses in both private and public health care facilities were targeted for this purpose. Informed consent were obtained from the participants who were also made to realize that the questions were meant for research purpose only, and that answers provided will be made confidential. One hundred and sixty nine of the questionnaires (94%) were retrieved out of which 155 were sufficiently completed and suitable for analysis. The questionnaires were entered into Excel spread sheet and later exported to PSPP GNU statistical software version 0.8.5 for analysis. Results were cross tabulated and chi square was used to test for degree of association between the variables. Associations were said to be significant when P value was found to be less than 0.05.

**RESULTS**

Most of our respondents (58%) practice in public hospitals while 25% practice in private hospitals, and the rest work at both settings (Figure 1). Seventy six (49%) of the respondents are younger than 30 years old while 67 (43%) have less than 5 year post basic qualification experience (Table 1). Majority (56.1%) still have confidence in the efficacy of CQ while another 18.1% were not sure whether the drug is no longer efficacious, P=0.01. Many of the nurses above 40 years of age (69.1%) and majority of those who have between 11 to 20 years of post-qualification experience (71.4%) still have confidence in CQ efficacy P=0.001. Sixty three (40.7%) of the respondents have experienced ACT treatment failures with their patients during the course of their practice which were eventually treated with another antimalarial. Majority of those who still have confidence in the efficacy of CQ were those that have experienced treatment failure with ACT (68.3%), Table 1.

**TABLE 1: ASSOCIATION BETWEEN CONFIDENCE IN EFFICACY OF CQ AND AGE, YEARS OF EXPERIENCE AND ACT TREATMENT FAILURE EXPERIENCE.**

| | Confidence in the efficacy of CQ | | | |
|---|---|---|---|---|
| | Yes (%) | No(%) | Not sure(%) | Total(%) |
| **Age** | | | | |
| <30 | 33(43.4) | 29(38.2) | 14(18.4) | 76(100) |
| 30-40 | 25(67.6) | 7(18.9) | 5(13.5) | 37(100) |
| >40 | 29(69.1) | 4(9.5) | 9(21.4) | 42(100) |
| Total | 87(56.1) | 40(47.1) | 28(18.1) | 155(100) |
| P=0.01 | | | | |
| **Years of experience** | | | | |
| <5 | 27(40.3) | 27(40.3) | 13(19.4) | 67(100) |
| 5-10 | 22(66.7) | 8(24.2) | 3(9.1) | 33(100) |
| 11-20 | 20(71.4) | 1(3.6) | 7(25.0) | 28(100) |
| >20 | 18(66.7) | 4(14.8) | 5(18.8) | 27(100) |
| P=0.001 | | | | |
| **ACT treatment failure experience** | | | | |
| Yes | 43(68.3) | 12(19.0) | 8(12.7) | 63(100) |
| No | 36(49.3) | 22(30.1) | 15(20.6) | 73(100) |
| Not sure | 8(42.1) | 6(31.6) | 5(26.3) | 19(100) |
| P=0.15 | | | | |

The younger nurses (30.1%) and those with less than 5 years of experience (28.45%) had fewer experience with ACT treatment failure compared to the older and more experienced nurses. But the older and more experienced nurses were more sure of their perception of ACT treatment failure with only 2 (4.8%) of those above 40 and only 1 (3.8%) of those with 11 to 20 years of experience in the not sure category.

Our study showed that 70 (45.2%) of our respondents still prescribe CQ (P=0.02). It was also discovered that CQ was mostly prescribed by those who had previous ACT treatment failure experience (54.3%) with their patients, P=0.03; as well as those who believe that ACT resistance malaria is now in circulation (44.3%). Fifty (32.3%) of the nurses admitted that they had come across self-recognized fake ACT before (Figure 2), out of which 40% now prescribe CQ, (Table 3).

**TABLE 2: RELATIONSHIP BETWEEN ACT TREATMENT FAILURE EXPERIENCE AND AGE AS WELL AS YEARS OF EXPERIENCE OF RESPONDENTS**

| | ACT treatment failure experience | | | |
|---|---|---|---|---|
| | Yes (%) | No (%) | Not sure (%) | Total (%) |
| **Age** | | | | |
| <30 | 23(30.1) | 40(52.6) | 13(17.1) | 76(100) |
| 30-40 | 20(54.1) | 13(35.1) | 4(10.8) | 37(100) |
| >40 | 20(47.6) | 20(47.6) | 2(4.8) | 42(100) |
| P=0.06 | | | | |
| **Years of experience** | | | | |
| <5 | 19(28.4) | 35(52.2) | 13(19.4) | 67(100) |
| 5-10 | 17(51.5) | 13(39.4) | 3(9.1) | 33(100) |
| 11-20 | 14(50.0) | 13(46.4) | 1(3.8) | 28(100) |
| >20 | 13(48.2) | 12(44.4) | 2(7.4) | 27(100) |
| P=0.10 | | | | |

TABLE 3: PATTERN OF CHLOROQUINE PRESCRIPTION AMONG RESPONDENTS.

| | Still prescribe CQ to patients | | |
|---|---|---|---|
| | Yes (%) | No (%) | Total (%) |
| **Age** | | | |
| <30 | 30(39.5) | 46(60.5) | 76(49.0) |
| 30-40 | 24(64.9) | 13(35.1) | 37(23.9) |
| >40 | 16(38.1) | 26(61.9) | 42(27.1) |
| Total | 70(45.2) | 85(54.8) | 155(100) |
| P=0.02 | | | |
| **Years of experience** | | | |
| <5 | 26(38.8) | 41(61.2) | 67(43.2) |
| 5-10 | 17(51.5) | 16(48.5) | 33(21.3) |
| 11-20 | 14(50.0) | 14(50.0) | 28(18.1) |
| >20 | 13(48.2) | 14(51.9) | 27(17.4) |
| Total | 70(45.2) | 85(54.8) | 155(100) |
| P=0.57 | | | |
| **ACT treatment failure experience** | | | |
| Yes | 38(54.3) | 25(29.4) | 63(40.7) |
| No | 24(34.3) | 49(57.7) | 73(47.1) |
| Not sure | 8(11.4) | 11(12.9) | 19(12.3) |
| P=0.01 | | | |
| **Encounter fake ACT** | | | |
| Yes | 28(40.0) | 22(25.9) | 50(32.3) |
| No | 24(34.3) | 47(55.3) | 71(45.8) |
| Not sure | 18(25.7) | 16(18.8) | 34(21.9) |
| P=0.03 | | | |
| **ACT resistance is in circulation** | | | |
| Yes | | | |
| No | 31(44.3) | 13(15.3) | 44(28.4) |
| Not sure | 16(22.9) | 39(45.9) | 55(35.5) |
| P=0.00 | 23(32.9) | 33(38.8) | 56(36.1) |

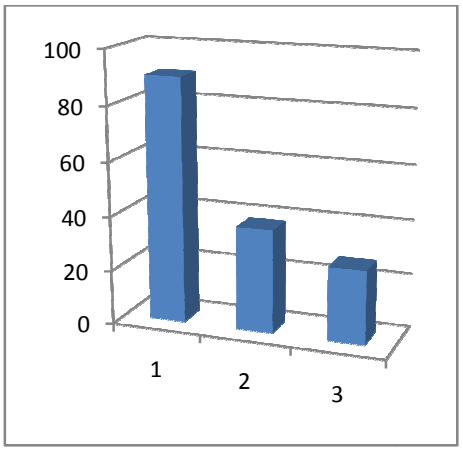


**FIGURE 1.PLACE OF PRACTICE.**
**(1) PUBLIC HOSPITAL = 90 (58%), (2) PRIVATE HOSPITAL= 38 (25%), (3) BOTH PUBLIC AND PRIVATE= 27 (17%).**

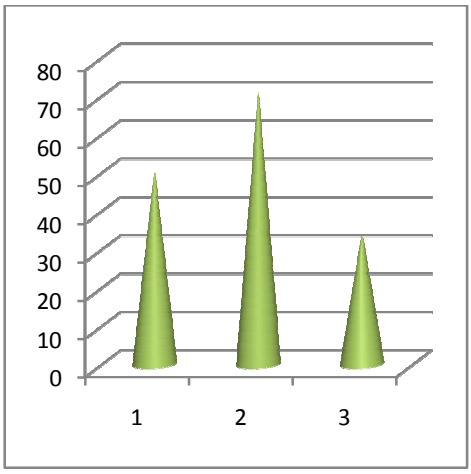


**FIGURE 2: HAD COME ACROSS RECOGNIZED FAKE ACT BEFORE?**
**(1) YES =50 (32.3%), (2) NO= 71 (45.8%), (3) NOT SURE =34 (21.9%)**

## DISCUSSION

The WHO recommends regular antimalarial treatment efficacy trials so as to keep an eye on impending Plasmodium resistance. Clinical efficacy trials with standardized protocol are the best way of doing this. In this study, we sampled the opinion of important stakeholders in the field of malaria

treatment and control, nurses, to find out their perception of ACT efficacy as well as the level of confidence they still have in chloroquine.

The number of nurses who still prescribe CQ in this study is still high, 70(45.2%). This is similar to studies in Kenya and Tanzania which also found poor compliance with the new antimalarial treatment policy. The relationship between the confidence in the efficacy of CQ and its prescription rate with age and years of experience of our respondents may actually be related to the long years of experience they had with it, before it became a failed drug. Adherence to CQ by healthcare workers in similar Nigerian study was attributed to its cheaper price and ease of availability (17).

The numbers of respondents who still have confidence in CQ suggest that the drug is not a 100% failed drug. But then, is important to effectively educate healthcare workers about the rationale behind the policy change in the first place. It is important to let all stakeholders appreciate the fact that only 25% failure rate was what informed the change, and that some degree of treatment success is still expected, which does not warrant early return to its use.

Perception of nurse's ACT treatment failure as recorded in this study will need further investigation, especially when it is associated with CQ prescription. The recent claim of 3 cases of ACT treatment failure by Ajayi and Ukwaja (24) also supported this nurse's suspicion. High prevalence of this perception among the more experienced healthcare workers calls for concern because the impression can readily be transmitted to the younger ones who they are expected to instruct and mentor. A similar study in Tanzania also found out that personal experience among healthcare worker including doctors, was responsible for poor adherence to new antimalarial treatment policy. The same study also showed that healthcare workers easily bring their experience to bear when prescribing drugs for their patients (21).

Experience of our respondent's with fake and substandard antimalarial drugs further suggests that the menace of unwholesome ACT is still a reasonable treat in Africa and this should be further investigated in a more formalized study. The issue of fake and substandard ACT calls for urgent remedial attention since it is found in this study to be associated with increased CQ prescription by health care workers.

We will like to recommend that standard methods should be developed to evaluate and document the personal experience of healthcare workers regarding antimalarial treatment failure. Records from such data can serve as an adjunct to the standard clinical efficacy trial. When healthcare workers realize that their experience contributed to decision that lead to a change in treatment policy, they are more like to adhere to such a joint decision compare to when they perceive the policy emanated from a group of sponsored researchers alone.

One limitation of our study is that there was no documented laboratory evidence of malaria in patients that were said to have experienced treatment failure with ACT by our respondents. But then this is also not sufficient to dismiss the claims of these respondents because most malaria cases in Africa are still being treated without laboratory evidence.

**Conflict of interest declaration-**

I , Dr Efunshile AM hereby declare on behalf of my co-authors that none of us has any potential conflict of interest whatsoever.

**Author's contribution-**

Deign the study-AME, OO, DI, CNI, AA

Administer the questioner-OO

Analyse the results- AME, AA

Writing of the manuscript- AME, OO, DI, CNI, AA

Read and approve the final manuscript- AME, OO, DI, CNI, AA

***Significance for public health***

Despite the change in the malaria treatment policy in Nigeria over the last 10 years, our study showed that a large proportion of the healthcare workers still prescribe chloroquine. This practice was significantly associated with the believe that ACT resistance malaria is now in circulation as well as the experience of the workers with ACT treatment failure. Urgent measures have to be taken to investigate the believe of this group of healthcare workers so as to reinforce confidence in ACT in order to sustain the progress made so far in the fight against malaria.

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# PREVALENCE OF *ENTAMOEBA HISTOLYTICA* IN STOOL SPECIMENS AT MUHONDO HEALTH CENTER, RWANDA

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*ABSTRACT*

***Entamoeba histolytica* is a protozoan parasite causing amebiasis. It occurs usually in the large intestine and causes internal inflammation as its name means (histo = tissue, lytic = destroying). Between 40 and 50 million people are infected worldwide, mostly in tropical countries, in areas of poor sanitation. The infection occurs by ingestion of mature cyst in fecally contaminated food, water or hands. The disease shows different symptoms including vomiting, abdominal pain, nausea, watery and bloody diarrhea. While the infection becomes extra-intestinal, it may cause abscess in other organs such as liver, kidney, brain and lungs.**
**The present study was carried out to determine the prevalence of *E. histolytica* in stool specimens at Muhondo Health Center. A total of 103 fecal specimens were collected over a period of three months. Out of 103 specimens, only 26 (25.2%) were positive for *E. histolytica*. Out of the 26 specimens positive for *E. histolytica*, 17 (16.5%) and 9 (8.7%) were from males and females respectively. Furthermore, of the 26 Specimens positive for *E. histolytica*, 15 (14.6%) and 11 (10.7%) were from people ≤ 15 and >15 years of age respectively. *Entamoeba histolytica* was more prevalent 26 (25.2%) than other parasites including *Giardia* with 15 (14.6%), *Ascaris sp* with 5 (4.9%), *Trichomonas intestinalis* with 16 (15.5%) and *Entamoeba coli* with 1 (1%). In order to reduce *Entamoeba histolytica* contamination and infections, the following recommendations were pointed out: (i) improving personal hygiene (washing hand before eating and after using latrines) ; (ii) avoiding fecal contamination of food, water, and utensils ; and (iii) boiling drinking water before consumption.**

**Key words*: Parasites*, Prevalence, *Entamoeba histolytica*, amebiasis, Stool specimens, Muhondo Health Center.**

# PREVALENCE D'*ENTAMOEBA HISTOLYTICA* DANS LES ECHANTILLONS DE SELLES AU CENTRE DE SANTE DE MUHONDO

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**Résumé**

***Entamoeba histolytica* est un parasite protozoaire provoquant l'amibiase. Il se produit généralement dans le gros intestin et provoque une inflammation interne comme son nom le signifie (histo = tissu, lytica = destruction). Entre 40 et 50 millions de personnes sont infectées dans le monde, principalement dans les pays tropicaux, dans les zones de mauvaises conditions d'hygiène.**
**L'infection se produit par ingestion de kyste mature dans des aliments, l'eau ou les mains contaminés par des matières fécales. La maladie montre différents symptômes, notamment du vomissement, des douleurs abdominales, de la nausée, une diarrhée aqueuse et sanglante. Quand l'infection devient extra- intestinale, elle peut provoquer des abcès dans d'autres organes tels que le foie, les reins, le cerveau et les poumons.**
**La présente étude a été réalisée afin de déterminer la prévalence d'*E. histolytica* dans les echantillons de selles au Centre de Santé de Muhondo. Un total de 103 échantillons de selles ont été recueillis sur une période de trois mois. Au cours de l'étude, sur 103 échantillons, seulement 26 (25,2%) étaient positifs pour *E. histolytica*. Sur les 26 échantillons positifs pour *E. histolytica*, 17 (16,5%) et 9 (8,7%) venaient des mâles et les femelles respectivement. En outre, de ces 26 échantillons positifs pour *E. histolytica*, 15 (14.6%) et 11 (10.7%) venaient des gens de ≤ 15 ans et de >15 ans respectivement.**

***E. histolytica* était plus fréquent 26 ( 25,2% ) que les autres parasites, y compris *Giardia* avec 15 ( 14,6% ) , *Ascaris sp* avec 5 ( 4,9% ), *Trichomonas intestinalis* avec 16 ( 15,5% ) et *Entamoeba coli* avec 1 ( 1 % ) .**
**Afin de réduire la contamination et les infections d'*Entamoeba histolytica*, les recommandations suivantes ont été mentionnées : (i) améliorer l'hygiène personnelle (lavage des mains avant de manger et après avoir utilisé les latrines), (ii) éviter la contamination des aliments, de l'eau et des ustensiles avec des matière fécales, et ( iii ) faire bouillir l'eau potable avant la consommation .**
**Mot Clés: Parasites, Prevalence, *Entamoeba histolytica*, amibiase, les échantillons de selles, Centre de Santé de Muhondo.**

## INTRODUCTION

*Entamoeba histolytica* is an eukaryotic, anaerobic, parasitic protozoan that is a member of the genus *Entamoeba. Entamoeba histolytica* may lead to amebic dysentery, illness characterized by fulminating dysentery, diarrhea, weight loss, fatigue, abdominal pain, vomiting and amebomas (1). *Amebiasis* ranks third among parasitic diseases leading to death Worldwide and it is second to malaria as a protozoan cause of death (2).

It is distributed worldwide and poses an especially serious health threat in tropical and subtropical developing areas and it is also a problem in the developed world in travelers, immigrants, and men who have sex with men (3). Worldwide intestinal amebiasis is frequent, with approximately 500,000,000 persons infected every year (4, 5).
*Entamoeba histolytica* may live as cysts and trophozoites. Cysts are typically found in formed stool whereas trophozoites are typically found in diarrheal stool. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts in fecally contaminated food, water, or hands. Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine (3).

Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen of individuals who are asymptomatic carriers, passing cyst in their stool (6). Both trophozoites and cysts are found in the intestinal lumen, but only trophozoites invade tissue. In animals, depletion of intestinal mucus, diffuse inflammation, and disruption of the epithelial barrier occur before trophozoites actually come into contact with the colonic mucosa. Trophozoites attach to colonic mucus and epithelial cells by a galactose-inhibitable lectin (7).

Transmission occurs via the fecal-oral route, either directly by person-to-person contact (such as by diaper-changing or sexual practices) or indirectly by eating or drinking fecally contaminated food or water (8). The major objective of this study was the determination of the prevalence of *Entamoeba histolytica* in stool specimens at Muhondo Health Center.

## MATERIALS AND METHODS

A total of 103 non-duplicate stool specimens were collected at Mohondo Health Center located in Northern Province (Gakenke district) over a period of three months (1st of May to 31st July 2014). Stool specimens from patients with diarrhea or complaining of dull and persistent lower part abdominal pains were analyzed in laboratory department of Muhondo Health Center. The informed consent of the experimental subjects and the approval of the appropriate ethical committee had been obtained.

A wide mouth screw-capped container pre-labeled with the individual's code, sex, and age was distributed to each patient for collection of a fecal sample. The people enrolled for this study were examined for the presence of common intestinal parasites by microscopy based on characteristics of *E. histolytica*.

Stool specimens were taken from each person and processed the same day with direct smears (saline and iodine), and examinations by concentration (flotation) with zinc sulfate were prepared from each sample and examined microscopically at low (20x) and high (40x) magnifications. These examinations were carried out in accordance with NCCLS recommendations (9).
The identification of *E. histolytica* trophozoites was made by the characteristic movement of the protozoan and the presence of phagocytized red blood cells (10). Trophozoites were more frequently observed in fresh stool specimens that contain mucus, pus, and trace amounts of blood **(**11**).** The identification of amebic cysts of *E. histolytica* and other commensal and pathogenic parasites was based on morphologic characteristics (12).
The data were presented in form of tables and complimented with graphs and chart and analyzed using Microsoft Office (word and Excel) to get the trend of *E. histolytica* during the study period.

## RESULTS

The patients were classified in two age groups (≤ 15 years and >15 years). The study showed that 53 (51.5%) people were > 15 years while 50 (48.5%) were ≤ 15 years (Table 1). Patients were also classified

based on gender. Results showed that 52 (50.5%) and 51(49.5 %.) patients were male and female respectively (Table 2).

The majority of examined specimens (103) were negative for *entamoeba histolytica* because 77 (74.8%) and 26 (25.2%) specimens were negative and positive respectively (Table 3).

**TABLE 1: STUDY PARTICIPANTS ACCORDING TO AGE GROUP**

| Age | Frequency | Percentage (%) |
|---|---|---|
| ≤ 15 years | 50 | 48.5 |
| > 15 years | 53 | 51.5 |
| Total | 103 | 100 |

**TABLE 2: STUDY PARTICIPANTS ACCORDING TO GENDER**

| Gender | Frequency | Percentage (%) |
|---|---|---|
| Male | 52 | 50.5 |
| Female | 51 | 49.5 |
| Total | 103 | 100 |

**TABLE 3: PREVALENCE OF *ENTAMOEBA HISTOLYTICA* IN STOOL SPECIMENS**

| Results | Frequency | Percentage (%) |
|---|---|---|
| Positive to *E.histolytica.* | 26 | 25.2 |
| Negative to *E.histolytica* | 77 | 74.8 |
| Total | 103 | 100 |

The study also checked the prevalence of *Entamoeba histolytica* compared to other common parasites. Results showed that *Entamoeba histolytica* was more prevalent 26 (25.2%) than other parasites including *Giardia* with 15 (14.6%), *Ascaris sp* with 5 (4.9%), *Trichomonas intestinalis* with 16 (15.5%) and *Entamoeba coli* with 1 (1%) (Table 4).

**TABLE 4: PREVALENCE OF *E. HISTOLYTICA* COMPARED TO OTHER PARASITES**

| Parasites | Frequency | Percentage (%) |
|---|---|---|
| *Entamoeba histolytica* | 26 | 25.2 |
| *Giardia lamblia* | 15 | 14.6 |
| *Ascaris sp* | 5 | 4.9 |
| *Trichomonas intestinalis* | 16 | 15.5 |
| *Entamoeba coli* | 1 | 1 |
| Other parasites (*yeast,Trichuris,Taenia eggs*) | 8 | 7.8 |
| Negative cases | 32 | 31 |
| Total | 103 | 100 |

Our main objective which was the determination of the prevalence of *E.histolytica* among the people attending MUHONDO Health Center was achieved. This study showed that people ≤ 15 years of age are more affected by *E. hystolytica* than people above 15 years. Lastly, *E. hystolytica* was prevailing at Muhondo Health Center along with other parasites (*Giardia*, *Ascaris*, *Trichomonas, and E. coli*).

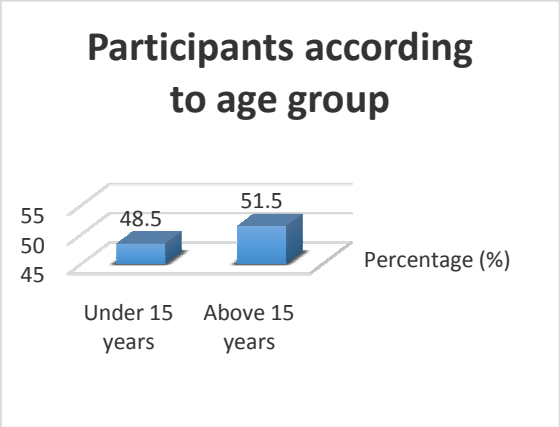


*Figure1: Study participants according to age group*

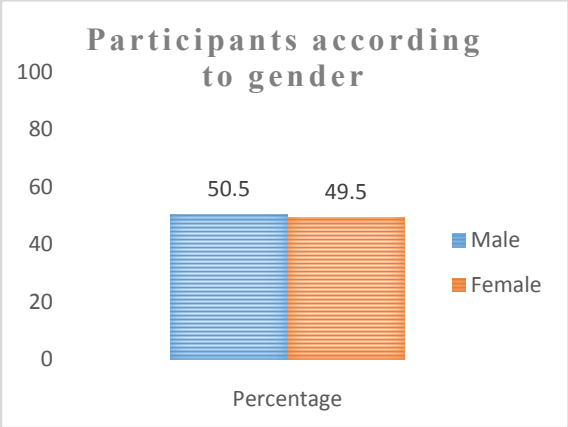


*Figure 1: Study participants according to gender*

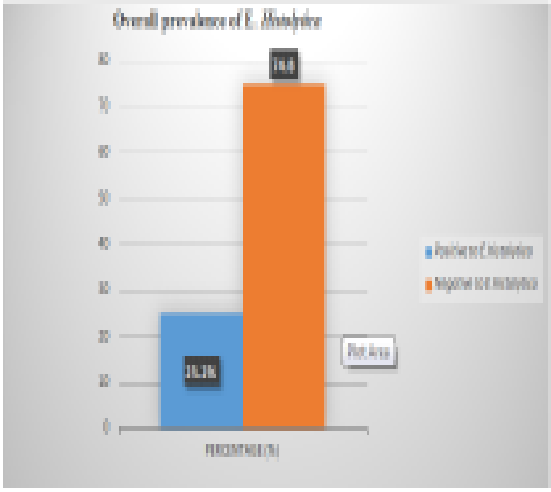

*Figure 2: The overall prevalence of Entamoeba histolytica in stool specimens*

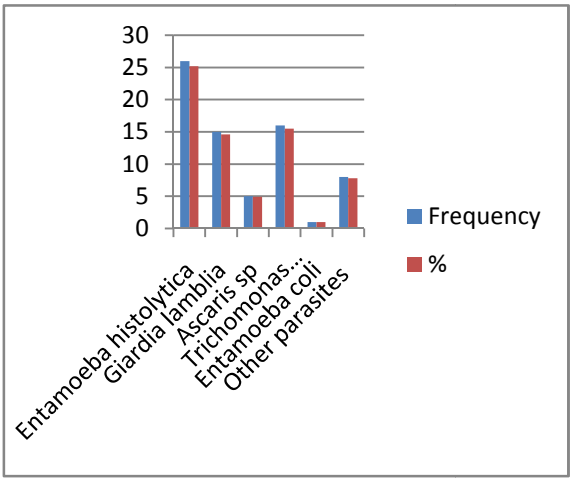


***Figure 4. Prevalence of E. histolytica compared to other parasites***

**DISCUSSION**

The study showed that *Entamoeba histolytica* was prevalent in 26 (25.2%) patients. This suggests that people around Muhondo Health center are exposed to fecally contaminated food and water which concurs with the study of Lupi *et al,* 2009 stating that transmission occurs via the fecal-oral route, either directly by person-to-person contact or indirectly by eating or drinking fecally contaminated food or water (7).

The results revealed that the majority of people affected by *E. histolytica* are in the age group ≤ 15 years. This is in agreement with study carried out by Brown *et al,* 1991 where *E. histolytica* had 30.82% in people ≤ 15 years of age and 17.34% in age group of 31 to 45 years (13). According to Cook et al. (2009), there is positive correlation between increased rates of *E. histolytica* infection with young age, wet season, female gender, and severe malnutrition (14).

The study results showed that men are more affected by *E. histolytica* (16.5%) than women (8.7%). This concurs with the studies of Haque *et al,* 2006 and Brown *et al,* 1991 which showed that the infection of *E. histolytica* was more prevalent in male hosts (22.36%) as compared to female ones (20.9%) (13).

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# SERO-PREVALENCE STUDY OF PARASITIC INFECTIONS AMONG HIV POSITIVE AND NEGATIVE PATIENTS IN LAGOS, NIGERIA

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**ABSTRACT**

**Background: Diseases caused by opportunistic pathogens are the major clinical signs of HIV infected and AIDS patients with parasitic infection being part of the common causes of morbidity and mortality.**

**Objectives: This was a cross-sectional study to determine the sero-prevalence of serum antibodies to three parasitic infections namely *Entamoeba histolytica, Schistosoma sp. and Toxoplasma gondii,* which are opportunistic infections among HIV/AIDS patients.**

**Methods: One thousand and eighty patients that attended three healthcare institutions in Lagos were recruited for the study through convenience sampling method. Venous blood was collected from the recruited patients and screened for HIV infection as well as the presence of serum antibodies to three parasitic infections. All positive sera samples were confirmed for HIV infection.**

**Result: The results revealed that 65/1080 (6%) of the recruited patients were HIV sero-positive. In addition, 5/65 (7.7%) of the HIV positive patients had *E. histolytica* co-infection, 1/65 (1.5%) had *Schistosoma sp.* co-infection while 2/65 (3.1%) had *T. gondii* co-infection. The results also indicated that the proportion of patients with *E. histolytica* was significantly higher among HIV sero-positive patients than the sero-negative patients (P = 0.031).**

**Conclusion: The study showed the opportunistic potential of the three parasitic infections among HIV/AIDS patients in the study area.**

**Keywords: [HIV, AIDS, Seropositive, Seronegative, *Toxoplasma gondii*, *Entamoeba histolytica*, *Schistosoma haematobium*}**

# LA SEROPREVALENCE DES INFECTIONS PARASITAIRES CHEZ LES PATIENTS SEROPOSITIFS ET LES PATIENTS VIH NEGATIFS A LAGOS, NIGERIA.

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**RÉSUMÉ**

**CONTEXTE : Les maladies causées par les pathogènes opportunistes sont les principaux signes cliniques des patients infectés par le VIH et des Patients infectés par le VIH et le SIDA avec les infections parasitaires faisant partie des causes fréquentes de morbidité et de mortalité.**

**OBJECTIFS : Ceci fut une étude transversale pour déterminer la séroprévalence des anticorps sériques aux trois infections parasitaires à savoir *Entamoeba histolytica, Schistosoma sp*. et *Toxoplasma gondii*, qui sont les infections opportunistes chez les patients VIH/SIDA.**

**METHODES : Mille huit cent patients qui ont à trois établissements de sante à Lagos ont été recrutés pour l'étude à travers la méthode d'échantillonnage de commodité. Sang veineux a été recueilli des patients recrutés et dépistés pour les infections du VIH ainsi que la présence des anticorps sériques aux trois infections parasitaires. Tous les échantillons de sérums positifs ont été confirmés pour l'infection au VIH.**

**RESULTAT : Les résultats ont montré que des patients 65 sur 1 080 (6%) recrutésétaientséropositifs pour le VIH. En outre, 5 sur 65(7,7%) des patients séropositifs avaient l'infection *E.histolytica*,1 sur 65 (1,5%) avait la coinfection *Schistosoma sp.*alors que 2 sur 65(3,1%) avaient la coinfection *T.gondii*. Les résultats ont également indiqué que la proportion de patients avec *E.histolytica* était significativement plus élevée chez les patients VIH séropositifs que les patients séronégatifs (P=0,031).**

**CONCLUSION : L'étude a montré le potentiel opportuniste des trois infections parasitaires chez les patients VIH/SIDA dans la zone d'étude.**

**MOTS CLES : {VIH,SIDA, Seropositif, *Seronegatif, Toxoplasma gondii, Entamoeba histolytica, Schistosoma haematobium*}**

## INTRODUCTION

Infections caused by these parasites *Toxoplasma gondii, Schistosoma sp.* and *Entamoeba histolytica* have been characterized as opportunistic infection in human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) patients around the world (1, 2, 3). The major clinical manifestation of HIV infection as well as in AIDS patients is diseases caused by opportunistic pathogens and parasitic co-infections constituted part of the common causes of morbidity and mortality( 4, 5).

*Toxoplasmosis* is a typical disease that affects humans, with most cases asymptomatic. While the prevalence of *Toxoplasma* antibodies correspond with age, it does not correspond with sex; variations and differences exist in each country. In part of sub-Saharan Africa with moist weather, variations in toxoplasmosis prevalence ranges between 50% and 70% and the estimated population risk and infection is one third and half among worldwide population (6). The incidence of sero-positivity varies in humans, showing previous exposure and location in addition to eating habits (7, 8). Previous studies demonstrated the common cause of focal lesions of the brain as a result of *Toxoplasma* encephalitis and thereby complicating the direction of AIDS due to the reactivation of a latent infection (9, 10). In advanced stage with *Toxoplasma* encephalitis recrudescent toxoplasmosis usually manifest as cerebral abscess impairing the immune responses to infections causing the infective reactivation in AIDS patients through immune suppression (2, 11, 12). Patients with *Toxoplasma* and HIV co-infections have between 30% to 40% risk of developing *Toxoplasma* encephalitis especially amongst those with significant immunosuppression (CD4 count less than 200 cells/μl) (13).

Schistosomiasis is a parasitic disease cause by *Schistosoma sp.* and it is mainly found in the tropical and sub-tropical areas of the world. Due to inadequate potable water supply and limited water resource development, schistosomiasis is prevalent in sub-Saharan Africa, Asia and South America (14). Earlier studies demonstrated that urinary schistosomiasis occurs more than intestinal schistosomiasis in Nigeria (15, 16). Both HIV/AIDS and schistosomiasis causes significant disease burden in sub-Saharan Africa with frequent overlap in their epidemiological characteristics[17]. While few studies have examined interaction between the two diseases (3, 15, 16, 17, 18, 19), studies have shown that both diseases exert bi-directional effects on one another (20, 21).

*Entamoeba histolytica* primarily inhabits the large intestine where the trophozoites or active forms live. Studies have shown that morphologically identical pathogenic and non-pathogenic strains of amoebae that are genetically, biochemically and immunologically distinguishable exist (22, 23, 24). However, the same strain can change its behavior within the same host from time to time. Hence, there is no doubt that immunosuppression of the host can turn a harmless commensal infection into a dangerously invasive one (1, 25, 26). Invasive amoebiasis (IA) is a significant parasitic infection that is associated with significant morbidity and mortality world-wide in individuals that resides or travels to endemic areas and it accounts for 40,000 to 100,000 deaths annually. IA had previously been reported in HIV infected / AIDS patients (27, 28, 29, 30).

While some work had been conducted on these parasitic infections in Nigeria, few of such studies had been carried out comparatively in HIV infected and non-infected individuals in the country. Hence, the objective of this study was aimed at determining the sero-prevalence of these three parasitic infections among HIV seropositive and sero-negative patients using serodiagnostic approach to determine the presence of serum antbody to these infections.

**Research Method and Design**
**Study design**
This was a cross-sectional based study that was designed to carry out sero-prevalence of serum antibodies to three parasitic infections namely *Entamoeba histolytica, Schistosoma sp. and Toxoplasma gondii* using serodiagnostic approach. Details of the study design and population have been published previously[31]. Briefly, patients attending three health-care institutions in Lagos, Nigeria namely, General Hospital Ikeja, Sexually Transmitted Diseases Clinic Yaba, and the Central Public Health Laboratory Yaba were recruited for the study through the convenient sampling method between January 1996 and December 1997. The selected institutions serve as: (i) provider-based facility, (ii) referral centre for HIV and STD patients, and (iii) client based facility especially for people who intend to be familiar with their HIV and STD statuses.

Blood samples for screening of HIV, *Toxoplasma gondii, Schistosoma sp,* and *Entamoeba histolytica* were obtained with consent from 1080 patients out of 200 patients interviewed. Written informed consent or thumbprints were received from all recruited individuals and consent could not be obtained from 920 patients who declined to participate and were excluded from the study. Both patients who refused to be recruited for the study and those who were sero-positive in the study were given adequate clinical services.

**Laboratory procedures**
Blood sera of the patients were screened for HIV-1 and HIV-2 using Cambridge Biotech Corporation Recombigen HIV-1 and HIV-2 rapid test device while confirmation of positive cases was done with Immunocomb 11 and Bio-rad Novapath HIV-1 immunoblot for HIV-2 and HIV-1, respectively.

Diagnosis of parasitic infections from blood sera of sampled patients was done by Indirect Haemaglutination (IHA) method. The IHA principle involves the use of reagent made of formalized sheep blood cells, which are sensitized by a soluble antigen of the parasite and made to react with antibodies present in the patient with the formation of a haemagglutination i.e. a reddish-brown film that is observed in the wells of a U-microplate. In the absence of specific antibodies, the sensitized red blood cells will deposit, by forming a ring in the bottom of the well. Cellognost® kits from Behring Diagnostic Inc. were used for the screening of IgG antibodies to *Schistosoma sp.* and *Entamoeba histolytica,* while Toxocell IHA kits from Biokit SA were used for screening of *T. gondii* IgG antibodies. *Toxoplasma* kits from Randox Laboratories Ltd. were used for the detection of IgM antibodies to *T. gondii* by the Enzyme Linked Immunosorbent Assay (ELISA) method. Titer values were interpreted specific to each parasite according to the manufacture's guideline for interpretation as earlier documented (32, 33, 34, 35, 36, 37).

Manufactures recommended cutoff titer of 1:16 (Low) and 1:32 was used for the evaluation of *Entamoeba histolytica* and *Schistosoma sp.*, while 1:64 (Toxo IgG) and 0.9 (Toxo IgM) was used for *Toxoplasma gondii*

**Data analysis**
The obtained data were analyzed using the EPI-INFO Statistical Package, version 6.0. Statistical significance of the proportions of categorical data was estimated using Fisher's exact test for contingency table. All tests were two-tailed and P-value of $<0.05$ was taken as statistical significant.

**RESULTS**
Of the sampled patients (aged 4 to 62 years), 36/1080 (3.3%) were males and 29/1080 (2.7%) were females. Titer values ranged from 1:16 to 1:4096. Out of the total patients screened for HIV in the study, 65 (6%) were sero-positive. Among the 65 patients with confirmed HIV sero-positive results, 8 (12.3%) had three parasitic co-infections while among the 1015 patients without HIV infections, 34 (3.3%) had the three infections (Table 1).

Sero-diagnosis assay from the study showed that 5 (7.7%) of patients with HIV sero-positive result had serum antibodies to *E. histolytica* (Table 1). Titer values of serum antibodies to *E. histolytica* infection ranged from 1:16 to 1:512 for the sampled patients. A significant titer value range of 1:32 to 1:512 was found in 5 (0.5%) HIV sero-negative patients, while 3 (4.6%) HIV sero-positive patients had a significant titer value of 1:256 to 1:512 for *E. histolytica* serum antibody (Table 2).

Only 4 (0.4%) HIV sero-negative patients and 1 (1.5%) HIV sero-positive patients had serum antibodies to *Schistosoma sp.* infection (Table 1). Among HIV sero-negative patients 3 (0.3%) had a titer of 1:16 while only 1 (0.1%) patient had a higher titer of 1:256. Titer value in the HIV sero-positive patient was 1:16 (Table 2).

**TABLE 1: SAMPLED PATIENTS WITH SERUM ANTIBODIES TO PARASITIC INFECTIONS**

| Parasitic infection | Patients with serum antibody | | Total |
|---|---|---|---|
| | HIV+ N = 65 (%) | HIV- N = 1015 (%) | N = 1080 (%) |
| *E. histolytica* | 5 (7.7) | 24 (2.4)* | 29 (2.7) |
| *Schistosoma sp.* | 1 (1.5) | 4 (0.4)** | 5 (0.5) |
| *T. gondii* | 2 (3.1) | 6 (0.6)*** | 8 (0.8) |
| Total | 8 (12.3) | 34 (3.3) | |

*P = 0.031, ** P = 0.27, ***P = 0.082

Serum antibodies to *T. gondii* infection were found in 2 (3.1%) of HIV sero-positive patients and in 6 (0.6%) of the sero-negative patients with titer value range of 1:16 to 1: 128 (Table 1). Significant titer value of ≥ 1:64 Toxo kit and >0.9 Toxo IgM kit interpretations was recorded in 1 (0.1%) HIV sero-negative patient and 2 (3.1%) HIV sero-positive patients (Tables 2).

Result from the study also showed that more patients within the age range 21-30 years in both HIV sero-positive and sero-negative patients had serum antibody to *T. gondii* infection (Table 3). Also, more females than males presented with antibodies to *T. gondii* infection in HIV sero-positive 2 (3.1%) and HIV sero-negative 5 (0.5%) patients. (Table 4).

**TABLE 2: TITER VALUES OF SAMPLED PATIENTS SHOWING SERO-POSITIVITY FOR PARASITIC INFECTIONS**

| Serodiagnosed Parasitic infections | HIV Sero Status | Titre values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 | 1:1024 | 1:2048 | 1:4096 | Total |
| *E. histolytica* | sero- | 19 (1.9) | 1 (0.1) | | 2 (0.2) | 1 (0.1) | 1 (0.1) | | | | 24 |
| | sero+ | 2 (3.1) | | | | 2 (3.1) | 1 (1.5) | | | | 5 |
| *Schistosoma sp.* | sero- | 3 (0.3) | | | | 1 (0.1) | | | | | 4 |
| | sero+ | 1 (1.5) | | | | | | | | | 1 |
| *T. gondii* | sero- | 2 (0.2) | 3 (0.3) | 1 (0.1) | | | | | | | 6 |
| | sero+ | | | 1 (1.5) | 1 (1.5) | | | | | | 2 |

**TABLE 3: AGE DISTRIBUTION OF SAMPLED PATIENTS SHOWING SERO-POSITIVITY FOR PARASITIC INFECTIONS**

| Age group (Years) | Sero-positive for invasive *E. histolytica* | | Sero-positive for *Schistosoma sp.* | | Sero-positive for *T. gondii* | |
|---|---|---|---|---|---|---|
| | HIV sero- | HIV sero+ | HIV sero- | HIV sero+ | HIV sero- | HIV sero+ |
| **1-10** | 3 (0.3) | | | | | |
| **11-20** | 5 (0.5) | 1 (1.5) | 2 (0.2) | | 1 (0.1) | |
| **21-30** | 5 (0.5) | 2 (3.1) | 2 (0.2) | 1 (1.5) | 3 (0.3) | 2 (3.1) |
| **31-40** | 6 (0.6) | 2 (3.1) | | | 2 (0.2) | |
| **41-50** | 3 (0.3) | | | | | |
| **>50** | 2 (0.2) | | | | | |

**TABLE 4: SEX DISTRIBUTION OF SAMPLED PATIENTS SHOWING SERO-POSITIVITY FOR PARASITIC INFECTIONS**

| Sex | Sero-positive for invasive *E. histolytica* | | Sero-positive for *Schistosoma sp.* | | Sero-positive for *T. gondii* | |
|---|---|---|---|---|---|---|
| | HIV sero- | HIV sero+ | HIV sero- | HIV sero+ | HIV sero- | HIV sero+ |
| **Males** | 14 (1.4) | 3 (4.6) | 4 (0.4) | | 1 (0.1) | 0 (0) |
| **Females** | 10 (1.0) | 2 (3.1) | | 1 (1.5) | 5 (0.5) | 2 (3.1) |

## DISCUSSION

The presence of significant serum antibody to *E. histolytica* and *T. gondii* among HIV sero-positive patients in the study has further exposed these two parasitic organisms as possible opportunistic infections in HIV infected / AIDS patients as previously reported (1, 2). Medical practitioners in this region should focus attention on the diagnosis of these organisms and clinical management of infection with these organisms as well as other endemic diseases like malaria and tuberculosis that may interact or be opportunistic in HIV infected and AIDS patients.

The significant titer value of 1:32 or more recorded in this study in HIV sero-positive as well as in HIV sero-negative patients is an indication of the presence of invasive *E. histolytica.* Clinical manifestations associated with *E. histolytica* infection could result in asymptomatic and symptomatic infection with or without tissue invasion. *E. histolytica* may remain a harmless commensal in its host or may become pathogenic due to certain factors not fully understood; however, immunosuppression had been described as a factor that could turn harmless commensal *E. histolytica* into a dangerously invasive one. Majority of infection with *E. histolytica* are asymptomatic and such individuals will have a negative or weak serologic response and will primarily pass cysts in their stools [38, 39]. In one study, asymptomatic HIV-1 infected individuals with high anti-Eh titer were reported to be at risk of IA, perhaps as a result of exacerbation of subclinical amebiasis (27). In another study, the main presenting symptoms of IA were reported as fever, chronic diarrhea, and abdominal pain (29).

Only 1.5% of the HIV sero-positive patients in the study had serum antibody to *Schistosoma sp.* infection as compared with 0.6% of HIV sero-negative patients that had serum antibody. Both urogenital and intestinal schistosomiasis are chronic inflammatory disease caused by a water-borne parasitic blood fluke with about 220 million people infected in sub-Saharan Africa, and more people especially children at risk (17). The presence of *Schistosoma sp.* serum antibodies with titer value of 1:16 in only one HIV sero-positive patients in this study does not exclude a possible interaction between *Schistosoma sp.* and

HIV in endemic areas like Nigeria since both agents co-inhabit the blood where they both cause pathological symptoms. Further studies are progressing to investigate the effect of one on the other in infected patients. Few of such studies that examined the interaction between *Schistosoma sp.* and HIV co-infection especially in areas where dual endemicity is most prevalent tried to understand the pathogenesis and immune responses in co-infection as well as clinical studies of responses to antiparasitic and antiretroviral drugs for HIV/AIDS disease progression (3, 17, 40).

Indications for the presence of *T. gondii* infection was found in two patients that were sero-positive for HIV and one patient that was sero-negative for HIV with a significant titer value. In the diagnosis of *T. gondii* infection, a negative IgM indicates absence of infection in the past 6 months while a combination of positive IgM and IgG titer indicates acute infection (41, 42, 43). A positive IgG test with a negative IgM indicates chronic infection (12). This result thus supports the presence of *T. gondii* infection in HIV infection in the area. A sero-prevalence of 58% was reported for Toxo-IgG antibodies among HIV patients without neurological complications in Lagos, Nigeria (44). Although more females than males in this study presented with serum antibodies to *T. gondii* infection, studies have shown that there is little or no difference in the prevalence of *T. gondii* infection between sexes (6).

**Limitation**

Limitation of this study includes the inability to investigate factors like education, nutrition, environmental and behavioral attitudes that can influence the rate differences between HIV infected and HIV non-infected patients in the study. Furthermore, serology positive tests for parasitic infections in the study could not be confirmed with more specific test due to limited resources. Another limitation is the low sensitivity and specificity of serological diagnosis of parasitic infections (45). In addition, sample sizes of HIV positive for uncommon parasites were very small.

**Recommendation**

Information on the prevalence of these diseases among HIV infected patients is scanty in the area; with a population of over 150 million and HIV prevalence of 3.1% among adults ages 15–49 reported in 2012[46], more studies are required on these diseases by location in different part of the country.

**Conclusion**

We have therefore been able to further determine the sero-prevalence as well as demonstrate the presence of the three parasitic infections in the area most especially among HIV infected patients using serodiagnosis technique which is considered to be the most available and affordable routine method of diagnosing diseases like Toxoplasmosis in resource poor countries (37) and as well help guide therapeutic and management policies for infected patients in the area.

**Ethical considerations**

**Ethical clearance**

Ethical approval for the study was obtained from the Federal Ministry of Health Authority.

**Informed consent**

Study objectives were explained to all participants. Participation was voluntary and confidentiality of study information was guaranteed.

**Competing interests**

None

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# INTESTINAL HELMINTHS IN SOME CASES OF ACUTE APPENDICITIS OPERATED IN BAMENDA, CAMEROON

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**ABSTRACT**

**Background: Acute appendicitis is the most frequent infectious surgical abdominal emergency and previous studies have noted the presence of parasites in the appendicular lumen.**

**Objective: This study was done to determine the involvement of intestinal worms in the etiology of acute appendicitis.**

**Materials and Methods: This was a prospective and descriptive study concerning cases of confirmed and operated acute appendicitis between 15th April, 2013 and 14th April, 2015 at the People's Clinic, Ngomgham, Bamenda, Cameroon. The appendicular content was macroscopically examined for parasites and formol-ether concentration technique was carried out for ova detection.**

**Results: A total of 112 patients were operated for acute abdominal pain within the study period. There were 74 (60.8%) cases confirmed with acute appendicitis of which 45 (60.1%) were males and 29 (39.2%) were. The most affected age group was the 21 to 40 years (50%). The removed appendices appeared congestive in 30 (40.5%) cases, suppurated in 27 (40.5%) cases and gangrenous in 17 (23.0%) cases.There was no relationship between the appearance of the removed appendix and the gender of participants (P>0.05).Three different helminth ova were identified in the 74 samples. In decreasing prevalence, the parasite trend was 8 (10.8%) *Ascaris lumbricoides*, 5 (6.8%) *Enterobius vermicularis*, 3 (6.0%), *Ankylostoma duodenale*, Adult *Ascaris lumbricoides* and *Enterobius vermicularis* were seen in 1 and 3 cases respectively.**

**Conclusion: A small percentage of parasitic worm eggs were found in the appendicular content, though a good portion of patients took medications against parasites before surgery. Intestinal worms could not be incriminated in the causation of the appendicitis; nevertheless, one adult ascaris was found as an evident cause of appendicular lumen obstruction.**

**Key words: Intestinal Helminthes, Acute appendicitis, Bamenda**

# LES HELMINTHES INTESTINAUX DANS CERTAINS CAS D'APPENDICITES AIGUES OPERES A BAMENDA, CAMEROUN

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**RÉSUMÉ**

**Contexte: L'appendicite aigue est la plus fréquente des urgences chirurgicales abdominales d'origine infectieuse et des études ont démontré la présence des parasites dans la lumière appendiculaire.**

**Objectif: Le travail a été réalisé pour investiguer l'implication des helminthes intestinaux dans l'étiologie del'appendiciteaigue.**

**Méthodologie : Il s'agissait d'une étude prospective et descriptive concernantles cas confirmés et opérésd'appendicite aiguedu 15 Avril 2013 au 14 Avril 2015 à Peoples Clinic, Ngomgham à Bamenda. Le contenu appendiculaire a étéexaminé macroscopiquement pour la détection des parasitesadultes pendant que la technique de concentration par le formol-éther était utilisée pour la détection des œufs.**

**Résultats : Un total de 112 patients présentant des douleurs abdominales ont été opérés. Il y avait 74 (60.8%) cas d'appendicites aigues confirmés desquels 45 (60.1%) étaient de sexe masculin et 29 (39.2%) de sexe féminin. Le groupe d'âge le plus affecté était celui compris entre 21 et 40 ans (50%). Les appendices enlevés étaient congestifs dans 30 (40.5%) cas, suppurant dans 27 (40.5%) cas et gangreneuxdans 17 (23.0%) cas. Il n'y avait pas de relation entrel'apparencede_l'appendice enlevé le sexe des participants (P>0.05). Les œufs de 3 types d'helminthes ont été identifiés des 74 spécimens. Il s'agissait par ordre décroissant de prévalence de 8 (10.8%) *Ascaris lumbricoides*, 5 (6.8%) *Enterobius vermicularis* et 3 (6.0%) *Ankylostoma duodenale*. Les adultes d'*Ascaris lumbricoides* et d'*Enterobius vermicularis* ont été observés dans 1 et 3 cas respectivement.**

**Conclusion : Un petit pourcentage de cas avait des œufs de vers intestinaux dans le contenu appendiculaire. Mais une grande proportion des patients avait pris un vermifuge avant la chirurgie. La parasitose intestinale ne pouvait pas êtreincriminéecommecause d'appendicite aigue ; néanmoins un adulte d'ascaris a été identifié comme cause évidente d'obstruction de la lumière appendiculaire.**

**Mots clés : Appendiciteaigue, contenu appendiculaire, vers intestinaux, obstruction appendiculaire.**

## INTRODUCTION

Acute appendicitisis the most frequent infectious surgical abdominal emergency andusually suspected in all cases of acute abdomen pain which makes it a serious public health problem[1]. It is admitted that appendicular mucosal lesions are responsible for the pathology and pressure in the appendix caused by obstruction at its base is known to be the main mechanism [2]. Obstruction favoursbacterial multiplication and development of the pressure within the appendix [2].The diagnosis is essentially clinical though laboratory and ultrasonography investigations are often requested and done [3-6]. Presence of a foecalithe,tumour, or foreign body on ultrasonography and macroscopically on surgical specimens would confirm the assertion [6,7]. But there are cases of appendicitis without the presence of any obstruction. Therefore other mechanisms could be at the base.

More than one dozen different species of intestinal helminths infect humans, particularly in the tropical and subtropical parts of the developing world. However, four nematodes stand out due to their widespread prevalence and distribution that result in hundreds of millions of human infections. These include the large roundworm (*Ascarislumbricoides)*, the whipworm (*Trichuristrichiura)*, and two species of hookworm (*Necatoramericanus*and*Ancylostomaduodenale)* [8]

In Cameroon intestinal helminthiasis are among the most important parasitic diseases. These infections are more prevalent in the Southern part of the country [9]and about 5.6 million people are infected with *A.lumbricoides*, 6.5 million with *T. trichiura*, and 2.6 million with *N. americanus*[10]These parasites co-exist in most parts of the country with *Schistosoma species* [11] causing great parasitic burden in infected children.

Numerous studies have reported the presence of parasites in the appendicular lumen which is continuous with the lumen of the coecum [4, 12, 13]. Parasitism as a predisposing factor of acute appendicitis is preventable. Bamenda is a highly populated rural town where few campaigns for the elimination of intestinal worms have been known to be carried out. This study was done to determine the involvement of intestinal worms in the etiology of acute appendicitis. Such information would be of great contribution in elaborating an appendicitis prevention programme.

## MATERIALS AND METHODS

### Study design and ethical considerations

This was aprospective and descriptive study carried out from April, 2013 toApril, 2015 at thePeople's Clinic Ngomgham in Bamenda- Cameroon. It included all persons aged 5years and above that presented with abdominal pain clinically diagnosed as acute appendicitis, and who consented to be included in the study.Patients or their guardians were made to understand that it was not a hospital obligation to take part in the study, neither was it a prerequisite for receiving medical attention. Written informed consent/parental assent forms were signed. An ethical clearance was obtained from theInstitutional Review Board (IRB) of the Regional Delegation of Health prior to the study.

### Sample collection and processing

After the routine clinical examination and ultrasonography (if requested by the physician), patients were operated upon and acute appendicitis.Those operated for appendicular abscess were not retained.Appendicectomy specimen were emptied of their contentsandanalysed in the parasitology laboratory.Detection of adult parasites was done macroscopically. For detection of parasite ova, formol-ether concentration technique was carried out. A gram of each faecessample was emulsified in 3 mLof 10% formol water. Four mLs of formol water was further added to the preparation and mixed. The emulsified preparation was therefore sieved and the filtrate was collected and transferred to a centrifuge tube, then 4 mLs of ethyl acetate was added to the preparation. The tube was therefore stoppered and its content mixed for a minute. The stopper was gently removed and the preparation centrifuged at 3000 rpm for one minute. The layer of faecal debris from the side of the tube was gently loosened with a stick and the supernatant was discarded. The content of the sediment was transferred to a slide, covered with a coverslip and observed using the X10 andX40 objectives. A drop of iodine was run under the slide to increase visibility of parasite ova. Slides were read within 24 h of preparation.More details on this technique is described elsewhere [14].

Data from the examinations were retained for analysis; they includedpatients' biodata, whether or not antiparastic drugsweretaken prior to operation, ultrasonography results, parasites found in the appendicular content, and the pathology results. The Chi-Square test was used to determine the significance of results obtained at significant level of 0.05.

## RESULTS

A total of 112 patients were operated for acute abdominal pain within the study period. There were 74 (60.8%) cases of confirmed with acute appendicitis of which 45 (60.1%) were males and 29 (39.2%) were females. The mean age was 21.4years andthe age range was 5 years to 53years. The most affected age group was the 21 to 40 years (50%) followed by the 11 to 20 years (36.5%). Table 1 shows the Distribution of study participants according to age and sex.

**TABLE 1: DISTRIBUTION OF STUDY PARTICIPANTS ACCORDING TO AGE AND SEX.**

| Age group, (years) | Number (%)* of participants | | Total n (%) |
|---|---|---|---|
| | Males | Females | |
| 5 – 20 | 17 (37.8) | 10 (34.5) | 27 (36.5) |
| 21 – 40 | 23 (51.1) | 14(48.3) | 37(50.0) |
| >40 | 5 (1.1) | 5(17.3) | 10 (13.5) |
| Total | 45 (100.0) | 29 (100.0) | 74 (100.0) |

*Percentages based on number of participants

Ultrasonography was carried out on 42 participants of which 19 (42%) showed a positive result. The removed appendices retained for examination appeared congestive in 30(40.5%) cases, suppurated in 27(40.5%) cases and gangrenous in 17(23.0%) cases.These pathologies are shown in Table 2. There was no significant relationship between the appearance of the removed appendix and the gender of participants (P>0.05).

**TABLE 2: APPEARANCE OF REMOVED APPENDIX IN STUDY PARTICIPANTS ACCORDING TO AGE AND SEX.**

| Appearance of removed appendix | Number (%)* of participants | | Total n (%) |
|---|---|---|---|
| | Males | Females | |
| Congestive | 17 (37.8) | 10 (34.5) | 30 (40.5) |
| Suppurated | 23 (51.1) | 14 (48.3) | 27(36.5) |
| Gangrenous | 5 (11.1) | 5(17.2) | 17(23.0) |
| Total | 45 (100.0) | 29 (100.0) | 74 (100.0) |

*Percentages based on number of participants

Appendicular lumen obstruction was found in 46 (62.2%) of the retained cases, there were 29 (39.2%) foecalith cases and 16 (21.6%) cases showed a swollen appendix.One case showed a 22cm long appendix as presented in Figure 1 with an adult *Ascarislumbricoides* within the lumen as shown in Figure 2

**Figure 1**: Appendix with a small portion of *Ascarislumbricoides*.

**Figure 2**: Adult *Ascarislumbricoides* extracted from appendicular lumen

The pathology report confirmed all the cases that were retained. Three differenthelminth ova were identified in the 74samples examined.In decreasing prevalence, the parasite trend was8 (10.8%)*Ascarislumbricoides*, 5 (6.8%) *Enterobiusvermicularis*, 3 (6.0%) *Ankylostomaduodenale*as shown in Table 3

**TABLE 3. PREVALENCE OF INTESTINAL HELMINTHEFROM APPENDICECTOMY SPECIMEN CONTENTS**

| Parasites | Number (%)* of specimen with parasites |
|---|---|
| *Ascarislumbricoides* | 8 (10.8) |
| *Enterobiusvermicularis* | 5 (6.8) |
| *Ankylostomaduodenale* | 3 (6.0) |

* Percentages based on total examined samples

Adult *Enterobiusvermicularis*was seen in 2 (2.7%) samples while 1(1.3%) showed an adult *Ascarislumbricoides*. The worm had been cut in two after ligation of the appendicular stump. A purse string suture was applied below the stump, the first suture released, the worm extracted and the purse string tied to avoid spillage from the coecum. It should be noted that 27 (36.5%) patients said they had taken antihelminticmedications prior to

consultationwhile 11(14.9%) other had taken metronidazole.

## DISCUSSION

Two units were chosen for the study to maximize the number of cases. To limit bias as much as possible, the study was prospective, the sampling was consecutive, and the clinical investigations were carried out by the same people.

Majority (73.4%) of our participants were aged 21 to 40years. This age range has been observed in other studies [14].In this study, acute appendicitis waspredominant in males, as reported in previous works [14]. Ultrasonography was positive in42% of patients examined. As found in previous reports [19], the positivity of ultrasonography should not be considered as the only conclusive revelation of appendicitis. It is sometimes doubted and depends on the manipulator [15].

In the present study, the surgically removed appendices were most congestive or suppurated. This observation is consistent with a previous report [16]. The appendicular lumen obstruction was found in a high numberof cases (62.2%). This observation was also reported in a study carried out in Niger by Marouma et al. [17].

Some parasites where found in the appendicular content but only one could be incriminated as being responsible for acute appendicitis. This is consistent with observations by Halkic et al. [12] and Flamant et al. [16] respectively.Nineteen patients showed evidence of parasitic helminthinfection.The parasite most frequently involved in was *Enterobiusvermicularis* in the form of the eggs or adult as it has been observed in other studies [12,18]. This is probably due to the localization of the adult worm in the coecum. Our study has not confirmed the reports from other works which had found *Entamoebahistolytica* and *Schistosomamansoni*directly implicated as causes of acute appendicitis [15,18,19]

This study found a case of luminal obstruction by adult *Ascarislumbricoides*.the worm could be considered responsible for acute appendicitis in consistence with a previous observation [1]. In some other reports the percentages of such obstruction ranged from 5 to 20 % [13]. The obstruction effect of this large worm is obvious, the worm being often larger than the orifice at the base of the appendix. The mechanisms by which smaller parasites cause appendicitis still remain to be clearly elucidated.

## CONCLUSION

A small percentage of parasites were found in the appendicular content in this study. Nothing in this study could lead to a formal conclusion that the parasite eggs seen in the appendicular content could have contributed to the acute appendicitis.However, adult *Ascarislumbricoides* by its size is an evident cause of the obstruction of the lumen and obviously can be incriminated in the causation of acute appendicitis.

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**Conflict of interest Statement:** The authors declared no conflict of interest in the present manuscript.

**Authors' Contributions**:

PTC –Study conception, appendicectomy, sample collection and analysis and compilation of results

KFHL –Laboratory investigation and substantial review of the manuscript for final publication

KFA and GML – Verification of results and data management

LNS–Ultrasonography

TS–Verification of data

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# ANTIFUNGAL AND TOXICOLOGICAL ACTIVITIES OF COMPOUNDS FROM TRAVELLER'S TREE (*RAVENALA MADAGASCARIENSIS* SONNERAT)

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**ABSTRACT**

**This study was done to investigate the antifungal and toxicological activities of extracts from the leaves of traveller's tree (*Ravenala madagascariensis*). Different concentrations (i.e. 25 - 200 mg/ml) of the extracts prepared using ethanol; n-Hexane, hot water and cold water were tested against some selected human pathogenic fungi using agar well diffusion method. The *in vivo* effects of the extracts on vital organs such as liver, kidney and some haematological parameters (Pack cell volume, Erythrocyte sedimentation rate, Red blood cell count, White blood cell count, Hemoglobin, Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils) were determined using experimental rats. The haematological analyses revealed that there were no significant differences (p≤0.05) between the values of haematological parameters obtained from the treated animals and the control groups before treatment and at the end of the treatment. The extracts appeared haematologically not toxic to the experimental rats, but deleterious effects were observed on the vital organs such as liver and kidney of the experimental rats. This may be due to the presence of higher percentage of Cyanogenic glycoside (47%), thus suggesting that the extracts could be potentially deleterious to human health when consumed orally.**

**Key Words: Antifungal activity, Toxicological effect, *in vivo*, Haematological parameters**

# LES ACTIVITES ANTIFONGUES ET TOXICOLOGIQUES DES COMPOSES DE L'ARBRE DU VOYAGEUR (*RAVENALA MADAGASCARIENSIS* SONNERAT)

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**RESUME**
**Cette recherche a été faite d'examiner les activités antifongiques et toxicologiques des extraits pris des feuilles de l'arbre du voyageur (*Ravenalamadagascariensis*). Les diverses concentrations (c. – a – d 25 a 200mg/ml) des extraits préparées en employant l'éthanol; l'en - Hexane, l'eau chaude et l'eau froide ont été testés contre certains champignons pathogènes pour l'homme selon la méthode de diffusion d'agar. Les effets *in vivo* des extraits sur les organes vitaux tel que le foie, le rein, et certaines paramètres hématologiques (Volume de cellule paquet, Vitesse de sédimentation des érythrocytes, Sang nombre des globules rouges, Sang nombre des globules blancs, Hémoglobine, Lymphocytes, Neutrophiles,Monocytes, Eosinophiles, et Basophiles) ont été déterminés utilisant des rats expérimentaux. Les analyses ont révélé qu'il n'y avait pas de différence significative (p≤0,05) entre les valeurs des paramètres hématologiques obtenus des animaux traités et le groupe témoin avant le traitement et a la fin du traitement. Les extraits ont apparu hématologiques pas toxiques aux rats expérimentaux, mais les effets délétères ont été observés sur les organes vitaux tels que le foie, le rein, des rats expérimentaux. Ceci peut être dû à la présence de pourcentage plus élevé des glycosides cyanogeniques (47%), suggérant ainsi que les extraits pourraient être potentiellement délétères pour la santé humaine lorsqu'ils sont consommés par voie orale.**

**Mots- clés: L'activité antifongue, L'effet toxicologique, *in vivo*, Les paramètres hématologiques.**

## INTRODUCTION

Plant materials remain an important resource to combat diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role in health care system of the developing countries. Natural products of plant origin may possess a new source of antimicrobial agents with possibly novel mechanisms of action. They may be effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Schulz *et al.*, 2001(1).

*Ravenala madagascariensis Sonn* of the family Sterilitziaceae and commonly called as traveller's tree, is a native of Madagascar (South Africa), but often found cultivated in Indian gardens. It is a palm-like tree with simple alternate leaves forming a fan-like crown. Its blue seeds are used for food and sugar is obtained from the sap, its wood is used for construction, the leaves for thatching, and the leafstalk contains water which travellers would drink to quench their thirst (McLendon Chuck, 2000(2). This plant is widely used in folklore medicine in the treatment of diabetes, kidney stone and diarrhoea (Sowmayanath, 2008(3). Therefore, it is of great interest to screen this plant to validate its use in traditional medicine because systematic screening of such plant may result in the discovery of novel active compounds. The study was designed to: Determine the *in vitro* effects of the leaf extracts of traveller's tree on selected human pathogenic fungi; and Determine the effect of the plant extracts on the liver, kidney and hematological parameters of experimental rats i.e. safety of the extracts for human consumption using experimental animals.

## MATERIALS AND METHODS

### Collection, Identification and Preparation of plant materials

Fresh leaves of traveller's tree (*Ravenala madagascariensis* Sonn.) used for this experiment were collected from Akure in Ondo state, Nigeria on 30th August, 2012 by 10:25am, using a scalpel. The plant was identified by Dr O. A. Obembe of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State. The identity of the plant sample was authenticated at the Herbarium of the University of Ibadan, Nigeria. Voucher specimens were deposited in the Herbarium of the Federal University of Technology Akure. The leaf samples were air-dried at room temperature for four weeks and later milled into powder using a grinding machine (ATLAS) (Das *et al.,* 2005(4). The powdered sample was stored in a sterile polythene bag and kept at 28±2°C for 4 days for subsequent analyses

### Preparation of plant extracts

A 400g portion of the powdered sample was mixed with 2 litres of n-Hexane (99.99%) and ethanol (95%$^{v}/_{v}$) respectively. Aqueous extract was prepared by mixing 400g of the powdered leaves with 2 litres of cold distilled water (28±2°C) and hot distilled water (100°C), respectively. The mixtures were allowed to stand for 72h with constant stirring, and then filtered with a clean white muslin cloth. The ethanol and n-Hexane extracts were later air-dried at 28±2°Cfor 48h and reconstituted in 30% Dimethylsulphoxide (DMSO) by weighing 10g each of ethanol and n-Hexane extracts into 50ml of 30% DMSO to make a concentration of 200mg/ml. The reconstituted ethanol and n-Hexane extracts were allowed to stand for 24h before sterilization.

### Collection and maintenance of test organisms

The clinical isolates of the pathogenic fungi used for this study were obtained from the Science Laboratory Department of Rufus Giwa Polytechnic, Owo, while the typed cultures were collected from Federal Institute of Industrial Research Oshodi (FIIRO) Lagos, Nigeria. They were maintained in double strength of Sabouraud Dextrose agar slant at four weeks interval.

### Evaluation of antifungal activity of plant extracts

The antifungal effect of the extracts were carried out on the clinical isolates and typed cultures of *Candida albicans, Trichophyton mentagrophyte, Microsporium cannis Aspergillus niger* and *Rhizopus stolonifer* using agar diffusion method as described by Leonard *et al.,* (2007(5). Four holes were made on Sabouraud dextrose agar (SDA) plates using sterile 5mm diameter cork borer and equal volumes of the extracts were transferred into the holes using a sterile needle and syringe. Dimethylsulphoxide and water was used as blank control, while standard antifungal agent (20mg/ml of ketoconazole) was used as positive control. The plates were allowed to stand for 15minutes for pre-diffusion of the extract to occur and were incubated at 28$^{O}$C for 36h. Thereafter, the diameter of zones of inhibition that developed was measured in millimeters.

### Toxicological testing of the extracts

A total number of 25 albino rats were used to determine whether any of the extracts will be toxic to humans. Five animals in each group of four different groups and another five as a control group were used for each of the extracts as five replicates. Prior to the experiment, the animals were weighed and stabilized for a period of 7days by giving them water and grower's mash obtained from Guinea feed Nig. Ltd. This was done to ascertain that the animals were apparently healthy. Different types of the extract were administered orally to each of the four groups of rats for a period of 14 days, according to Laurence *et al*. (2002(6) and Oladunmoye (2007(7). Clean water and grower's mash were administered to the control group. During the days of extracts' administration, the animals were observed for clinical presentations like salivation, nervousness, vomiting and diarrhoea and none was observed. After the expiration of fourteen days, the animals were sacrificed and blood samples were collected to test for blood parameters; Packed Cell Volume (PCV), white blood cell (Total WBC) Erythrocyte sedimentation rate (ESR) and haemoglobin estimation (Hb), to detect the level of toxicity of the extracts to the used animals according to Cheesbrough (2004).

### Statistical Analysis of Data

Data obtained from the study were subjected to one way statistical analysis of variance (ANOVA) with five replicates and treatment means were separated

using Least Significant Difference (LSD) at 95% confidence intervals using SPSS window 7 Version 16.

**RESULTS**

The results of antifungal screening of the different extracts against the test isolates are shown in Table 1. All the fungi tested showed no sensitivity to the plant extracts as shown in this table. The mean weights of the rats treated with four different extracts and their mean weight were not significantly different from the values obtained from the control for these parameters at $p \leq 0.05$. The mean weight of the kidney and the liver of the experimental animals (rats) after treatment with four different extracts, the mean weight of the kidneys were significantly different from the values obtained from the control for these parameters but not significantly different from each other ($p \leq 0.05$) with group E (control) having the highest weight of 0.54g and group C having the lowest weight of 0.37g.The mean weighs of the liver of rats, after treatment with four different extracts were significantly not different from the values obtained from the control. Group A was significantly different from other groups ($p \leq 0.05$), with group E (control) having the highest weight of 4.78g and group A (animals treated with cold water extract) having the lowest mean weight of 2.90g (Table 2).

**TABLE 1**: **ANTIFUNGAL EFFECTS OF EXTRACTS (200MG/ML) FROM *R. MADAGASCARIENSIS* AGAINST CLINICAL ISOLATES AND TYPED CULTURES OF THE TEST ORGANISMS.**

| Test organisms | Zones of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Ketoconazole (20mg/ml) | 30% DMSO | Ethanol extract | n-Hexane extract | Hot water extract | Cold water extract |
| *Candida albicans* | 25.00 | - | - | - | - | - |
| *Aspergillus niger* | 20.00 | - | - | - | - | - |
| *Trichophyton mentagrophyte* | 15.00 | - | - | - | - | - |
| *Microsporium cannis* | 19.00 | - | - | - | - | - |
| *Aspergillus fumigatus* | 15.00 | - | - | - | - | - |
| *Aspergillus flavus* | 16.00 | - | - | - | - | - |
| *Mucor mucedo* | 12.00 | - | - | - | - | - |
| *Rhizopus stolonifer* | 18.00 | - | - | - | - | - |
| *Candida albicans* (ATCC 10231) | 16.00 | - | - | - | - | - |
| *Aspergillus niger* (ATCC 16404) | 20.00 | - | - | - | - | - |
| *Trichophyton mentagrophyte* (ATCC9533) | 16.00 | - | - | - | - | - |
| *Microsporium cannis* (ATCC 18375) | 17.00 | - | - | - | - | - |
| *Rhizopus stolonifer* (ATCC 62417) | 10.00 | - | - | - | - | - |

**Values are means of five replicates ± Standard error ; - = No inhibition ; Error : Values with different superscripts on the same row are significantly different (p ≤0.05) ; A = Cold water extract; B = Hot water extract; C = Ethanol extract; D = n-Hexane extract; E=control**

The results of haematological analysis of the blood samples obtained from the rats before the ingestion. In general, the mean values obtained for Pack cell volume (PCV), Erythrocyte sedimentation rate (ESR), Hemoglobin (HB), Lymphocyte (LYM), Neutrophils (NEU), Monocytes (MON), Eosinophils (EOS) and Basophils (BAS) differed from one another but were not significantly different from each other. While the values obtained for the RBC and WBC counts were significantly different from each other including the control group ($p \leq 0.05$) as indicated in Table 3. The results of haematological analysis of the blood samples obtained from the rats before the commencement of the treatment, the mean values obtained for ESR, PCV, RBC, HB, LYM and NEU were not significantly different from each other but ignificantly different from the control group (E). While the values obtained for MON, EOS and BAS show no different from each other and also with the control group only the WBC count show that group CI and the control group are significantly different from each other and also from the other group ($p \leq 0.05$) presented in Table 4.

Table 5 shows the result for haematological analysis of the blood sample obtained from the rats after 14 days of treatment, the mean values obtained for ESR, PCV, RBC, WBC, HB, LYM, NEU, MON and EOS did not show any significant differences from each other within the group and the control but, only group DI show significant different in BAS values from other groups including the control group. Moreover, there were no significant differences between the control group and the rest of the group ($p \leq 0.05$).

**TABLE 2: MEAN WEIGHTS (GRAMS) OF THE KIDNEY AND LIVER OF ANIMALS AFTER 14 DAYS OF TREATMENT WITH FOUR DIFFERENT EXTRACTS**

| Organs | A | B | C | D | E | LSD |
|---|---|---|---|---|---|---|
| Kidney | $0.38^{b} \pm 0.02$ | $0.42^{b} \pm 0.01$ | $0.37^{b} \pm 0.04$ | $0.41^{b} \pm 0.03$ | $0.54^{a} \pm 0.05$ | 0.067 |
| Liver | $2.90^{b} \pm 0.32$ | $4.67^{a} \pm 0.11$ | $4.18^{a} \pm 0.13$ | $3.83^{a} \pm 0.08$ | $4.78^{a} \pm 0.11$ | 0.66 |

**Each value is a mean weight of five replicates ± Standard**

**TABLE 3: RESULTS OF HEMATOLOGICAL ANALYSIS OF RATS BEFORE ORAL INGESTION**

| Group | ESR mm/hr. | PCV % | RBC 10000rbc/ $mm^3$ | WBC 50wbc/ $mm^3$ | HB g/100ml | LYM % | NEU % | MON % | EOS % | BAS % |
|---|---|---|---|---|---|---|---|---|---|---|
| A | $0.61^{a} \pm 0.01$ | $42.00^{a} \pm 0.10$ | $1168.50^{a} \pm 1.19$ | $1669^{b} \pm 1.24$ | $14.00^{a} \pm 0.03$ | $66.00^{a} \pm 0.12$ | $21.00^{a} \pm 0.02$ | $6.00^{a} \pm 0.01$ | $3.00^{b} \pm 0.01$ | $3.00^{a} \pm 0.01$ |
| B | $0.60^{a} \pm 0.02$ | $42.33^{a} \pm 0.11$ | $1015.30^{e} \pm 1.30$ | $1502^{d} \pm 1.12$ | $14.11^{a} \pm 0.04$ | $70.00^{a} \pm 0.14$ | $22.00^{a} \pm 0.03$ | $5.00^{a} \pm 0.01$ | $2.00^{c} \pm 0.01$ | $1.00^{c} \pm 0.00$ |
| CI | $0.70^{a} \pm 0.00$ | $40.67^{a} \pm 0.13$ | $1066.70^{d} \pm 1.12$ | $1523^{d} \pm 1.30$ | $13.56^{a} \pm 0.00$ | $63.00^{a} \pm 0.16$ | $28.00^{a} \pm 0.04$ | $6.00^{a} \pm 0.01$ | $2.00^{c} \pm 0.01$ | $1.00^{c} \pm 0.00$ |
| CII | $0.56^{a} \pm 0.01$ | $39.57^{a} \pm 0.10$ | $1048.50^{d} \pm 1.00$ | $1696^{a} \pm 1.22$ | $13.19^{a} \pm 0.02$ | $64.00^{a} \pm 0.20$ | $20.00^{a} \pm 0.02$ | $9.00^{a} \pm 0.03$ | $4.00^{a} \pm 0.02$ | $3.00^{a} \pm 0.01$ |
| CIII | $0.65^{a} \pm 0.01$ | $39.50^{a} \pm 0.08$ | $1057.67^{d} \pm 1.22$ | $1496^{d} \pm 1.41$ | $13.17^{a} \pm 0.04$ | $70.00^{a} \pm 0.19$ | $23.00^{a} \pm 0.03$ | $5.00^{a} \pm 0.01$ | $1.00^{d} \pm 0.00$ | $1.00^{c} \pm 0.00$ |
| CIV | $0.51^{a} \pm 0.02$ | $40.50^{a} \pm 0.14$ | $1050.00^{d} \pm 1.12$ | $1584^{c} \pm 1.40$ | $13.50^{a} \pm 0.03$ | $65.00^{a} \pm 0.15$ | $23.00^{a} \pm 0.03$ | $7.00^{a} \pm 0.02$ | $3.00^{b} \pm 0.01$ | $2.00^{b} \pm 0.00$ |
| CV | $0.65^{a} \pm 0.01$ | $42.00^{a} \pm 0.12$ | $1126.50^{b} \pm 1.00$ | $1549^{d} \pm 1.23$ | $14.00^{a} \pm 0.06$ | $67.00^{a} \pm 0.21$ | $24.00^{a} \pm 0.03$ | $6.00^{a} \pm 0.01$ | $2.00^{c} \pm 0.01$ | $1.00^{c} \pm 0.00$ |
| DI | $0.55^{a} \pm 0.01$ | $41.67^{a} \pm 0.13$ | $1092.00^{c} \pm 1.22$ | $1531^{d} \pm 1.42$ | $13.89^{a} \pm 0.00$ | $64.00^{a} \pm 0.17$ | $28.00^{a} \pm 0.04$ | $7.00^{a} \pm 0.02$ | $0.00^{e} \pm 0.00$ | $1.00^{c} \pm 0.00$ |
| DII | $0.56^{a} \pm 0.02$ | $39.50^{a} \pm 0.11$ | $1092.20^{c} \pm 1.25$ | $1533^{d} \pm 1.20$ | $13.17^{a} \pm 0.05$ | $69.00^{a} \pm$ | $22.00^{a} \pm 0.02$ | $6.00^{a} \pm 0.01$ | $2.00^{c} \pm 0.01$ | $1.00^{c} \pm 0.00$ |
| E | $0.50^{a} \pm 0.01$ | $39.83^{a} \pm 0.09$ | $1126.50^{b} \pm 1.32$ | $1595^{c} \pm 1.14$ | $13.28^{a} \pm 0.04$ | $68.00^{a} \pm 0.14$ | $21.00^{a} \pm 0.00$ | $8.00^{a} \pm 0.03$ | $2.00^{c} \pm 0.01$ | $1.00^{c} \pm 0.00$ |
| LSD | 0.20 | 3.92 | 17.80 | 23.55 | 1.12 | 4.10 | 1.02 | 1.52 | 0.81 | 0.54 |

**Mean values with different superscripts in the same column are significantly different (p= 0.05); WBC= White blood cell count, HB= Haemoglobin, LYM= Lymphocyte, NEU= Neutrophils, MON= Monocytes.**

**TABLE 4: THE HEMATOLOGICAL ANALYSIS AFTER THE INGESTION OF EXTRACTS**

| Tests/ Group | ESR mm/hr. | PCV % | RBC 10000rbc/mm$^3$ | WBC 50wbc/mm$^3$ | HB g/100ml | LYM % | NEU % | MON % | EOS % | BAS % |
|---|---|---|---|---|---|---|---|---|---|---|
| CI | 1.12$^a$ ± 0.01 | 28.50$^b$ ± 0.08 | 915.70$^b$ ±1.20 | 403.8$^b$ ± 1.31 | 9.50$^b$ ± 0.05 | 58.00$^b$ ± 0.16 | 30.00$^a$ ± 0.04 | 10.00$^a$ ± 0.02 | 1.00$^b$ ± 0.01 | 1.00$^a$ ± 0.01 |
| CII | 1.08$^a$ ± 0.01 | 31.50$^b$ ± 0.10 | 941.80$^b$ ± 1.12 | 434.80$^a$ ± 1.08 | 10.50$^b$ ± 0.03 | 60.00$^b$ ± 0.14 | 31.00$^a$ ± 0.02 | 8.00$^a$ ± 0.01 | 1.00$^b$ ± 0.01 | 0.00$^b$ ± 0.00 |
| CIII | 1.25$^a$ ± 0.00 | 30.00$^b$ ± 0.00 | 931.30$^b$ ± 1.14 | 462.30$^a$ ± 1.24 | 10.00$^b$ ± 0.01 | 59.00$^b$ ± 0.19 | 32.00$^a$ ± 0.05 | 9.00$^a$ ± 0.02 | 0.00$^c$ ± 0.00 | 0.00$^b$ ± 0.00 |
| CIV | 1.17$^a$ ± 0.02 | 33.50$^b$ ± 0.09 | 873.70$^b$ ± 1.10 | 460.80$^a$ ± 1.19 | 11.17$^b$ ± 0.02 | 61.00$^b$ ± 0.11 | 28.00$^a$ ± 0.00 | 10.00$^a$ ± 0.02 | 1.00$^b$ ± 0.00 | 0.00$^b$ ± 0.00 |
| CV | 1.03$^a$ ± 0.01 | 30.00$^b$ ± 0.10 | 972.70$^b$ ±0.09 | 468.00$^a$ ± 1.32 | 10.00$^b$ ± 0.04 | 62.00$^b$ ± 0.15 | 27.00$^a$ ± 0.01 | 9.00$^a$ ± 0.02 | 1.00$^b$ ± 0.01 | 1.00$^a$ ± 0.01 |
| DI | 1.20$^a$ ± 0.00 | 31.50$^b$ ± 0.09 | 965.50$^b$ ± 1.06 | 438.80$^a$ ± 1.22 | 10.50$^b$ ± 0.03 | 61.00$^b$ ± 0.00 | 29.00$^a$ ± 0.03 | 9.00$^a$ ± 0.02 | 0.00$^c$ ± 0.00 | 1.00$^a$ ± 0.01 |
| DII | 1.25$^a$ ± 0.01 | 30.67$^b$ ± 0.07 | 964.20$^b$ ± 1.09 | 473.00$^a$ ± 1.13 | 10.22$^b$ ± 0.00 | 61.00$^b$ ± 0.18 | 28.00$^a$ ± 0.00 | 10.00$^a$ ± 0.03 | 0.00$^c$ ± 0.00 | 1.00$^a$ ± 0.01 |
| E | 0.54$^b$ ± 0.00 | 40.83$^a$ ± 0.11 | 1200.70$^a$ ± 1.10 | 271.67$^c$ ± 1.24 | 13.61$^a$ ± 0.01 | 68.00$^a$ ± 0.13 | 22.00$^b$ ± 0.01 | 7.00$^a$ ± 0.01 | 2.00$^a$ ± 0.01 | 1.00$^a$ ± 0.01 |
| LSD | 0.40 | 3.35 | 96. | 23.42 | 1.40 | 5.10 | 4.00 | 1.40 | 0.85 | 0.72 |

**Mean values with different superscripts in the same column are significantly different (p= 0.05).**
**LEGEND: ESR= Erythrocyte sedimentation rate, PCV= Pack cell volume, RBC= Red blood cell count, WBC= White blood cell count, HB= Hemoglobin, LYM= Lymphocyte, NEU= Neutrophils, MON= Monocytes, EOS = Eosinophils; BAS = Basophils**

TABLE 5: THE HEMATOLOGICAL ANALYSIS AFTER THE TREATMENT WITH EXTRACTS.

| Group | ESR mm/hr. | PCV % | RBC(10000rbc/mm$^3$) | WBC 50wbc/mm$^3$ | HB g/100ml | LYM (%) | NEU % | MON % | EOS % | BAS % |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.55$^a$ ± 0.02 | 42.67$^a$± 0.11 | 1258.20$^a$ ± 1.34 | 319.80$^a$ ± 1.02 | 14.22$^a$ ± 0.03 | 65.00$^a$± 0.14 | 25.00$^a$± 0.03 | 7.00$^a$± 0.02 | 2.00$^a$± 0.01 | 1.00$^b$± 0.01 |
| B | 0.52$^a$ ± 0.02 | 42.67$^a$± 0.13 | 1239.30$^a$ ± 1.34 | 320.50$^a$ ± 1.02 | 14.22$^a$ ± 0.03 | 68.00$^a$± 0.17 | 22.00$^a$± 0.02 | 7.00$^a$± 0.02 | 2.00$^a$± 0.01 | 1.00$^b$± 0.00 |
| CI | 0.55$^a$ ± 0.02 | 42.50$^a$± 0.13 | 1181.80$^a$ ± 1.06 | 308.50$^a$ ± 1.00 | 14.17$^a$ ± 0.03 | 64.00$^a$± 0.13 | 26.00$^a$± 0.04 | 7.00$^a$± 0.02 | 2.00$^a$± 0.00 | 1.00$^b$± 0.00 |
| CII | 0.50$^a$ ± 0.02 | 42.00$^a$± 0.12 | 1208.82$^a$ ± 1.33 | 314.80$^a$ ± 1.01 | 14.00$^a$ ± 0.03 | 68.00$^a$± 0.16 | 21.00$^a$± 0.02 | 8.00$^a$± 0.03 | 2.00$^a$± 0.00 | 1.00$^b$± 0.00 |
| CIII | 0.55$^a$ ± 0.02 | 40.67$^a$± 0.11 | 1153.70$^a$ ± 1.20 | 323.00$^a$ ± 1.02 | 13.56$^a$ ± 0.02 | 68.00$^a$± 0.17 | 22.00$^a$± 0.01 | 7.00$^a$± 0.02 | 2.00$^a$± 0.01 | 1.00$^b$± 0.01 |
| CIV | 0.45$^a$ ± 0.01 | 39.67$^a$± 0.10 | 1136.00$^a$ ± 1.09 | 305.50$^a$ ± 1.02 | 13.22$^a$ ± 0.02 | 63.00$^a$± 0.12 | 27.00$^a$± 0.04 | 7.00$^a$± 0.02 | 2.00$^a$± 0.00 | 1.00$^b$± 0.01 |
| CV | 0.50$^a$ ± 0.02 | 42.50$^a$± 0.12 | 1140.50$^a$ ± 1.12 | 311.20$^a$ ± 1.01 | 14.17$^a$ ± 0.03 | 67.00$^a$± 0.16 | 24.00$^a$± 0.02 | 6.00$^a$± 0.02 | 2.00$^a$± 0.00 | 1.00$^b$± 0.01 |
| DI | 0.52$^a$ ± 0.02 | 42.00$^a$± 0.12 | 1182.50$^a$ ± 1.24 | 321.20$^a$ ± 1.02 | 14.00$^a$ ± 0.01 | 61.00$^a$± 0.11 | 27.00$^a$± 0.04 | 8.00$^a$± 0.03 | 2.00$^a$± 0.00 | 2.00$^a$± 0.01 |
| DII | 0.52$^a$ ± 0.02 | 40.50$^a$± 0.11 | 1171.80$^a$ ± 1.23 | 314.70$^a$ ± 1.01 | 14.17$^a$ ± 0.03 | 67.00$^a$± 0.16 | 24.00$^a$± 0.03 | 6.00$^a$± 0.02 | 2.00$^a$± 0.01 | 1.00$^b$± 0.01 |
| LSD | 0.08 | 2.10 | 100.50 | 35.40 | 0.86 | 5.2 | 2.61 | 1.24 | 0.32 | 0.30 |

## DISCUSSION

From the present study, the results obtained indicated that extracts of leaves of travelers trees did not inhibit the growth of the fungal isolates used in this work.
The non-susceptibility of all the fungi tested to four different extracts may be due to absence of antifungal compound(s) produced by the plant or due to the possession of fungi enzyme(s), that are capable of destroying the antifungal compounds that may be present and this has also justify the fact that the plant have not been reported in the literature to be used in treating fungal infections (Sowmayanath, 2008(3).

The statistical analysis of the mean weight of the liver show that there was no significant different in the effect of the extracts when compared to the control except for the water extract which showed a significant different and these may be due to ability of water to extract compounds that responsible for the decrease in the weight of the animals liver. The results revealed that for RBC there was no significant different ($p \leq 0.05$) between the values obtained for the different extract administered and the control after 14 days of administration. This indicates that the extract did not affect either the circulating red blood cells or the erythropoetic centers of the animals. Some workers Aniagu *et al*., (2005(8) have also shown that some extracts of plants do not have deleterious effects on RBC after 14 days of administration. This is also true for the WBC counts, the same trend was also observed for Hb content which indicates that the extract did not affect synthesis of hemoglobin by the animals. This is at variance to the statement of Osadebe and Okuneze, (2004(9) that, some plant extracts interfere with the synthesis of Hb by inhibition of the uptake and utilization of iron, but corroborates the statement of Esimone *et al*., (1998(10) that the statement of Osadebe and Okuneze is not applicable to all plants due to the differences in soil nutrients, geographical location and phytochemical compositions of plants. Thus the extract did induce production of the WBC as it was observed in the animals in group A and B which may be due to the presence of compound(s) that helps to improve the body immunity against diseases. These results also indicate that the extract is less toxic hematologically, at least to the rats, at the concentrations administered (200mg/ml

The histopathology of the liver of the experimental animals indicate a high level of distortion in the tissues of the liver of animals treated with cold water

extracts, there is distortion and degeneration of tissue of animals fed with the hot water extracts. The animals treated with the ethanol extract showed necrotic lesions in the tissue of their liver. The animal treated with n-hexane extract showed no histopathological defect on the liver tissue when compared with the control group fed with 30% DMSO which showed normal liver architecture and this corroborates the works of Sowmayanath, (2008(3).

The histopathology of the kidney of the experimental animals indicate that there is degeneration and a necrotic lesions in the organ (kidney) of the animals treated with cold water extract, those treated with hot water extract also showed a degenerated and degraded tissue, those that are treated with the ethanol extract showed a necrotic lesion in the tissue of the kidney and those treated with n-hexane extract is highly affected with necrotic lesions and severe degeneration of the kidney tissues when compared with the control group treated with 30% DMSO which showed the normal kidney cells architecture and this corroborates the works of Sowmayanath, (2008). Although the four extracts showed no deleterious effect on the blood of the experimented animals, but high deleterious effects were shown on the liver and the kidney of the experimental animals. This indicates that all the extracts could be potentially deleterious to human health at 200mg/ml concentration when consumed orally. Therefore, it is recommended that further research on determination and removal of toxic components of this plant extracts should be done alongside its antibacterial effects to see if it can be recommended to human for the treatment of bacterial infection(s), since it has no antifungal effects.

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# FACTORS INFLEUNCING NEONATAL SEPTICAEMIA IN MAIDUGURI, NORTH-EASTERN NIGERIA

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**ABSTRACT**

***BACKGROUND:*** **Neonatal septicaemia is a leading cause of morbidity and mortality worldwide, especially in the tropics. The risk factors vary, and the clinical features of neonatal septicaemia may be vague and nonspecific, therefore a high index of suspicion is vital to early diagnosis and treatment. The aetiological agents and their antibiotic sensitivity pattern have continued to change (in the same centre over time), hence the need to have undertaken this study.**

***AIMS AND OBJECTIVES:*** **The primary objective of the study was to determine the risk factors influencing the aetiology of neonatal septicaemia at the University of Maiduguri Teaching Hospital.**

***PATIENTS AND METHODS*****: The study was prospective and all the newborn that had clinical diagnosis of septicaemia were consecutively enrolled and admitted to the Special Care Baby Unit of the Department of Paediatrics of University of Maiduguri Teaching Hospital (UMTH). The patients were appropriately investigated including blood cultures, cerebrospinal fluid cultures and urine culture among others.**

***RESULTS:*** **One hundred and ten neonates were studied, of these 46(42.0%) had positive blood culture, while 64 (58.0%) were blood culture negative. Eighteen (39.1%) of the septicaemic neonates were inborn, while 28 (60.9%) were out born. The incidence of neonatal septicaemia among babies delivered at UMTH was 5.9/1000 live births and the male to female ratio among septicaemic neonates was 1.9:1. The common risk factors for NNS were prolonged rupture of membrane (PROM), prematurity and low socio-economic status of parents among others. Fever was the commonest clinical feature at presentation (87%), others include: poor feeding (64 %), excessive crying (33%), tachypnoea, hepatomegaly were some of the common examination findings. *Staphylococcus aureus* 16(69.6%) and *Streptococcus pyogenes* 5(21.8%) were the predominant Gram positive organisms isolated while *Escherichia coli* 9(39.1%) and *Klebsiella Pneumoniae* 7(30.4%) were the predominant Gram negative organisms isolated.**

***CONCLUSION*****: Mortality was high in infection associated with Gram negative organisms and in the presence of conditions/complications like urinary tract infections, tetanus and meningitis.**

# LES FACTEURS QUI INFLUENCENT SEPTICEME NEONATALE A MAIDUGURI AU NORD – EST DU NIGERIA

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**RESUME**

**CONTEXTE : Septicémienéonatale est une cause majeure de morbidité et de mortalité dans le monde entier, en particulier dans les tropiques. Les facteurs de risques varient, et les caractéristiques cliniques de septicémienéonatale peuvent être vague et non spécifiques, pas conséquent, un indice élevé du suspicion est important pour le diagnostic et le traitement précoce. Les agents étiologiques et leur modèles sensibles aux antibiotiques ont continué à changer (dans le même centre finalement), d'où la nécessité d'avoir entrepris cette étude.**

**OBJECTIF : L'objectif principal de l'étude était pour déterminer les facteurs de risques qui influencent l'étiologie de septicémienéonatale à l'hôpital d'enseignement universitaire de Maiduguri.**

**PATIENTS ET METHODES : L'étude était éventuelle et tous les nouveaux – nés qui ont eu le diagnostic clinique de septicémie ont été inscritsconsécutivement et ils ont été admis à l'unité de soins spéciaux de bébé du Département de Pédiatrie de l'hôpital d'enseignement universitaire de Maiduguri (UMTH). Les patients ont été examinés correctement y compris l'hémoculture, la culture liquide céphalorachidienne et la culture d'urine parmi d'autres.**

**RESULTATS : Cent dix nouveau – nés ont été étudiés, de ceux – ci, 46 (42,0%) ont eu l'hémoculture positif, alors que 64 (58,0%) étaientnégatif à l'hémoculture. Dix – huit (39,1%) des nouveaux – néssepticémiquesétaientinnées, alors que 28 (60,9%) étaient hors nés. L'incidence de septicémienéonatale chez les bébés mis au monde à UMTH était5,9/1000 naissances vivantes et le ratio male –**

**femelle parmi les nouveau – néssepticémiques était 1, 9 :1. Les facteurs des risques communs pour NNS étaient membrane de rupture prolongée (PROM) prématurité et de faible statut socio – économique des parents parmi d'autres. La fièvre était la caractéristique clinique le plus commun lors de la présentation (87%) d'autres comprennent : une mauvaise alimentation (64%), pleure excessif (33%), tachypnée, hépatomégalieétaient des résultats de l'examen commun. *Staphylococcus aureus* 16 (69,6%) et *Streptococcus pyogenes* 5 (21,8%) étaient les organismes Gram positifs prédominantisolés alors que *Escherichia Coli* 9 (39,1%) et *Klebsiella Pneumoniae*7 (30, 4%) étaient les organismes Gram négatifsprédominantisolés.**

**CONCLUSION : La mortalité à été élevé dans l'infection associé aux organismes Gram négatifs et en présence des conditions /complications comme les infections des voies urinaires, le tétanos et la méningite.**

## INTRODUCTION

Infections are common problems of the newborn especially children in the developing countries.(1) Severe bacterial infections such as neonatal septicaemia constitute a major cause of morbidity and mortality in the newborn, accounting for 15 to 40% of neonatal morbidity. (1, 2) It is one of the commonest causes of admissions into the neonatal intensive care units of developing countries.(3) The incidence of neonatal septicaemia varies widely between the developed world and developing countries and also varies from one nursery to another.(1) The characteristics of neonates studied also influences the incidence. For example the prevalence rate is 3-10 fold higher in preterm than in full term neonates.(1) Also, the incidence is higher in low birth weight (LBW), (4, 5) than normal weight babies, and in males than females.(6, 7) Other factors are the levels of obstetric and nursery care available, the presence of predisposing factors like lack of good water supply, poor socio economic status, delivery at home or unhygienic environment.(8-10) The common risk factors for NNS in the developed world include prematurity and peripartum colonization of the birth canal by group B β-haemolytic streptococcus (GBS).(7) In one study from South-Western Nigeria, common risk factors were lack of good obstetric care, poor nursery practices, low socio economic status, poor housing conditions, poor personal hygiene, delivery at home/unhygienic environment, prematurity and complications of labour,(7) and most of these are preventable. The factors that influence the likelihood of neonatal infections can be classified into three groups ; Maternal, Neonatal and Environmental.(7,8)

The incidence of neonatal septicaemia in developed countries of Europe and North America ranges between .95/1000 live birth to 3/1000 live birth.(11) Unlike the values reported from developed countries, the reports from Nigeria like other developing countries are higher, ranging from 5.5/1000 live births to 35/1000 live births. (12-14)

The burden of aetiologic organisms causing NNS varies from place to place, and over time, even in the same centre or region.(1,3,6) It is therefore important to maintain local vigilance, so as to detect shifts in pattern early enough to intervene effectively.

This prospective study aimed to determine the risk factors, the characteristics and outcome of neonates with diagnosis of neonatal septicaemia at UMTH, Maiduguri North-Eastern region of Nigeria. Similar prospective study has not been carried out to determine the risk factors that influences the aetiology of neonatal septicaemia in this North-Eastern region so the need to conduct such study.

## PATIENTS AND METHODS

This was a prospective study conducted on patients admitted into the Special Care Baby Unit (SCBU) over a period of twelve months from January 1st to December 31st 2012. Total of one hundred and ten (110) patients who met the inclusion criteria were studied.

Ethical clearance was obtained from the Research and Ethics Committee of the University of Maiduguri Teaching Hospital and informed consent was obtained from the parents or the patients care giver. The babies were consecutively recruited from both the in-born and out-born units of the SCBU who presented with risk factors and features and/or diagnosis of neonatal septicaemia. All patients who met the set criteria were evaluated at admission; parameters such age, sex, maternal age, maternal education, place of antenatal care and delivery, cord care, symptoms such fever, poor feeding, respiratory difficulty and convulsion among others were recorded on to the study proforma.

Samples including blood, urine, cerebrospinal fluid, umbilical swab and other septic foci were appropriately taken for cultures before antibiotics were commenced on the patients.

All data were entered into SPSS version 16.0 (SPSS Inc. Chicago. USA. Soft ware) and analysed. Tables were used for data presentation and association were tested using Chi-square and Fishers exact test where appropriate, while statistical significance were set at $p<0.05$.

## RESULTS

Forty two (38.2%) of the 110 neonates were inborn and 68 (61.8%) were out born and the age of the neonates at admission ranged from 0-28 days with a mean of 5.33± 5.29 days. Forty nine (44.5%) presented within the first 72 hours of life, while 61(55.5%) presented after 72hrs of life. Eighteen (16.4%) of the newborn were preterm while 92 (83.6%) were full term. The mean gestational age was 38.03±2.30weeks and the range was between 30-43 weeks.

The weight at admission ranged between 1150gm - 4300gm with a mean weight of 2842.27 ± 734.23g. Forty six (41.8%) of the newborns that were studied had blood culture proven septicaemia, of whom 18(39.0%) were inborn and 28(61.0%) were out born. The remaining 64(58.2%) had negative blood culture.

Table I. Shows the distribution of the neonates in relation to place of delivery and frequency of septicaemia.

**TABLE I: PLACE OF DELIVERY AND FREQUENCY OF NEONATAL SEPTICAEMIA**

| Place of delivery | No of neonates in each group | No of culture proven septicaemia (%) |
|---|---|---|
| **UMTH** | **42** | **18(42.9)*** |
| **Gen Hospital** | **14** | **2(14.3))** |
| **PHC** | **2** | **0(0.0)** |
| **Private Hospital** | **10** | **6(60.0)** |
| **Home** | **42** | **20(47.6))** |
| **Total** | **110** | **46(42.0)** |

*** Of this 10 mothers had ANC in UMTH; the rest had no ANC but only came in during labour.**

Table II shows factors that may influence the risks of neonates to come down with neonatal septicaemia. These includes mode of delivery, characteristics of liqour mode of umbilical cord care.

Table III. Shows the relationship between parental socio economic class and neonatal septicaemia.

**TABLE II: MODE OF DELIVERY, CHARACTER OF LIQUOR AND CORD CARE IN RELATION TO NEONATAL SEPTICAEMIA**

| Factors | Number of neonates in the group | No of neonates with culture proven septicaemia (%) | $\chi^2$ | p-value |
|---|---|---|---|---|
| **Mode of delivery** | | | | |
| **SVD** | **95** | **41(43.2)** | **5.081** | **0.279** |
| **Breech** | **2** | **0(0.0)** | | |
| **Vacuum** | **1** | **0(0.0)** | | |
| **Forceps** | **3** | **0(0.0)** | | |
| **C/S** | **9** | **5(55.6)** | | |
| **Liqour Character** | | | | |
| **Clear** | **92** | **36(39.1)** | **6.236** | **0.182** |
| **Cloudy liquor** | **6** | **2(33.3)** | | |
| **meconium stained** | **4** | **2(50.0)** | | |
| **Foul smelling** | **4** | **4(100.0)** | | |
| **blood stained** | **4** | **2(50.0)** | | |
| **Cord care** | | | | |
| **Methylated spirit** | **52** | **23(44.2)** | **8.960** | **0.255** |
| **Warm old rag** | **5** | **2(40.0)** | | |
| **Cow dung use** | **5** | **4(80.0)** | | |
| **Charcoal** | **12** | **4(33.3)** | | |
| **n warm compression** | **11** | **6(54.5)** | | |
| **Tooth paste** | **22** | **5(22.7)** | | |
| **Dettol** | **2** | **1(50.0)** | | |
| **Soap** | **1** | **1(100.0)** | | |

**TABLE III: PARENTAL SOCIO-ECONOMIC CLASS AND FREQUENCY OF NEONATAL SEPTICAEMIA**

| Socio-economic class | Number of neonates in group | No of neonates with culture proven septicaemia (%) | $\chi^2$ | p-value |
|---|---|---|---|---|
| I | 7 | 4(57.1) | | |
| II | 7 | 1(14.3) | | |
| III | 25 | 9(36.0) | 3.920 | 0.417 |
| IV | 26 | 13(50.0) | | |
| V | 45 | 19(42.2) | | |
| Total | 110 | 46(42.0) | | |

Table IV. shows various factors(gestational age, prolonged rupture of membrane, age at admission, weight at birth and sex) in relation to the occurrence of neonatal septicaemia.

Ten (55.6%) of the 18 preterm newborns that were screened had blood culture proven septicaemia. There were ninety two term neonates (gestational age ≥ 37 weeks), 36 (39.1%) of whom had blood culture proven neonatal septicaemia. One of the babies born at ≥37weeks was post term ($\chi^2$ = 1.669, p = 0.196). Septicaemia was significantly more likely to occur following PROM ($\chi^2$=6.587, p=0.01). Preterm delivery, low birth weight and gender however, were not significantly associated with septicaemia.

**TABLE IV: RISK FACTORS IN RELATION TO NEONATES WITH SEPTICAEMIA**

| Factors | Number of neonates | No of neonates with culture proven septicaemia (%) | $\chi^2$ | p-value |
|---|---|---|---|---|
| Gestational age | | | | |
| Preterm | 18 | 10(55.6) | 1.669 | 0.196 |
| Term | 92 | 36(39.1) | | |
| PROM | | | | |
| YES | 21 | 14(66.7) | 6.587 | 0.010* |
| NO | 89 | 32(36.0) | | |
| Weight | | | | |
| LBW | 30 | 16(53.3) | 2.248 | 0.134 |
| NBW | 80 | 30(37.5) | | |
| Sex | | | | |
| Male | 73 | 32(43.8) | 0.363 | 0.547 |
| Female | 37 | 14(37.8) | | |

*Statistical significance set at p<0.05

**ISOLATES IDENTIFIED ON BLOOD CULTURE STUDIES**

The proportion of organisms isolated includes 23 (50%) Gram positive and the remaining half 23 (50%) were Gram negative. Out of the Gram positive organisms, Staphylococcus aureus accounted for 16 (69.6%) of the isolates, Streptococcus pyogenes accounted for 5 (21.8%), while *Staphylococcus epidemidis* and *Streptococcus pneumoniae* responsible for one isolate each. The Gram negative organisms consisted of *Escherichia coli* 9 isolates (39.2%), *Klebsiella pneumoniae* 7 (30.5%) while *Coliforms* 5 isolates (21.7%) and *Haemophilus influenzae* and *Salmonella spp* responsible for one isolates each.

**OUTCOME OF BABIES**

Of the 110 neonates studied, 46 (42.0%) neonates were blood culture proven septicaemia. Thirteen (28.00%) of the 46 neonates with positive blood culture died, while 20(31.3%) neonates out of 64 with blood culture negative septicaemia died. The overall mortality among neonates studied was 33(30.0%). ($\chi^2$ =0.860, P=0.804). Two (9.09%) of the 22 neonates with early onset septicaemia died while 11 (45.93%) of the 24 neonates with late onset neonatal septicaemia died. There was statistically significant difference ($\chi^2$=4.420, P =0.035). Ten (31.25%) of the 32 male neonates with blood culture positive septicaemia died compared to 3 (21.43%) of the 14 females neonates with positive blood culture. ($\chi^2$ =0.270, P=0.605).

Four (40.00%) of the 10 preterm neonates died compared to 9 (25.00%) of the 36 full term neonates. ($\chi^2$ =0.460, P=0.499). Six (37.50%) of the 16 low birth weight (LBW) neonates died compared to 7(23.33%) of the 30 normal birth weight babies. ($\chi^2$ =0.560, P= 0.454).

The overall mortality rate was 28.3%. Mortality was high among male than female, also higher among preterm low birth weight than term normal birth weight neonates. Though it was not statistically significant ($\chi^2$ = 1.190, p = 0.274)

## DISCUSSION

This study has revealed that neonatal septicaemia is an important cause of morbidity and mortality among neonates admitted at our centre, supporting the finding in previous studies.(13,16) The incidence of neonatal septicaemia of 5.9/1000 live births in this study is quite high compared to incidences reported from developed countries, (11) and some earlier studies in Nigeria,(10) the possible reason for such differences while in the developed countries, pregnant mothers are well informed, easy access to antenatal care delivery often at low cost, but lower than other reports,(13, 17) because these studies were also from lower resource setting like Nigeria.

Factors responsible for neonatal septicaemia were not different from other studies reported in Nigeria,(14) and elsewhere.(18) Maternal risk factors such as fever and prolonged rupture of membrane (PROM) which were common in this study have been reported by some workers.(18) Another important risk factor includes parental socio-economic status, majority of the neonates with septicaemia in this study were from low socio-economic (class IV and V), (18) and most of the other factors were also found in this category of social class. In this study neonatal septicaemia was seen more common among those that were delivered at home, which was also reported in other parts of Nigeria. (7) All the bacterial isolates in this study were mono microbial, and have been implicated previously in neonatal septicaemia.(7,19) The predominant organisms includes *Staphylococcus aureus, Streptococcus pyogenes* among the Gram positive agents and *Escherichia coli, Klebsiella pneumoniae* among the Gram negative agents in this study, had been reported by other workers in Nigeria.(7,19) Other organisms were *Staphylococcus epidemidis* and *Streptococcus pneumoniae* among the Gram positive bacteria and *Coliforms, Haemophilus influenzae* and *Salmonella spp* among the remaining Gram negatives. However there were equal proportion of both Gram positive and Gram negative organisms in this study which differs with findings in previous report from the same centre done over ten years earlier which showed Gram positive as predominant organisms.(13) The reason probably, the earlier report was a retrospective study with so many flaws as oppose to the present study which is prospective study and precaution were taken to adhere strictly the inclusion criteria set for the study.

Also, in this study, among the Gram negative organisms, *Escherichia coli* was commoner than *Klebsiella Pneumoniae*. This differ from earlier report by Ambe *et al* (13) in which *Klebsiella spp* was the predominant Gram negative pathogen. This affirms the well-known phenomenon of a periodic changes in the pattern of bacterial pathogens of neonatal septicaemia in a given environment over time, but differs from report from other centres in Nigeria.(14, 18)

The six deaths from early onset (EONNS) were due to Gram negative bacteria, out these, 4 were preterm low birth weight neonates, other two were term neonates. This is in keeping with a report from a centre in Nigeria.(10) Two had severe birth asphyxia and *E. coli* infection, one had meningitis due *E. coli*, and one had meningitis due *Haemophilus influenzae*. There were two normal birth weight babies with the EOS that died; one had severe birth asphyxia with *coliforms* septicaemia and one with meningitis due to *Klebsiella* infections.

Late onset neonatal septicaemia (LONNS) were responsible for seven death. Five cases of tetanus, out of which 3 cases were due to *staphylococcus aureus* septicaemia, one case due *Klebsiella* septicaemia and one case due to *Streptococcus pyogenes*. There was a case of preterm with *streptococcus pyogenes* UTI and another preterm with *E. coli* UTI. The overall mortality in this study was 13/46 (28.3%) which high. However, it was lower than the mortality reported elsewhere in Nigeria.(13,19) Possibly the high index of suspicion, concerted effort on the side of the health care team and vigilance on ensuring use of good quality and highly potent drugs in the treatment of index cases might likely explain the low mortality rate in our study report.

## CONCLUSION

There is unacceptable high burden of neonatal septicaemia in the region. The common risk factors identified in the study includes lack of antenatal care services, maternal illnesses such as maternal fever, antepartum haemorrhages, eclampsia, home delivery and poor cord such as use of hot compression, use of charcoal and application of cow dung and application of Maclean(tooth paste). Prematurity and prolong rupture of amniotic membrane beyond 18hrs were highly associated neonatal septicaemia. The common organisms responsible for neonatal septicaemia identified in this study include *Staphylococcus aureus*, *Streptococcus pneumoniae* among the Gram positive organisms and *Escherichia coli* and *Klebsiella pneumoniae* among the Gram negatives. Mortality was 28.3% in this study and was high among the neonates with Gram negative septicaemia. Mass education of pregnant mother at utilization of health care facility for booking, regular antenatal care, safe delivery and good cord care practices will reduce un wanted morbidity and mortality.

## LIMITATION

Due to cost of financing of the research work we could not afford to repeat the blood culture, which could have been necessary in accordance with scientific standard.

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**CONTRIBUTORS**: Pius S conceive the research and with other authors collected and analyze the data, wrote the draft and critical review of the final article.

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# MICROBIOLOGICAL AND KINETIC DETECTION OF GRAM NEGATIVE BACILLI PRODUCING EXTENDED-SPECTRUM- B-LACTAMASES (ESBL) IN EMERGENCIES AND REANIMATION UNITS OF UNIVERSITY HOSPITAL CENTER, YALGADOOUEDRAOGO, BURKINA FASO

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**ABSTRACT**

**Background: Epidemiology of extended-Spectrum- β-lactamases has become worldwide, and our aim was to establish the prevalence of isolates producer in university hospital center Yalgado OUEDRAOGO particularly in reanimation and emergencies units.**

**Material and methods: Prospective study was drive during July 2009 to march 2012 in order to collect strains resisting to third generation of cephalosporin during diagnosis analysis of biological specimens. Susceptibility of bacteria to antimicrobial agents was evaluated by disc diffusion method. Production of extended-spectrum β-lactamases has been investigated by double disc diffusion and kinetic methods.**

**Results: 259 isolates which resisted at least to one of third generation of cephalosporins were collected. Among them 188 (72, 58 %) were positive to synergy test by a double disc diffusion method. The MICs of ceftriaxone determined by E-test were under than 50µg/ml, 100µg/ml et 256µg/ml for respect 81,57°/◦ ; 55,26°/◦ et 39,74°/◦ of isolates. Hydrolyze of β-lactam ring by bacterial extract followed at spectrophotometer showed speeds running at 0 to 0,090UAb.mn$^{-1}$ for both isolates. Extract of 171 bacterial strains positives to synergy test had hydrolyzed at least one of oxy-iminocephalosporins and were identified as producing extended- spectrum β-lactamases. Spices reported by this study were *99 Escherichia coli (57,89%) ; 28 Klebsisella pneumonia (16,37%) ; 15 Enterobactersp (8,77%) ; 19 Pseudomonas aeruginosa (11,11%) ; 4 Citrobactersp (2,33%) 2 Acinetobactersp (1,16%), 3 Proteus mirabilis (1,75%) and 1 Salmonella typhi (0,05%).***

**Conclusion: This study showed that bacterial resistances by extended- spectrum β-lactamases are a reality in University Hospital center YalgadoOuedraogo. It calls about antibiotics prescription and hospital hygiene in order to reduce emergence and propagation of new resisting bacterial.**

**Keys words: microbial and kinetic analysis, Gram negative bacilli, extended-Spectrum- β-lactamase, emergencies, reanimation.**

# DETECTION MICROBIOLOGIQUE ET CINETIQUE DE BACILLES A GRAM NEGATIF PRODUCTEURS DEB-LACTAMASES A SPECTRE ELARGI DANS LES UNITES D'URGENCE ET DE REANIMATION AU CENTRE HOSPITALIER UNIVERSITAIRE, YALGADO OUEDRAOGO, BURKINA FASO

**Résumé**

**Objectif : En raison du caractère mondial de l'épidémiologie actuelle des β-lactamases à spectre élargi (BLSE), notre objectif a été d'évaluer la prévalence des souches productrices au Centre Hospitalier Universitaire YalgadoOuedraogo en particulier dans les unités d'urgence et de réanimation.**

**Matériel et méthodes : Un criblage des isolats résistants aux céphalosporines de troisième génération a été conduit lors d'une étude prospective entre Juillet 2009 et Mars 2012. Les souches d'intérêt étaient les bacilles à Gram négatif isolés au décours de l'analyse des échantillons biologiques à visée diagnostic au profit des malades hospitalisés. La production de BLSE a été recherchée par des approches microbiologiques et cinétiques.**

**Résultats : 259 isolats résistant à au moins une C3G ont été collectés dont 188 (72, 58 %) présumées productrices de BLSE objectivées par une image de synergie. La CMI de la ceftriaxone recherchée était supérieure à 50µg/ml, 100µg/ml et 256µg/ml pour respectivement 81,57°/◦ ; 55,26°/◦ et 39,74°/◦ des isolats. La vitesse moyenne d'hydrolyse des noyaux β-lactames par les extraits bactériens était supérieure à 0,090UAb.mn$^{-1}$ pour l'ensemble des souches. A l'issue des analyses cinétiques les extraits de 171souches bactériennes précédemment positives au test de synergie ont hydrolysé au moins une oxy-imino céphalosporine. Ces souches ont été retenues comme productrices de BLSE. Les espèces rapportées sont:*99 Escherichia coli***

*(57,89%) ; 28 Klebsisellapneumoniae(16,37%) ; 15 Enterobactersp (8,77%) ; 19* **Pseudomonas aeruginosa** *(11,11%) ; 4 Citrobactersp (2,33%)* **2Acinetobactersp** *(1,16%), 3 Proteus mirabilis (1,75%) and 1 Salmonella typhi (0,05%).*

**Conclusion : Cette étude a montré que le péril BLSE est une réalité au CHU Yalgado OUEDRAOGO. Elle interpelle sur les mesures d'hygiène hospitalière et sur les mesures de prescription des antibiotiques.**

**Mots clés : Détection microbiologique et cinétique, bacilles à Gram négatif, β-lactamases, spectre élargi, Urgences, Réanimation**

## INTRODUCTION

Infectious diseases are a major cause of loss of productive years of life in the world, and more than 45% of deaths in low-income countries [1]. Bacterial infections are responsible for 70 % of cases of mortality caused by microorganisms. [2] These data are certainly linked to the occurrence of bacterial resistance and could be unfortunately constantly increased with the emergence of new resistances. Control of bacterial resistance is justified nobly motivated and constitute a public health priority for WHO. The mechanisms of resistance are widely documented. The enzymatically resistance consist in the production of enzymic proteins forwhoseβ-lactamases constitute a large family that inactive β-lactams antibiotics. The involved form of thoseenzymes called extended spectrum β-lactamases (ESBL) allow bacteria to resist to third or fourth generation cephalosporins [3]. ESBLs, worldwide documented are carried by several species of Gram negative bacilli for which a large proportion adapted to humans are responsible to clinical therapeutic failure. Molecular characterization of bacterial strains allows us to know the types of β-lactmases encoded. It is established that among main ESBL, *blaCTX-M* is more described than *bla* TEM and *bla* SHV. Faced with the global expansion of extended spectrum β-lactamase, clinical laboratories are most than ever called to screen isolates producing. Molecular methods conduce to determine*bla*-type but don't access to kinetic parameters which indicate the affinity between these enzymes and β-lactam antibiotics.Through this study we aimed to contribute to clear bacterial resistance byestablishing extended-spectrum β-lactamase profile of Gram negative bacilli that resist oxy-iminocephalosporins at University Hospital Center Yalgado, Ouedraogousing microbiological and kinetic methods.

## I.MATERIALS AND METHODS

### I.1.Clinical specimens and bacterial strains

Bacterial strains collected during a prospective study from July 2009 to March 2012 were Gram negative bacilli that resist third generation of cephalosporin. They were isolated during diagnosis analysis of biological specimens. Different clinical specimens such as blood, urine, pus, vaginal swam, ascitic fluid, peritoneal fluid, and stool and rachis fluid samples were collected from hospitalized patients of emergencies and reanimation units. Samples were taken from infected patients who presented infectious evident symptoms like fever and purulent urine. Isolates were identified using conventional method [4] Identification of isolates was achieved using API 20E test trips (BioMerieux S.A., Marcy l'Etoile, France).

### I.2. Antibiotic susceptibility testing and ESBL detection

Antibiotic susceptibility was tested by disk diffusion method [5], with antibiotic disks used to test Gram negative bacilli particularly monobactam: Aztreonam (30 µg), third generation cephalosporin like cefotaxim (30 µg), ceftriaxon (30 µg), ceftazidim (30 µg), and fourth generation cephalosporins: cefepim (30 µg). Antibiotics were tested on Petri plates containing Muller Hinton agar. Measurements of inhibition area determine the clinical categories (CA-SFM, 2012). Isolates that were resistant at least to one of the antibiotics in clinical test, using NCCLS methods [6] were collected, purified and conserved at -80°C for furthers analysis. In order to screen ESBL phenotypical profile, isolates were submitted for synergy test[7] between third generation of cephalosporins disks (cefotaxime or ceftazidime) and amoxicillin plus clavulanic acid.

In addition, MICs of ceftriaxone, antibiotic frequently used in clinical routine in our sanitary centers was performed as recommended by guide E-test AB BIODISK.

### I.3. Extraction and kinetics activity of β-lactamase

From bacterial aliquot conserved 2µl of an inoculum were collected and suspended in 4ml of Luria Bertini solution. This bacterial suspension stirring, was cultured 24 hours overnight at 37°C. Bacterial culture resulting was centrifuged at 3000 rpmduring 30 minutes. The bacterial pellet which resulted was suspended in 500 µl of 100 mMphosphate buffer Ph7, and submited to physical method of freeze / thaw cycles [8,9].This treatment allowed to obtainperiplasmic contents and enzyme solution was obtained after a final centrifugation at 8000 rpm for 20 minutes. For certain bacterial strains, the enzyme production was induced with a solution of cefoxitin 20µg /ml during culture. Different bacterial extracts obtained were tested for β-lactamase activity with nitrocefin. A reaction medium consisting of 50 mM phosphate buffer pH7, enzyme (5 to 10µl) and nitrocefinat final concentration of 100 µM/ml was carried out in a spectrophotometer's tank. The extracts with a β-lactamase activity were tested with third-generation

β-lactams in order to determine their hydrolytic profile (ESBL profile). For this purpose, solutions of antibiotics were used as substrate in a reaction medium where the final concentration of antibiotic varied increasingly (25 .mu.m, 50 .mu.m, 100 .mu.m and 75µM). The enzymatic activities were monitored at UV / VIS double beam Uvikon 923, XL appropriate wavelengths: 482 nm (nitrocefin) to 235nm (ampicillin, benzylpenicillin), at 260 nm (cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefepime). All these experiments were performed at 30 °C in 50mM phosphate buffer pH7. The initial velocity ($V_0$) of hydrolysis of each compound (expressed as absorbance units per minute) was evaluated according to the relationship $V_0 = \Delta A_\lambda / \Delta t$ wherein $\Delta A_\lambda / \Delta t$ represents the slope at the origin of the curve of variation Absorbance at the wave length$_\lambda$relating to time.

**I.4. Determining the magnitude of kinetic parameters related to bacterial extracts**

Kinetic parameters Km and $V_m$ witch represent respectively Michaelis constant linked to substrates and maximal velocity of enzymatic reaction were determined byHanse linearization based onMichaelis equation.

## II. RESULTS

**II.1. Antibiotic susceptibility testing and ESBL detection**

After the isolation procedures, identification and antibacterial susceptibility testing, 259 bacilli Gram-negative resistant to at least one of third generation cephalosporin were collected. The bacterial strains identified using biochemical characteristics grouped in galleries (API 20E, minimum galleries) were distributed in the bacterial species as follows: *Escherichia coli* (n = 132), *Klebsiellapneumoniae* (n = 43), *Pseudomonas aeruginosa* (n = 34), *Enterobactersp*(n = 25), *Citrobacterspp* (n = 11) *Acinetobacterbaumannii* (n = 7), *Proteus mirabilis* (n = 6) and *Salmonella typhi* (n =1). On a Petri dish, these strains were found to be resistant to antibiotics targeted as cefotaxime, ceftriaxone, ceftazidime, cefepime, aztreonam and to a lesser extent to imipenem (Table IV). For these antibiotics to which we particularly interested, the diameter of the inhibition of bacterial growth zones varied between 0 and 22 mm. The tests synergy revealed among the 259 strains, 188 (72.58%) ESBL-producing objectified by a synergy picture (Figure 1). Antibiotic discsused in carrying out tests were Amoxicillin + clavulanic acid (20/10 ug) Ceftazidime (30µg) and Cefotaxime (30µg) cefepime (30µg), aztreonam (30µg). The distance between the antibiotic disks for the materialization of the synergistic picture in ESBL-producing strains varied from 10 to 15 mm.The study of theMICofceftriaxone ABBIODISKE-test,showed that the MIC was greater than 50 µg/ ml, 100 µg/ ml and 256µg/ml for 81.57%, respectively, 55.26% and 39.74% of isolates.

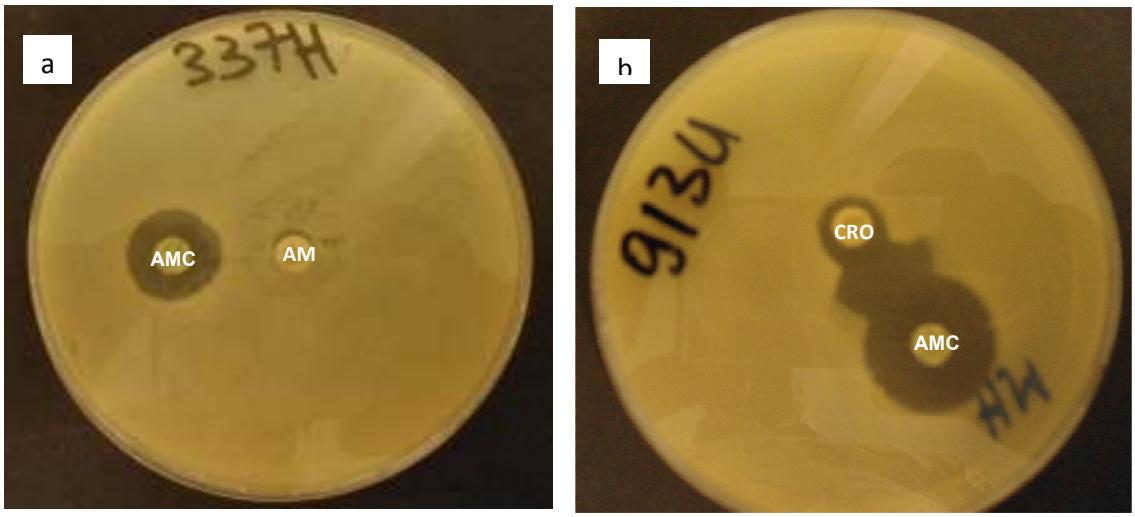


Figure: 1 a) Picture showing*E.coli*suspected as ESBL producing , b) Picture of sinergy showing ESBL production by *E. coli* on Petri plate

**II.2. Detection ofESBL bykinetic approach**

**II.2.1.Study**

**Hydrolytic Profile Of Bacterial Crude Extracts:** At the end of kinetic analysis of bacterial crude extracts, the rates of hydrolysis of the β-lactam nuclei ranged from **0 to 0,69Uabs.min$^{-1}$ for all extracts tested with nitrocefin.Table1 For total of 259 extracts analyzed, 227 showed a β-lactamase activity with Nitrocefin; while the activity was null for 32 extracts. TableII. In our experimental conditions, 171 bacterial extracts in addition to cefinase activity showed a β-lactamase**

activity with at least a third generation cephalosporin indicating that the vast majority of host strains would be extended spectrum- β-lactamase- producing. The figures below are some examples that show the progression of the hydrolysis of β-lactam antibiotics by bacterial crude extracts. In figures 2 and 3, the plots reflect changes in concentrations of the products formed (positive slope) or those of the disappearing substrate (negative slope).

| N° Extract | $V_0$(UAbs.mn$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | Nitrocefin (CPR) | Ceftriaxon(RO) | Cefotaxim(CTX) | Ceftazidin(CAZ) | Cefepim(FEF) | Imipenem(IPM) |
| 1226Uro | 0,360 | 0,028 | 0,072 | 0,018 | 0,042 | 0 |
| 450H | 0,123 | 0,047 | 0,056 | 0,030 | 0,029 | 0 |
| 176Uro | 0,073 | 0,037 | 0,063 | 0,014 | 0,051 | 0 |
| 224P | 0,598 | 0,046 | 0,0687 | 0,022 | 0,041 | 0 |
| 565P | 0,194 | 0,031 | 0,0615 | 0,011 | 0,021 | 0 |

**TABLEI: RATE OF HYDROLYSISOFSUBSTRATES WITHSOMEBACTERIAL EXTRACTS**

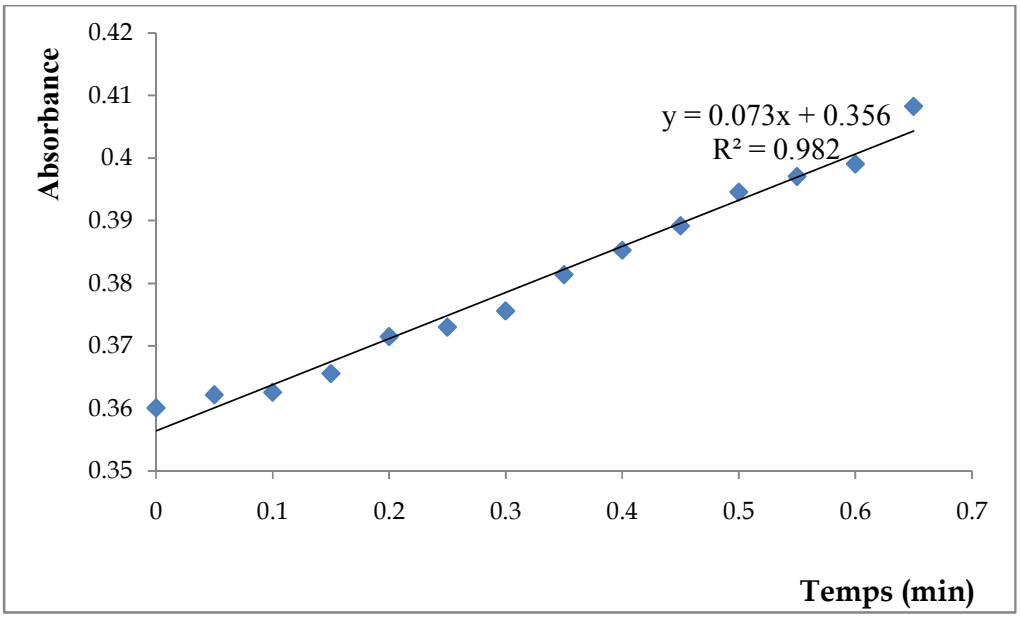


**FIGURE 2:MONITORING THE KINETICS HYDROLYSIS OF NITROCEFIN (100 µM )IN FUNCTION OF TIME WITH AN EXTRACT OF *S. typhi*176 URO. V0=0.073 UAbs.mn$^{-1}$**

**II.2.2. Determining the magnitude of thekinetic parameters bacterial crude extracts**
Linearization of Michaelis expression of the initial velocity$V_0$:$V_0 = \frac{Vm[S]}{Km+[S]}$ by Hanes's method allowed to obtain new expression of the write speed as $\frac{[S]}{V0} = \frac{1}{Vm}[S] + \frac{Km}{Vm}$ .This linearization leads to approach magnitude order of kinetic parameters Km and corresponding $V_m$. Table III gives an application example with the crude enzymatic extracts from the strain 224P.

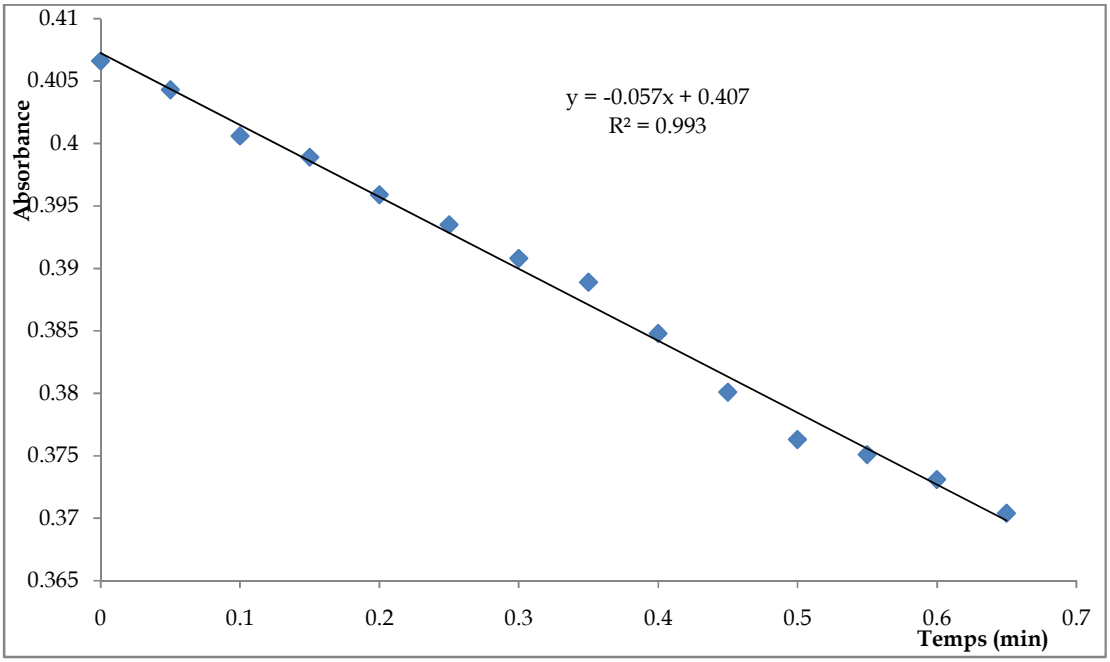


**FIGURE3:MONITORING OF THE KINETICS HYDROLYSIS OFCEFOTAXIM (25 µM) IN FUNCTION OF TIME WITH AN EXTRACT OF*ENTEROBACTER SP* 224P. $V_0$=0.057 UABS.MN$^{-1}$**

**TABLEII:HYDROLYSIS ANTIBIOTICS SUBSTRATES RATES BY BACTERIAL EXTRACTS**

| | Nitrocefin (CPR) | Cefotaxim (CTX) | Ceftriaxon (CRO) | Cefepim (FEP) | Ceftazidin (CAZ) | Imipenem (IPM) |
|---|---|---|---|---|---|---|
| **Extractsanalysednumber** | **259** | **227** | **227** | **98** | **227** | **74** |
| **Hydrolysis rates ofsubstrates (%)** | ***n=227*** **87,64** | ***n=142*** **62,55** | ***n=93*** **40,96** | ***n=16*** **16,32** | ***n=46*** **20,26** | ***n=0*** **0** |

**TABLEIII: VALUESLINKINGCONCENTRATIONSTO INITIALVELOCITIES OF HYDROLYSISOFCEFOTAXIME**

| V0 UAbs.mn$^{-1}$ | 1/V0 | [S]µM | [S]/V0 |
|---|---|---|---|
| **0.040** | **25** | **6,25** | **156,25** |
| **0.057** | **17,5438** | **25** | **438,595** |
| **0.084** | **11,9076** | **50** | **595,38** |
| **0.094** | **10,6382** | **75** | **797,865** |

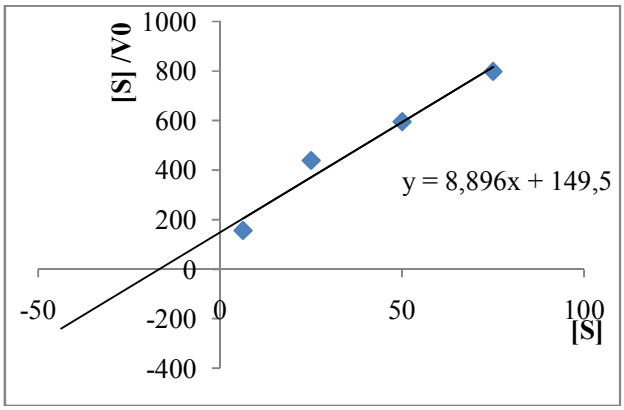


**FIGURE 4: PLOT [S] / $V_0$ ACCORDING TO [S] FOR CEFOTAXIME**

According to Hanse's linearization, the slope of the plot (Figure 4) intersects the axis at point $K_m$

/$V_m$and that of the point abscices $k_m$. Solving the equation y =8,896x + 149.5 =0 to determine the Km value. From the foregoing, the kinetic parameters Km and $V_m$ linked to enzyme extracted from the strain 224P for cefotaxim were: $k_m$ = 16,80µM and $V_m$ = 0,112 UAb.mn$^{-1}$. Similar analysis allowed determining Km and Vm relevant to ceftriaxon linked crude extract of strain 224P (*Enterobactersp).* For ceftriaxon parameters values were: Km 17,61µM and $V_m$ = 0.109 UAbs.mn$^{-1}$.

It was found that 171 bacterial strains have been implicated in both the synergy tests and kinetic analysis as producing ESBL. Note that of the 188 bacterial strains implicated by the synergy test 17 strains haven't any kinetic activity with third generation cephalosporin. Strains producing ESBL were retained in 66.02% of all collected resistant strains. The species were reported in order of quantitative importance: 99 *Escherichia coli* (57.89%); 28 *Klebsisellapneumoniae* (16.37%); *Enterobactersp* 15 (8.77%); 19 *Pseudomonas aeruginosa* (11.11%); 4 *Citrobactersp*(2.33%) 2 Accinetobactersp (1.16%) and *Proteus mirabilis* 3 (1.75%) and 1 *Salmonella typhi* (0.05%). Distribution of extended spectrum bacterial strains producing in biological samples are presented in table 4

**TABLE IV: DISTRIBUTION OFESBL-PRODUCING STRAINSBYBIOLOGICAL SAMPLE ANALYZED**

| **Strains** | **Urines** | **Stools** | **Blood** | **PV** | **Pus** | **LCR** | **Total** | **Pourcentage** |
|---|---|---|---|---|---|---|---|---|
| ***Escherichia coli*** | **71** | **00** | **06** | **06** | **14** | **02** | **99** | **57,89** |
| ***Klebsiellapneumoniae*** | **11** | **00** | **07** | **01** | **09** | **00** | **28** | **16,37** |
| ***Enterobactersp*** | **08** | **00** | **02** | **01** | **04** | **00** | **15** | **8,77** |
| ***Proteus mirabilis*** | **01** | **00** | **00** | **00** | **02** | **00** | **03** | **1,75** |
| ***Pseudomonas aeruginosa*** | **08** | **00** | **01** | **00** | **10** | **00** | **19** | **11,11** |
| ***Salmonella typhi*** | **01** | **00** | **00** | **00** | **00** | **00** | **01** | **0,05** |
| ***Citrobactersp*** | **04** | **00** | **00** | **00** | **00** | **00** | **04** | **2,33** |
| ***Acinetobacterbaumannii*** | **02** | **00** | **00** | **00** | **00** | **00** | **02** | **1,16** |
| **Total** | **106** | **00** | **16** | **08** | **39** | **02** | **171** | **100** |
| **Pourcentage** | **61,98** | **00** | **9,35** | **4,67** | **22,80** | **1,16** | **100** | |

**PV: vaginal swab. LCR: cerebrospinal fluid**

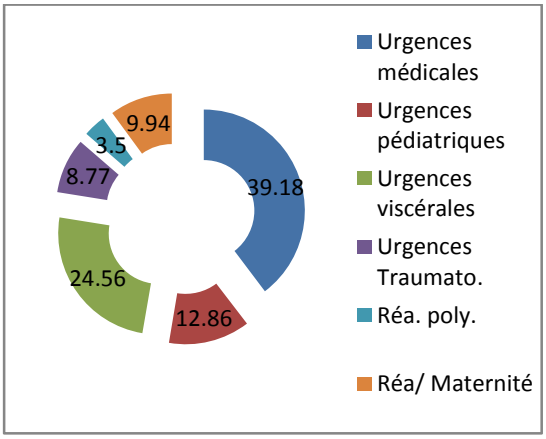


**FIGURE 5: DISTRIBUTION OF PROPORTIONS (%) OF BACTERIAL STRAINS IN CLINICAL UNITS**

## III. DISCUSSION

### III. 1. Antibiotic susceptibility testing and ESBL detection

Microbiological detection of ESBL production based on the synergy test is today a routine practice of laboratory diagnostics in low-income countries. Performed with the utmost care, it allows us to incriminate bacterial strains for which special attention should be given when prescribing antibiotics. ESBL phenotype of these strains is very often confirmed byan increase in MICs of antibiotics. Of 259 resistant strains we collected, 188 (56.7%) showed an ESBL phenotype on plates. The MIC values of ceftriaxone and cefotaxim we have determined for these strains were of the same order of magnitude as those determined by other authors for ESBL-producing strains [10, 11].However, factors such as the distance between the discs of antibiotics for the materialization of the synergy image, the additional production of other β-lactmases (AmpC, MBL) can influence the result of thesynergy test [12]. The study of the kinetic activity of the bacterial extracts has allowed incriminating 171 ESBL-producing strains among the 259. It was revealed that the extracts of 17 yet positive bacterial strains in synergy test hydrolyzed no third generation cephalosporins. The breakfast strains were therefore not producing ESBL [13]. Detecting the ESBL by the enzymatic method could be considered a correction method for the synergistic test. In addition, this same method, if it does not determine the type of beta-lactamase in the presence of genes as molecular methods, allows through the values of the kinetic parameters to specify the nature of the affinity of the enzyme for antibiotics.

### III.2. Frequency and biological distribution of ESBL producing strains

ESBL producing strains collected in our study are spread into bacterial species frequently reported by several authors. The species *Escherichia coli*, *Proteus mirabilis*, *Klebsiellapneumoniae*, *Enterobactersp.*,*Pseudomonas aeruginosa*, *Citrobactersp*, *Accinetobacterbaumannii* and *Salmonella typhi* we collected are very often cited by several authors as responsible for nosocomial infections [14]. ESBL-producing strains accounted for 66, 02% of all collected strains. This result was beyond those reported in Ghana (49, 3%)[15] ,SouthAfrica (36, 1%) [16] and (5, 4 to 25%) in Europe [17] .Among ESBL-producing bacteria species we have identified *E. coli* represented the major specie (57,89% of all our strains). This statement was similar to the results of other workers [18, 19, 20, and 21].Note that *Escherichiacoli*is the most representative of commensal enterobacteria. Feces contain 13 x. $10^6$ per gram in healthy subjects and this figure re ached in sick subjects to $10^{11}$ [22]. The anatomical proximity of urinary and genital openings with the anal opening could facilitate the ano-genital spread of this bacterium, which by ascending way affects various organs of the urogenital system. This observation is more pointed in the emergency and reanimation units, where the condition of patients is associated with a lower level of hygiene.

### III.3.Ecological niche of ESBL-producing strains detected

From results of our study, medical emergencies unit followed by those of Visceral Reanimation and Pediatric Emergencies appear to be most sources of ESBL producing strains. However it should be noted that in context of our country, the position of the medical emergencies unit can be relative, given its place in the organizational system of hospitals. This unit represents for many others the entry and where complementary diagnosis is often required before the transfer of patients. From the above, Visceral Emergency unit prove to be the ecological niche of primary importance among the short-term hospitalization. In this unit as its name suggests, pathologies consist of visceral infections or visceral diseases complicated infections due to reputed ESBL producing *Enterobacteriaceae*. Intensive care unit of motherhood takes third place after Pediatric Emergencies. In both units, promiscuity and of individual and hospital hygiene conditions could explain the persistence of nosocomial strains and spread of ESBL. Reanimation and Intensive Care Services are indexed as excellent places for selective pressure of resistant strains by the indiscriminate use of antibiotics [23]. However, from our results polyvalent reanimation unit, recorded fewer cases of infections related to ESBL producing strains. This could be related to good hygiene and safety care practices, but it should be noted that the samples referred to diagnosis from this service were infrequent. In France, in 2009, the relative risk of acquiring abacterial strain producing ESBL in clinical units of a university hospital was established as follows: Emergency (0, 46), Intensive care(1,78), Medicine(0,67), Surgery (0,97), Paediatrics(0,6), Haematology(3,07), Maternity(0,43)[24].

**Conclusion**

Detection of ESBL-producing bacterial strains is an important global concern. If nowadays molecular detection methods are the most recommended, it is indisputable that they remain inaccessible to low-income countries. Phenotypical procedures are common practices in diagnostic laboratories. The enzyme method of detection of the production of β-lactamases that we have proposed is similar to cefinase tests performed in the clinical laboratory. The improvement of our process is that it provides opportunities to obtain kinetic parameters of enzymes that assess their affinity with antibiotics substrates. Through both microbiological and kinetic methods, we were able to establish data on bacterial species frequently responsible for antibiotic resistance in emergency units at the University Hospital Center, Yalgado, OUEDRAOGO. The affinity of the bacterial extracts for cefotaxim suggests that the major part of the strains harbors CTX-M type ESBLs. Urine samples from units of medical emergencies and visceral surgery reanimation appeared respectively as sources and ecological purveyors of ESBL-producing strains. The challenge lies in the development of reliable mechanisms to control the spread of these strains that contribute to high morbidity and mortality in our hospitals. These mechanisms should take into account a good hospital hygiene policy and also antibiotics management.

**Acknowledgements**

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# DETERMINATION OF THE ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING AND THE NON-ESBL PRODUCING STRAINS OF *ESCHERICHIA COLI*

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## ABSTRACT

**Background: The extended spectrum beta lactamases producing bacteria are bacteria of great concern among Gram negative bacilli. *Escherichia coli* stand out as major carrier of this enzyme. The appropriate control of this resistance pattern depends on using the antimicrobial regimen of best choice. Therefore the value of the susceptibility profile of organism harboring this enzyme cannot be overemphasized.**
**Objectives: To determine the antimicrobial susceptibility of extended spectrum beta lactamases (ESBL) producing and the non-ESBL producing strains of *Escherichia coli* from clinical isolates of *Escherichia coli* in University of Maiduguri Teaching Hospital.**
**Methodology: Confirmed variants of *Escherichia coli* were screened and confirmed for ESBL possession. Subsequently, modified Kirby Bauer method was utilized to test for antibiotic susceptibility using the commercially available Oxoid single disc for some major antibiotics.**
**Results: A total of 172 strains of *Escherichia coli* were identified during the study period. Out of this number; 131 were identified as ESBL positive while a total of 41 were ESBL negative. The highest sensitivity for both the ESBL positive and ESBL negative strains of *Escherichia coli* was observed with Imipenem followed closely by Gentamicin.**
**Conclusion: The study reveals narrow choice of antibiotics for the ESBL positive isolates of *Escherichia coli* although Imipenem antibiotic still retains its sensitivity.**
**Key words: Cephalosporins, Resistance, Maiduguri, Nigeria.**

# LA DETERMINATION DE LA MODELE DE SENSIBILITE ANTIMICROBIEN BETA LACTAMASE A SPECTRE ETENDU (BLSE) SOUCHES PRODUCTRICES ET NON – SOUCHES PRODUTRICES DE BLSE *D'ESCHERICHIA COLI.*

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**RESUME :**
**Contexte : Les bêta lactamases à spectre étendu produisant bactérie sont des bactéries de grande inquiétude parmi les bacilles à Gram négatif. *Escherichia coli* se démarque en tant que porteur de cette enzyme. Le contrôle approprié de cette modèle de résistancedépend d'usage de régime antimicrobien de la meilleur choix. Donc, la valeur du profil de sensibilité d'organisme hébergeant cette enzyme ne peut êtresoulignée.**

**Objectifs : Déterminer la sensibilitéantimicrobienne des bêta lactamases à spectre étendu(BLSE) souches productrices et non productrices de BLSE d'*Escherichia coli* des isolats cliniques *d'Escherichia coli*à l'Universitéhôpital d'enseignement, Maiduguri.**

**Méthodologie : Des variantesconfirmées d'*Escherichia coli* ont été examinées et confirmées pour la possession de BLSE. Ensuite, méthodeKirby Bauer modifiée a été utilisée pour analyser la sensibilité antibiotique en utilisant le disque unique Oxoid disponible dans le commerce pour des antibiotiques majeurs.**

**Résultats : Un total de 172 souches *d'Escherichia coli* ont été identifiées au cours de la période d'étude. Sur ce nombre,131 étaientidentifiées en tant que BLSE positifs alors qu'un total de 41étaient BLSE négatifs. La plus haute sensibilité pour les souches BLSE positifs et les souches BLSE négatifs*d'Escherichia coli* était observées avec imipenème suivi étroitement par Gentamicine.**

**Conclusion : L'étuderévèle un choix limité des antibiotiques pour les isolats BLSEpositifs d'*Escherichia coli* bien que l'antibiotique imipenème conserve encore sa sensibilité.**

**Mots – clés : Céphalosporines, Résistance, Maiduguri,Nigeria.**

## INTRODUCTION

Extended spectrum beta lactamases are plasmid mediated enzymes that are capable of conferring bacterial resistance to the penicillins, first, second and third generation cephalosporins and aztreonam.(1) They do this by hydrolysis of these antibiotics but they are inhibited *in vitro* by beta lactamase inhibitors such as clavulanic acid.(1) The beta lactams are the most commonly used antimicrobial drugs accounting for almost 50% of antibiotic use.(2)

ESBLs have been reported worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa.(*3) However, they are more common in *Klebsiella pneumoniae* and *Escherichia coli*.(4) Carbapenems are the drugs of first choice in most infections due to ESBL producers but they need to be used judiciously to remain efficacious. (5)

This study aims to determine the antimicrobial susceptibility pattern of the ESBL and the non ESBL producing strains of *Escherichia coli* in our locality with the aim of providing a rationale antibiotic profile.

## METHODOLOGY

**Study Area:** The study was carried out in the department of Medical Microbiology and Parasitology University of Maiduguri Teaching hospital from January to June, 2014.

**Study Design:** Descriptive, Observational and Cross-sectional in design

**Sample Size:** A total of 172 strains of *Escherichia* coli were isolated during the study period

**Clinical Specimen:** The isolates were obtained from the following specimens; wound swabs, wound biopsies, aspirates, urine, cerebrospinal fluid, blood culture, sputum, ear swabs and eye swabs that were submitted to Medical Microbiology Department of University of Maiduguri Teaching Hospital (UMTH) for routine analysis.

**Sampling Method:** Non-probability, convenient sampling was used. All specimens that yielded the growth of *Enterobacteriaceae* during the study period were utilized.

**Bacterial Culture and Preliminary Identification:** The specimens were inoculated on MacConkey agar. They were then incubated at 18-24 hours under aerobic atmosphere at 37 $^{o}$C. Any isolate with the typical morphology of *Escherichia coli* is picked for additional studies. The morphology of *Escherichia coli* on MacConkey is that of a lactose fermenter; producing pink colored colonies that are 1-4 mm in diameter and slightly mucoid.(6) Gram staining and motility testing was done. *Escherichia coli* are Gram negative rods and motile.

**Bacterial Confirmation:** Suspected isolates of *Escherichia coli* were confirmed by the Microbact Gram negative identification system 24E ™ (Oxoid) according to the manufacturer's instructions.

**ESBL Screening:** Isolates were screened for ESBL production by using disc diffusion of cefotaxime (CTX) and ceftazidime (CAZ) placed on inoculated plates containing Muller Hinton agar according to Clinical and Laboratory Standard Institutes (CLSI) recommendations. (7)

**ESBL Confirmation:** Double disk synergy test was performed by placing ceftazidime (30 µg) and cefotaxime (30 µg) at a distance of 20 mm (centre to centre) from a disc containing amoxicillin (20 µg) plus clavulanate (10 µg); (augmentin; 30 µg ). Positivity for ESBL production was interpreted if there is a ≥ 5mm diameter for either antimicrobial agent tested in combination with clavulanic versus its diameter when tested alone as recommended by CLSI. (7)

**Susceptibility Testing:** The modified Kirby Bauer method was utilized. Antibiotic sensitivity testing was done using the commercially available Oxoid single disc comprising of ampicillin(10µg), amoxicillin/ clavulanic acid(10/20 µg), ciprofloxacin(5µg), trimethoprim/ sulphamethoxazole(1.25/23.75µg), gentamicin(10µg), ceftazidime(30µg), cefotaxime(30µg) and imipenem(10µg). The test was carried out on Mueller Hinton agar according to CLSI guidelines.(7)

**Ethical Consideration:** The study protocol was reviewed and approved by the Ethical Review Committee of UMTH

**Data Analysis:** Data analysis was carried out using the Microsoft Excel, computer software.

## RESULTS

A total of 172 isolates of *Escherichia coli* were identified during the study period. Out of this number 60 were screened as ESBL positive while 112 were screened as ESBL negative. However, following the confirmatory testing; 41 were identified as ESBL producers while 131 were identified as ESBL negative. The distribution of the various isolates based on the specimen is as shown in Table 1.

The antimicrobial susceptibility profile of the 131 ESBL negative *Escherichia coli* were as shown in Figure 1.

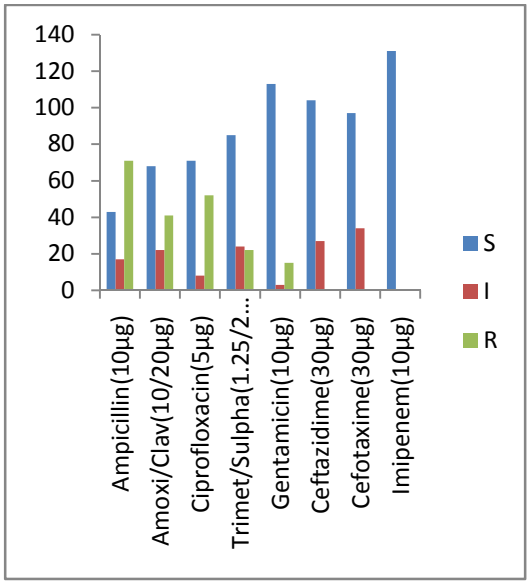
All the 131(100%) isolates were sensitive to imipenem. Gentamicin was the second most sensitive antimicrobial agent with 113(86%). The highest resistance of 71(54%) was observed with ampicillin, followed by ciprofloxacin with 52(40%).

The antimicrobial susceptibility profile of the 41 ESBL positive *Escherichia coli* were as shown in Figure 2. The highest sensitivity of 37(90%) was observed with imipenem, followed by gentamicin with 27(66%). However, the highest resistance was observed with ceftazidime and cefotaxime with 41(100%) and 35(85%) respectively.

**TABLE 1: DISTRIBUTION OF THE 172 ISOLATES OF *ESCHERICHIA COLI* BASED ON SPECIMEN ISOLATED**

| Specimen | No | % |
|---|---|---|
| Swabs | 39 | 22.7 |
| Urine | 68 | 39.5 |
| Blood | 18 | 10.5 |
| CSF | 16 | 09.3 |
| Pus | 31 | 18.0 |
| Total | 72 | 100 |

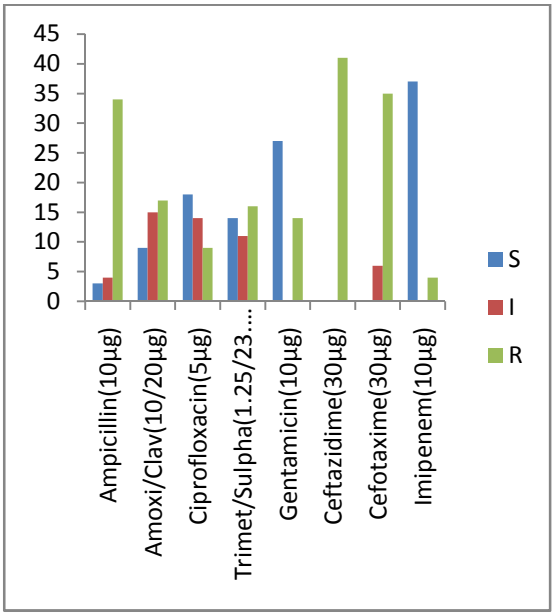
**FIGURE 1: SUSCEPTIBILITY PATTERN OF ESBL NEGATIVE STRAINS OF *ESCHERICHIA COLI* (N=131)**




**Legend: Amoxi/Clav = Amoxicillin/Clavulanic acid; Trimet/Sulpa = Trimethoprim/Sulphamethoxazole**

**S = Sensitive; I = Intermediate; R = Resistant**

**FIGURE 2: SUSCEPTIBILITY PATTERN OF ESBL POSITIVE STRAINS OF *ESCHERICHIA COLI* (N=41)**




**Legend: Amoxi/Clav = Amoxicillin/Clavulanic acid; Trimet/Sulpa = Trimethoprim/Sulphamethoxazole**

**S = Sensitive; I = Intermediate; R = Resistant**

## DISCUSSION

This study recorded a low resistance to imipenem but a high resistance to ampicillin and ciprofloxacin for the ESBL negative strains of *Escherichia coli*. This implies that imipenem are effective for the treatment of the ESBL positive strains but ampicillin and ciprofolaxacin are ineffective and may result in treatment failure if used. Nwadioha and colleagues reported a similar finding, (8) although it's in contrast to the work of Olanitola where ESBL negative *Escherichia coli* were found to have low resistance to ciprofloxacin and amikacin. (9)

This study recorded a low resistance of imipenem, gentamicin and ciprofloxacin but a high resistance of ceftazidime, cefotaxime and ampicillin for the ESBL positive strains of *Escherichia coli*. The finding is in agreement with the work of Kadar *et al* (10) in 2005, where 89% of the ESBL producers were susceptible to imipenem and meropenem. Even though, a different finding was noted by Okesola and Oni (11) in a study, to determine the susceptibility of carbapenems (imipenem and meropenem) and amikacin against the ESBL-producing *Klebsiella* isolates. The finding of a high sensitivity to imipenem is likely due to the fact that this antibiotic is expensive and not commonly prescribed hence selection for resistance is minimal compared to the other readily available antimicrobials. The clinical significance of the finding is that ampicillin and cephalosporins are not likely to be successful in treating the infection caused by ESBL positive isolates of *Escherichia coli*. However, trimethoprim/sulphamethoxazole can be used with caution when treating empirically for the urinary isolates of *Escherichia coli* as it has a relative sensitivity for ESBL positive *Escherichia coli* from this study.

Carbapenem (imipenem) class antibiotic was the most reliably effective empirical therapies for infection with these organisms, even though worrisome fact of resistance to imipenem of 9.8% (4/41) for ESBL positive *Escherichia coli* was observed.

The antibiotic susceptibility of the ESBL positive isolates of *Escherichia coli* revealed a limited group of effective antibiotics for the treatment of infections caused by this organism. The multi drug resistance observed in ESBL positive isolates might reflect the fact that, ESBLs have been associated with co-resistance to other agents including trimethoprim-sulphamethoxazole, gentamicin and ciprofloxacin. (12)

Cephalosporins, frequently used against *Enterobacteriaceae*, were widely recommended and abused in the past decade. (13) This study revealed the already documented resistance of ESBLs to cephalosporins in addition to likely quinolones co-resistance. This happens because some of the patients had exposure to both cephalosporins and quinolones or both resistances could be simultaneously adopted by plasmid-mediated mechanisms. (14) Therefore these agents cannot be use for empirical treatment of infections due to ESBLs producing organisms.

**CONCLUSION:** The study revealed a pan resistance of ESBL positive *Escherichia coli* isolates to the beta lactam agents in addition to the likely quinolone co-resistance. However, Imipenem retain its sensitivity for the ESBL positive isolates. In view of this drug resistance the practice of routine ESBL testing along with the use of the appropriate antibiotic following a conventional antibiogram would be useful for all cases which will help in the proper treatment of the patient and also prevent further development of bacterial drug resistance.

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# PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN BURNS AND PRESSURE ULCER PATIENTS

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**ABSTRACT**

**Methicillin resistant *Staphylococcus aureus* (MRSA) is a multidrug resistant bacterium that threatens the continued effectiveness of antibiotics worldwide. The objective of this study was to investigate the prevalence of MRSA and its antibiotic susceptibility pattern in patients with burns and bedsore. This was a cross- sectional study that was carried out at National Orthopaedic Hospital, Enugu, Nigeria. A structured questionnaire was used to obtain information on demographic and source of wounds. Pus from the wound was collected with swab sticks or 2ml syringe and analyzed bacteriologically, using mannitol salt agar sheep red cell blood agar. Isolates of *Staphyloccus aureus* were subjected to oxacillin and cefoxitin disc-diffusion assay and confirmed by chromogenic Brilliance MRSA 2 Agar; for identification of MRSA and MSSA. The MRSA and MSSA strains were tested for antimicrobial susceptibility patterns and multiple antibiotic index calculated. Of 104 wound swabs analyzed, 52 (50%) were *Staphylococcus aureus* isolates, while 21 (20.2%) were MRSA and 31 (29.8%) were MSSA. No significant differences were observed in the prevalence of MRSA among gender, duration of wounds, wound dressing interval and source of wound. There was an association between age, prolonged hospital admission MRSA infection. Methicillin-resistant *Staphylococcus aureus* isolates showed high resistance to ampicillin 90.5% followed by erytromycin 81% and ciprofloxacillin 71.4%.All the MRSA isolates were susceptible to vancomycin. All isolates of MRSA were resistant to β-lactams, aminoglycosides and quinolones group of antibiotic used. Minimum Inhibitory Concentration of vancomycin showed that the break point was between 0.5-2µg/ml and that of ampicillin was ranges from 4 µg/ml-128 µg/ml. MAR Index was >0.2 which indicates the resistance emanates from hospital. The high prevalence of MRSA and antibiotics resistance may increase the disease burden amongst these patients. It is necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in the hospitals and community settings, to prevent the spread of MRSA.**

**Keywords: MRSA, Brillience ChromAgar, ampicillin, vancomycin, multiple antibioticindex,**

# LA PREVALENCE ET LA MODELE DE SENSIBILITE AUX ANTIBIOTIQUES DE *STAPHYLOCOCCUS AUREUS* RESISTANT A LA METHICILLINE CHEZ LES PATIENTS BRULES ET LES PATIENTS ATTEINTS D'ULCERE DE PRESSION.

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**RESUME:**

***Staphylococcus aureus*résistant à la Méthicilline (MRSA) est une bactériemulti résistant qui présente une menace pour l'efficacité des antibiotiques dans le monde entier. Le but de cette étudeétait d'enquêter sur la prévalence de MRSA et sa modèle de sensibilité aux antibiotiques chez les patients brulés escarres. Ce fut une étude transversale qui a étéréalisée à l'hôpitalorthopédique national, Enugu, Nigeria. Un questionnaire structuréàétéutilisé pour obtenir l'informations sur démographique et source de blessures. Le pus de plaies a été recueilli avec bâtons d'écouvillons ou une seringue de 2ml et analysébacteriologiquement, utilisant Mannitol agar moutons de sel globules rouges gélose au sang. Isolats de *Staphylococcus aureus* ont été exposés au oxacilline et cefoxitine test de diffusion sur disque et a confirmé par Brillance MPSA 2 Agar chromogène ; pour l'identification de MRSA et MSSA. Les souches de MRSA et MSSA ont été analysées pour la modèle de**

**sensibilité antimicrobien et l'indice multiples antibiotiques calculés. Sur 104 des tampons enroulésanalysés, 52 (50%) étaient souches de *Staphylococcus aureus*, alors que 21 (20,2%) étaient MRSA et 31 (29,8%)étaient MSSA. Aucunesdifférencesconsidérables n'ont été observées dans la prévalence de MRSA parmi le sexe, la durée des plaies, l'intervalle de pansement et la source de plaie. Il y avait une association entre l'âge, l'hospitalisationprolongée d'infection MRSA. Les souches de *Staphylococcus aureus*résistants à la Méthicilline montraient une résistance plus élevéeà l'ampicilline 90,5% suivi par érythromycine 81% et ciprofloxacilline 71,4%. Tous les isolats de MRSA étaientprédisposés à vancomycine. Toutes les isolats de MRSA étaientrésistants au β – lactamines, les Aminoglycosides et le groupe des antibiotique quinolones utilisées. L'inhibitrice minimale de la concentration de vancomycine a montré que le point de rupture était 0,5 - 2µg/ml et celle d'ampicilline variait de 4µg/ml à 128µg/ml. L'indice MAR était >0,2 qui indique que la résistanceémane de l'hôpital. La haute prévalence de MRSA et la résistance des antibiotiques peut augmenter le fardeau de la maladie chez les patients. Il est nécessaire d'établir le système de surveillance de la sensibilité aux antimicrobiens et d'améliorer les programmes courants pour contrôler l'infection aux hôpitaux et milieux communautaires pour éviter la propagation de MRSA.**

**Mots – clés : MRSA,Brillance chrom Agar, l'ampicilline, vancomycine, multiples antibiotiques, l'indice.**

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that have acquired the ability to grow in the presence of methyl penicillin derivatives, including methicillin, oxacillin and nafcillin (1). The first report of methicillin-resistant *Staphylococcus aureus* (MRSA) was in 1961 after the introduction of methicillin in clinical settings (2). Subsequently, the spread was observed globally both as hospital acquired (HA-MRSA) and community acquired (CA-MRSA) infection within the population without any apparent risk factor (3). MRSA have proven particularly difficult to treat because they possess antimicrobial resistance gene known as *mecA* (4). Staphylococcal cassette chromosome mec (SCCmec) is a genomic island of unknown origin containing the antibiotic resistance gene *mecA* which is responsible for resistance to methicillin and other β-lactam antibiotics (5,6)

The reasons for the emergence of MRSA are multifactorial and can be attributed to host factors, infection control practices and antimicrobial pressures (7). Eileen and Venezia (7) attributed the use of levofloxacin and macrolides as promoters of MRSA spread within hospital environment. Hospital associated MRSA isolates often show multiple resistances to other commonly used antimicrobial agents, including quinolones, aminoglycosides, erythromycin, clindamycin, co-trimaxole and tetracycline (8,9).

Burn wound infection is a major complication in burn patients after initial period of shock and the chance of infection persist until complete wound healing (10). Thermal injury destroys the skin barriers that normally prevent invasion by microorganisms (4,11,12).The removal of epithelial layer of the skin in burns enhances a suitable site for bacterial colonization and multiplication and presents a more persistent and richer source of infection than surgical wound (13).

Pressure ulcers are more common among patients who are immobilized because of injury, acute illness or sedation. Their immobile state does not have to occur for long for bed sores to develop and the prevalence is particularly high in hospital setting especially in critical care unit (14). The nature of burn or bedsore involves removal of epithelial barrier. Patients with extensive burn injuries or sore are especially susceptible to infections with MRSA due to loss of the skin barrier prolonged antibiotic therapy and reduced immunological capacity such that the T cells cannot reach sites of infection (15). MRSA infections in burn or bedsore patients are classified as a secondary infection because of the traumatized skin (16).

Infections of these wounds may lead to systemic conditions such as septicaemia, pneumonia, endocarditis, deep-seated abscesses or multiple organ dysfunction syndrome (4). Transmission of organisms can be from the patient's own skin, gut and respiratory flora. Healthcare provider-to-patient transfer is common, especially when healthcare providers move from patient to patient without performing necessary hand-washing techniques in-between patients (17). The objective of this study were in two fold; to assess the prevalence of methicillin resistant *staphylococcus aureus* in burns/bed sore patients and determine the patterns of antibiotic resistance.

## MATERIALS AND METHODS

**Study Area:** The patients were recruited from National Orthopedic Hospital Enugu. This is a tertiary health care institution with 701 bed spaces that is dedicated to handling of trauma, burns and accident victims. The hospital is a regional center for burns and serves the whole of south east and south-south geographical region of Nigeria.

**Study population:** The subjects for this study were patients that had several degrees of burns and

patients that had developed bedsore due to prolonged illness. These patients were chosen because of long stay in hospital and have been administered with several antibiotics both topically, orally or injection. Those patients with burns and have not stayed a minimum of 30 days in the hospital were not selected. Those that cannot afford the hospital expenses but comes from their homes for treatment and dressing were included in the study. The participants were administered with a structured questionnaire to obtain information on demographic characteristics and risk factors that gave rise to the initial injury. Information was obtained either orally or via their medical records.

**Ethical Consideration:** Ethical Commitee of National Orthopaedic Hospital Enugu, Nigeria reviewed and approved the study protocol. The patients endorsed Informed Consent Forms and participation was voluntary.

**Collection of samples:** The surface of wound was first cleaned with a disinfectant to eliminate surface contaminants. The sides of the tissue surrounding the wound were carefully pressed with hand until pus comes out. This was then collected with a sterile swab or a 2ml syringe. The pus samples were collected in batches and were immediately transported to the laboratory for bacteriological analysis.

**Bacteriological Techniques:** Swab samples and/or pus were aseptically inoculated into Blood agar (enriched with 10% sheep red blood) and Mannitol Salt Agar plates and then incubated aerobically at 37°C for 24 hours. The cultures were examined and those with growth were identified using typical morphological characteristics and biochemical methods. Isolates that were Gram positive cocci in clusters, catalase positive and coagulase positive were confirmed as *S. aureus*.

**Identification of MRSA:** The *S aureus* isolates were subjected to secondary culture and antibiotic sensitvity to identify the MRSA and methicilin sensitive *Staphyloccus aureus (*MSSA) strains.The confirmatory antibiotics used were: Oxacillin disk (1 µg) and Cefoxitin disk (30µg),and culturing on Brilliance MRSA 2 Agar plate (oxoid, UK).

**Antibiotic susceptibility test:** All the MRSA and MSSA isolates were tested for antibiotic susceptibility by the disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (2012). The tested antibiotics were gentamicin (10µg), erythromycin (15µg), ciprofloxacin (5µg), levofloxacin (5µg), Rifampicin (2ug) clindamycin (2µg), Ceftriaxone (30µg), Ampicillin (10µg), and vancomycin (30µg).

**Minimum inhibitory concentrations (MIC):** The MIC was done using ampicillin and vancomycin. Preparation of antibiotic stock solutions was according to CLSI and antibiotic dilution range of 0.06-32 mg/L for vancomycin and 0.03-128 mg/L for ampicillin was prepared using sterile Muller Hinton broth in serial dilution. Macrodilution method was used for the MIC.

**Multiple Antibiotic Resistance index** (MAR): Isolates resistant to the tested antibiotics in at least three of the following classes: β-lactams, aminoglycosides and quinolones group of antibiotics were considered multi drug resistant. The MAR index of an isolate is defined as **a/b**, where **a** represents the number of antibiotics to which the isolate was resistance and **b** represents the number of antibiotics to which the isolates was subjected to.

**RESULTS**

Patients with burns and bedsore wounds recruited for this study, comprised of 104 subjects. The males were 53(50.9%) and female were 51(49.0%); with a mean age of 36.6 ± 20.7(range1-86 year-olds).Out of 104 wound swab/pus, 52 (50%) *S. aureus* isolates were obtained, and 21(20.2%) were identified as MRSA while 31(29.8%) were MSSA.

**Characteristics of patients:** The MRSA isolates were more in females than the males with 11(10.6%) and 10(9.6%) respectively. The presence of MRSA was independent of sex. The MRSA were obtained from all age groups with the highest 7(6.7%) from age group of 21- 40 and 61 and above respectively. The age group of ≤20 and 41-60 years had 3(2.9%) and 4(3.8%) MRSA isolates respectively. The duration of wound though not statistically significant shows that patients that had suffered the infection within 1-2 years had the highest MRSA isolates, 12(11.5%). Of the 70 patients with burns, 14 (13.5%) had MRSA isolates while 34 patients with bedsore, 7(6.7%) had MRSA. The analysis of wound dressing indicates that the interval for routine dressing of wound as chosen by individuals or family was not statistical significant. p=0.504.To assess whether the MRSA was hospital acquired or community acquired, the status of the patients in respect to hospital admission were analysed. Those that were on admission for 6months or more were 51 of which 15 (14.4%) were positive for MRSA while those that were on admission for less than 6months were 53 of which 6(5.8%) were MRSA. There was an association between hospitalization and MRSA infection (p=0.022) (Table 1)

**TABLE 1.CHARACTERISTIC OF PATIENTS WITH BURNS AND BEDSORE WOUNDS**

| VARIABLES | Total no. of subjects (n) | MRSA (%) | MSSA(%) | P-Value |
|---|---|---|---|---|
| **SEX:** | | | | **0.732** |
| **Male** | **53** | **10 (9.6)** | **17 (16.3)** | |
| **Female** | **51** | **11 (10.6)** | **14 (13.5)** | |
| **AGE:** | | | | **0.023** |
| **≤ 20** | **27** | **3 (2.9)** | **6 (5.8)** | |
| **21-40** | **32** | **7 (6.7)** | **10 (9.6)** | |
| **41-60** | **26** | **4 (3.8)** | **6 (5.8)** | |
| **61-above** | **19** | **7 (6.7)** | **9 (8.7)** | |
| **DURATION OF WOUND (MONTHS):** | | | | **0.078** |
| **<1** | **38** | **4 (3.8)** | **7 (6.7)** | |
| **1-2** | **53** | **12 (11.5)** | **20 (19.2)** | |
| **>2** | **13** | **5 (4.8)** | **4 (3.8)** | |
| **TYPE OF WOUND:** | | | | **0.297** |
| **Burns** | **70** | **14 (13.5)** | **20 (19.2)** | |
| **Bedsore** | **34** | **7 (6.7)** | **11 (10.6)** | |
| **INTERVAL FOR WOUND DRESSING:** | | | | **0.504** |
| **Daily** | **22** | **5 (4.8)** | **0** | |
| **2days** | **31** | **5 (4.8)** | **9 (8.7)** | |
| **3days** | **28** | **8 (7.7)** | **12 (11.5)** | |
| **4days** | **23** | **3 (2.9)** | **10 (9.6)** | |
| **HOSPITAL ADMISSION:** | | | | |
| **≥ 6months** | | | | **0.022** |
| **< 6months** | **51** | **15 (14.4)** | **19 (18.3)** | |
| | **53** | **6 (5.8)** | **12 (11.5)** | |

**Sources of wound**: The sources of injury through which the patients got the wound varied from patients to patients. The patients with home accidents such as hot water, home fire were 19 and 14 of which 6(5.8%) and 3(2.9%) had MRSA isolates respectively. Twenty-five patients that had injury due to car-(road) accident, 2(1.9%) with burns had MRSA while 3(2.9%) with bedsore had MRSA. Source of wound was not statistical significant, p=0.904 (Table 2)

**Prior antibiotic use**: The antibiotic usage among the patients was assessed to know the type of antibiotics consistently used as prescribed by the physicians. Ceftriazone with frequency of 98(16.3%) followed by Augmentin 84(14.5%) and Metronidazole 80(13.8%) were mostly prescribed. The least prescribed was Imipenem 3(0.5%). Patients are usually encouraged to combine drugs with metronidazole in very severe cases and as first line therapy. Antibiotics gels like gentamicin, penicillin ointment and other gels containing antibiotics were consistently used on the patients, especially those with burns (Table 3).

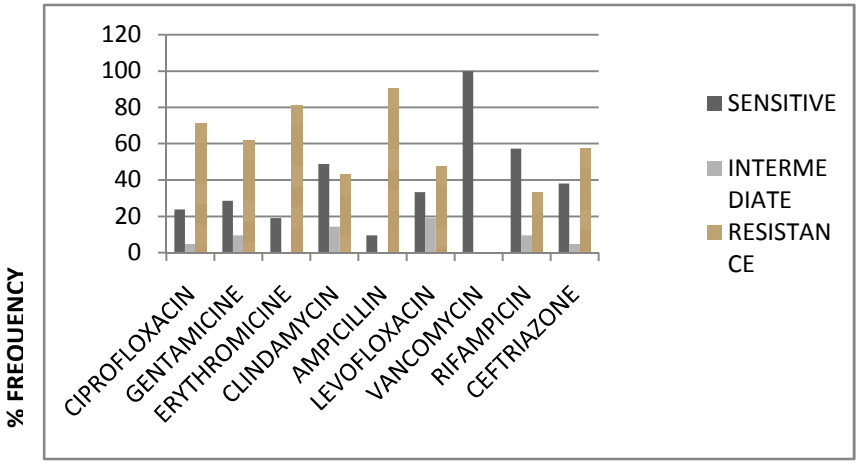
**TABLE 2: SOURCES OF WOUNDS AMONG THE SUBJECTS**

| Variables | Total no. of subjects (n) | BURNS (n = 70) | | BEDSORE ( = 34) | |
|---|---|---|---|---|---|
| | | MRSA (%) | MSSA (%) | MRSA (%) | MSSA (%) |
| Accident (road) | 25 | 2 (1.9) | 3 (3.9) | 3 (2.9) | 5 (4.8) |
| Home fire | 14 | 3 (2.9) | 4 (3.8) | 0 (0) | 0 (0) |
| Acid bath | 4 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Industrial fire | 9 | 2 (1.9) | 3 (2.9) | 0 (0) | 0 (0) |
| Hot water | 19 | 6 (5.8) | 5 (4.8) | 0 (0) | 0 (0) |
| Fuel/gas burn | 8 | 1 (1.9) | 3 (2.9) | 0 (0) | 0 (0) |
| Hot metal | 19 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Eletric burn | 2 | 0 (0) | 2 (1.9) | 0 (0) | 0 (0) |
| Other chemical than acid | 3 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Paralysis (spinal injury/stroke) | 5 | 0 (0) | 0 (0) | 2 (1.9) | 2 (1.9) |
| Other sickness (chronic diabetes) | 14 | 0 (0) | 0 (0) | 2 (1.9) | 4 (3.8) |
| | 104 | 14 (13.5) | 20 (19.5) | 7 (6.7) | 11 (10.6) |

**TABLE 3: FREQUENCY OF PRIOR ANTIBIOTICS THERAPY BY THE PATIENTS**

| Antibiotics therapy | Frequency | Percentage |
|---|---|---|
| Augmentin | 84 | 14.5 |
| Ceftriatriazone | 98 | 16.9 |
| Ciprofloxacillin | 61 | 10.6 |
| Clindamycin | 65 | 11.2 |
| Gentamicin | 67 | 11.6 |
| Ampiclox | 40 | 6.9 |
| Levofloxacillin | 50 | 8.7 |
| Cotrimaxole | 10 | 1.7 |
| Metronidazole | 80 | 13.8 |
| Erythromycin | 20 | 3.5 |
| Imipenem | 3 | 0.5 |
| Total | 578 | 100 |

**Susceptibility pattern**: The MRSA isolates exhibited multi resistance pattern as the 9 selected antibiotic showed different variation of resistance to the MRSA isolates. The antibiotic vancomycin was sensitive to all MRSA isolates. The highest resistance was Ampicillin with 19(90.5%) MRSA isolates. This was followed by Erythromycin, Ciprofloxacillin and Ceftriazone with 17(81.0%), 15(71.4%) and 12(57.2%) respectively (Fig 1a).The antibiotic susceptibility pattern was compared to MSSA and almost the same pattern were observed on MSSA with ampicillin having highest resistance 11(35.5%) (Fig 1b).




**1 a: Antibiotic susceptibility pattern of MRSA Isolates**

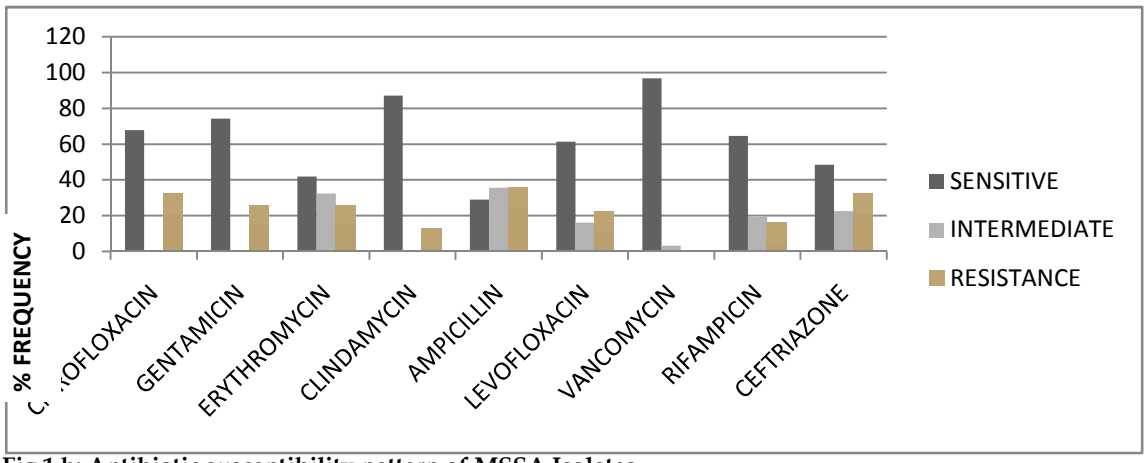



**Fig 1 b: Antibiotic susceptibility pattern of MSSA Isolates**

**MIC of MRSA:** The Minimum Inhibitory Concentration (MIC) of Vancomycin and Ampicillin to MRSA isolates were determined to assess the level of their resistance and sensitivity (Table 4). MIC of Ampicillin indicates that 10 MRSA isolates had >128µg/ml while 5 had MIC of 64µg/ml. Low MIC values of 4µg/ml was exhibited by 2 MRSA isolates to ampicillin. The MIC of Vancomycin shows that all the isolates were between ≤ 0.5µg/ml and 2µg/ml.

**MAR Index analysis of the isolates**: Multiple Antibiotic Resistance index of the isolates ranged from 0.33 to 0.77. It indicates that all MRSA isolates were hospital acquired.

**DISCUSSION**

*Staphylococcus aureus* is the predominant bacteria responsible for burn and pressure sore infection and it is perhaps the most common cause of health care associated infections worldwide (19).In this study, the MRSA prevalence of 20.1% was considered to be high against the study in Maiduguri, Northeastern Nigeria with a prevalence level of 12.5% (20) and was however lower than the report in Ibadan (30.4%), Ilorin (34.7%) and Jos (43%) all in Nigeria(21,22,23). Although MRSA prevalence is known to vary with geographical location, type of health institution, studied population and method of detection employed (20). It is clear that MRSA has become a global nosocomial pathogen with attendant therapeutic problems.

**TABLE 4: ESTIMATION OF MIC VALUE IN AMPICILLIN AND VANCOMYCIN**

| MRSA Isolate | Ampicillin (µg/ml) | Vancomycin (µg/ml) |
|---|---|---|
| 1 | 32 | 1 |
| 2 | 128 | 1 |
| 3 | 64 | 0.5 |
| 4 | 4 | 2 |
| 5 | 128 | 1 |
| 6 | 64 | 1 |
| 7 | 128 | 1 |
| 8 | 128 | 0.5 |
| 9 | 128 | 1 |
| 10 | 8 | 1 |
| 11 | 16 | 1 |
| 12 | 128 | 0.5 |
| 13 | 128 | 0.5 |
| 14 | 16 | 1 |
| 15 | 64 | 1 |
| 16 | 128 | 1 |
| 17 | 4 | 1 |
| 18 | 128 | 2 |
| 19 | 64 | 1 |
| 20 | 128 | 1 |
| 21 | 64 | 1 |

**TABLE 5: MAR INDEX ANALYSIS OF THE ISOLATES**

| Groups of isolates | Total no. of isolate | No. of antibiotics to which the isolates where resistance (a) | MAR Index |
|---|---|---|---|
| 1 | 2 | 3 | 0.33 |
| 2 | 4 | 4 | 0.44 |
| 3 | 11 | 5 | 0.55 |
| 4 | 3 | 6 | 0.66 |
| 5 | 1 | 7 | 0.77 |

The prevalence rate of 13.5% and 6.7% of burns and pressure ulcer respectively were relatively high which in a similar study by Nery Silver Pireet *et al* (24), identified a rate of 43.5% MRSA in patients with pressure ulcer and concluded that the rate of MRSA colonization in pressure ulcer is a risk factor which lead to prolonged hospitalization or poor prognosis. In a study at Ibadan, Nigeria, Adetoye *et al* (21) identified 30.4% colonization of MRSA in wound patients but fail to show whether they are burns or bedsore.

There was no association between sex and infection though prevalence of MRSA was 9.6% in males and 10.6% in females. This was similar to other findings where there is no significant different (25).There was association when the age of patients were compared. MRSA infection affected participants of all ages with highest in those age groups of 21-40 and $60^{+}$ with the prevalence of 6.7%. This was agreed with the report of Bodh *et al* (26) who showed that MRSA was high in 10-40 year and those above 60 years but the difference was not statistically significant. Madani (27) reported that MRSA affected all age groups, but almost half (45.9%) of the patients were in the "extremes of age" group (< 1 or > 60 years). The problem with this outcome is that MRSA will induce severe disease irrespective of age which will eventually worsen their condition.

The duration of wound plays an important role in the outcome of infectious agents colonizing both burns and pressure ulcer. For instance, pressure ulcers result from long periods of uninterrupted pressure exerted on the skin, muscle and bone. In this study, MRSA was isolated more from the patients that had suffered from wound infection within 1-2 years, with prevalence of 11.5%. Earlier studies suggested that duration and intensity of predisposing illness leads to development of pressure ulcer (28). Therefore, conditions of associated with prolonged and impaired healing may enhance the colonization of MRSA in these group of patients. However, the intervals of wound dressing as chosen by individual or their family members as a result of their socioeconomic condition, where most of them cannot afford daily dressing expenses were not statistically significant likewise the source of wound. There was a significant association between length of admission in the hospital and MRSA infection as those that were on hospital admission for≥ 6months had the highest prevalence of 14.4%.This might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure (29).

It is a common practice in health sector that allows changes in antibiotic usage by physician to achieve immediate healing. Looking at the previous antibiotic usage, some of these prescription by physicians showed that ceftriaxone had the highest frequency of 16.3% followed by Augmentin with 14.5%.Some of these prescription may in turn be disadvantageous due to the induction of resistance to the microorganism and since MRSA are resistance to all β-lactams including cephalosporin (30). The overuse and misuse of antibiotics are major contributing factors for bacterial resistance; therefore antibiotics must be prescribed only when indicated and the drug chosen should have the narrowest spectrum of activity and be given at an appropriate dose and duration. High multi antibiotic resistance rate were observed in the MRSA isolates. The most culprits were Ampicillin, Erytromycin and Ciprofloxacin. Similar facts have been demonstrated in the study by some reports where Ampicillin was highly resistance (31,32,33). This high resistance may be due to the expression of chromosomal *mecA* gene that specifies the production of an abnormal penicillin binding protein (PBP) which has low affinity for binding β-lactam antibiotics. All MRSA isolates were susceptible to Vancomycin. This may be due to its efficacy against bacteria resistant to β- lactamase antibiotics. Crandon et al (34) stated that vancomycin inhibits peptidoglycans biosynthesis, binding to the D-alanyl-D alanine peptide subunit and is unaffected by bacterial β –lactamases. The antibiotic susceptibility pattern was compared to MSSA and almost the same pattern were observed on MSSA with ampicillin having highest resistance. Furthermore, a high level of multiple drug resistance was observed in both MRSA and MSSA isolates.

The gold standard for antimicrobial susceptibility testing has been the Minimum Inhibitory Concentration (MIC). The MIC was done using ampicillin and vancomycin because of their

extremities in their resistance and susceptibility pattern. The MIC value of vancomycin was 0.5 - 2µg/ml and that of Ampicillin was 4 – 128µg/ml. This indicates that Ampicillin has high resistance and less of vancomycin is required to inhibit the growth of MRSA. MIC values lower than 4 µg/mL indicate that the staphylococcus is susceptible to vancomycin (35,36) and is more effective antimicrobial agent. Therefore continuous monitoring of antibiotic usage on burns and bedsore patients should be encouraged to know the best antibiotic to use to avoid therapy failure.

Multi Antibiotic Resistance index analysis reveals that all the 21 MRSA isolates had a high MAR index value greater than 0.2.The antibiotic susceptibility reveal that all the isolates were resistant to all the β-lactams, aminoglycosides and quinolones group of antibiotic tested fulfilling the criteria to be designated as Multi Drug Resistance (37).From the study, MAR index ranges from 0.33 to 0.77 as compared to work done by Subramani and Vinesh (37).Therefore bacteria having MAR index >0.2 originates from an environment where several antibiotics are used(38),indicating that all MRSA were hospital acquired. This may be due to prolonged use of antibiotics either topical or systematically followed by poor infection control mechanism and hygiene.

**Limitations of the study:** In this study, characterization of MRSA into Hospital acquired and Community acquired was difficult, because the patients have been hospitalized during the course of injury. Secondly, the number of times the patients changed antibiotics were not ascertained due persistent buying of these antibiotics outside prescriptions by the physician which makes it difficult to control the antibiotic usage among the patients. Secondly, antibiotic genetic characterization of MRSA isolates was not carried out due to unavailability of reagents in our environment.

**CONCLUSSION:** The prevalence of MRSA isolates is increasing in hospital settings and showed multiple drug resistance to the beta-lactams and commonly prescribed antibiotics. Vancomycin is the most effective agent against isolated MRSA and MSSA strains. These data show that antimicrobial resistance is increasing among *Staphylococcus aureus* strains in our locality. This increase highlights the value of prudent prescribing of antibiotics (including vancomycin) and avoiding their irrational use. It is necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in our hospitals to prevent the spread of resistant microorganisms including MRSA especially with patients with burns and bedsore.

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## ORIGINAL ARTICLE

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# IDENTIFICATION AND DETECTION OF ANTIBIOTIC SUSCEPTIBILITY OF THE MOST COMMON ANEROBES CAUSING INFECTION IN SURGICAL HOSPITAL, FACULTY OF MEDICINE ZAGAZIG UNIVERSITY, EGYPT

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**ABSTRACT**

**Objectives: Anaerobic infections are considered to be the most difficult organisms to be identified in the microbiology laboratory. It requires strict conditions, proper sampling , long time and laboratory skills. In addition most of them are mixed infections having both aerobic and anaerobic organisms. Choice of the proper antibiotic for treating these anaerobes is live saving for the patient.**

**Methods: Identification of anaerobic organisms using MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) as a recent tool for identification together with API 20A (as a reference method). Antibiotic susceptibility test was done for the anaerobic isolates using Agar Dilution Method. With the the most commonly used antibiotic in our hospital which are Amoxacillin/Clavulonic acid, clindamycin, metronidazole and Imipenem.**

**Results: Anaerobic infections constitutes 21.7% of total 249 specimen from different surgical departments. *Bacteroids spp.* (41%) were the most prevalent anaerobic organisms followed by peptostreptococcus (26.9%). MALDI TOF MS system and API achieved 100% agreement for identification of *Porphoryomonas spp.* and *Fusobacterium*, while near results were obtained for other isolates. *Bacteroid spp.* shows the highest rate of resistance to clindamycin (69%). Excellent results were obtained for Imipenem and metronidazole. Most of resistance to Amoxacillin/Clavulonic acid is related to *Bacteroid spp.* and *Fusobacterium spp.***

**Conclusion: MALDI TOF MS System is a useful tool for identification of. Anerobes are showing higher rates of resistance to commonly used antibiotics thus detection of resistant strains is vital for proper selection of antibiotics.**

**Key words: Anaerobes, MALDI TOF System, API 20, Agar Dilution Method, Zagazig.**

# L'IDENTIFICATION ET LA DETECTION DE SENSIBILITE AUX ANTIBIOTIQUES DES AENEROBIES LES PLUS COURANTES QUI CAUSENT L'INFECTION A L'HOPITAL CHIRURGICAL, FACULTE DE MEDECINE, L'UNIVERSITE DE ZAGAZIG, L'EGYPTE.

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**RESUME :**

**Objectifs : Des infections anaérobies sont considérées d'être les plus difficiles a identifier dans le laboratoire microbiologie. Elle nécessite des conditions strictes, l'échenillage approprie, un long temps et compétences de laboratoire. En outre, la plupart d'entre eux sont des infections mixtes ayant a la fois les organismes aérobies et anaérobies. Le choix d'antibiotique approprie pour le traiter ces anaérobies est un choix de sauvetage pour le patient.**

**Méthodes : L'identification des organismes anaérobies utilisant MALDI TOF(Méthode d'identification de micro – organismes au moyen de la spectrométrie de masse Matrix assisté Laser Désorption Ionisation) comme un outil récent pour l'identification ainsi que API 20 A(en tant qu'une méthode de référence). Le test pour la sensibilitéa un antibiotique a été fait pour les isolats anaérobies en utilisant Méthode Agar de dilution. Avec l'antibiotique la**

**plus couramment utilisée dans notre hôpital qui sont Amoxicilline/ Acide clavulanique, clindamycine, métronidazole et l'imipenème.**

**Résultats : Les infections anaérobies constituent 21,7% du nombre total de 249 échantillons des départements chirurgical différents. *Bacteroidsspp* (41%) étaient les organismes anaérobies les plus prévalents suivi par peptostreptococcus (26,9%). Le système MALDI TOF MS et API ont été d'accord pour l'indentification de *Porphoryomonasspp* et *Fusobacterium*, alors que les résultatsa peu près ont été obtenus pour les autres isolats. *Bacteroidspp*. adémontré le taux le plus élevé de la résistance au clindamycine (69%). Les résultats excellents ont été obtenus pour imipenème et métronidazole. Plus de résistance a Amoxicilline/ acide clavulanique est liée au*Bacteroidspp*.et*Fusobacterium spp.***

**Conclusion : Le système MALDI TOF MS est un outil utile pour l'identification d'anaérobies montrant des taux plus élevés de la résistance aux antibiotiques les plus couramment utilisées ainsi la détection de souches résistantes est essentielle pour la sélection d'antibiotiques.**

**Mots – cles : Anaérobies, Le système MALDI TOF, API 20, Méthode Agar de Dilution, Zagazig.**

## INTRODUCTION

Anaerobes are considered as common cause of bacterial infections. Anaerobic bacteria is very sensitive organisms that require special methods for collection, transportation and cultivation. As a result, most of anaerobic infections are not properly diagnosed (1)

Treatment of anaerobic infections is a major concern, not only because they are usually overlooked during diagnosis, but also due to the progressively raising resistance rates among anaerobic genera (**2).** Continued surveillance of anaerobic sensitivity is thus essential to detect changes in susceptibility patterns (3)(4).

Because the laboratory diagnosis of anaerobes requires special techniques, extensive experience, and they consume much time and expenses, there is always a search for newer diagnostic options (5)**.** Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry **(MALDI-TOF MS)** is a rapid and inexpensive technology used nowadays for identification for most bacterial strains (6)**.**

This study aimed to identify the most common anaerobic organisms that cause infection in surgical hospital, Zagazig University, Egypt, to compare MALDI-TOF MS and API 20A technique that are used for routine anaerobes identification and to detect the antibiotic sensitivity patterns for the isolated organisms using the standard agar dilution method.

## MATERIALS AND METHODS

**Consent:** Consents for all patients were obtained prior to sampling.

**Inclusion criteria**: Patient admitted to surgical hospital, Zagazig University with an infection that clinically suggested anaerobic infection like: deep infection, bad odor, foul Discharge and crepitation. The quality of the obtained sample was assessed according to the Algorithms for Wound Specimens and Q score described by the study of Sharp (1997) (7).

**Exclusion criteria:** Lesions that don't show previous manifestations of anaerobic infections and failure to obtain proper consent.

**Specimen collection**

Specimens were collected as described by Sinha, 2007 (8). For **diabetic foot infection:** After full laboratory investigation**,** X- ray of the foot was done to check the presence or absence of osteomyelitis. All procedures were done in the operating room, under complete aseptic condition. Sedatives were given and samples included purulent discharge, necrotic infected tissues and infected bone parts. **Appendicular abscess:** During exploration of the abdomen and under general anesthesia, aspiration of peritoneal fluid in sterile syringe was done before any surgical steps. **Psoas abscesses:** Under local anesthesia and complete aseptic condition, ultrasound guided aspiration of pus in sterile syringe was performed. **Surgical site infection:** The area was wiped with sterile saline then with 70% alcohol. Material from the wound was collected by aspiration and necrotic tissue were excised **(8).**

Specimens were transported to the lab within 2 hours. Tissue specimens were homogenized using a vortex Bead Beating. Grinding stainless steel beads (>2 mm) were added to the sample to disturb the tissue, and then was repeatedly vortexed. To overcome excessive heat produced, the specimens were interspersed with cooling on ice (9)**.**

All samples were examined by Gram stain, cultures were done on non-selective blood agar for aerobic culture and on neomycin blood agar for anaerobic culture. A single

colony of each morphotype was examined microscopically by Gram stain preparations, evaluation of enzyme catalase production and aero-tolerant test. Aero- tolerance testing was done on chocolate agar and incubated in carbon dioxide(10).

*Bacteroids fragilis* ATCC 25285 for gram negative anaerobes and *Eubacterium lentum* ATCC43055 for gram positive bacteria was included as a control strain in each run.

**MALDI-TOF MS identification**

**Samples Preparation**

A portion of a single colony was applied directly to a disposable target slide (bioMérieux, Marcy-L'Etoile, France) composed of a polypropylene carrier with a stainless steel layer and was lysed by direct application. One µl of matrix solution (3.1% cyan-4-hydroxycinnamic acid, bioMérieux) was applied and allowed to dry at room temperature prior to mass spectrometric analysis.

Isolates were prepared for mass spectrometry analysis at the Vitek MS preparation station, and the isolate information was transferred to the Vitek MS acquisition station using Myla v2.4 middleware. The total sample preparation time was approximately 1 min per isolate.

Samples were then analyzed using the Vitek MS MALDI-TOF mass spectrometer in linear positive-ion mode, across the mass-to-charge ratio range of 2,000 to 20,000 Da. Each spot was irradiated with 500 laser shots at 50 Hz. Target plates were calibrated and quality controlled both before and after data acquisition by using Escherichia coli ATCC8739. A sample containing matrix only (negative control) was assayed for quality control purposes.

After the acquisition of spectra, data were transferred from the Vitek MS acquisition to the Vitek MS analysis server and identification results were displayed using Myla v2.4 middleware. The total processing and data analysis time was approximately 20 min for a single isolate.

**Data Analysis**

The Vitek MS identification system is based on comparison of the characteristics of the spectra obtained with the Vitek MS v2.0 database. This database was built using spectra for known strains for each claimed species. Based on this representative data collection, a weight is assigned to each peak for each species according to its specificity. A single identification is displayed with a confidence value from 60.0 to 99.9.

Results of MALDI-TOF MS and API 20A were categorized as: 1) identical identification to the species level or identical identification to the genus level (if either or both techniques identified to the genus level only), 2) discrepant results, 3) unreliable.

**API 20A:** Identification of microorganisms was done according to the manufacture protocol (BioMerieux SA, France).

**Antibiotic sensitivity testing:** We selected the four most commonly used antimicrobials to treat clinically suspected anaerobic infections in our hospital. These antibiotics were Amxacillin/Clavulonic acid, Clindamycin, Mitronidazole and Imipenem.

**The Agar dilution Method:** The method was done according to the Clinical Laboratory Standard Institute (CLSI) recommendation for testing anaerobic bacteria. For the antibiotic sensitivity discs, Brucella agar (Difco, Becton Dickinson, Sparks MD21152, USA) supplemented with 5% lysed sheep blood, 5 mg/L haemin and 1 mg/L vitamin K was used. Briefly, appropriate dilutions of antimicrobial solutions were added to Brucella blood agar that had been allowed to equilibrate in a water bath to 50–55°C. The agar and antibiotic solution were mixed thoroughly, and the mixture was poured into Petri dishes on a level surface to result in an agar depth of 3–4 mm. Each bacterial culture was adjusted to a turbidity equivalent to that of a 0.5 McFarland standard (~1–9×$10^8$ CFU/mL for most species) and was then diluted 1:10 in sterile Mueller–Hinton broth. A 5 µl aliquot of each diluted bacterial suspension was spotted onto the agar surface using an automatic pipette within 15 min of preparation. All plates were incubated in an anaerobic jar for 48 h. MICs for all isolates were interpreted using the The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI break points (11).

**RESULTS**

According to MALDI-TOF results, out of 249 lesions 50 (20%) were sterile, 145 (58.2%) showed growth of aerobic organisms only, and only 54 (21.7%) revealed anaerobic organisms

upon culturing. Those 54 lesions were distributed as following; 27 from diabetic foot (that represented 18% of all diabetic foot lesions), 14 surgical wound aspirates (that represented 25% of all surgical wound aspirates), 8 appendicular abscess (26.6% of all appendicular abscess aspirates) and 5 psoas abscess aspirates (38.4% of all psoas abscess aspirates).
Of the 54 lesions, 28 (52%) showed mixed aerobic anaerobic organisms

The polymicrobial nature of anaerobic infection was greatest in psoas abscess aspirates as ratio of isolates number to the cases was 1.6, followed by diabetic foot (1.5) and surgical wound aspirates which was 1.4, lastly appendicular abscess aspirates (1.25). Four different anaerobic genera were cultured from different clinical samples. The most common anaerobic isolates were *Bacteroides spp.* 32 (41%) as shown in fig.1 *and Peptostreptococcus* spp. 21 (27%). All genera and species were identified by MALDI-TOF with a score of 85–90% (table 1).

Comparison between API 20 and MALDI-TOF for identification of different anaerobic genera revealed 100% agreement in identification of *Porphoryomonas spp* and Fusobacterium spp. However, It was 98% for *Bacteroid ssp,* 94% for *Peptostreptococcus spp,* and only 79% for *Prevotella* (table 2).

The antibiotic susceptibility pattern and antibiotics MICs of the anaerobes were determined as shown in Tables 3 and 4; respectively. *Bacteroide spp*. were the most sensitive to metronidazole (94%). *Peptostreptoccus spp*. were the most sensitive with (100%) sensitivity to imipenium, metronidazole and amoxicillin-clavulonic acid. The most effective antibiotics for *Porphoryomonas spp* were imipenen and amoxicillin-clavulonic acid (100%). *Prevotella spp*. was 100% sensitive to metronidazole and amoxicillin-clavulonic acid. However, *Fusobacterium spp.* Showed 100% sensitivity to imipenem and metronidazole (table 3).

Antibiotic sensitivity to metronidazole and imipenem were the highest among all antibiotics (94.9%) and (93.6%); respectively. However, only 45 (57.7%) isolates were susceptible to clindamycin with *Bacteroids non-fragilis* showing the highest resistance (four out of five). Seventy (89.6%) isolates were susceptible to amoxicillin-clavulonic acid (table 3).

The MICs of tested antibiotics were listed in table 4. MICs $_{50}$ and MIC $_{90}$ were determined for all strains. MICs $_{50}$/ MIC $_{90}$ of clindamycin were the highest, as clindamycin $MIC_{90}$ for *bacteroids, prevotella* and *fusibacterium* exceeded 256 ug/ml. *Bacteroids* showed high level of resistance against both amoxicillin clavulonic acid and clindamycin. Metronidazole was the most active antibiotic as $MIC_{90}$ didn't exceed 2ug/ml for any strain.

**TABLE 1: ANAEROBES DISTRIBUTION AMONG THE DIFFERENT SURGICAL INFECTIONS CATEGORIES**

| | Surgical infection categories | | | | |
|---|---|---|---|---|---|
| | Diabetic foot | Surgical sites infection | Appendicular abscess | Psoas Abscess | Total |
| *Bacteroides spp. NO. (%)* | 12 (30%) | 8 (40%) | 7 (70%) | 5 (62.5%) | 32 (41%) |
| *B. fragilis*<br>*B.thetaiotaomicron*<br>*B.vulgatus* | 8<br>3<br>1 | 8<br>0<br>0 | 6<br>0<br>1 | 5<br>0<br>0 | |
| *Peptostreptococcus spp. NO. (%)* | 18 (45%) | - | - | 3 (37.5%) | 21 (27%) |
| *P.asaccharolyticus*<br>*P. anaerobius* | 16<br>2 | | | 2<br>1 | |
| *Porphorymonas spp. NO. (%)* | 2 (5%) | 7 (35%) | 3 (30%) | - | 12 (15.5%) |
| *P. asaccharolytica*<br>*P. uneonis* | 2<br>0 | 6<br>1 | 3<br>0 | | |
| *Prevotella spp. NO. (%)* | 5 (12.5%) | 4 (20%) | - | - | 9 (11.5%) |
| *P.melaninogenica*<br>*P. bivia* | 4<br>1 | 4<br>0 | | | |
| *Fusobactrium . spp. NO. (%)*<br>*F.Nucleatum* | 3 (7.5%)<br>3 | 1( 5%)<br>1 | - | - | 4 (5%) |
| Total | 40 | 20 | 10 | 8 | 78 |

**TABLE (2) COMPARISON BETWEEN RESULTS OF API 20 AND MALD-TOF IN IDENTIFICATION OF ANAEROBIC ISOLATES**

| | Identified by API 20 (NO.) | Identified by MALDITOF (NO.) | Kappa (P-value) |
|---|---|---|---|
| *Bacteroides spp.* | 31 | 32 | 0.98 (<0.001) |
| *Peptostreptococcus spp.* | 19 | 21 | 0.94(<0.001) |
| *porphorymonas spp.* | 12 | 12 | 1.0(<0.001) |
| *Prevotella spp.* | 6 | 9 | 0.79(<0.001) |
| *Fusoacterium spp.* | 4 | 4 | 1.0(<0.001) |

**TABLE 3: SUSCEPTIBILITY PATTERN OF ANAEROBIC ISOLATES FROM DIFFERENT SURGICAL INFECTION CATEGORIES**

| | Amoxacillin clavulonic acid | | | Clindamycin | | | Metronidazole | | | Imipenem | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R | S | I | R |
| Bacteroides spp NO.( %)<br>*Bacteroid fragilis*<br>*Non-Bacteroid Fragilis* | 25 (78)<br>20<br>5 | 2 (6)<br>2<br>0 | 5 (16)<br>5<br>0 | 10 (31)<br>9<br>1 | - | 22 (69)<br>18<br>4 | 30 (94)<br>25<br>5 | - | 2 (6)<br>2<br>0 | 29 (90.6)<br>24<br>5 | - | 3 (9.3)<br>3<br>0 |
| *Peptostreptococcus spp* NO. (%) | 21 (100) | - | - | 18 (86) | - | 3 (14) | 21 (100) | - | - | 21 (100) | - | - |
| *Porphorymonas spp.* NO. (%) | 12 (100) | - | - | 9 ( 75) | - | 3 (25) | 10 (83) | - | 2 (17) | 12 (100) | | - |
| *Prevotella spp* NO. (%) | 9 (100) | - | - | 6 (67) | - | 3 (33) | 9 (100) | - | - | 7 (78) | - | 2 (22) |
| *Fusobactrium spp* NO. (%) | 3 (75) | - | 1 (25) | 2 (50) | - | 2 (50) | 4 (100) | - | - | 4 (100) | - | - |
| Total NO. (%) | 70 (89.6) | 2 (2.5) | 6 (7.9) | 45 (57.7) | | 33 (42.3) | 74 (94.9) | | 4 (5.1) | 73 (93.6) | | 5 (6.4) |

**TABLE 4: MICS LEVELS OF DIFFERENT ANTIBIOTICS TESTED ON ANAEROBIC ISOLATES**

| Organism / Antibiotics | Amoxacillin clavulonic acid MIC range ( MIC50/ $MIC_{90}$) µg/ml | Clindamycin MIC range (MIC50/ $MIC_{90}$) µg/ml | Metronidazol MIC range (MIC50/ $MIC_{90}$) µg/ml | Imipenem MIC range (MIC50/ $MIC_{90}$) µg/ml |
|---|---|---|---|---|
| *Bacteroides spp*<br>*Bacteroid fragilis*<br><br>*Non-Bacteroid Fragilis* | <0.06 - >256<br>(0.5/ 32)<br>0.25- 2<br>(<0.06/0.5) | <0.06 - >256<br>(>256/>256)<br>0.06- > 256<br>(>256/>256) | 0.25 – 16<br>(0.5/1)<br>0. 5- 16<br>(<0.06/1) | 0.125- 16<br>(0.125/2)<br>0.125- 16<br>(0.5/2) |
| *Peptostreptococcus spp.* | <0.06 -4<br>(0.125/2) | 0.06- 8<br>(0.25/16) | 0.25- 4<br>(0.5/1) | 0.06- 2<br>(<0.06/0.5) |
| *Porphorymonas spp.* | <0.06 - 4<br>(0.125/1) | 0.06- 8<br>(1/8) | 0.06- 4<br>(0.125/2) | 0.06- 2<br>(0.125/0.5) |
| *Prevotella spp* | <0.06 -2<br>(<0.06/1) | 0.25- >256<br>(1/ >256) | <0.06- 2<br>(.125/0.5) | <0.06->256<br>(0.25/>32) |
| *F.Nucleatum spp* | <0.06->32<br>(0.125/>32) | 0.5- >256<br>(1/>256) | <0.06->2<br>(0.5/1) | <0.06- 2<br>(0.06/0.5) |

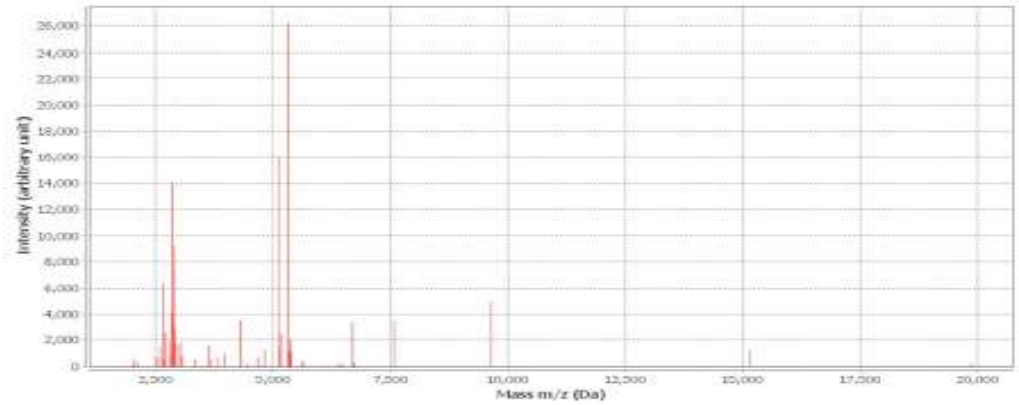


**FIG1: MALDI-TOF CURVE OF *BACTERIOD FRAGILIS***

## DISCUSSION

Anaerobic bacteria is part of the human flora; however, it can cause variety of life threatening infections. Culture and identification of anaerobes in the microbiology laboratory are difficult and require strict conditions, long time, and laboratory skills in isolation. Also, traditional methods do not always capable to differentiate between closely related species (12). The alternative recent techniques as Mass Spectrometry and molecular techniques such as real-time polymerase chain reaction, sequencing and microarrays provide fast and accurate diagnostic tools. However, Molecular techniques are not applied as a routine tool as they are expensive, and need technical expertise (13).

Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a useful tool for identification of different micro-organisms including anaerobes (12).

The identification of anaerobes by MALDI-TOF MS offers several advantages in comparison with the conventional routine methods. Most importantly, reducing the period required to identify an organism from days to few minutes that will improve the patient clinical outcome (14). Also, It has a great significance in the identification of biochemically inert, fastidious and slow growing anaerobic cocci (15).

The results of our work demonstrated that infections caused by anaerobic bacteria constituted 21.7% of different infection categories in surgical department, and 52% of these anaerobic infections were caused by mixed aerobic and anaerobic bacteria. This high frequency of mixed aerobic and anaerobic infection is explained by a symbiotic relationship with aerobic or facultative bacteria

as these species may consume oxygen to the level that allow the anaerobes to survive and exert their virulence to cause anaerobic infection (16).

Our study revealed that MALDI-TOF diagnosis of different surgical specimens identified ten species within five genera. This is nearly the same result obtained by the study of Jamal et al., 2013 (17) who identified fourteen species within five genera of anaerobic clinical isolates. In this study, *Bacteroides spp.* were the most frequent species (41%) isolated from the different surgical infection. This result was also demonstrated by the studies performed by Knoester and his colleagues,2012 (18) and Jamal et al., 2013 (17) which revealed that *Bacteroides* species constituted more than one-third of the isolates that were identified by MALDI-TOF MS.

The second prevalent genus isolated in this work was *peptostreptococcus spp.* (27%). followed by ***Peptostreptococcus spp.*** then came *Prevotella spp* and lastly *Fusobacterium spp*. Frequency of different anaerobic species are widely variable between different studies, Knoester and his colleagues ,2012 (18) demonstrated that *Propionibacterium* (15%), *Prevotella* (13%) were the second frequently isolated genera. In contrast, the study of Scola et al., 2011 demonstrated that the most common anaerobes were *Propionibacterium spp*. (12%), followed by *Fusobacterium spp.* (6%) and *Bacteroides spp.*(12). This difference may be due to differences in site of infection and different bacterial flora that cause these infections in the case of presence of risk factors.

There are several predisposing factors that favour anaerobic bacterial infection in diabetic patients as metabolic and physiological disturbance, vascular occlusive disease and peripheral neuropathy (19). In addition, immune deficient mechanisms as defective leukocyte chemotaxis, phagocytosis, and intracellular killing are important risk factors (20).

In agreement with the study done by El-Tahawy, 2000 (20) , The diabetic anaerobic infection was polymicrobial as 40 bacterial isolates were cultured from 27 cases resulting in an average of 1.5 organisms per lesion. In our study, anaerobic isolates in diabetic foot constitutes 18 % of the diabetic foot infections. However, Ng et al., 2008 (21) isolation rate of anaerobes was 79% which is far more than that of the present study. Also, Edmiston et al., 2002 (19) concluded that anaerobic pathogens were recovered from 87% of diabetic foot infections. This different finding most probably due to different sampling methods, type of transport media, different transportation time of samples.

The anaerobic genera isolated by our work from diabetic foot infections are in line with other studies done by Ng et al., 2008 (21) and Lipsky 1997 (22) which demonstrated that *peptostreptococci spp* were the predominant isolates. However, El-Tahawy, 2000 (20) found that *Bacteroides fragilis* were responsible for 92% of anaerobic diabetic foot infections. In contrast, Edmiston et al., 2002 (19) found that *Bacteroides* and *Peptostreptococcus* representing the predominant anaerobic isolates. This discrepant frequency of anaerobic species isolation could be due to different ranges of diabetic soft-tissue infections from mild ulcer and cellulitis to chronic osteomyelitis.

Surgical site infections (SSIs) infection is the infection of skin or/and soft tissues at the surgical incision site that occurs within 30 days after the operation (23). Surgical site infections are the third frequent nosocomial infections reported and responsible for a quarter of all nosocomial infections (24).

In the present study, 25% of cultures from SSIs revealed positive culture for anaerobes, which is higher than that obtained by studies of Rao et al., 2013 (24) , and Reddy, 2012 (25) which found that anaerobic infection of SSI was rare (3.4%). While we detected the Polymicrobial nature of these infections in 50% of the cases, Rao et al., 2013 (24) found that 35.2% of lesions were polymicrobial in nature.

The predominant anaerobic bacteria isolated from SSI and in line with the study done by Reddy , 2012 (25) was *Bacteroides spp.* While Rao et al., 2013 (24) results revealed that *Peptostreptococcus* species (2%) were the most frequently isolated species. However, the predominance of anaerobes bacilli contradicts previous reports that aerobic cocci were the primary contributor to SSI (26). Also, The importance of anaerobes such as *Peptostreptococcus spp., Prevotella spp., Finegoldia* and *Peptoniphilus* has been reported (27). This discrepant result may due to the various bacterial flora responsible for surgical site infections and different categories of surgical wounds that include clean, contaminated and dirty lesions (25).

Complicated intra-abdominal infection is a common problem, with appendicitis alone affecting more than 300,000 patients/year and consuming 11 million hospital days (28). In our study, 26.6% of appendicular abscess cases were due to anaerobic infection. In association

with the results obtained by study of Solomkin et al., 2010**,** the major pathogen isolated by our work from appendicular abscess cases was *Bacteroides spp*. (70%), followed by *porphorymonas spp.* (35%).

In our study, anaerobic infection was demonstrated in 38.4% of the patients with psoas abcess and *Bacteroides spp*. were the most frequently isolated pathogen as it is responsible for 62.5% of these infection, followd by *peptostreptococcus spp*. (37.5%). However, Adelekan et al., 2004 (29) found that *clostridium difficile* was the most common anaerobic pathogen isolated from psoas abcess cases. This means that bacterial flora are responsible for theses two types of infection in this study.

In agreement with results obtained by Knoester et al., 2012 (18), Jamal et al., 2013 (17), and Veloo et al. (2011) (30**)** , we demonstrated that all isolates (100% ) could be identified to the species level with MALDI-TOF MS system. In addition, Garner et al., 2014 (31) study revealed that the MALDI-TOF MS system provided the correct identification for 92% isolates to species level and 94% isolates to the genus level. However, Justesen et al. (2011) (32), found that the species level identification with the MALDI-TOF MS system was 43.8–49% .

However, Li et al., 2014 (33) and Scola et al., 2011 (12) found that MALDI-TOF MS system was effective for certain common species or genera, with 100% identification level for *Bacteroides fragilis*, and 80% for *Prevotella spp* but identification levels were above 50% for *Propionibacterium spp*., and 21.6% for *Fusobacterium spp.* This could be explained by Absence of reference spectra of unidentified isolates in the system database (34).

The agreement between MALDI-TOF MS system and API 20 A in identification varies with different anaerobic genera or species. In this study, both tests achieved 100% agreement (Kappa; 1.0) for identification of *Porphoryomonas spp*. and *Fusobacterium spp.* In addition, the comparison between both tools in identification of *Bacteroides spp.* and *Peptostreptococcus spp*, demonstrated very good agreement (kappa; 0.98). However, the least degree of agreement between both techniques was in identification of *Prevotella spp*. (kappa; 0.79). This finding is in accordance with previous reports of this technique's efficacy in identifying anaerobes which demonstrated that MALDI-TOF MS system is more accurately and quickly than conventional commercial techniques (35),(36),(14).

In this study, there was a discrepancy between MALDI-TOF MS system and API in identification of 8% of all isolates (33% of *Prevotella spp*., 9.5% of *Peptostreptococcus spp,* and 3% of *Bacteroides spp*) . Also, Knoester etal., 2012 (18) demonstrated that the discrepant result was found in 11% of the isolates. The isolates with discrepant results in the previous study were subjected to identification by 16S rRNA gene sequencing, and revealed that MALDI-TOF MS did not result in major errors (18) .However, the limitation of our study is the small number of anaerobic genera and species that were isolated and tested from different surgical infections.

The fact that anaerobes are fastidious in nature and thus difficult to be isolated and diagnosed makes them often overlooked. As a result, treatment of anaerobic infections is usually empirical; Although the type of anaerobic bacteria causing certain infection can be suspected, resistance of anaerobes to antibacterial drugs is a continuously growing problem and may even develop while the patient is receiving therapy (37). Reports around the world are reporting an increase in anaerobes resistance to antimicrobial (38).

MIC distribution of the antimicrobial agents tested is in table (4), in our hospital, these four drugs are the antibiotics of choice to treat clinically suspected anaerobic infection.

Clindamycin was considered the gold standard for anaerobic infection treatment since 1960, However, resistance to clindamycin has steadily increased among anaerobes in the last 15 years (39)**.** According to our result, about one third of all the isolates were resistant to clindamycin. *Bacteroid spp.* strains showed the highest rate of resistance (69%) especially *Bacteroid fragilis.* While, one third of *Prevotella spp*. in this study were resistant to clindamycine, other studies showed that *Prevotella spp*. resistance to clindamycin ranges between (31%-70%) (40), (41). In this study, 25% of *Porphorymonas spp*. were resistant to clindamycin, compared to 1% in Belgium (40),(41), (42),(43).

Half of *Fusobacterium spp.* isolated by our work were resistant to clindamycin. However, resistance of *Fusibacterium spp.* to clindamycin has been detected in other places of the world to be in the range of 0-20%, this could be explained by the difference in geographical distribution and pattern of antibiotic usage in different hospitals, (44), (45). *Peptostreptococcus spp.* species resistance to clindamycin in our study was 14%, near to the resistance of 11% detected in a study in Taiwan Hospital (3).

Our results represented that *Peptostreptococcuss spp., Porphoryomonas spp.*, and *Fusibacterium spp.* had excellent sensitivity to imipenem with 100% sensitivity among the isolated strains. These results matches the results of Al-Jebouri and Al-Hadeethy 2014 (46) in Iraq. About 10% of *Bacteroid spp.* strains were resistant to Imipenem. Resistance of B*acteroids spp.*to imipenem had also been also in earlier works done by (Hecht, 2004) (39) and (liu et al 2008)(3). Resistance of *provetella spp.* raised to 25% in another study performed by l-Jebouri and Al-Hadeethy 2014 (46).

Metronidazole has an excellent antimicrobial activity among most of anaerobes, this was supported by the study of Liu et al., 2008 (3). However, resistance of *Bacteroid fragilis* had been reported in several countries (47), (48), (4).

Our results showed that all *Fusobacterium*, *Porphoyromonas spp.*, P*eptostreptococcus spp.* and *Prevotella spp.* isolates were sensitive to Amoxacillin clavulonic acid. However, only 78% of *Bacteroids spp.* were sensitive. In a study done by Jamal et al., 2015 (49) showed that the drug gave excellent activity against *Fusibacterium spp.*, *Porphoyromonas spp.* and *Peptostreptococcus spp.*

*Bacteroid fragilis* MIC $_{50}$/MIC $_{90}$ in this study for amoxicillin clavulonic acid were (0.5/ 32) clindamycin (>256/>256) and Imipenem (0.125/2) were higher than those detected in Kuwait (0.75- 8), (4 >256) and (0.125-1) respectively(44), and these values were much higher than MIC $_{50}$/MIC $_{90}$ for amoxicillin clavulonic acid (0.016- 0.5) and clindamycin (0.016- >256) in Netherland (49). While MIC $_{50}$/MIC $_{90}$ for metronidazole (0.5/1) were lower than (0.75-2) in Kuwait both values, however, are much higher than that in Netherland (0.064-0.75). MIC $_{50}$/MIC $_{90}$ for *Bacteroid Non-Fragilis* were characteristically high for clindamycin (>256/>256) indicating higher level of resistance than elsewhere, while values for other drugs were within given ranges (44), (49).

*Peptostreptococcus spp.* showed the best sensitivity profile for all drugs as their MIC $_{50}$/MIC $_{90}$ has been reserved within acceptable ranges in relation to other studies (49).

*Prevotella spp.* in our study showed high level of resistance to clindamycin as MIC $_{50}$/MIC $_{90}$ were (1/ >256) and imipenem MIC $_{50}$/MIC $_{90}$ were (0.25/>32). This high resistant level to clindamycin has been detected before in previous studies (45), (44), (49) while, *provetella spp.* in this study were all sensitive to amoxacillin clavulonic acid and metronidazole.

*F. Nucleatum spp.* MIC$_{50}$/MIC$_{90}$ were (0.125/>32) for amoxicillin calvulonic acid, (1/ >256) for clindamycin, (0.5/1) for metronidazole and (0.06/0.5)for imipenem. Our results for *Porphorymonas spp.* MIC$_{50}$/MIC$_{90}$ were (0.125/1) for amoxicillin calvulonic acid, (1/8) for clindamycin, (0.125/2) for metronidazole and (0.125/0.5) for imipenem. Values for *F.Nucleatum spp* and *Porphorymonas spp.* were higher than previous studies (44), (49).

Analysis of MIC $_{50}$/MIC $_{90}$ values for this study reveals that in general they are much higher than other studies and this can be explained in view of the following: 1) resistance is a continuously growing problem and as more recent studies are introduced, the more incidence of resistance could be detected. 2) Chosen drugs are the most commonly used drugs in the hospitals and high level of resistance is expected to be detected. 3) Misuse of antibiotic is still a problem.

We conclude that anaerobes are common causes of infection in surgical unit, In addition, MALDI-TOF is an accurate rapid test for diagnosis. Unfortunately there is increasing tendency toward developing resistance in many species, thus routine testing for antibiotic sensitivity is a must to treat affected patients. We also recommend continuous monitoring of patterns of resistance in our hospitals and elsewhere.

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# EVALUATION OF MICROBIAL QUALITY OF SELECTED BLISTER-PACKED PARACETAMOL TABLETS AND PARACETAMOL SYRUPS MARKETED IN NIGERIA.

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**ABSTRACT**

**Ten brands of blister-packed paracetamol tablet and twenty brands of paracetamol syrup marketed in Nigeria were evaluated for their microbial quality. While no microbial contaminant was isolated from all blistered-packed paracetamol tablets, ten of syrups were contaminated with organisms such as *Escherichia coli, Klebsiellas spp, Pseudomonas aeruginosa* and *Staphylococcus aureus* at 14.3, 21.4, 21.4 and 42.9% occurrence respectively. *Penicillium spp* was isolated from two brands. Antibiotic susceptibility profile revealed all bacterial isolates to be multidrug resistant with *Escherichia coli* resistant to all antibiotics tested, while *Staphylococcus aureus* isolates were sensitive to Oxacillin, Cefuroxime and vancomycin. *Pseudomonas aeruginosa* isolates were sensitive to ofloxacin and gentamycin while *Klebsiella* isolates were sensitive to ofloxacin and nitrofurantoin. The study concluded that compliance with the provisions of good manufacturing practice as well as good quality control play role in determining the microbial bioburden of pharmaceutical products while isolation of multi-drug resistant organisms calls for establishment and adherence to antibiotics use policy in Nigeria.**

**Key Words: Blister-pack, multidrug resistance, good manufacturing practice, quality control, bioburden.**

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**Résumé:**

**Dix marques de comprimé de paracétamol boursouflée-emballés et vingt marques de sirop de paracétamol commercialisés au Nigeria étaient évaluées pour leurs qualités microbienne. Bien qu'aucun contaminant microbien était isolé à partir de tous les comprimés de paracétamol boursouflée-emballés, dix de sirops étaient contaminés par des organismes tels que :** *Escherichiacoli*, *Klebsiellaspp*, *Pseudomonasaeruginosa*et *de Staphylococcusaureus*à14,3, 21,4, 21,4et42,9% respectivement**en l'occurrence.***Penicilliumspp*était isoléà partir de deuxmarques. Profilde sensibilité antibiotiquea révélé **qu'***Escherichia*coli**est**résistantes àtouslesantibiotiques examinés, tandis que**les**isolâtesde *Staphylococcusaureus*étaient sensibles àl'*oxacilline*, *céfuroximeet la vancomycine*. **Les** Isolâtesde*Pseudomonasaeruginosa*étaientsensiblesà l'ofloxacineetlagentamicinependant **que les** isolâtesde*Klebsiella*étaientsensiblesà l'ofloxacinela nitrofurantoïne**.** Alors, on peut concluque la conformitéauxdispositionsdebonnes pratiques de fabricationvis-à**-vis le** contrôlede bonne qualité prends part dansla détermination de lacharge-biologie microbiennedesproduits pharmaceutiques pendant que isolement des organismes résistants aux **nombreux** médicaments demandentpour l'établissementet l'adhésionau politique d'utilisation des antibiotiquesau Nigeria**.**

Mots clés: **Boursouflée-emballés,** activité de l'eau**, les bonnes pratiques**de fabrication**, le contrôle de qualité,**la charge-biologie microbienne**.**

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## INTRODUCTION

Pharmaceutical preparations can be classified into sterile and non-sterile products. While sterile products are expected to be absolutely free of all microbes, non-sterile products are not to be free of all forms of microbes although there are limits as to the permissible levels of these contaminants in such pharmaceutical products(1).

Microorganisms possess diverse metabolic activities and are likely to present a variety of hazards (for examples, infections, toxicity, degradation of formulations) both to the user and to the stability of the products, if allowed to persist. Limits have therefore been set for the presence of microorganisms in medicines by commissions as European Pharmacopoeia commission, which vary depending on the product and its intended use. However, microbial contamination over and above these pharmacopoeia levels is a global problem which is still being reported in distributed medicines worldwide (2). The most commonly reported microbial hazards found in liquid medicines as syrups are pseudomonads and their related Gram-negative rods, with spores (bacterial and fungal) predominating in dry tablets, capsules and cosmetic powders. Occurrence of true pathogens as S*almonella* spp in syrups has been reported (3, 4).

The risk (likelihood of harm actually occurring) associated with delivery of contaminated products is less clearly determined. It will depend upon the type of microorganisms present, the infective dose (dependent on the ability of the formulation to encourage microbial survival and level of preservative protection built with it), the route of administration of the product and the host's resistance to infection (including the immune status or the degree of tissue damage at the site of application).

The presence of the species as *Escherichia* spp, *Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp, *Enterococcus* spp, *Micrococcus* spp, *Salmonella* spp, *Staphylococcal* spp, *Bacillus* spp, *Aerobacter* spp, *Aspergillus* spp as well as *Penicillium* spp in syrups, capsules and tablets within and outside Nigeria has been reported (3, 5-11).

The nature of the ingredients, the quality of the vehicle and the care and attitude of personnel involved in their handling will determine the incidence of microflora in non-sterile products (12). However, since raw materials, manufacturing environment, packaging materials and personnel have been implicated as potential sources of contaminants in pharmaceutical products, it implies that pharmaceutical preparations can be contaminated at the point of production and packaging by the manufacturer or use by the consumers.

This study aimed at comparing the microbial quality of some blister-packed paracetamol tablets to that of paracetamol syrups marketed in Nigeria as well as identifying the source(s) of such contaminants and the potential health hazard implication of such contaminants on the populace.

## MATERIALS AND METHODS

***Media***

Nutrient broth, nutrient agar, MacConkey agar, Mannitol salt agar, Cetrimide agar, Mueller-Hinton agar, Salmonella-Shigella agar, Saboraud Dextrose agar; all ATER products from Topley House 52, Wash Lane, Bury, Lanchashire BL96AS, UK.

***Preparation of tablet dispersion***:

Two tablets of each brand of blister-packed paracetamol were aseptically removed directly into 10mL sterile normal saline and dispersed. Tablet dispersions were mixed in a vortex mixer for 5 minutes to dislodge possible microbial cells. The solid particles settled down and the supernatants were used.

***Determination of microbiological quality of tablets***:

One milliliter aliquot of each brand's dispersion was seeded in nutrient agar, poured and allowed to set in plates (in duplicates). The procedure was repeated using MacConkey agar medium, Mannitol salt agar medium, cetrimide agar medium, Salmonella-Shigella agar and Saboraud Dextrose agar medium. The Saboraud Dextrose agar plates were incubated at 25 $^{o}$C for 72-96 hours while the other agar plates were incubated at 37 $^{o}$C for 48 hours before they were observed for growth.

***Determination of microbiological quality of syrups:***

1 in 10, 1 in 100 and 1 in 1000 dilutions of each paracetamol syrup in sterile peptone water was made and 0.1mL of each dilution was seeded in nutrient agar medium, MacConkey agar medium, Mannitol salt agar, Cetrimide agar medium, Salmonella/Shigella agar medium as well as Saboraud Dextrose agar medium contained in sterile Petri dishes (in duplicates). All the plates were incubated at 37$^{o}$C for 24-48 hours except the Saboraud Dextrose agar plates that were incubated at 25$^{o}$C for 72-96 hours before they were observed for growth.

Bacterial isolates from the plates that showed growth were subjected to standard microbiologic identification tests based on colony morphology, and conventional biochemical tests to confirm their identity and/or purity.

***Antibiotic susceptibility profiling***:

Susceptibility of the Gram-negative and Gram-positive isolates to eight and three antimicrobial agents respectively was tested by the disc diffusion technique according to the guidelines by the Clinical and Laboratory Standards Institute (13). The Gram-negative antibiotic disc contained augmentin (30 µg); ofloxacin (5 µg); gentamycin (10 µg); nalidixic acid (30 µg); nitrofurantoin (200 µg); cotrimoxazole (25 µg); amoxycillin (25 µg) and tetracycline (25 µg) while the Gram-positive antibiotic single disc comprised of oxacillin (1µg); cefuroxime (30µg); and vancomycin (30 µg).

Four or five colonies of each test organism taken from a nutrient agar culture plate was inoculated into 10 mL of sterile distilled water using a sterile loop. The suspension was thoroughly mixed with a spin mixer. The resulting suspension was adjusted to a turbidity of 0.5 McFarland standard. This was then applied to the surface of oven-dried Mueller Hinton agar and spread evenly with a sterile swab stick. The inoculated plates were incubated at 37$^{o}$C for 20 minutes for acclimatization and growth of the inocula. Antibiotic discs (Abtek, Liverpool, UK) were then lightly but firmly pressed onto the surface of the plates using a pair of sterile forceps. The plates were then refrigerated at 4$^{o}$C for thirty minutes to ensure adequate diffusion of antibiotics. *E. coli* ATCC 25922 was used as control strain. All plates were incubated at 37$^{o}$C for 18 hours. The diameters of inhibition zones were measured in millimetres and interpreted according to CLSI manual.

**RESULTS**

Of the ten selected brands of blister-packed paracetamol tablet used for the study, only one was imported from India while the remaining nine were manufactured in Nigeria. All the brands used have their identities revealed by indicating the date of manufacture, expiry date, NAFDAC number as well as the address of the manufacturer as shown in Table 1.

The container disclosures of all the twenty brands of paracetamol syrup, manufactured in Nigeria, used for the study are as shown in Table 2.However, of the twenty brands of paracetamol syrup used for the study, 50% were free of microbial contaminants while 50% were contaminated with microbial loads ranging from $2 \times 10^1$ in $S_{13}$ to $1.05 \times 10^4$ cfu/mL in $S_{18}$ as shown in Table 3.

A total of 14 bacterial isolates comprising of *Staphylococcus aureus,* 42.9%; *Klebsiella spp*, 21.4%; *Pseudomonas aeruginosa*, 21.4%; and *Escherichia coli*, 14.3% as shown in Table 4 were isolated from this study.

Antibiotic susceptibility profiles revealed all *Klebsiella* isolates to be sensitive to ofloxacin, and nitrofurantoin, with 2 strains sensitive to gentamycin, an aminoglycoside, and 1 strain with intermediate sensitivity. They were however resistant to other antibiotics tested namely: cotrimoxazole, amoxicillin, tetracycline, augmentin and nalidixic acid. All *Escherichia coli*, on the other hand, were resistant to all antibiotics tested while one strain of *Pseudomonas aeruginosa* was sensitive to ofloxacin and gentamycin while showing intermediate sensitivity to tetracycline. One strain was also sensitive to tetracycline with intermediate sensitivity to ofloxacin. One other strain showed intermediate sensitivity to ofloxacin and nitrofurantoin as shown in Table 5

All *Staphylococcus aureus* isolated were sensitive to oxacillin and cefuroxime, both ß-lactam antibiotics with varying sensitivity to vancomycin as shown in Table 6

**TABLE 1: CONTAINER DISCLOSURE OF EACH BRAND OF BLISTER-PACKED PARACETAMOL TABLET USED FOR THE STUDY**

| SAMPLE | MANUFACTURING DATE | EXPIRY DATE | NAFDAC NUMBER | ADDRESS OF MANUFACURER |
|---|---|---|---|---|
| $T_1$ | + | + | 04-0633 | + |
| $T_2$ | + | + | 04-0411 | + |
| $T_3$ | + | + | 04-1957 | + |
| $T_4$ | + | + | 04-0101 | + |
| $T_5$ | + | + | 04-0975 | + |
| $T_6$ | + | + | 04-1853 | + |
| $T_7$ | + | + | 04-5762 | + |
| $T_8$ | + | + | 04-1217 | + |
| $T_9$ | + | + | 04-1207 | + |
| $T_{10}$ | + | + | 04-4686 | + |

**TABLE 2: CONTAINER DISCLOSURE OF PARACETAMOL SYRUP SAMPLES USED FOR STUDY**

| Brand Name | Manufacturer's Address | Batch Number | Production Date | Expiry Date |
|---|---|---|---|---|
| $S_1$ | + | LO66P | + | + |
| $S_2$ | + | 452 | + | + |
| $S_3$ | + | PL7108 | + | + |
| $S_4$ | + | TPS008 | + | + |
| $S_5$ | + | 9169 | + | + |
| $S_6$ | + | L4909 | + | + |
| $S_7$ | + | P510001 | + | + |
| $S_8$ | + | 242 | + | + |
| $S_9$ | + | 08001 | + | + |
| $S_{10}$ | + | 08760507 | + | + |
| $S_{11}$ | + | 0254 | + | + |
| $S_{13}$ | + | PL 001 | + | + |
| $S_{14}$ | + | P05 | + | + |
| $S_{15}$ | + | S m 3325 P | + | + |
| $S_{16}$ | + | 014251 | + | + |
| $S_{17}$ | + | IZ1686 | + | + |
| $S_{18}$ | + | 9011 | + | + |
| $S_{19}$ | + | PA0405 | + | + |
| $S_{20}$ | + | 00111 | + | + |

**TABLE 3: MICROBIAL QUALITY OF EACH PARACETAMOL SYRUP USED FOR STUDY**

| Paracetamol sample | Total viable count (cfu/mL | Organism(s) isolated |
|---|---|---|
| $S_1$ | $3 \times 10^1$ | *Staphylococcus aureus* |
| $S_2$ | $2 \times 10^2$ | *Penicillium spp* |
| $S_3$ | Nil | |
| $S_4$ | Nil | |
| $S_5$ | $6.75 \times 10^2$ | *Staphylococcus aureus* |
| $S_6$ | Nil | |
| $S_7$ | Nil | |
| $S_8$ | Nil | |
| $S_9$ | Nil | |
| $S_{10}$ | Nil | |
| $S_{11}$ | $1.9 \times 10^2$ | *Staphylococcus aureus* |
| | | *Penicillium spp* |
| $S_{12}$ | Nil | |

| Paracetamol sample | Total viable count (cfu/mL | Organism(s) isolated |
|---|---|---|
| $S_{14}$ | Nil | |
| $S_{15}$ | $1.6 \times 10^2$ | *Pseudomonas aeruginosa*<br>*Klebsiella spp*<br>*Staphylococcus aureus* |
| $S_{16}$ | $2.45 \times 10^2$ | *Pseudomonas aeruginosa*<br>*Klebsiella spp*<br>*Staphylococcus aureus* |
| $S_{17}$ | $2 \times 10^1$ | *Escherichia coli* |
| $S_{18}$ | $1.48 \times 10^4$ | *Pseudomonas aeruginosa*<br>*Klebsiella spp*<br>*Staphylococcus aureus* |
| $S_{19}$ | $1.3 \times 10^2$ | *Escherichia coli* |
| $S_{20}$ | Nil | |

**TABLE 4: PERCENTAGE OCCURRENCE OF ISOLATED BACTERIA IN PARACETAMOL SYRUP SAMPLES**

| ISOLATED ORGANISM | P PERCENTAGE PREVALENCE |
|---|---|
| *Staphylococcus aureus* | 42.9% |
| *Klebsiella spp* | 21.4% |
| *Pseudomonas aeruginosa* | 21.4% |
| *Escherichia coli* | 14.3% |

**TABLE 5: ANTIBIOTIC SENSITIVITY PATTERNS OF GRAM-NEGATIVE BACTERIAL ISOLATES OBTAINED FROM PARACETAMOL SYRUP SAMPLES**

| Samples | ANTIBIOTICS/ZONE OF INHIBITION IN MILLIMETERS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | COT | AMX | TET | AUG | OFL | GEN | NAL | NIT |
| $S_{15(a)}$ | 0(R) | 0 (R) | 9 (R) | 7 (R) | 21 (S) | 14 (I) | 0 (R) | 24 (S) |
| $S_{15(b)}$ | 0 (R) | 6 (R) | 20 (S) | 0 (R) | 13 (I) | 0 (R) | 0 (R) | 0 (R) |
| $S_{16(a)}$ | 0 (R) | 0 (R) | 11 (R) | 0 (R) | 24 (S) | 17 (S) | 0 (R) | 18 (S) |
| $S_{16(b)}$ | 0 (R) | 0 (R) | 10 (R) | 0 (R) | 14 (I) | 0 (R) | 0 (R) | 16 (I) |
| $S_{17(c)}$ | 0 (R) | 13 (R) | 0 (R) | 0 (R) | 11 (R) | 0 (R) | 0 (R) | 9 (R) |
| $S_{18(a)}$ | 0 (R) | 0 (R) | 14 (R) | 0 (R) | 16 (S) | 20 (S) | 0 (R) | 17 (S) |
| $S_{18(b)}$ | 0 (R) | 0 (R) | 16 (I) | 0 (R) | 17 (S) | 16 (S) | 0 (R) | 10 (R) |
| $S_{19(c)}$ | 0 (R) | 0 (R) | 0 (R) | 0 (R) | 12 (R) | 0 (R) | 0 (R) | 10 (R) |

**KEY:(a)-*Klebsiella*spp.; (b)- *Pseudomonas aeruginosa;* (c) - *E. coli;* S - Sensitive; I - Intermediate; R - Resistance**

**TABLE 6: ANTIBIOTIC SENSITIVITY PATTERNS OF GRAM-POSITIVE BACTERIAL ISOLATES OBTAINED FROM PARACETAMOL SYRUP SAMPLES**

| SAMPLES | ANTIBIOTICS/ZONE OF INHIBITION IN MILLIMETERS | | |
|---|---|---|---|
| | OX | CXM | VA |
| $S_1$ | 13 (S) | 25 (S) | 15 (I) |
| $S_5$ | 14 S) | 24 (S) | 15 (I) |
| $S_{15}$ | 14 (S) | 24 (S) | 15 (S) |
| $S_{16}$ | 15(S) | 23 (S) | 15 (I) |
| $S_{18}$ | 21 (S) | 34 (S) | 17 (S) |

**KEY: S – Sensitive; OX = Oxacillin; I – Intermediate; CXM = Cefuroxime; R – Resistant; VA = Vancomycin**

## DISCUSSION

In the microbiological evaluation of non-sterile products, two tests are important namely: the extent of contamination test and the nature of the contaminant test. These tests are complementary and every non sterile product must pass the two tests before being released for customer use. While the extent of contamination test sets allowable limits for microbial contaminants in the product, the nature test addresses objectionable pathogenic organisms whose presence in the product will render the product unsuitable for use irrespective of their number in the product. These two tests were used in this study to determine the suitability for use or otherwise of some blister-packed paracetamol tablets and paracetamol syrups marketed in Nigeria.

Tablets are compacts drug delivery systems with low water content which usually afford them good protection against microbial contamination. Spoilage and clinical infections resulting from microbial contamination of tablets under hot and humid conditions of the tropics have been reported (7, 14). Tablets also undergo deleterious changes as discoloration, weakening of tablets matrixes and decreases in the potency of active ingredients when improperly stored (15). Potential contamination of tablets may arise from heavy microbiological burden in raw materials, though this is usually drastically reduced by lethal drying stage of wet granulation (16). However, the decreasing use of direct compression in manufacturing of tablets in pharmaceutical industries implies that some contaminants may survive up to the compression stage. The compression of formulation is known to effect some level of microbial destruction but this depends on the compression pressure applied, the properties of the contaminating organisms, and the formulation involved (17, 18). The effects of these variables in turn are believed to depend on the mechanism of microbial kill which has been proposed to include high localized heat shearing forces during compression (18, 19). The shear stresses manifested during compression depend largely on the principal mode of consolidation of the formulation which can be by fragmentation of plastic flow, with plastic having been shown to be a highly effective mechanism for microbial kill even at low compression pressures (17, 20). Binding agents employed in formulations are known to undergo a high degree of plastic deformation during compression and are forced into their inter-particulate spaces where they increase the area of contact between the particles and form strong solid bounds. All these factors played role in the result obtained for tablets used for this study as none of the tablets used for the study was contaminated by any form of microbial contaminant.

Syrups, on the other hand, usually consist of active ingredient, sugar, vehicle and/or preservative.

The ability of the bacteria isolated in this study to survive in syrup can be attributed to water activity of those brands of paracetamol syrup studied.

Reduced water activity will greatly assist in the prevention of microbial proliferation in pharmaceutical products. Because the water activity requirements for different Gram-reactive bacteria, bacterial spores, yeast and moulds have been described (12), the appropriate microbial limit testing program for products of differing water activities can be established. For instance, Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli,* and *Salmonella* species will not proliferate or survive in products with water activities below 0.91 while Gram-positive bacteria like *Staphylococcus aureus* will not proliferate below 0.86, and *Aspergillus niger* will not proliferate below 0.77. Furthermore, most osmophilic yeast and xerophilic fungi will not proliferate below 0.60 (21).

Suffice it to say that the water activity of the product is a function of the organism to be excluded from the product. In this study in particular, it can be said that the water activity of all the paracetamol tablets and

some brands of paracetamol syrup, where neither bacteria nor fungi were isolated, was below 0.60.

Of the 50% contaminated paracetamol syrup samples used, $S_{15}$, $S_{16}$ and $S_{18}$ were heavily contaminated with 3 strains of bacteria namely: *Klebsiella spp, Pseudomonas aeruginosa* and *Staphylococcus aureus* while $S_{19}$ was contaminated with only *Escherichia coli* and other samples with *Staphylococcus aureus* with the exception of $S_2$ and $S_{13}$ that were contaminated with *Penicillium spp*.

*Klebsiella* spp are found in the respiratory, intestinal, and urinogenital tracts of animals and humans. However, when *Klebsiella* moves outside the gut, it can cause a serious infection. Thus, its presence in the assayed samples is also an indication of unhygienic conditions, and may have originated from pharmaceutical personnel. The presence of *Staphylococcus aureus* does not always mean that the consumption of medicines are potentially being hazardous to users as not all the strain of *Staphylococcus* sp. can necessarily produce enterotoxin where higher infectious dose ($10^5$-$10^6$CFU/mL) is required (6). *Staphylococcus aureus* may however cause a significant deterioration in the health status of patients, particularly those who are immunologically compromised and of infants with an immature immune system (22). *Staphylococcus* sp. might transmit from hands of handler during the preparation of drugs.

The presence of *Escherichia coli* is a good indicator of fecal contamination resulting from water supply. Incidence of infantile diarrhoea associated with *E. coli* as a result of poor water supply in Nigeria has been reported (23). However, the presence of all the isolated organisms in this study has rendered the product from which they were isolated unfit for human use, being indicator organisms according to the United States Pharmacopoeia (USP). Organisms as *Salmonella* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* have been recommended as indicators of pathogenic microorganism contaminants of syrups (24).

Antibiotic sensitivity profiles revealed all isolates to be multidrug resistant as they were resistant to two or more antibiotics used for the study. Ofloxacin and Nitrofurantoin are the drugs of choice as far as this study is concerned as majority of the isolates displayed highest degree of susceptibility to them. However, all the Gram-positive isolates were sensitive to all the antibiotics against which they were tested in this study.

Quality control, as part of quality assurance, involves the sampling and testing of starting materials, intermediate, bulk and finished products and packaging materials to ensure compliance with appropriate standards and specifications. Quality Assurance is the totality of the process undertaken to ensure absolute quality of a product. Quality Assurance is Quality Control plus Good Manufacturing Practices (GMP), i.e. the product is manufactured through laid down procedures, contain necessary ingredients in correct proportions and of right purity, packed in proper containers and with proper labels. The pharmaceutical industries have an obligation to design, test and produce dosage forms that provide the consumer with products having the attributes of quality, purity, uniformity of content, stability, safety and physiological availability. These requirements necessitate the total involvement of corporate personnel with formal system of checks and balances. It is only through well organized, adequately staffed and accurately performed process and dosage form control before, during and after production that adequate quality assurance of the product can be achieved (25).

The presence of antibiotic-resistant organisms in paracetamol syrup as shown in this study is of concern as they pose danger to children especially the immunocompromised ones in whom they can aggravate illness as a result of secondary infection (26).

## CONCLUSION

It can be concluded that since no organism was isolated from all paracetamol tablets studied while organisms were isolated from some paracetamol syrup samples studied, it follows that manufacturing processes to which both the drug substance and the excipients were subjected have effect on the microbial quality of the final dosage form. While none of the blistered-packed paracetamol tablets studied pose threat health-wise to the consumers, isolation of antibiotic-resistant contaminants from some samples of paracetamol syrup studied emphasize a need for rational use of antibiotics in the country.

Moreover, product of good microbial quality can be guaranteed through quality control and adherence to the provisions of good manufacturing practices. Also, low water activity can aid reduction in vulnerability of formulations to microbial contamination provided the pharmaceutical products are made from ingredients of good microbial quality, when manufacturing environments do not foster microbial contamination, when there are processes that inherently reduce the microbial content, to mention but a few.

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# RABIES IN NIGERIA: A REVIEW OF LITERATURE

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**ABSTRACT**

**Rabies, also known as hydrophobia is an acute, viral disease of all warm blooded animals including man. It is caused by the rabies virus (RABV), a bullet–shaped, enveloped RNA virus, 45-100 nm in diameter & 100-430 nm in length with projections and helical nucleocapsid, one of the better known encephalitis viruses of the family *Rhabdoviridae* and genus Lyssavirus type 1**

**It is a major public-health problem in most parts of the developing world. The domestic dog (*Canis familiaris*) plays a principal role (accounting for over 99%) as a reservoir and transmitter of the disease to humans. Developing countries account for almost all the reported human deaths (99.9%) and most cases of human post-exposure treatments. Rabies is an important public health problem especially in the developing countries and this articles aims to draw attention to this neglected disease.**

**Key words: *rhabdoviridae*, rabies**

# LA RAGE AU NIGERIA: UNE REVUE DE LA LITTERATURE.

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**RESUME:**

**La rage , également connu sous hydrophobie, est une maladie virale aiguë de tous les animaux a sang chaud y compris l'homme. Elle est causée par le virus de la rage (RABV), une forme de balle, virus enveloppé à ARN,45 – 100nm de diamètre et 100 – 430 nm en longueur avec des saillies et des nucléocapsidehélicoïdale, l'un des virus encéphalite mieux connu de la famille *Rhabdoviridae* et le genre *Lyssavirus* type 1.**

**C'est unproblème majeur de Sante Publique dans la plupart des régions du monde développant. Le chien domestique (*Canis familiaris)* joue une rôle majeur (représentant plus 99%) comme une réservoir et émetteur de la maladie aux humains. Les pays en voie de développementreprésentent presque tous les décèsrapportés des humains (99,9%) et la plupart des cas de traitement post – exposition de l'homme. La rage est un problème important de Santé publique particulièrement dans les pays en voie de développement et cet article vise à attirer l'attention sur cette maladie négligée.**

**Mots – clés: *rhabdoviridae*, la rage.**

## INTRODUCTION

Rabies has existed for more than 4,300 years making it one of the most typical zoonoses known through the ages (1). The antiquity of rabies is illustrated by the ancient origins of terms describing the disease. For instance, the word "rabies" is a Latin word derived from the Sanskrit "rabhas" meaning "to do violence."

Early recognition of the infectivity of the saliva of rabid dogs led Roman writers to describe the infectious material as a poison, for which the Latin word was "virus" (2). Lyssa virus, the genus to which rabies and rabies-related viruses belong, owes its name to the Greek "lyssa" or "lytta," meaning "madness." The first recorded description of canine rabies apparently was made by Democritus in 500 B.C. In his Natural History of Animals, Aristotle's writings on rabies described dogs suffering from a madness causing irritability and how following their bite other animals became diseased. Little has changed in the epidemiology of rabies, as dogs and other carnivores remain the common sources of human infection in most areas of the world where the virus is enzootic.

In the 1930s it was thought that if dog rabies were eliminated, the human problem would be solved and by the late 1950s it appeared that rabies had indeed lost much of its potential as a public health problem, having been significantly reduced by mass dog vaccination programs. A turning point was reached in 1958, when rabid canine cases had been reduced by such a degree that they were surpassed by the increasing number of cases in wild animals but it was discovered that rabies in wild animals is equally a threat to the human populace as it is in dogs. The situation continued to improve until 1960s; when"only" 16 human rabies related deaths were recorded as against 113 in the previous decade (3).

**Rabies in Nigeria**

Rabies was first reported in Nigeria in 1912 and about 10,000 annual human cases are reported in Nigeria (4) making the disease a persistent endemic problem. Despite efforts to control rabies, the disease continues to be a major scourge of dogs and cats in plateau state and in Nigeria in general (4).

**AETIOLOGY**

Rabies is caused by a single stranded, negative-sense RNA virus of the order *Mononegavirales,* family *Rhabdoviridae* and genus Lyssavirus (5). It is a neurotropic virus that is generally transmitted through bites from infected animals to susceptible host species including humans (6).

**Rabies Virus Characteristics**

Based on antigenic characterization of panels of Lyssaviruses, seven genotypes (GTs) have been identified (7). These are Classical rabies (RABV) GT1, Lagos bat Virus (LBV) GT2, Mokola virus (MOKV) GT3, Duvenhage virus (DUVV) GT4, European bat Lyssavirus type-1 (EBLV-1) GT5, European bat Lyssavirus type-2 (EBLV-2) GT6 and Australian bat Lyssavirus (ABLV) GT7. There are several emerging Lyssaviruses recently identified in Eurasia and these include Aravan (ARAV), Khujand (KHUV) (Kuzmin et al., 2005), Irkut (IRKV) and West Caucasian bat virus (WCBV) (8). These were incorporated into the genus as putative species including Rochambeau virus (RBUV), but no phylogenic relatedness to Lyssaviruses (9)

The virus is contained within a bullet-shaped bilayered envelope. The genome encodes five structural proteins viz:Viral nucleocapsid protein (N), Phosphoprotein (P), Envelope matrix protein (M), Glycoprotein (G), RNA polymerase (L). The Polymerase, Nucleoprotein and Phosphoprotein form a complex with the genome to form an inner nucleocapsid. The matrix protein forms the inner side of the bilayered lipid envelope and the glycoprotein forms the outer layer and spike-like projections, the target of virus neutralizing antibody (Wunner et al, 1988)*

The Lyssavirus genus is separable into two distinct phylogroups based on genetic analysis of the G gene, and sequences of representative virus isolates, immunogenicity and their virulent properties [11]. Phylogroup I comprises of GTs 1, 4, 5, 7, ARAV, KHUV and IRKV, whereas phylogroup II is composed of GTs 2 and 3. Recent reports suggest that WCBV does not reside in either of the two phylogroups[12] based on genetic distances and the absence of cross-reactivity, hence the proposal to place WCBV in a newly formed phylogroup III (12).

**EPIDEMIOLOGY**

Rabies is a worldwide threat of which the domestic dog (*Canisfamiliaris*) is the main source of exposure and primary vector for this important human disease (13). Global estimates indicate that approximately ten million persons are bitten by animals around the world yearly and considered for prophylaxis and treatment against rabies. The disease causes 55,000 annual mortalities with 56% (30,800/55,000) and 44% (24,200/55,000) occurring in Asia and Africa respectively (14).

In spite of its endemic nature, the true picture of the disease burden has not been well understood. The disease recently gained tremendous public interest of which several efforts were made by government to assess the magnitude of the problem through surveillance approach, mass vaccination programmes and awareness campaigns in hot zones of high rabies activities. Despite all efforts made by health authorities nationwide to curb increasing preponderance of the disease, reports of cases still

persist (15). However, myriads of factors have been highlighted by some studies to be responsible for the persistent increase in cases both in animal and human populations in Nigeria. These factors include; socio-economic disposition, awareness and knowledge of the disease, vaccine and vaccine related factors, weak surveillance system, game activities involving dogs, slaughter dog meat consumption, lack of accurate data on the true impact of the disease and finally lack of government commitment to control measures amongst others

**TRANSMISSION**

The virus is transmitted in most cases by bites and to a lesser extent by contamination of cuts, wounds and mucous membranes with saliva from rabid animals. Non-bite related exposures to the virus of apparent highest risk are those from large amounts of aerosolized rabies virus, organs (i.e., corneas) transplanted from patients who died of rabies, and contact of saliva or nervous tissue from a rabid animal with mucous membranes or scratches (16).

**PATHOGENESIS**

The Lyssaviruses have a predilection for neural tissue (neurotrophism) where they migrate to the central nervous system and cause severe signs. Following a bite wound, the virus may remain inactive or replicate in local nervous tissues (and possibly skeletal muscle). The virus then spreads to neuromuscular junctions and neurotendinal spindles after a variable period (days or weeks). By retrograde (centripetal or axoplasmic) flow in peripheral nerves, transport of the virus to the central nervous system (CNS) needs a minimum of 21 days. After progression in CNS, the virus moves rapidly to the brain. The virus enters the spinal cord or brain stem ipsilateral to the initial inoculated site. The infection then spreads to contralateral neurons and ascends bilaterally in the spinal cord or brain stem to the forebrain. The damaged motor neurons can cause typical flaccid paralysis and ascending paralysis. Viral invasion leads to inflammation and degeneration of nervous tissue. From the CNS the virus spreads centrifugally to other tissues such as heart, cornea, adrenal glands, etc via peripheral, sensory and motor nerves. Visceral and somatic portions of cranial and spinal cord nerves and the autonomic nervous system are affected. The virus spreads via cranial nerves to salivary glands which indicates that the brain has been already infected. Viremia is not detectable; the virus affects the neural system and results in mental status changes and respiratory failure which is fatal (17).

**DIAGNOSES**

The confirmation of suspected rabies cases involveslaboratory tests. Rabies diagnoses based on clinical presentation is unreliable as there are no truly pathognomonic symptoms of the disease (18) Definitive diagnosis of rabies in the laboratory usually requires various histological and virological techniques (19).

**Direct Rapid Immunohistochemstry Test (DRIT)**

The DRIT is a biotin streptavidin-HRP system of immunohistochemical test that detects rabies virus antigen in fresh frozen, glycerol preserved and other archived brain tissues. It is an unlicensed procedure designed by the CDC for consideration as a potential confirmatory measure of the direct fluorescent antibody test, according to the national standard operating procedure for the diagnosis of rabies in animals. In addition, the RIT may be used to enhance field surveillance among suspect wildlife, particularly in support of national, regional, state, or local oral vaccination programs

A study carried out by Lembo et al., 2006, in Tanzania result in the conclusion that DRIT showed a sensitivity and specificity equivalent to those of the DFA. The test is simple, requires no specialized equipment or infrastructure, and can be successfully performed on samples preserved in glycerol solution for 15 months or frozen for 24 months and in variable conditions of preservation. These qualities make it ideal for testing under field conditions and in developing countries. Although further laboratory and field evaluations are required, results are promising and highlight the potential value of the DRIT for countries with limited diagnostic resources. First, this technique could greatly enhance epidemiologic surveillance in remote areas where rabies incidence data are difficult to obtain. Second, the test could improve the ability to respond to outbreaks with effective management decisions. Third, it could be extremely valuable in guiding decisions regarding rational use of rabies PEP.

**Histopathology**

As described by Adlochi Negri in 1903, definitive diagnosis for rabies include demonstration of intracytoplasmiceosinophilic inclusions which are round or oval in shape, eosinophilic with basophilic granules in rabies infected tissues. These granules now referred to as negri bodies can be demonstration in histological sections or fresh bilateral smears of samples from hippocampus (Ammon's horn), brain stem and the cerebellum after staining with seller, haematoxylin& eosin or Mann. This method which

has been proven to have 50-80% reliability in detecting antigens in infected animals (20) has been superseded by other methods.

**Fluorescent antibody test (FAT)**

This is the current OIE and WHO prescribed method for rabies virus detection (21) because of its reliability and sensitivity. It is a quick test with result of test being obtainable within two hours but the expensive nature of equipments such as fluorescent microscope and the expensive antibody conjugate makes its usage a challenge in Africa.

**PREVENTION AND CONTROL**

**Pre exposure immunization**

In humans, especially high-risk groups such as rabies laboratory workers, animal control facilities workers and veterinarians, pre-exposure immunization is recommended. Persons living in or traveling to areas of the world where rabies in dogs is poorly controlled and post-exposure treatment may be difficult to obtain should also receive pre-exposure immunization against rabies. Although it almost certainly confers some degree of protection against an in apparent contact with rabies virus, the intent of preimmunization is to eliminate the need for immune serum and reduce the number of vaccine doses to two booster injections should the worker or traveler sustain a bite or wound exposure to rabies virus.

**Post exposure immunization**

Post exposure immunization comprise of administration of anti-rabies immune globulin and vaccine. When indicated, treatment should begin within 24-48 hrs of an animal bite.

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# LABORATORY INFORMATION MANAGEMENT IN A CENTRAL NIGERIAN HOSPITAL: NON-COLLECTED OR UNDELIVERED REPORTS AS QUALITY INDICATOR

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**ABSTRACT**

**Background: Reporting laboratory reports to the requesting physician is one vital component of the clinical laboratory testing process. Poor management of information generated in the laboratory, such as non-collection/non-delivery of test reports, can adversely affect patient care and safety.**

**Aim: To determine the proportion and financial impact of some laboratory test reports not collected or delivered to the requesting physician.**

**Methods: A review of laboratory records of requests and collected reports of malaria parasite, urine microscopy, culture and sensitivity, and blood culture from June 2014 to December 2014 was carried out, and data analyzed.**

**Results: A total of 5321 laboratory requests comprising 4506 malaria parasites (MP), 414 urine microscopy, culture and sensitivity (urine m/c/s), and 410 blood culture were made, processed and reports generated. Of these, 1040 (19.6%) were not collected or delivered to the requesting physician. Urine m/c/s with 37.9% (157/414) accounted for the highest test-specific non-collected reports, closely followed by blood culture with 37.7% (151/401) and MP with 16% (732/4506). ICU with 54.6% (18/33) and A&E with 21% (149/710) accounted for the highest department-specific non-collected or undelivered reports. The cost of all non-collected or delivered reports was N1, 442,560 or 29.3% of the cost of the total requests during the study period.**

**Conclusion: The proportion of non-collected or undelivered test reports as seen in this study is huge, and indicates a poor laboratory information management system. There is therefore, need to institute and implement appropriate laboratory quality management system to improve patient care and reduce wastage of resources.**

**Key Words: Information management, Laboratory Report, Central Nigeria**

# GESTION DE L'INFORMATION DE LABORATOIRE DANS UN HOPITAL CENTRAL NIGERIAN: RAPPORTS NON COLLECTES OU NON LIVRES COMME INDICATEUR DE QUALITE

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**RESUME:**

**Contexte : Rapporter des rapports de laboratoire a un médecin prescripteur est une composante vitale du processus d'essais cliniques en laboratoire. La mauvaise gestion de l'information généré dans le laboratoire tel que non collection/non livres des rapports de test**

**Objectif : Pour déterminer la proportion et l'impact financier de certains rapports des tests de laboratoire qui ne sont pas collectés ou livrés au médecin prescripteur.**

**Méthodes : Un examen des dossiers de laboratoire des demandes et des rapports collectés du parasite du paludisme, de la microscopie urine, mise en culture et antibiogramme et hémoculture du juin 2014 à décembre 2014 a été effectué et les donnéesanalysées.**

**Résultats : Un totale de 5 321 demandes laboratoires comprenant 4 506 parasites du paludisme (MP), 414 microscopie urine, mise en culture et antibiogramme (urine m/c/s) et 410 hémoculture ont été faites, traitées et les rapports générés. De ceux – ci, 1 040 (19,6%) n'étaient pas collectés ou livrés au médecin prescripteur. L'urine m/c/s avec 37,9% (157/414) représentait le rapport le plus élevés non collectésspécifiques au test, suivi de près par l'hémoculturеavec 37,7% (151/401) et MP avec 16% (732/4 506). L'unité de soins intensifs avec 54,6% (18/33) et l'unité d'accident et d'urgence avec 21% (149/710) représentait le départementspécifique avec les rapports les plus élevés non livrés. Le coût de tous les rapports non collectés ou non livrés était N1 442 560 ou 29,3% du coût des demandes totales au cours de la période d'étude.**

**Conclusion : La proportion des rapports des tests non collectés ou non livrés comme on le voit dans cette étude est énorme, et cela indique une mauvaise information system de gestion. Donc il est nécessaire d'instituer et de mettre en œuvre un system de gestion de laboratoire de bonne qualité et approprié pour améliorer les soins des patients et pour réduire le gaspillage des ressources.**

**Mots –clés : Gestion d'information, le rapport laboratoire, le Centre duNigeria.**

## INTRODUCTION

Clinical laboratory investigations play a crucial role in the diagnosis and treatment of diseases. Measurement of laboratory testing processes, outcomes or laboratory's contribution to patient care can be achieved via implementation of a number of quality indicators (1,2,3). Among others, timely collection of laboratory reports and their delivery to the requesting physician to make an informed decision on patients' management is an important indicator of quality clinical laboratory services (1,4). Failure of such reports to reach the requesting physician does not only affect the quality of patient care but also unnecessarily results in waste of financial health resources. Such non-collection/delivery of the laboratory reports may indicate ineffectiveness of the laboratory service or considerable lack of medical need for such test requests.

There is a growing need to introduce measurable and evidence based indicators of laboratory efficiency and its contribution to clinical effectiveness into every segment of the health care system (5,6). Although there is paucity of literature on non-collected laboratory reports as indicator of quality laboratory information management, the few available have shown substantial numbers of non-collected/undelivered laboratory reports and the associated huge waste of laboratory budget on them (7,8). There is no documented information of any study on non-collected laboratory reports in Nigeria. It is in the light of this that this novel study was conducted to determine the proportion of some types of microbiology laboratory test reports that remained non-collected by or undelivered to the requesting physicians in National Hospital Abuja

## METHODOLOGY

The study was designed to determine the proportion and financial impact of laboratory reports of malaria parasites (MP), urine microscopy, culture and sensitivity (Urine m/c/s) and blood culture investigations that were not collected from the microbiology laboratory. Laboratory data on laboratory requests by physicians from the various service department/units along with the respective non-collected reports of the same tests from June 2014 to December 2014 were retrieved from the laboratory manual information system and analysed. The direct financial impact of the non-collected reports per test type was calculated using investigations price list of microbiology laboratory. Data collected were entered into and analyzed using Microsoft Excel. Results were presented as frequencies and percentages.

## RESULTS

During the study period, a total of 5321 laboratory requests for the three investigations were performed and reports produced. Of these, 1040 (19.6%) were non-collected laboratory reports **(Table 1)**. The percentage test-specific distribution of non-collected reports was as follows: Urine m/c/s- 37.9% (157/414), Blood culture-37.7% (151/401), and MP-16% (732/4506) **(Table 1)**. ICU accounted for 54.6% (18/33) of the department-specific distribution of all non-collected reports, while A&E, Paediatrics, Internal medicine, GOPD, Surgery, O&G, and others (oncology, special treatment clinic, haematology out-patient clinic, out-patient specialist clinic) constituted 21% (149/710), 19.9% (444/2233), 17.7% (42/237), 15.0% (139/926), 9.9% (11/111), 9.1% (49/537) and 35.2% (188/537) respectively.

Of the non-collected MP reports based on department-specific request, A&E accounted for 18.2% (115/631), while GOPD, Paediatrics and Internal Medicine accounted for 14.7% (135/918), 14.2% (232/1633) and 12.6% (26/207) respectively **(Table 1)**. While each of non-collected reports of urine m/c/s and blood culture constituted 69.2% of each respective test request for ICU, both were 53.3% for Internal Medicine, 50.0% for GOPD, and 42.5% and 32.7% respectively for A&E **(Tables 1)**.

The financial impact of all requests for all test types in this study was ₦4,928,020 out of which ₦1,442,560 (29.3%) represented the cost of non-collected reports **(Table 2)**. Non-collected MP reports accounted for 16.7% (₦344,960/2,066,120) of the cost, urine m/c/s 40.2%

(₦145,600/361,900) and blood culture 38.1% (₦952,000/2,500,000) **(Table 2).**

**TABLE1. DEPARTMENT- AND TEST- SPECIFIC DISTRIBUTIONS OF LABORATORY REQUESTS AND NON-COLLECTED REPORTS.**

| Department | No of MP test | | No of urine m/c/s tests | | No of blood culture tests | | Overall no (%) of all tests | |
|---|---|---|---|---|---|---|---|---|
| | Req | Non-col | Req | Non-col | Req | Non-col | Req | Non-col |
| Paediatrics. | 1633 | 232 (14.2%) | 311 | 106 (34.1%) | 289 | 106 (36.7%) | 2233 (42.0%) | 444 (19.9%) |
| Surgery. | 89 | 3 (3.4%) | 11 | 4 (36.1%) | 11 | 4 (36.4%) | 111 (2.1%) | 11 (9.9%) |
| Internal Medicine. | 207 | 26 (12.6%) | 15 | 8 (53.3%) | 15 | 8 (53.3%) | 237 (4.5%) | 42 (17.7%) |
| O&G | 531 | 47 (8.9%) | 3 | 1 (33.3%) | 3 | 1 (33.3%) | 537 (10.1%) | 49 (9.1%) |
| GOPD | 918 | 135 (14.7% | 4 | 2 (50.0%) | 4 | 2 (50.0%) | 926 (17.4%) | 139 (15.0%) |
| ICU | 7 | 0 (0.0%) | 13 | 9 (69.2%) | 13 | 9 (69.2%) | 33 (0.6%) | 18 (54.6%) |
| A&E | 631 | 115 (18.2%) | 40 | 17 (42.5%) | 49 | 17 (34.7%) | 710 (13.3%) | 149 (21.0% |
| Others | 500 | 174 (34.8%) | 17 | 10 (58.5%) | 17 | 4 (23.5%) | 534 (10.0%) | 188 (35.2%) |
| Total | 4506 | 732 (16.0%) | 414 | 157 (37.9%) | 401 | 151 (37.7%) | 5321 (100%) | 1040 (19.6%) |

**Req- Requested, Non-col-Non-collected, O&G-Obstetrics and Gynaecology, GOPD-General Out-Patient Department, ICU-Intensive Care Unit, A&E-Accident & Emergency unit, Others-oncology, special treatment clinic, haematology out-patient clinic, out-patient specialist clinic.**

**TABLE 2. FINANCIAL IMPACT OF EACH AND ALL TEST TYPE(S) REQUESTS/NON-COLLECTED REPORTS**

| Test type | Cost (₦) of total test request | Cost (₦) of non-collected results | % cost of results non-collected |
|---|---|---|---|
| MP | 2,066,120 | 344,960 | 16.7% |
| Urine m/c/s | 361,900 | 145,600 | 40.2% |
| Blood culture | 2,500,000 | 952,000 | 38.1% |
| Total | 4,928,020 | 1,442,560 | 29.3% |

## DISCUSSION

In this study, 19.6% of the laboratory reports were not collected. This rate is higher than 2.1% and 13% reported from studies on non-collected biochemistry reports in Croatia (7) and Pakistan (8) respectively. Although reports of urine sample would appear to be the most non-collected in this study, the case of blood culture, where almost 38% were not collected, is particularly worrisome, considering that whenever blood culture is indicated the condition is usually life-threatening. MP reports also were substantially not collected despite malaria being endemic and a major cause of morbidity and mortality especially in children.. The relatively low percentage recorded for MP test reports

compared to others in this study might be due to the comparatively shorter turn-around time (maximum of 24 hours) for the test in our laboratory during which the illness is still largely acute and both the physician and patients have strong desires to know the result. The turn-around times for the other two tests are longer (3-5 days), during which many clinical features would have substantially abated following treatment, thus making the desire for results weaker.

ICU accounted for the highest (54.6%) of the non-collected laboratory reports in terms of department-specific distribution, followed by A&E. These two departments/units often deal with critically or acutely ill patients, and therefore, would have been expected to be anxious of requested clinical laboratory rest reports. ICU still recorded the highest percentage of non-collected reports (69.2%) for each of the other two tests followed by Medicine (GOPD and A&E .

Although this study did not look into the reasons for the non-collected reports, being essentially a laboratory based review study, it is likely the habit of some physicians ordering a barrage of necessary and un-indicated tests may be partially responsible, as they later found no need for the reports of the un-indicated tests. It could also be that reports of some of the tests had earlier been communicated across the phone and the physician felt no need for the hard copies. In our institution patients/patients' relations and ward staff (in most cases of in-patients) come to collect the reports of laboratory tests from the laboratory, and this process may contribute to the issue of non-collected reports. Studies have widely reported inappropriate use of the clinical laboratory in clinical practice. (9,10,11).

The total cost of the non-collected reports was about 29% of the total value of all the tests requested. This is higher than the 13 % recorded in a similar study in Pakistan.[8] This constitutes a huge economic loss to the patients who had already paid for such test. The institution also incurred some indirect costs in carrying out those tests. To determine the true cost to the institution and the real factors responsible for this high rate of non-collected results, a well designed prospective study would be required.

**CONCLUSION**

Information management is one of the quality system essentials, and the test report is a critical component of information from the laboratory, and represents the end product of the clinical laboratory processes with respect to testing. Therefore, the non-delivery of laboratory reports to the requesters as seen in this study is a major non-conformity and has the potential to seriously impinge on quality patient care and safety. There is therefore, need for laboratories to institute and implement appropriate quality management system.

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