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ANTIBIOTIC RESISTANCE AND *NDVB* GENE EXPRESSION AMONG BIOFILM PRODUCING *PSEUDOMONAS AERUOGINOSA* ISOLATES

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ABSTRACT

A novel antibiotic resistant mechanism among biofilms is glucan-mediated sequestration in which *ndvB* gene encodes a glucosyltransferase involved in the formation of this glucans. We studied the biofilm formation and antibiotic susceptibility pattern of *P. aeruginosa* isolated from clinical samples, and measured the expression of *ndvB* gene among biofilm forming isolates and their planktonic counterparts. The study was conducted on 92 *P. aeruginosa* isolates. Biofilm was measured using tissue culture plate method. Antibiotic susceptibility of biofilm positive isolates and planktonic counterparts for ciprofloxacin, tobramycin and gentamycin was tested using tube microdilution method. Expression of *ndvB gene* was measured using Syper green real time PCR. We found that 44 isolates (47.8%) of *P. aeruginosa* were biofilm positive. The biofilm formation was high among urine, endotracheal tube aspirate and burn isolates compared to isolates of wound specimens, with statistically non-significant differences. None of biofilm forming isolates was susceptible to the 3 antibiotics compared to the presence of susceptible isolates among the planktonic counterpart (18/40.9% for ciprofloxacin, 12/27.3% for tobramycin and 13/29.5% for gentamycin). Expression of *ndvB* gene was significantly high in biofilm isolates than their corresponding counterpart, with significant correlations with minimal biofilm inhibitory concentration (MBIC) values of cibrofloxacin (r=+ 0.65, *p*001), tobramycin (r= + 0.54 *p*0[illegible]) and gentamycin (r=+ 0.77, *p*001).From this study we concluded that biofilm formation is an important character of *P. aeruginosa* that is a main cause of antibiotic resistance especially in isolates from catheterized urine , wound and endotracheal tube aspirate. *NdvB* gene expression is a mechanism of resistance to antibiotics in *P. aeruginosa* biofilms.

Key Words: *P. aeruginosa*, biofilm, antibiotic resistance and *ndvB* gene.

LA RESISTANCE AUX AINTIBIOTIQIQUESET L'EXPRESSION GENIQUE *NDVB* PARMI LES BIOFILMS PRODUISANT *PSEUDOMONAS AERUGINOSA* ISOLAT

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RESUME

Un mécanisme de résistance aux antibiotiques chez les nouveaux films biologiques est glucane médiation par séquestration dans laquelle le gène codant pour la glycosyltransferase *ndvB* impliqués dans la formation de ces glucanes. Nous avons étudié la formation des biofilms et le profil de la sensibilité de *P.aeruginosa* aux antibiotiques isolé d'échantillons clinique et on a mesuré l'expression de gène de *ndvB* parmi les isolats qui forment les biofilms et leurs homologues planctoniques. L'étude a été menée sur 92isolats de *P.aeruginosa*. Biofilm a été mesuré en utilisant la méthode du tissu plaque de culture. Sensibilité aux antibiotiques des biofilms isolats positifs et homologues planctoniques pour la ciprofloxacine, la tobramycine, et la gentamycine a été teste en utilisant la méthode tube de micro dilution. L'expression de *ndvB* a été mesurée en utilisant la PCR en temps réel vert super. Nous avons trouvé que 44 isolats (47.8%) de *P.aeruginosa*étaient biofilm positifs. La formation de biofilm était élevé parmi les urines, sonde endotrachéale aspirée, et isolats brulure par rapport aux isolats de spécimens

de plaies avec des différences statistiquement non – significatives. Aucun des isolats formant des biofilms était sensible aux trois antibiotiques par rapport à la présence des isolats sensibles parmi les homologues planctoniques (18/40,9%pour ciprofloxacine, 12/27,3% pour tobramycine et 13/29,5% pour gentamycine). L'expression de gène de *ndvB* était significativement plus élevée chez les isolats biofilm que leurs homologues correspondants, avec des corrélations significatives avec un minimum biofilm concentration inhibitrice (CMI) des

valeurs de ciprofloxacine (r= + 0,65, *p*<0,05), tobramycine (r= + 0,54 *p*<0,05) et gentamycine (r= + 0,77 *p* < 0,001). De cette étude nous avons conclu que la formation des biofilms est un caractère important de p. aeruginosa qui est une cause principale de la résistance aux antibiotiques en particulier dans les isolats provenant de l'urine cathétérisées, plaie et sonde endotrachéaleaspirée. L'expressiondugène de*ndvB*est un mécanisme de résistance antibiotique chez biofilms de *P.aeruginosa*.
Mots clés : *P.aeruginosa*, biofilms, résistance aux antibiotiques, etgène de *ndvB*.

INTRODUCTION

Pseudomonas aeruginosa is a key opportunistic pathogen characterized by high-level antibiotic resistance and biofilm formation (1).Biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix adherent to an inert or living surface. Biofilm-producing organisms are more antimicrobial resistant than organisms without biofilm. In some extreme cases, the concentrations of antimicrobials required to kill biofilm positive organisms can be three- to four-fold higher than for biofilm negative bacteria, depending on the species and drug combination (2). Biofilms have great importance for public health as they are the main cause of nosocomial infections, especially implant-based and chronic infections (3).

Antibiotic resistance in biofilms is due to a combination of many factors that act together to result in a level of resistance that is much higher than that of planktonic bacteria (4,5). One factor of these is the altered expression of specific genes involved in the biofilm. Some of these genes may function to protect biofilm cells from antibiotics. N*dvB gene,* encodes the glucosyltransferase enzyme that responsible for the formation of cyclic glucans **(6).** The glucans are cyclic polymers of 12 to 15 β- (1 → 3) -linked glucose molecules with phosphoglycerol substitutions (7). Inactivation of *ndvB* gene blocked glucan synthesis without affect the kinetics of biofilm formation, the architecture of the biofilms or growth of bacteria **(6).** However, *ndvB* mutants of PA14 strains exhibited increased sensitivity to the aminoglycosides tobramycin and gentamicin and the fluoroquinolone ciprofloxacin (6,7). This study aimed to detect the biofilm formation and antibiotic susceptibility pattern of *Pseudomonas aeruginosa* strains isolated from clinical samples, and to measure the expression of *ndvB* gene among biofilm forming isolates.

MATERIALS AND METHODS

Bacterial strains

They were isolated from patients hospitalized in surgery , burn units, and intensive care unit, at Zagazig University Hospitals, during the period from February 2015 –August 2015. Isolates were obtained from 274 specimens including catheterized urine, endotracheal tube aspirates (ETAs) and exudates of burn and septic wound. The specimens were analysed in the Lab. of Microbiology& Immunology Department, Zagazig University Hospitals .The isolates were identified as *P. aeruoginosa* by *API 20NE* and stored at -70°C. The study was approved after receiving permission from Zagazig University Ehical Committee and a written consent was taken from each patient or his relative.

Quantitation of biofilm

It was done by tissue culture plate method as described previously (8) using sterile 96 well-flat bottom polystyrene tissue culture plate with a lid (Sigma, USA). Methicillin –sensitive *staphylococcus aureus (MSSA)* ATCC-25923 and *P. aeruginosa* ATCC-27853 were used as negative and positive control organisms , respectively (9). Sterile broth was inoculated in negative control wells. The absorbance at 570 nm was determined using Microtiter plate reader (Spectra III, SIT measurement, Australia). All samples were tested in triplicate and the results were averaged. The interpretation for biofilm production was done according to specific criteria (10).

Antibiotic susceptibility testing

Antimicrobial agents

They included gentamycin, tobramycin and ciprofloxacin, provided as standard powder from Sigma Company, USA,. Antibiotics were serially diluted in cation- adjusted Muller Hinton broth (CAMHB) according to Clinical Laboratory Standard Institute (CLSI) (11).

Biofilm susceptibility assay

It was performed as previously described **(**12**)** with certain modifications to make the procedure compatible with our clinical microbiology laboratory. Biofilm positive strains were subcutured twice on tryptic soy agar (TSA, Oxoid) with 5% sheep blood after retrieval from -70°C storage and then gown overnight in CAMHB. After dilution of this culture to 0.5 McFarland, 100 µl was transferred to all except the well No 12 of a round-bottom 96-well microtiter plate with a

lid (Sigma, USA) followed by incubation at 37°C for 20-24 h with no movement. After incubation, the microtiter plate was rinsed by sterile distilled water and 100 µl of CAMHB with serial two fold dilutions of antibiotics (512 - 1µg/ml for gentamycin and tobramycin and 128 - 0.25µg/ml for ciprofloxacin) were added to corresponding wells from 1-10 in each row. In each row, well No 11 was used as growth control while well No 12 was used as sterility control. Each row represents an antibiotic susceptibility test of one antibiotic against one isolate of the organism. The plate was incubated at 37°C for 20-24 h. After antibiotic incubation, the microtiter plate was again rinsed three times in sterile water then 100 µl antibiotic-free CAMHB was added to each well. Each plate was sonicated at room temperature for 5 min The optical density at 650 nm (OD_{650}) was measured on microtiter plate reader before and after incubation at 37°C for 6 h. Adequate biofilm growth for growth control wells was defined as a mean OD650 difference that is ≥0.05**.** The biofilm inhibitory concentration (MBIC) was the lowest concentration of antibiotic that resulted in an OD_{650} difference ≤ 10% of the mean of two readings of growth control wells (12)**.**

Planktonic susceptibility assay
Conventional minimum inhibitory concentration, MIC, of the planktonic bacterial population was measured using standard broth microdilution method according to CLSI guidelines (11). MIC was the lowest concentration of antibiotic at which there is no visible growth.

Interpretations: In the statistical analysis MIC (or MBIC) values at ≤ 1 µg/ml for tobramycin or gentamicin, and ≤ 0.25 µg/ml for ciprofloxacin were considered as 1 µg/ml and 0.25 µg/ml respectively and which have MIC (or MBIC) values at > 512 µg/ml for tobramycin or gentamicin, and > 128 µg/ml for ciprofloxacin were considered as 1024 µg/ml and 256 µg/m respectively . The susceptible strains have tobramycin, gentamicin or ciprofloxacin MIC (or MBIC) values at ≤ 4µg/ml, ≤ 4 µg/ml, and ≤ 1 µg/ml respectively and resistant strains have MIC (or MBIC) values at ≥ 8 µg/ml, ≥ 8 µg/ml, and ≥ 2 µg/ml respectively (13).

Real- Time polymerase reaction: It was done to measure the expression of antibiotic resistance *ndv B* gene in 18 biofilm forming *P. aerurginosa* strains and their planktonic counterpart. Total bacterial RNA was isolated from the biofilm bacteria and planktonic counterpart. Briefly, the planktonic cells were cultivated in LB broth at 37°C to early stationary phase that corresponds to optical density of 0.7 at 600 nm , while the biofilm cells were grown on M63 agar plates overnight at 37°C followed by another night at room temperature as described previously (14). RNA was extracted using IQeasy TM plus CTB RNA Extraction Mini Kit (iNtRON Biotechnology, Inc, Korea) according to manufacturer instructions. Reverse Transcription was done using Maxime RT PreMix Kit (iNtRON Biotechnology, Inc, Korea) in which 0.1 µg template RNA and distilled water were added into the Maxime RT PreMix tubes that contain random primer to a total volume of 20µl. Complementary DNA synthesis was performed at 45° C for 60 min and RTase inactivation step at 95 °C for 5 min, using PCR Thermal cycler (Biometra, Germany) .

Complementary DNA was measured with SYBR green real-time PCR using superReal premix plus kit (TIANGEN, China). To measure *the ndvB* gene expression, a pair of primers specific for *ndvB* gene (5′-GGCCTGAA CATCTTCTTCACC- 3′ [forward]) and (5′-GATCTTGCCGACCTTGAAGAC -3′ [reverse]) was used to amplify and quantify cDNA corresponding to its mRNA. For a control, the primers (5′-GATCCGGAACAGGTGGAAGAC-3′ [forward]) and (5′-TCAGCAGTTCC ACGGTACCC-3′ [reverse]) were used to amplify and quantify mRNA of the constitutively expressed *bacterial rpoD* gene (15)**.** Each 20µl quantitative real-time PCR mixture contained 10µl SuperRealPreMix Plus, 0.5 µl Rox dye , 100µM of each primer and 2 µl cDNA (1µg cDNA) . The cycler (Stratagene Mx3000P qPCR real-time PCR) was programmed as following; 95 °C for 15 min, and 40 cycles each cycle consists of 95°C for 60 sec., 56°C for 30 sec. and 72° C for 30 sec.

Statistical analysis: Continuous variables were expressed as the median (Range), and the categorical variables were expressed as a number (percentage). Paired data were analyzed using the **Mann-Whitney U (MWU)** test. Percent of categorical variables were compared using Chi-square test (χ^2) with Fisher's exact correction. Spearman's rank correlation coefficient (r) was calculated, (+) sign was indicator for direct relationship & (-) sign was indicator for inverse relationship. All tests were two sided, *p*-value <0.05 was considered significant. All statistics were performed using SPSS 22.0.

RESULTS

During the study period, 92 *p. aeruginosa isolates* were identified from 274 clinical specimens. They were recovered from 24 (33.3%) out of 72 wound exudates, 16 (44.4%) out of 36 burn exudate, 33 (29.5%) out of 112 urine and 19 (35.2 %) out of 54 ETA specimens. 44(47.8%) out of these 92 isolates were biofilm positive by tissue culture plate method. The biofilm formation was high among urine, ETA and burn isolates compared to isolates of wound specimens, however with statistically non-significant difference, $P>0.05$ (Table 1). In urine specimens, 15 biofilm positive and 4 biofilm negative *P. aerruoginosa* strains were isolated from patients catheterized for more than 4 days, while 5 biofilm positive and 9 negative were from paients catheterized for less than or equal to 4 days ($p< 0.05$)). In ETA , 10 biofilm positive and 3 biofilm negative *P. aerruoginosa* strains were isolated from intubated patients for more than 4 days, while 1 biofilm positive and 5 negative were from ETA with intubation of less than or equal to 4 days ($p< 0.05$) .

After measuring MIC/ MBIC in µg /ml of the 44 biofilm positive *P. aeruginosa* isolates and their planktonic counterpart to the 3 antibiotics, it was found that none of biofilm form were susceptible to ciprofloxacin, tobramycin and gentamycin while 18 strains (40.9%), 12 strains (27.3%) and 13 strains (29.5%) of the planktonic counterpart were susceptible to the 3 antibiotics respectively with high statistic significant differences between biofilm form and their planktonic counterpart , $P<0.001$ for each. The differences between median (range) of biofilm positive cells MBIC and those of their planktonic counterpart MIC for the 3 antibiotics were statistically significant (Table 2). .

The expression of *ndvB* gene was measured in 18 biofilm isolates by SYBR green real time PCR It was higher among biofilm form in contrast to negligible expression in their planktonic counterpart and the difference between their median (range) of their expression was statistically significant (Table 3). According to our findings of statistical analysis for evaluating the association between the expression of *ndvB* gene and the antibiotic resistance in biofilm forming isolates, there were significant correlations between *ndvB* gene expression and MBIC values of ciprofloxacin (r=+ 0.65, $P< 0.001$), tobramycin (r= + 0.54 ,$P<$ 0.05) and gentamycin (r=+ 0.77 ,$P< 0.001$).

TABLE (1): DISTRIBUTION OF BIOFILM +VE AND BIOFILM –VE *P. AERUGINOSA ISOLATES* IN EACH CLINICAL SPECIMEN

Source of specimen	N	Biofilm negative (N=48)		Biofilm positive (N=44)	
		No.	%	No.	%
Wound	24	17	70.8%	7	29.2%
Urine	33	13	39.4%	20	60.6%
ETA	19	8	42.1%	11	57.9%
Burn	16	10	62.5%	6	37.5%
χ^2		6.96			
*p-value		0.073			

Qualitative data are presented as number (%),χ^2: Chi-square test, *$p>0.05$ is non-significant.

TABLE (2): COMPARISON BETWEEN PLANKTONIC BIOFILM +VE MIC AND BIOFILM +VE MBIC OF CIPROFLOXACIN, TOBRAMYCIN AND GENTAMYCIN (MG/ML)

Antibiotic	Planktonic MIC (N=44)		Biofilm +ve MBIC (N=44)		MWU*	
	Median	(Range)	Median	(Range)	Z	#p-value
Ciprofloxacin	16	(0.25 – 128)	96	(32 – 256)	-5.92	<0.001
Tobramycin	96	(1.0 – 512)	512	(64 – 1024)	-4.80	<0.001
Gentamycin	128	(1.0 – 1024)	512	(8 – 1024)	-4.14	<0.001

***MWU: Mann-Whitney U Test; #p< 0.001 is highly significant.**

TABLE (3): RELATIVE EXPRESSION OF NDVB GENE IN RELATION TO CONTROL GENE IN BIOFILM +VE ISOLATES AND PLANKTONIC COUNTERPARTS

	Planktonic (N=18)		Biofilm +ve (N=18)		*MWU test	
	Median	(Range)	Median	(Range)	Z	#p-value
Relative cDNA conc.	0.0008	(0.0006 – 0.0329)	5.1060	(2.0110 – 7.4370)	-5.11	<0.001

***MWU: Mann-Whitney U Test; #p< 0.001 is highly significant.**

DISCUSSION

Our finding that 47.8% of isolated *P. aeruginosa* strains were biofilm positive is in consistent with Hassan *et al* (16**)** and contrasting with Gupta *et al* (17) who found that all *P. aeruginosa* isolates were biofilm producers. In our study, biofilm production was 29.2% from wound, 37.5%from burn, 60.6% from urine and 57.9% from ETA specimens . We didn't find any statistically significant differences in biofilm production among the *P. aeruginosa* strains recovered from different specimens, which comes in agreement with the result of Mikucionyte *et al* (18).

However, different clinical results were reported by different authors. It was100% or 95% among isolates from ETAs in ICUs (19,20)**,** 76.4% among urine isolates of catheterized patients (21)and 40% or 60 % among chronic infected wound isolates (22,23**).**

Microorganisms must adhere to exposed surfaces of devices for long period to be irreversibly attached, once irreversibly attached, it produce extracellular polysaccharide matrix to develop a biofilm (24). In our study, instrumentation either by urinary catheter or endotracheal tube for more than 4 days considered as risk factor as it increased the risk for acquiring biofilm formation than in instrumented patients less than 4 days, which agrees with many reports from different studies (24-26).

None of 44 biofilm cells were susceptible to ciprofloxacin, tobramycin and gentamycin while 18, 12 and 13 strains of the planktonic counterparts were susceptible to the 3 antibiotic respectively. This result is more or less in agreement with a previous study carried out in ICU of Zagazig University Hospitals (27). In the same direction, Sepandj *et al* (28) found that, out of 8 strains, by using MIC results 8, 6 and 7 isolates were sensitive to ciprofloxacin, tobramycin and gentamycin respectively while, by measuring minimal biofilm eliminating concentration (MBEC), they found that sensitive isolates were one for each antibiotic.

In previous studies, several *P.aeruginosa* genes were identified that contribute to biofilm-specific antibiotic resistance by screening for mutants with increased antibiotic sensitivity when growing in biofilms (6,29,30). One of These genes, *ndvB,* encodes a glucosyltransferase enzyme involved in the formation of cyclic glucans (6**).**

In our study, the expression of *ndvB* gene was significantly higher among biofilm +ve *P. aeruginosa* isolates than their planktonic counterparts. There were also significant correlations between the gene expression and MBIC values of ciprofloxacin, tobramycin and gentamycin .These results are in consistent with the results reported on mutants of *P. aeruginosa* growing in biofilm (6,7,15) where **Mah *et al* (6)** reported that biofilm form of *P. aeruginosa* PA 14 had increased antibiotic resistance especially to the antibiotic tobramycin, compared to the isogenic *ndvB* mutant. Those authors proposed that this resistance was due to the ability of crude periplasmic carbohydrate extracts to interact physically with tobramycin forming molecular complex and prevent antibiotic from reaching to its site of action. The proposed mechanism is in agreement with the known properties of cyclodextrins, cyclic glucans derived from starch, as good chelating agents forming inclusion complexes with a wide variety of hydrophobic guest molecules (31) **.**

Furthermore, other studies have reported increased sensitivity of double mutant *ndvB/pa1874-1877* and *ndv B/PA0756-0757* to gentamycin and tobramycin (1,29). The relation between increased *ndvB* gene expression and synthesis of glucans in planktonic and biofilm cells have been studied by direct chemical analysis (7), and it was found that total amount of glucans was higher in biofilm culture which was in agreement with up-regulation of *ndvB* gene expression. The chemical structure of *ndvB* – dependent *P. aeruginosa* glucans was identified and it was able to interact with the aminoglycoside kanamycin, most probably by forming molecular complexes stabilized by ionic interaction. Importantly the cyclic nature and

the high negative charge of the cyclic B-(1...3)-glucans is in a good agreement with their properties to bind to positively charged antibiotics (7).

Conclusion: *P. aeruginosa*is has the ability to form biofilm that is the a cause of antibiotic resistance. Increasesd *NdvB* gene expression is an important mechanism of resistance in *P. aeruginosa* biofilm , and increased MBIC of biofilm positive *P. aeruginosa isolates to ciprofloxacin , tobramycin and gentamycin was associated with increased expression* of this gene.

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MOLECULAR DETECTION OF SALMONELLA SPECIES FROM SELECTED VEGETABLES SOLD IN A NORTH-CENTRAL NIGERIAN SETTING

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ABSTRACT

It is vital to study and understand the genetic basis to the virulence of different *Salmonella* strains in other to fully grasp the facts behind the unique capabilities of these pathogenic agents to causing diseases in both humans and animals. In this study, the conventional microbiological culture methods were used to isolate pure *Salmonella* strains from 120 vegetable samples of five different types; which were all obtained at seven different popular markets in the Jos Metropolis of North-Central Nigeria. 25 (20.8%) pure isolates were obtained from 120 samples after initial culture and sub-cultures; with 24 (20%) of the pure isolates testing positive as being pathogenic after biochemical analysis. From the 25 pure isolates, the same 24 which tested positive for biochemical tests were also successfully amplified by PCR technique with the *Salmonella invA* virulence gene. The result shows that 96% of the pure isolates were positive for the *Salmonella invA* gene. The PCR product which was very specific is a 250bp fragment of DNA which was visualized in 1.5% agarose gel. This finding shows that virulent *Salmonella* strains pose a major health hazard and public health concern to the affected population. Our study shows that there is a high prevalence rate of virulent *Salmonella* strains in North-Central Nigeria. It is thus concluded that although both the conventional culture and biochemical methods of isolating *Salmonella species* are most useful for obtaining pure isolates and identifying pathogenic strains, however, the PCR technique remains the most specific and sensitive; especially when the rapid identification and detection of virulent strains of *Salmonella species* are of utmost importance.

Key words: Virulence, *inv*A gene, PCR, North Central Nigeria

LA DETECTION MOLECULAIRE DES ESPECES DE SALMONELLES DE LEGUMES SELECTIONNES VENDUS AU NORD CENTRAL D'UN CADRE DU NIGERIA

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RESUME

C'est important d'étudier et comprendre la base génétique de la virulence des souches différentes de *Salmonelles* afin de saisir pleinement les faits derrière les capacités de ces agents pathogènes à l'origine de maladies chez les humaines et les animaux. Dans cette étude, les méthodes classiques de culture microbiologiques ont été utilisées pour isoler des souches de *Salmonelles* pures de 120 échantillons de légumes de cinq types différents ; qui ont été obtenus aux sept marchés populaires différents dans la métropole de Jos au nord – central du Nigeria. 25 (20,8%) isolats purs ont été obtenus de 120 échantillonsaprès la culture initiale et la sous – culture ; dont 24 (20%) des isolats purs ont ététestés positifs comme pathogènesaprès l'analyse biochimique. De 25 isolats purs, les mêmes 24 qui ont été testés positifs pour des tests biochimiques ont également été amplifiés par la technique PCR avec succès avec le gène de virulence de *Salmonelle invA*. Le résultat montre que 96% des isolats purs étaient positifs du gène de*Salmonelle invA*. Le produit de PCR qui était trèsspécifique est un fragment de 250bpde l'ADN qui a été visualisé en 1,5% gel d'agarose. Ce résultat montre que les souches de *Salmonelles* virulentes présentent un risque majeur pour la santé et le souci de la santépublique à la population touchée. Notre étude montre qu'il y a un taux élevé de prévalence de la virulence des souches de *Salmonelles* dans le centre – nord du Nigeria. Il est donc conclu que, bien que la culture conventionnelle et les méthodes biochimiques d'isoler des *espèces de Salmonelles* sont les plus utiles pour l'obtention des isolats purs et identifier les souches pathogènes, cependant, la technique PCR reste la plus spécifique et sensible ; en particulier lorsque l'identification rapide et la détection de souches virulentes *d'espèces Salmonelles* sont d'une importance capitale.

Mots clés : Virulence, le gène*invA* , PCR, Centre – nord du Nigeria.

INTRODUCTION

Salmonella is a bacterium of the genus *enterobacterecea*. There are many strains of this group of bacteria [1, 2]; with many commonly found in the environment, while some others have specific animal hosts. Salmonellosis is a generally and commonly distributed food borne disease all over the world [1, 3, 4,]. Salmonella infection and thus Salmonellosis is acquired through contaminated animal products, contaminated water, contaminated fruit and vegetables, and also via the feco-oral route through person-to-person contact [1, 3, 4, 5]. Salmonellosis is usually characterized by abdominal cramp, diarrhea, fever, headache, nausea and vomiting among others [2, 3, 4]. Usually, individuals with compromised immunity, the geriatrics, and pediatrics; especially children under five years of age are the most prone to this disease [2, 3, 4]. Complications associated with Salmonellosis include loss of body fluid (electrolytes); due to persistent diarrhea which may lead to death if not well managed. Also, 'Reactive Arthritis' may result from *Salmonella* infection [4].

Globally, several millions of people are infected with *Salmonella species* annually [1]; and with many strains of this bacterial now developing resistance against antimicrobial agents, Salmonellosis has thus become a disease of major Public Health concern [1,17]. There are different serotypes of *Salmonella species*; but the two strains which are both zoonotic and pathogenic, and most common worldwide are the *Salmonella typhimurium* and *Salmonella enteriditis* [1].

Studies have shown the prevalence of *Salmonella species* in different poultry and animal products [6, 7]. Some studies in Nigeria [8, 9, 10] and at different parts of the world [11, 12, 13, 14] have indicated the pollution and or contamination of most common vegetables by *Salmonella species*. In North-Central Nigeria where the occupation is predominantly farming, most small scale farmers cultivate vegetable farms; and the produce from these farms are generally marketed at the different markets within the respective local communities. The cultivated vegetables are mostly watered manually with contaminated waters from nearby streams or water bodies. These farms are mostly manured with infected and or contaminated poultry droppings and animal dung [15, 16] which serves as a rich source of nitrogen to the growing crops.

Conventional culture methods for the growth and isolation of bacteria are at best most useful for advanced molecular diagnostics and biochemical identification of pathogenic strains. The use of polymerase chain reaction (PCR) serves as the most reliable method of identifying and detecting pathogenic *Salmonella species* and their virulence gene.

MATERIALS AND METHODS

This study was carried out in Jos, the capital of Plateau State, North-Central Nigeria. The Jos Metropolis comprises of three different local government areas; namely, Jos North, Jos South, and Jos East local governments respectively. The occupation of the people on the Jos Plateau is predominantly farming. This farming is largely encouraged by the naturally fertile soil which enhances the growth of most vegetables and food crops. The seven major markets in the Jos Metropolis were visited for the sampling of the various vegetables used in this study. These markets are as follows: Gadabiu market, Terminus market, Faringada market, Gyel market, Bukuru market, Building Materials market, and the Angwan Rukuba market. The selected vegetables for the study include Cabbage, Carrot, Lettuce, Garden egg, and Cucumber respectively. All samples were aseptically collected into sterile poly bags, and adequate precautions were taken to avoid human contamination before transportation to the Laboratory for testing. A total of 120 samples of the above listed vegetables were randomly selected and used in this study.

CULTURE ISOLATION OF SALMONELLA BACTERIA

All the media used in this study, which includes Peptone water, Xylose Lysine Deoxycholate agar, Salmonella-Shigella agar, Brain-Heart Infusion media, and Nutrient agar, were all prepared according to methods as described by Ochei *et al* [18]; and they were all sterilized at 121˚C for 15 minutes; except for the *Salmonella-Shigella* agar which was boiled for 15 minutes as this does not require autoclaving. All samples collected were aseptically swabbed and cultured; and incubated at 37˚C for 24 hours in the selected medium until pure isolates were identified and biochemically tested by the Urease test, Simmons Citrate test, and the Andrade's Peptone sugar test[18]. The pure *Salmonella* isolates which serves as stock were refrigerated at 2˚C - 8˚C and then used for DNA – PCR analysis.

SALMONELLA DNA EXTRACTION

1.5mls Eppendorf tubes were labeled based on the number of pure isolates obtained; which was 1 to 25, and these were well arranged on a rack. 1ml of the pure isolates suspended in Brain-Heart infusion broth was then dispensed into the 1.5ml already labeled Eppendorf tubes. This was centrifuged at 14,000 rpm (rate per minute) for 3 minutes using the Eppendorf Cold Centrifuge until a supernatant and pellets are obtained – this process is known as 'Cell Washing'. The supernatant was discarded, and the pellet was adjusted to 100µl using normal phosphate buffered saline with the aid of a micropipette. This was vortexed and properly dissolved. 5µl of the digestion buffer was then dispensed into each of the Eppendorf tubes. A further 5µl of Proteinase K was also added to the buffer and this was well vortexed to aid the even distribution of the added buffer and enzyme. The mixture was then incubated at 55˚C for 20 minutes at the end of which the mixture appears transparent. 70µl of the genomic lysis buffer was then added to the mixture and this was thoroughly mixed.

The mixture was transferred to a Zymospin mini spin column tube and then centrifuged at 12,000 rpm for 1 minute. 200µl of DNA pre-wash buffer was added to the spin column and again centrifuged at 12,000 rpm for 1 minute. To enable the absolute washing away of all debris, 400µl of DNA wash buffer was dispensed into the spin column and centrifuged at 12,000 rpm for 1 minute. The mixture was then transferred to a clean micro-centrifuge tube where ≥50µl of DNA elution buffer was added. The tube was allowed to stand at room temperature (RT) for 2 to 5 minutes before being centrifuged at top speed for 30 seconds in order to elute the DNA. The eluted DNA was then taken for amplification reaction in an Eppendorf Thermocycler.

PCR AMPLIFICATION AND PRIMER SETS

A reverse and forward specific *Salmonella invA* primer of 250bp was used for the PCR of our quantified pure DNA extracts. This specific *Salmonella invA* gene has the following bases: 5'-GTG AAA TTA TCG CCA CGT TCG GGC AA -3' and 5' – TCA TCG CAC CGT CAA AGG AAC C – 3' respectively. The amplification mixture consist of the 'Master Mix' which is made up of 3µ of 10X PCR buffer, 1µl of $MgCl_2$, 1µl of dNTPs (10Mm), 1.25µl each *of invA Forward and invA Reverse polymerase*. 5µl of the sample was added, and the total volume of the mixture was made up to 25µl.

PCR amplification was carried out in an Eppendorf Master-Gradient Thermocycler. The stages of amplification involves: incubation at 94˚C for 60 seconds, followed by 35 cycles of denaturation at 94˚C for 60 seconds, this is followed by annealing at 64˚C for 30 seconds, and elongation of the annealed primers at 72˚C for 30 seconds; finally, there was the extension of the amplified sequences at 72˚C for 7 minutes.

AGAROSE GEL ELECTROPHORESIS

The amplified DNA products from the *Salmonella* specific PCR were analyzed on 1.5% w/v agarose gel; stained with ethidium bromide. 10µl of each amplified product was mixed with 2µl of 6X loading dye and then loaded unto the agarose gel. A 250 base pair (bp) ladder was used as a marker for the PCR product; and a current of 120volts for 30 minutes was passed through the electrophoretic tank. The amplified products for the positive control which was obtained from animal source yielded result at the expected band size of 284bp; while the pure isolates from the plant sources yielded base pairs of varied bands, with fourteen of the pure isolates yielding a consistent base pairs of 260.

RESULTS

This study was carried out in order to shed more light on the *Salmonella species* contamination of commonly available vegetables; and the danger this contamination poses to the public health of the affected populations. Of the 120 samples analyzed from the seven selected markets in the Jos Metropolis, 25 samples were concluded pure isolates after repeated cultures and sub-cultures. But with biochemical analysis, 24 of the pure isolates tested positive for pathogenecity. The DNA of the pure isolates which were re-suspended in Brain-Heart infusion broth was extracted and used for PCR. The percentage prevalence of the cultured and sub-cultured pure isolates was 20.8% representing 25/120 samples; while the percentage prevalence of those isolates which were biochemically positive and also successfully amplified with the virulent *invA* Forward and Reverse *Salmonella* gene was 20% which represents (24/120) samples. 24 of the 25 pure isolates tested positive for *Salmonella invA* specific gene and this represents a percentage of 96%; which clearly indicates a high prevalence rate of the virulent strains of *Salmonella species* in

this study. This prevalence rate poses a very severe danger to the public health of the affected population; thus affecting their socio-economic and general wellbeing (Table 1; and Figures 1 and 2).

***TABLE 1*: SUMMARY OF *SALMONELLA SPECIES* ISOLATES FROM VEGETABLES SOLD IN SEVEN SELECTED MARKETS IN THE JOS METROPOLIS OF PLATEAU STATE, NORTH-CENTRAL NIGERIA.**

Market Locations	Cabbage	Carrot	Lettuce	Garden egg	Cucumber	Total
Jos North	**2**	**1**	**2**	**1**	**2**	**8**
Jos South	**2**	**1**	**2**	**1**	**1**	**7**
Jos East	**4**	**4**	**1**	**0**	**1**	**10**
Total	**8**	**6**	**5**	**2**	**4**	**25**

FIGURE 1: PREVALENCE OF *SALMONELLA SPECIES* IN THE EXAMINED VEGETABLES.

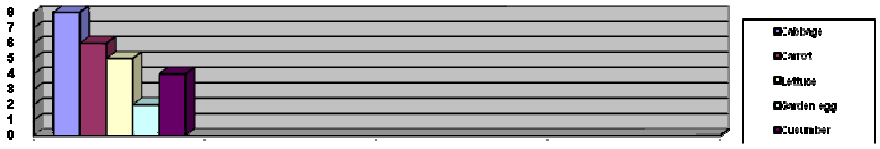
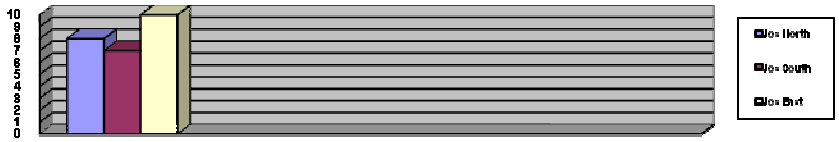


FIGURE 2: PREVALENCE OF *SALMONELLA SPECIES* IN THE THREE LOCAL GOVERNMENT AREAS OF THE JOS METROPOLIS IN PLATEAU STATE, NORTH-CENTRAL NIGERIA.



DISCUSSION

The repeated bouts of *Salmonella* infections, especially with the non-typhoidal *Salmonella* strains has become an issue of public health concern worldwide [1]. Though most cases of Salmonellosis are usually mild, this disease may sometimes lead to fatal outcomes [2, 4]. Poor hygiene practices; such as inadequate washing of hands, fruits and vegetables, contaminated food and water, and contaminated animal products, are all responsible for the transmission of Salmonellosis [1, 3, 4]. Also, with the rapid emergence of antimicrobial resistance strains of *Salmonella species* [17], it is of utmost importance that healthy individuals do not take routine antimicrobial medications in mild to moderate cases; safe in geriatrics, infants or children below five years of age, and immune-compromised individuals [1].

Safe ways of handling fruits and vegetables before personal and or public consumption have been well enumerated and outlined by various public health authorities and cooperate organs [1, 19, 20]; and now, the onus lies on the individuals to safeguarding their personal and thus, the health of the public through the strict observance and adherence to the universal and basic rules of hygiene and food safety.

Different studies have shown very high prevalence rate of *Salmonella* contamination of vegetables [8, 14] and different animal products [6, 21, 22]. The prevalence rates from these studies compares favorably with the 20% of the 120 study samples and the 96% of the 25 pure *Salmonella* isolates of DNA-PCR *Salmonella invA* gene detectable results obtained in this current study. The high virulence rate of 96% obtained in this study however poses a severe health risk to the affected populations. The observed contamination of the studied vegetables in this study emerged most probably as a result of either the manure (poultry droppings or animal dung) used to grow the crops; or the water sources used for the irrigation of the crops; which in most instances harbors the pathogen. Our study reveals that though the use of the conventional culture methods are most useful for obtaining pure *Salmonella* isolates, and the application of biochemical tests helps in identifying pathogenic strains; which is indeed most vital in poor resource settings and especially in low and middle-income countries where PCR technique materials may not be readily available. However, the PCR technique remains the most specific and the most sensitive; especially when the rapid identification of virulent strains of *Salmonella species* are of utmost importance.

CONCLUSION

25 (20.8%) pure culture isolates were obtained in this study; out of a total of 120 samples. 24 (96%) of the pure isolates tested positive for the *invA Salmonella* virulence gene. This indicates a very high prevalence rate of virulent *Salmonella* strains in the study area. And though the rich and fertile nature of the soil on the Jos Plateau, North-Central Nigeria favors the growth of very large varieties of vegetable crops, the use of contaminated poultry droppings and animal dung as manure, and the use of contaminated water sources for the irrigation of the farm lands, coupled with poor personal hygiene practices, have all led to the increased reports in the cases of Salmonellosis. It is therefore advised that urgent public health steps be taken by concerned authorities towards public education on food safety, observance of basic personal hygiene like hand washing, and information on known sources of *Salmonella* contaminants, so as to foster a healthy and a productive society through awareness and the observance of basic preventive measures.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors participated in this study. The final manuscript was well read and approved by all authors.

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PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND EXTENDED SPECTRUM B–LACTAMASE PRODUCERS AMONG BACTERIA ISOLATED FROM INFECTED WOUNDS IN A TERTIARY HOSPITAL IN IBADAN CITY

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RUNNING TITLE: MRSA AND ESBL PRODUCING BACTERIA

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ABSTRACT

Wound colonization by microorganisms is most frequently polymicrobial and incidences of high level resistance among bacterial isolates from wounds have been reported. Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) producing Gram-negative bacteria both constitute serious challenge to physician in their choice of antibiotic treatment of infections caused by these bacteria. This study determined the antibiotic susceptibility profiles and prevalence of MRSA and ESBL producers among wound bacterial isolates from a tertiary hospital in Ibadan City.

Forty (40) clinical bacterial isolates from five wound sources were collected from the Microbiology unit of the University College Hospital (UCH), Ibadan and were authenticated with standard bacteriological techniques. Antibiotic susceptibility test was done by disc-diffusion method using 19 antibiotics belonging to 12 classes. MRSA strains were detected by their resistance to cefoxitin and/or oxacillin antibiotics. Presumptive ESBL production was by double-disc synergy test using 30 µg cefotaxime and ceftazidime around 20/10 µg amoxicillin-clavulanic acid discs. ESBL confirmation was by minimum inhibitory concentration (MIC) using agar-dilution method.

The authenticated isolates include *Proteus* spp (47.5%), *Staphylococcus aureus* (27.5%), *Pseudomonas aeruginosa* (12.5%), *Klebsiella* spp (7.5%), *Acinetobacter baumanii* (2.5%) and *E. coli* (2.5%). Distribution of the isolates collected according to wound sources includes: acute soft tissue wounds (35%), leg ulcer (32.5%), surgical wounds (17.5%), burn wounds (12.5%) and diabetic foot ulcer (2.5%). Distributions according to patients' gender are: male (65%), female (35%), and according to age-groups are: 0 – 19 years (22.5%), 20 – 39 years (35%), 40 – 59 years (32.5%) and ≥ 60 years (10%). All (100%) the isolates were multidrug resistant (MDR) being resistant to ≥ 3 classes of antibiotics. Percentages of isolates resistance to each of the antibiotic include: piperacillin, piperacillin-tozobactam and amoxicillin-clavulanic acid were 100%, ceftazidime, cefuroxime, cefixime, aztreonam, sulphamethoxazole-trimethoprim, erythromycin, chloramphenicol and doxycyclin were > 70%, cefoxitin (62.5%), Nitrofurantion (52.5%), ciprofloxacin (45%), ofloxacin (35%), perfloxacin (37.5%), gentamicin (32.5%) and imipenem (2.5%). Of the 11 *Staphylococcus aureus* collected, 54.5% were detected to be MRSA strains while ESBL production was detected in 55.2% of the Gram negative isolates.

This study revealed 100% MDR phenotype constituting high level of MRSA strains (54.5%) and ESBL producers (55.2%) among Gram-positive and Gram-negative bacterial wound isolates respectively. Hence, this calls for caution in the use of extended spectrum antibiotics in treating patients with infected wounds.

LA PREVALENCE DES STAPHYLOCOQUES AUREUS RESISTANTS A LA METHICILINE ET LES PRODUCTEURS DE B – LACTAMASE SPECTRE ETENDU PARMI LES BACTERIES ISOLEES DE PLAIES INFECTEES DANS UN HOPITAL TRETIAIRE A LA VILLE D'IBADAN.

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TITRE COURANT: SARM ET BLSE BACTERIES PRODUCTRICES.

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RESUME

La colonisation de la plaie par des microorganismes est le plus souvent poly microbiennes et l'incidence de haut niveau de la résistance par des isolats de plaies ont été rapportés. Staphylocoque aureus résistant à la Méthicilline (SARM) et bêta–

Lactamases spectre étendu (BLSE) produisant des bactéries à Gram – négatif, les deux constituent un sérieux défi pour le médecin dans le choix du traitement antibiotique des infections causées par ces bactéries. Cette étude a déterminé les profils de sensibilité aux antibiotiques et la prévalence des producteurs de SARM et BLSE parmi les isolats bactériens des plaies d'un hôpital tertiaire dans la ville d'Ibadan. Quarante (40) isolats bactériens clinique provenant de cinq sources de plaies ont été recueillis de l'unité de microbiologie de l'University College Hospital (UCH), Ibadan et ont été authentifiés avec des techniques bactériologiques standard. Test de sensibilité aux antibiotiques a fait par la méthode de diffusion sur disque en utilisant 19 antibiotiques appartiennent à 12 classes. Les souches SARM ont été détectés par la résistance aux antibiotiques cefoxitin et /ou oxacilline. La production de BLSE présomptif était par le test de synergie double dique en utilisant 30µg cefotaxime et ceftaxidime autour de 20/10 µg disques d'acide amoxicilline – clavunique. La confirmation de BLSE a été par la concentration minimale inhibitrice (CMI) en utilisant agar · méthode de dilution. Les isolats authentifiés comprennent Proteus spp (47,5%), Staphylococcus aureus (27,5%), Pseudomonas aeruginosa (12,5%), Klebsiella spp (7,5%), Acinetobacter baumannii (2,5%),et E. Coli (2,5%).

La distribution des isolats collectés selon des sources de plaies comprend: plaies aigües des tissus mous (35%), ulcère de jambe,(32%) les plaies chirurgicales (17,5%), les plaies de brule (12,5%)et les ulcères du pied diabétique (2,5%). La répartition selon le sexe des patients sont : male(65%), femelle (35%), selon les groupes d'âge sont :0 –19 ans (22,5%),20 – 39ans (35%), 40 – 59 ans (32,5%) et ≥ 60 ans (10%). Tous (100%) les isolats étaient multiresistants (MDR) etant resistant a ≥ 3 classes d'antibiotiques. Les pourcentage de la résistance des isolats à chaque antibiotique comprend: piperacilline, piperacilline ·tozobactam et acide amoxicilline – clavulanique etaient 100%, ceftazidime, cefuroxime, cefixime, aztreonam, sulphamethoxazole – trimethoprim, erythromycin, chloramphenicol et doxycyline etaient >70%, cefoxitin (62,5%), nitrofurantoïne (52,5%), ciprofloxacine (45%), l'ofloxacine (35%),perfloxacin (37,5%), gentamicine (32,5%), et imipénème (2,5%). Du 11 Staphylocoque aereus recueillis, 54,5% ont été détectés comme des souches de SARM alors que la production de BLSE a été détectée dans 55,2% des isolats Gram négatif. Cette étude a révélé 100% phénotype constituant un niveau élevé des souches de SARM (54,5%) et les producteurs de BLSE (55,2%) chez les Gram – positif et Gram – négatif des isolats bactérien de plaies. Par conséquent, il faut la prudence dans l'utilisation des antibiotiques à spectre étendu dans le traitement des patients avec plaies infectées.

INTRODUCTION

Human or animal skin if not broken or damaged, prevents agents of infection from entering into the body. However, when the dermis of the skin is damaged usually by chemical or mechanical injury, the subcutaneous tissue becomes exposed and thus provides a moist, warm, and nutritious environment for the colonization and proliferation of microbes (1, 2). The term wound, is given to a compromised skin and can be classified either as open or closed wound (1, 3). In open wound, the skin is torn, cut, punctured or as a result of avulsion, thereby exposing the subcutaneous layer of the body (3). In the case of closed wound, the skin developed hematoma either through blunt force trauma resulting into contusion or through internal blood vessel pathology resulting into ecchymosis, purpura and petechiae (3). Wounds can also be categorized as accidental, pathological and post-operative wounds depending on its nature (4, 5).

Microbial contamination of wounds usually occur either at the time of injury that lead to the wound or as a result of improper handling of the wound by the patient concern (4). Sources of nosocomial bacterial contaminants in wounds could be exogenous or endogenous. Exogenous sources are other sources of microbial contamination order than the patient's own bacterial flora and they include: contaminated objects that caused the wound in the case of accidental wound, carelessness of patient with wound resulting in bacterial contamination from the hospital environment, contaminated medical materials, surgical equipments and use of poorly or non sterile gloves by the surgeon in the case of post-operative wounds (4, 5). Endogenous sources on the other hand are bacterial contamination from the patient's own microbial flora such as *Staphylococcus aureus* from the skin (6) and coliforms from the anus, which can contaminate open wounds when hands are not adequately washed after using the toilet. If these contaminating bacteria persist, grow and multiply and become established at the site of invasion will result into wound infection (2).

Several factors influence the diversity of microorganisms that can be found in any wound. Such factors includes: the type and location of the wound, the depth and level of tissue perfusion, and the host immune response (3). In most instances, wound colonization is polymicrobial involving numerous microorganisms that may be pathogenic (7, 8, 9). Infected wound has actively multiplying pathogenic organisms with clinical signs of infection such as pain, redness, oozing of pus and yellowing of the wound site. This usually prolongs wound healing and the patient concern suffers increased trauma with increased treatment costs (2, 3).

The rapid increase and spread of multidrug resistant (MDR) bacteria particularly involving nosocomial infections has added a tedious dimension to the problem facing the physicians in the treatment of wound infections among in-patients (2). Of the several antibiotics of choice that can be used in the treatment of infected wounds, extended-spectrum beta-lactam antibiotics such as the third generation cephalosporins are reserved for treatment of serious and life threatening wound infections. However, several studies have reported high level bacteria resistance to this class of antibiotic (10, 11, 12). Several resistant determinants have been detected in bacterial isolates from infected wounds and the distribution of the types detected is usually based on the level of dissemination among bacterial isolates in that

location (11, 12, 13). Most interesting and widely studied resistant determinants are the beta-lactamases, particularly the extended-spectrum beta-lactamases (ESBLs). ESBLs are different variants of beta-lactamase enzymes derived from the classical beta-lactamase enzymes by mutation at one or multiple points in their gene sequences and are known to mediate resistance against all beta-lactams especially the extended-spectrum beta-lactam antibiotics including the third generation cephalosporins (14). *Staphylococcus aureus* that are resistance to oxacillin and currently cefoxitin are multidrug resistant strains denoted as methicillin-resistant *Staphylococcus aureus* (MRSA) because they were previously detected to show resistance to methicillin, an improved penicillin derivative antibiotic against penicillinases producing Gram-positive bacteria (15). MRSA are usually one of the commonest nosocomial agents that are responsible for high morbidity and mortality in hospitals especially in the newborn nurseries (15, 16). Occurrence of MRSA and ESBL-producing Gram-negative bacteria within hospital setting have been established and their emergent as causative agents of nosocomial wound infections have been reported in many countries including Nigeria (2, 10, 12, 17). For effective treatment of wound infections in this multiple antibiotic resistance era, there is need for continuous isolation and screening of bacterial isolates from wound infections (18) with the view to assist physicians in making rational selection of antibiotics in the treatment of wound infections.

This study therefore phenotypically determined the susceptibility profiles, percentage frequency of MRSA strains and ESBL producers among bacterial isolates from nosocomial wound infections in a tertiary hospital in Ibadan City, Southwest Nigeria.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF ISOLATES

Forty bacterial isolates were collected through the Microbiology unit of the University College Hospital (UCH) Ibadan by random sampling within one month, on sterile nutrient agar slants. It was checked on the laboratory record that the bacterial isolates collected were from patients suffering from different wounds types that were infected while still on admission in the hospital wards. Information on the patients' age, sex and the type of wounds was also obtained from the laboratory record. The clinical isolates were re-identified using cultural characteristics, Gram staining and standard biochemical test to further confirm their identities.

ANTIBIOTIC SUSCEPTIBILITY STUDY

The clinical isolates were subjected to antibiotic screening using the disc-diffusion method as described by Etok *et al*. (2012) (12) and result interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines (2012) (19). The isolates, after dilution to 0.5 McFarland standard suspensions were inoculated on the surface of Mueller Hinton agar plate by surface spreading using sterile swab sticks to give a monolayer of bacterial cell over the agar surface. With the aid of sterile forceps the standard antibiotic discs were place on the inoculated agar surface and after 30 minutes of pre-incubation diffusion the agar plates were incubated in an inverted position for 24 hours at 37^0C. The following antibiotics were tested: piperacillin (30 µg), piperacillin-tozobactam (110 µg), oxacillin (1 µg), ceftazidime (30 µg), cefuroxime (30 µg), cefixime (5 µg), cefoxitin (30 µg), aztreonam (30 µg), amoxicillin-clavulanic acid (20/10 µg), imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), perfloxacin (5 µg), nitrofurantoin (300 µg), chloramphenicol (30 µg), doxycycline (30 µg), sulphamethoxazole-trimethoprim (25 µg) and erythromycin (30 µg). Isolates resistant to three or more classes of antibiotics will be considered multidrug resistant strain (20).

DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) STRAINS

All the *Staphylococcus aureus* isolates collected were subjected to antibiotic susceptibility test by disc-diffusion using oxacillin (1 µg) antibiotics as described by Etok *et al.* (12) as well as cefoxitin (30 µg) as described by CLSI guidelines (19). Isolates resistant to either oxacillin, cefoxitin or both were taken to be MRSA strains.

DETECTION OF EXTENDED-SPECTRUM β-LACTAMASES (ESBL) PRODUCTION

Presence of ESBL production among the Gram-negative bacterial isolates was first determined by double-disk synergy test using 30 µg cefotaxime and ceftazidime disc arranged 20mm centre to centre around 20/10 µg amoxicillin-clavulanic acid disc on Mueller Hinton agar plate already inoculated with 0.5 McFarland standard bacterial suspensions as described by Okesola and Fowotade (10). Those with positive result were then further confirmed by minimum inhibitory concentration determination involving the two cephalosporins alone and the cephalosporins with inhibitor (clavulanic acid) using agar dilution method as described by Ogbolu *et al.* (11). The agar plates were incubated in an inverted position for 24 hours at 37^0C. The results were interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines (19).

RESULTS

DISTRIBUTION OF BACTERIAL ISOLATES ACCORDING TO WOUND TYPES

The number and percentage distribution of clinical isolates collected and the type of wound infections from which they were isolated is presented in tables 1 and 2. Among the isolates, *Proteus* spp (47.5%)

recorded the highest collection followed by *Staphylococcus aureus* (27.5%), *Pseudomonas aeruginosa* (12.5%) and *Klebsiella* spp (7.5%) while both *Acinetobacter baumanii* and *E. coli* recorded 2.5% collection each. *Proteus* spp make up 80% of the isolates from burn wounds, 42.9% from surgical wounds and 53.8% from leg ulcer. *Staphylococcus* spp. make up 20% of isolates from burn wounds, 42.9% from acute soft tissue wounds, 14.3% from surgical wound and 23.1% from leg ulcer. *Klebsiella* spp make up 100% isolates from diabetic foot ulcer and 15.4% from leg ulcer while *E. coli* make up 14.3% of isolates from surgical wound infections.

DISTRIBUTION OF THE CLINICAL ISOLATES ACCORDING TO PATIENT'S GENDER AND AGE GROUPS

In this study all the *E coli*, *Klebsiella* spp, and *A. baumanii* and 63.2%, 80%, 45.5% of *Proteus* spp, *P. aeruginosa* and *S. aureus* collected respectively, were from infected wounds of male patients. Wound isolates from female patients constitute 54.5% of *S. aureus*, 36.8% of *Proteus* spp and 20% of *P. earuginosa* (table 3). Among the patients with *Staphylococcus* spp. infected wounds in this study, 45.5% of the patient fall within the age group 20 – 39 years, 27.2% fall within the age group 40 – 59 years and 18.2% within age group >60 years while *E. coli* infected wound was found to occur in one patient in the age group >60 years. Also, among the patients with *P. aeruginosa* infected wound, 40% were in the age group 0 – 19 years and 20 – 39 years while only one patient in the age group 20 – 39 years had *A. baumanii* infected wounds. Among the patients with *Proteus* spp infected wounds, 31.6% belongs to the age group 0 – 19 years, 26.3% to 20 – 39 years, 36.8% to 40 – 59 years and 5.3% to age group >60 years.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The isolates showed varied antibiotic susceptibility profile to the different antibiotics used in this study as shown in table 4. All the clinical isolates exhibited multidrug resistant phenotype, being resistant to three or more classes of antibiotics.

The isolates exhibited 100% resistance to amoxicillin-clavulanic acid, piperacillin and piperacillin-tozobactam. Percentages of the isolates that showed resistance to sulphamethoxazole-trimethoprim, erythromycin, chloramphenicol, doxycyclin, cefixime, ceftazidime and aztreonam were greater than 70%.

High level of resistance (>50%) was observed among the Gram positive isolates against cefixime, ceftazidime, aztreonam, cefoxitin, amoxicillin-clavulanic acid, ciprofloxacin, ofloxacin, perfloxacin, doxycycline, chloramphenicol, sulphamethoxazole-trimethoprim, piperacillin and piperacillin-tozobactam while their susceptibility to nitrofurantoin and imipenem was 100%. Majority (>70%) of the Gram negative bacteria were resistant to amoxicillin-clavulanic acid, piperacillin, piperacillin-tozobactam, sulphamethoxazole-trimethprim, and doxycycline while most (97.5%) were sensitive to imipenem. *E. coli* showed 100% resistance to sulphamethoxazole-trimethoprim, erythromycin, chloramphenicol, doxycycline, perfloxacin, ofloxacin, ciprofloxacin, piperacillin, piperacillin-tozobactam but 100% susceptibility to aztreonam, cefoxitin, ceftazidime, nitrofurantoin, cefixime, gentamicin and imipenem. *Proteus* spp., *Klebsiella* spp. and *A. baumanii* showed 100% susceptibility to imipenem while *P aeruginosa* showed 80% susceptibility.

MRSA AND ESBL PHENOTYPE

Six (54.5%) out of the 11 strains of *Staphylococcus aureus* were methicillin resistant as they showed resistance to either oxacillin, cefoxitin or both (Table 5). The result of the ESBL detection is presented in table 5. A total of 16 (55.2%) organisms produced ESBL out of 29 Gram negative organisms that were tested. *E. coli*, *A. baumanii* and *Klebsiella* spp. had 100% ESBL production while *Proteus* spp., and *P. aeruginosa* had 42.1% and 60% respectively.

TABLE 1: PERCENTAGE FREQUENCY OF BACTERIAL ISOLATES FROM WOUND INFECTION

Clinical Isolates	Number (N)	Percentage (%)
***Proteus* spp.**	**19**	**47.5**
Staphylococcus aureus	**11**	**27.5**
Pseudomonas aeruginosa	**5**	**12.5**
***Klebsiella* spp.**	**3**	**7.5**
Acinetobacter baumanii	**1**	**2.5**
Escherichia coli	**1**	**2.5**

TABLE 2: DISTRIBUTION OF BACTERIAL ISOLATES ACCORDING TO WOUND TYPES

Wound types	Isolate Number/%	Bacteria isolated	Percentage (%)
Burns	5 (12.5%)	*Proteus* spp	80
		Staphylococcus aureus	20
Acute soft tissue	14 (35%)	*Staphylococcus aureus*	42.9
		Proteus spp	35.7
		Pseudomonas aeruginosa	21,4
Surgical wound	7 (17.5%)	*Proteus* spp	42.9
		Pseudomonas aeruginosa	28.5
		Staphylococcus aureus	14.3
		Escherichia coli	14.3
Diabetic foot ulcer	1 (2.5%)	*Klebsiella* spp	100
Leg ulcer	13 (32.5%)	*Proteus* spp	53.8
		Staphylococcus aureus	23.1
		Klebsiella spp	15.4
		Acinetobacter baumanii	7.7

TABLE 3: NUMBER AND PERCENTAGE DISTRIBUTION OF BACTERIAL ISOLATES ACCORDING TO PATIENTS' AGE GROUPS AND GENDER

Bacterial isolates	Gender distribution		Patients' age group			
	Male	Female	0 – 19 years	20 – 39 years	40 – 59 years	>60 years
Staphylococcus aureus	5 (45.5%)	6 (54.5%)	1 (9.1%)	5 (45.5%)	3 (27.2%)	2 (18.2%)
Escherichia coli	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Proteus spp.	12 (63.2%)	7 (36.8%)	6 (31.6%)	5 (26.3%)	7 (36.8%)	1 (5.3%)
Pseudomonas aeruginosa	4 (80%)	1 (20%)	2 (40%)	2 (40%)	1 (20%)	0 (0%)
Acinetobacter baumanii	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Klebsiella spp.	3 (100%)	0 (0%)	0 (0%)	1 (33.3%)	2 (66.7%)	0 (0%)

DISCUSSION

Wound colonization by microorganisms especially bacteria has been found to be a major contributing factor to delay or non-healing of wounds which could result to increased trauma and financial burden for the patient and the entire healthcare institution as a whole (2, 12). Therefore, correct identification of the etiological agents and the selection of effective antibiotics against the causative organisms are very important for effective management of patients with infected wounds.

In this study, more of the isolates were recorded to occur in wound infections of male patients (65%) than the females (35%), and microbial colonization of the wounds occurred more among patients in the age group 20 – 39 and 40- 59 years. These findings corresponded with previous reports on patients' gender distribution of bacterial isolates from wound infections in hospitals in Nigeria (3, 4).

TABLE 4: NUMBER AND PERCENTAGE ANTIBIOTIC RESISTANCE OF BACTERIAL ISOLATES

Antibiotics	*S. aureus* (n=11)	*E. coli* (n=1)	*Proteus* spp. (n=19)	*Klebsiella* spp. (n=3)	*A. baumanii* (n=1)	*P. aeruginosa* (n=5)	Total (N=40)
SXT	9(81.8%)	1(100%)	11(56%)	3(100%)	1(100%)	4(80%)	29(72.5%)
E	5(45.5%)	1(100%)	17(89%)	3(100%)	1(100%)	5(100%)	32(80%)
C	6(54.5%)	1(100%)	14(74%)	2(67%)	0(0%)	5(100%)	28(70%)
DO	7(63.6%)	1(100%)	16(84%)	3(100%)	1(100%)	5(100%)	33(82.5%)
PEF	6(54.5%)	1(100%)	5(26%)	1(33%)	0(0%)	2(40%)	15(37.5%)
TZP	11(100%)	1(100%)	19(100%)	3(100%)	1(100%)	5(100%)	40(100%)
PRL	11(100%)	1(100%)	19(100%)	3(100%)	1(100%)	5(100%)	40(100%)
IPM	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(20%)	1(2.5%)
GEN	3(27.3%)	0(0%)	7(37%)	1(33%)	0(0%)	2(40%)	13(32.5%)
CXM	11(100%)	0(0%)	15(79%)	3(67%)	1(100%)	4(80%)	34(85%)
OFL	6(54.5%)	1(100%)	5(26%)	1(33%)	0(0%)	1(20%)	14(35%)
AMC	11(100%)	1(100%)	19(100%)	3(100%)	1(100%)	5(100%)	40(100%)
NIT	0(0%)	0(0%)	14(74%)	2(67%)	0(0%)	5(100%)	21(52.5%)
CPR	6(54.5%)	1(100%)	8(42%)	1(33%)	0(0%)	2(40%)	18(45%)
CAZ	11(100%)	0(0%)	14(74%)	1(33%)	1(100%)	2(40%)	29(72.5%)
CRX	5(45.5%)	1(100%)	16(84%)	2(67%)	0(0%)	4(80%)	28(70%)
OX	4(36.4%)	ND	ND	ND	ND	ND	ND
FOX	6(54.5%)	0(0%)	14(74%)	1(33%)	0(0%)	4(80%)	25(62.5%)
AT	11(100%)	0(0%)	17(89%)	3(100%)	1(100%)	3(60%)	35(87.5%)

SXT-sulphamethoxazole-trimethoprim, E-erythromycin, C-chloramphenicol, DO-doxycycline, PEF-perfloxacin, TZP-piperacillin/tozobactam, PRL-piperacillin, GEN-gentamicin, IPM-imipenem, CXM-cefixime, OFL-ofloxacin, AMC-amoxicillin-clavulanic acid, NIT-nitrofurantoin, CPR-ciprofloxacin, CAZ-ceftazidime, CRX-cefuroxime, OXA-oxacillin, FOX-cefoxitin, AT-aztreonam, ND-Not done

In this study, six different bacteria belonging to six different genera: *Proteus* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* spp, *Escherichia coli, Acinetobacter baumanii*, were collected. This is similar to reports from other part of the country where similar bacterial isolates have been isolated from infected wounds (9, 21). *Proteus* spp (47.5%) was the most prevalent, followed by *Staphylococcus aureus* (27.5%). This result is in conformity with that reported by Etok *et.al.* (12) where they found *Proteus* spp. to be the most common isolate (33.3%) followed by *Staphylococcus aureus* (20%). We found *Proteus* spp (80%) to be most prevalent in burn wound infection as opposed to *Staphylococcus aureus* (42.9%) which was prevalent in acute soft tissue infection. Similar organisms were identified in acute soft tissue infections which include cutaneous abscesses, traumatic wounds, and necrotizing infection in which microbiological investigations showed that *Staphylococcus aureus* is the single causative bacterium in approximately 25 to 30% of cutaneous abscesses (22). This could be

explained based on the fact that *Staphylococcus aureus* constitute the normal skin flora.

This study also showed variation in the susceptibility of bacterial isolates to different antibiotics. The result of the antibiogram revealed that gentamicin and the fluoroquinolones were effective against the clinical isolates, carbapenems such as imipenem, are still the most active class of antibiotic in the treatment of MDR infections as all the organisms except one were susceptible to it. High level resistance was observed against some of the antibiotics such as co-trimoxazole, piperacillin, piperacillin-tozobactam, doxycycline, erythromycin, chloramphenicol and amoxicillin-clavulanic acid particularly among the Gram-negative organisms. This may be due to high level of abuse through self-medication, of the penicillin and aminopenicillin, tetracycline and macrolide classes of antibiotics in this part of the world (23, 24).

TABLE 5: NUMBER AND PERCENTAGE OF ISOLATES WITH MDR, ESBL -PRODUCING AND METHICILLIN RESISTANCE PHENOTYPE

Organisms	N/% of MDR isolates	N/% of ESBL producers	N/% MRSA
S. aureus **(n = 11)**	**11 (100%)**	**NA**	**6(54.5%)**
Escherichia coli **(n = 1)**	**1(100%)**	**1(100%)**	**NA**
Acinetobacter baumanii **(n = 1)**	**1(100%)**	**1(100%)**	**NA**
Proteus **spp. (n = 19)**	**19(100%)**	**8(42.1%)**	**NA**
Pseudomonas aeruginosa **(n = 5)**	**5(100%)**	**3(60%)**	**NA**
Klebsiella **spp (n = 3)**	**3(100%)**	**3(100%)**	**NA**
Total (N = 40)	**40 (100%)**	**16 (40%)**	**6(15%)**

n – Number, % - Percentage, NA – Not Applicable, MDR – Multidrug resistance, ESBL – Extended-Spectrum Beta-Lactamase, MRSA – Methicillin Resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* have been reported to cause high mortality and morbidity especially in surgical units and newborn nurseries (16). MRSA are known to show resistance to multiple classes of antibiotics including the beta-lactams, aminoglycosides, macrolides and fluoroquinolones (16). The prevalence of MRSA compared with the 11 *Staphylococcus aureus* collected in this study was moderately high (54.5%) and this suggest that possible increase in their prevalence in the future is eminent if care is not taking to curtail their spread.

Production of extended-spectrum β-lactamases have been reported in both community and hospital settings amongst Gram-negative bacterial isolates (10, 11) and this has led to the campaign for appropriate and rational use of extended-spectrum antibiotics so as to minimize cases of antibiotic resistance. In this study, although ESBL production varied among the organisms, it was produced in varied percentages among the individual Gram-negative bacteria tested. This confirms wide spread reports of ESBLs among various species of Gram-negative bacteria (25, 26). Of the 29 Gram-negative bacterial isolates tested for the production of ESBLs, only 55.2% produced the enzymes. This percentage prevalence is comparable with previous reports in Nigeria (10, 11).

In conclusion, strict antibiotics policy should be implemented in hospitals nationwide to reduce the spread of highly resistant bacteria. This when effectively enforced will help to improve health condition and reduce the cost of treatment of life threatening diseases. Prevention they say is better than cure, adequate measures should be placed on preventive procedures such as hand washing, disinfection, good nursing practice and good surgical techniques amongst others, in the hospitals to reduce bacterial contamination and spread.

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NOSOCOMIAL IMIPENEM-RESISTANT *ACINETOBACTER BAUMANNII* INFECTIONS IN INTENSIVE CARE UNITS: INCIDENCE AND RISK FACTORS ASSESSMENT

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ABSTRACT

Imipenem-resistant *Acinetobacter baumannii* (*A. baumannii*) (IRAB) has emerged as a challenging nosocomial pathogen particularly in intensive care units (ICUs). Studying the risk factors associated with IRAB infection is of paramount importance for appropriate control of IRAB spread. The aim of this study was to assess the incidence rate and possible risk factors associated with nosocomial IRAB infections in ICUs. A prospective cohort study was carried out in surgical and emergency ICUs of a tertiary care hospital in Egypt. All patients who developed nosocomial *A. baumannii* infection from the start of January 2014 to the end of December 2015 were included. Isolates were identified as *A. baumannii* using API 20NE and E-test was used to define IRAB. Out of 146 *A. baumannii* isolates, 11 were found to be IRAB (7.5% incidence rate), of them 72.7% (8/11) were found to be multidrug resistant (MDR). Univariate analysis demonstrated that hospital stay before ICU admission [Relative risk (RR) 3.51, 95% confidence interval (CI) 1.0-12.7, P= 0.04)], longer ICU stay (P= 0.005), exposure to emergent surgery (RR 17.5, CI 7.39-41.4, P= 0.000), the presence of central venous catheter (RR 3.26, CI 1.0-10.6, P= 0.04) and previous carbapenem use (RR 4.05, CI 1.12-14.6, P =0.02) were significant risk factors for IRAB infection. In conclusion, a relatively high IRAB incidence was recorded in ICUs of our hospital. Hospital stay before ICU admission, longer ICU stay, exposure to emergent surgery, the presence of central venous catheter and previous carbapenem use were significant risk factors for IRAB infection. Rationale use of carbapenems in ICUs should be considered.

Key words: Imipenem-resistant, *Acinetobacter baumannii*, Intensive care units

LES INFECTIONS *ACINETOBACTER BAUMANNII*NOSOCOMIALES RESISTANTS A L'IMIPENEME DANS LES UNITES DE SOINS INTENSIFS: L'INCIDENCE ET LES FACTEURS DE RISQUE EVALUATION.

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RESUME

***Acinebacter Baumannii* (*A. baumannii)* résistant à l'imipenème (IRAB) a émergé comme une pathogène difficile en particulier dans les unités de soins intensifs (USI). L'étude des facteurs de risque associe à l'infection IRAB est d'une importance capitale pour le contrôle de la propagation de l'IRAB. Le but de cette étude était d'évaluer le taux d'incidence et les facteurs de risque possibles associesà des infections nosocomiales d'IRAB dans les USI. Une étude de cohorte prospective a été menée dans une USI chirurgicale et d'urgence d'un hôpital de soins tertiaire en Egypte. Tous les patients qui ont développé l'infection *A. baumannii* depuis le début de janvier 2014 jusqu'à la fin de décembre 2015 ont été inclus. Les isolats ont été identifiés comme *A. baumannii* en utilisant API 20NE et E – test a été utilisé pour définir l'IRAB. Sur 146 isolats d'*A. baumannii*, 11 ont été trouvés d'être IRAB (7,5% taux d'incidence), d'entre eux, 72,7% (8/11) ont été trouvés multi résistantes (MDR). Analyse univariée a montré que l'hospitalisation avant l'admission USI [Risque relative (RR)3,51, 95% intervalle de confiance (CI) 1,0-12,7, P=0.04)], long séjour à USI (P=0.0005), l'exposition à la chirurgie d'urgence (RR 17,5 CI 7,39-41.4, P= 0,000), la présence d'un cathéter veineux central (RR 3,26, CI 1,0-10,6 P=0.04) et l'usage de carbapenemprécédente (RR 4,05 CI 1,12-14.6 P=0,02) étaient des facteurs de risque importants d'infection IRAB. En conclusion, un incident relativement élevé d'IRAB a été enregistréà USI de notre hôpital. Séjour à l'hôpital avant l'admission à l'unité de soins intensifs, long séjour à USI, l'exposition à la chirurgie d'urgence, la présence d'un cathéter veineux central, et l'utilisation de carbapenemprécédenteétaient des facteurs de risque important pour l'infection d'IRAB. L'utilisation rationnelle des carbapanemes en USI devrait êtreconsidéré.**

Mots clés : Résistant àImipenème, *Acinebacterbaumannii*unité des soins intensifs.

INTRODUCTION

Acinetobacter spp., in particular *Acinetobacter baumannii* (*A. baumannii*), are opportunistic pathogens frequently involved in nosocomial infections. These occur mostly in intensivecare units (ICUs). These infections can range from urinary tract infections (UTI) to pneumonia and septicemia (1). The ability of these bacteria to survive for long periods on dry

inanimate surfaces as well as their ability to persist on human skin can potentiate the risk of cross-contamination in hospital settings resulting in endemic and/or epidemic outbreaks of infection (2,3). This could result in secondary morbidity particularly in patients with serious underlying diseases, but whether these infections have an attributable mortality, is controversial (4).

Isolates of *A. baumannii* are well known for their antibiotic resistance and multidrug-resistant (MDR) strainshave emerged globally (5). Carbapenems are often considered the antibiotics of last choice for treating infections caused by *A. baumannii*. Unfortunately, carbapenem-resistant *A. baumannii*(CRAB) has become a worldwide issue (6). Moreover, these isolates are often MDR, which constitutes a great challenge to the treating physician, who finds himself facing an untreatable organism.

In spite of the extreme difficulty recorded in controlling CRAB in hospital settings, successful trials have been reported previously (7,8). This indicates that the strict implementation of infection control measures, rationale use of antimicrobials according to a sound policy, regular antimicrobial resistance surveillances, as well as risk factor assessment, may help control or contain the threat of this challenging organism.

This work aimed to assess the incidence and possible risk factors associated with nosocomial infections caused by imipenem-resistant *A. baumannii* (IRAB) in ICUs of a tertiary care hospital in Egypt.

MATERIALS AND METHODS

Study population and Case definition

A prospective cohort study was conducted in the emergency and the surgical ICUs of Zagazig University Hospitals, a tertiary care hospital in the eastern governorate of Egypt. Patients who had *A. baumannii* infections from the start of January 2014 to the end of December 2015 were included in the study. Only the initial *A. baumannii* isolate from each patient was included. The clinical significance (colonization or infection) of each *A. baumannii* isolate was assessed according to Centre for Disease Control (CDC) criteria (9,10). For patients with an indwelling bladder catheter, UTI was diagnosed with detection of pyuria(10 leukocytes/mm^3), growth of $\geq 10^5$ CFU/ml bacteria (with no more than two species) in urine culture, and the presence of clinical signs of infection (fever 38˚C, leukocytosis, abnormal macroscopic appearance of urine, presence of urinary nitrites). In mechanically ventilated patients, pneumonia was diagnosed by the presence of a new or progressive infiltrate in chest X-ray with the presence of purulent endotracheal aspirates, supported by a growth of $\geq 10^5$ CFU/ml bacteria in a quantitative culture of deep endotracheal aspirate. For non-ventilated patients, the diagnosis of pneumonia was made when patients had a compatible chest X-ray and purulent sputum, with Gram stain and sputum culture yielding a pathogenic microorganism. Surgical site infection (SSI) was diagnosed by the presence of purulent discharge and presence of suggestive clinical signs (incision site pain, tenderness, localized swelling, redness or heat, spontaneous opening of the incision) supported by microbiologic results of specimen analysis. Diagnosis of sepsis was made by the presence of positive blood cultures and sepsis criteria. Patients colonized with *A. baumannii* as well as patients from whom *A. baumannii* isolates had been recovered within 48 h of admission to ICU were excluded. Data were recorded on individual forms for each patient. The form included age, gender, diagnosis, length of hospital stay before ICU admission (if any), length of ICU stay, comorbidity (diabetes mellitus, renal insufficiency, dialysis, hepatic failure, malignancy, immunosuppression, neutropenia, chronic lung disease, malnutrition and anoxic encephalopathy), emergent surgical operations, ventilator support, physical examination findings, hematological and biochemical test results, antibiotics given to the patient, culture and antimicrobial susceptibility test results, and time between admission to ICU and isolation of the first positive *A. baumannii* culture. Carbapenem uptake during 14 days (for at least 24 h) prior to isolation of the *A. baumannii* was considered (11).For all included patients, written or verbal informed consent was obtained from the patients themselves, or their guardians. Patients were assigned as IRAB cases if they had imipenem-resistant *A. baumannii* infections and as ISAB cases if they had imipenem-sensitive *A. baumannii* infections.

Microbiologic examination

This was carried out at the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University.Identification of isolates as *A. baumannii* was performed using API 20NE (Bio-Mérieux, Marcy L´Etoile, France). The minimum inhibitory concentration (MIC) of the identified isolates for imipenem was determined using E-test (Bio-Mérieux, Marcy L´Etoile, France) according to the manufacturer's instructions. Isolateswereassigned as IRAB if theyhad MIC values ≥ 8 µg/ml, while those with MIC values <8 µg/ml were assigned as ISAB (12). In addition, all identified isolates were tested against the following antibiotics; amikacin,

gentamicin, aztreonam, cefipime, ceftazidime, ciprofloxacin, levofloxacin, piperacillin—tazobactam, trimethoprim—sulfamethoxazole, tigecycline and colistin by disc diffusion method. All discs were supplied from Bioanalyse (TibbiMalzemelerSanayiveTicaret Ltd. Sti., Turkey) except tigecycline was from Oxoid, England. Isolates were identified as being MDR if resistance to at least one agent in more than three antimicrobial categories was detected (13).

Statistical analysis

Potential risk factors were analyzed by univariate analysis. Independent Student's t-test, Chi-square test and Fisher's exact tests were used when appropriate to compare proportions. A P value of <0.05 was considered statistically significant.

RESULTS

One hundred and forty-six non-repeated *A. baumannii* were isolated from an equal number of patients during the study period. IRAB was isolated from 11 (7.5%) patients while 135 isolates (92.5%) were ISAB. The isolation frequency of both IRAB and ISAB from different nosocomial infections is presented in **Table 1**. Lower respiratory tract infection (LRTI) and SSI represented the main infections from which IRAB were isolated (36.4% isolation frequency for each), followed by UTI (18.2%) and then blood stream infection (9.1 %). No significant difference was found between IRAB and ISAB regarding the type of infection **(Table 1)**.

TABLE 1: ISOLATION FREQUENCY OF IMIPENEM-RESISTANT *A. BAUMANNII* (IRAB) AND IMIPENEM-SENSITIVE *A. BAUMANNII* (ISAB) FROM DIFFERENT NOSOCOMIAL INFECTIONS

Type of infection	IRAB (n = 11) No. (%)	ISAB (n = 135) No. (%)	Total (n = 146) No. (%)	P
Surgical site infection (SSI)	4 (36.4)	45 (33.3)	49 (33.56)	0.8
Urinary tract infection (UTI)	2 (18.2)	41 (30.4)	43 (29.5)	0.4
Lower respiratory tract infection (LRTI)	4 (36.4)	29 (21.5)	33 (22.6)	0.3
Blood stream infection	1 (9.1)	20 (14.8)	21 (14.4)	0.6

The susceptibility profiles of IRAB and ISAB isolates to the tested antimicrobials in disc diffusion method are presented in **Table 2**. All IRAB isolates were susceptible to colistin, followed by tigecycline (81.8%), while they were all resistant to cefepime. Multidrug resistance was detected in 72.7% of IRAB isolates (8/11).

Univariate analysis of different factors associated with IRAB infection (Table 3) demonstrated that five factors were recognized significant in IRAB infection. These included; hospital stay before ICU admission (RR 3.51, CI 1.0-12.7, P= 0.04), longer ICU stay (21.5±6.1 day with IRAB, compared to 16.1±5.6 day with ISAB, P= 0.005), exposure to emergent surgery (RR 17.5, CI 7.39-41.4, P= 0.000), the presence of central venous catheter (RR 3.26, CI 1.0-10.6, P= 0.04) and previous carbapenem use (RR 4.05, 1.12-14.6 CI, P=0.02).

TABLE 2: ANTIMICROBIAL SUSCEPTIBILITY RATES OF IMIPENEM-RESISTANT *A. BAUMANNII* (IRAB) AND IMIPENEM-SENSITIVE *A. BAUMANNII* (ISAB) ISOLATES TO THE TESTED ANTIMICROBIALS IN DISC DIFFUSION TEST

Antimicrobial	IRAB (n=11) No. (%)	ISAB (n=135) No. (%)	P
Amikacin	2 (18.2)	72 (53.3)	0.24
Gentamycin	1 (9.09)	64 (47.4)	0.01*
Aztreonam	2 (18.2)	78 (57.8)	0.01*
Cefipime	0 (0.0)	17 (12.6)	-------
Ceftazidime	4 (36.4)	87 (64.4)	0.06
Ciprofloxacin	3 (27.3)	92 (68.1)	0.006**
Levofloxacin	6 (54.54)	113 (83.7)	0.16
Piperacillin-tazobactam	5 (45.5)	97 (71.85)	0.06
Trimethoprim-Sulfamethoxazole	2 (18.2)	77 (57)	0.12
Tigecycline	9 (81.8)	127 (94.1)	0.12
Colistin	11 (100)	135 (100)	-------

*significant, **highly significant

TABLE 3: UNIVARIATE ANALYSIS OF RISK FACTORS FOR IMIPENEM-RESISTANT *A.BAUMANNII* (IRAB) INFECTIONS

Risk factors	IRAB (n = 11)	ISAB (n = 135)	RR (95% CI)	P
Mean age in years ±SD	49 ± 11.2	46 ± 10.1		0.4
Gender Males (n=74) Females (n=72)	 6 (8.1) 5 (6.9)	 68 (91.9) 67 (93.1)	 1.17 (0.37-3.66)	 0.8
Presence of comorbidity Yes (n=59) No (n=87)	 5 (8.5) 6 (6.9)	 54 (91.5) 81 (93.1)	 1.2 (0.4-3.8)	0.7
Hospital stay before ICU admission Yes (n=63) No (n=83)	 8 (12.7) 3 (3.6)	 55 (87.3) 80 (96.4)	 3.51 (1.0-12.7)	 0.04*
Mean length of ICU stay in days ± SD	21.5 ± 6.1	16.1 ± 5.6		0.005**
Emergent Surgery Yes (n=45) No (n=101)	 6 (13.3)	 39 (86.7)	 17.5 (7.39-41.4)	0.000**

	5 (5.0)	96 (95.0)		
Total parenteral nutrition				
Yes (n=45)	**6 (13.4)**	**39 (86.6)**	**2.69 (0.87-8.37)**	
No (n=101)	**5 (5.0)**	**96 (95.0)**		**0.1**
Enteral nutrition				
Yes (n=21)	**2 (9.5)**	**19 (90.5)**	**1.32 (0.31-5.7)**	
No (n=125)	**9 (7.2)**	**116 (92.8)**		**0.7**
Endotracheal tube				
Yes (n=43)	**5 (11.6)**	**38 (88.3)**	**2.0 (0.64-6.19)**	
No (n=103)	**6 (5.8)**	**97 (94.1)**		**0.3**
Central venous catheter (CVC)				
Yes (n=51)	**7 (13.7)**	**44 (86.2)**	**3.26 (1.0-10.6)**	
No (95)	**4 (4.2)**	**91 (95.7)**		**0.04***
Urinary catheter				
Yes (n=90)	**9 (10.0)**	**81 (90)**	**2.8 (0.62-12.5)**	
No (n=56)	**2 (3.6)**	**54 (96.4)**		**0.2**
Surgical drain				
Yes (n=13)	**2 (15.4)**	**11 (84.6)**	**2.27 (0.55-9.43)**	
No (n=133)	**9 (6.8)**	**124 (93.2)**		**0.3**
Arterial line				**0.3**
Yes (n=45)	**5 (11.1)**	**40 (88.9)**	**1.87 (0.6-5.81)**	
No (101)	**6 (6.0)**	**95 (94.0)**		
Nasogastric tube				
Yes (n=46)	**5 (10.9)**	**41 (89.1)**	**1.81 (0.58-5.63)**	
No (n=100)	**6 (6.0)**	**94 (94.0)**		**0.3**
Previous carbapenems use				
Yes (n=58)	**8 (13.8)**	**50 (86.2)**	**4.05 (1.12-14.6)**	
No (n=88)	**3 (3.4)**	**85 (96.6)**		**0.02***

RR; Relative risk, CI; Confidence interval,*significant, **highly significant

DISCUSSION

In this work,146 *A.baumannii* were isolated from an equal number of patients during the study period. IRAB was isolated from 11 patients accounting for 7.5% isolation frequency. In USA and Canada, the incidence of imipenem resistance in *Acinetobacter* spp. ranged from 6-8%. Higher levels were recorded in Latin America and Europe (10% and 16%, respectively) (14,15,16). In Asian countries, the prevalence ranged from 2% to 26% (17,18). Marked increase in CRAB incidence was reported in a previous study from South Africa (19).Variable results were reported from Arabian countries, where the incidence of CRAB ranged from 5.4% in Kingdom Saudi Arabia (20) to 37.2% in Kuwait (21) to as high as 58% in Manama (22). In a previous Egyptian study, a very high incidence was recorded in an ICU of a university hospital in Upper Egypt (71.4%) (23). These discrepant results may be contributed to the different study designs where one center evaluation was performed in some

studies (23), whereas, other studies were nation-wide (18).

In our study, the highest isolation frequency of IRAB was from LRTI and SSI (36.4% each), followed by UTI (18.2%) and then blood stream infection (9.1%). A number of previous studies have reported LRTI as the main infection yielding IRAB (11,18,19,24). A similar finding was also reported previously in Egypt (23). This was not unexpected, as previous studies demonstrated that as much as two-thirds of hospitalized patients have their respiratory tract colonized with *A. baumannii* (25) and that infection by MDR *A. baumannii* is strongly correlated to this initial colonization (26), particularly when excessive manipulations are encountered as in ICU environment.

Among the obtained IRAB isolates, 72.7% were found to be MDR being resistant to three or more different classes of antimicrobials. This is supported by a previous study where most of the examined CRAB isolates were found to be resistant to all tested antimicrobials (27). On the other hand, all IRAB isolates were found susceptible to colistin and 81.8% were susceptible to tigecycline. This comes in accordance with what was reported in South Africa concerning the susceptibility pattern of CRAB (19).

Accumulating evidence suggested that *A. baumannii* isolates are difficult to eradicate in hospital settings as they rapidly adapt to the hospital environment and become endemic with a remarkable ability to contaminate hospital equipment and to be transmitted via contact and even airborne methods. This constitutes a great threat to patients as well as to their physicians particularly with mostly an untreatable organism such as IRAB (28). This makes the study of the factors that could be associated with the acquisition of such an organism of paramount importance as it could help control its spread.

Previous studies had assigned different risk factors to be significantly associated with IRAB acquisition. These included; prolonged hospital stay, prolonged ICU stay, exposure to and duration of different invasive procedures, exposure to emergent surgical procedure, the presence in a unit accommodating patients infected with IRAB and previous exposure to broad-spectrum antimicrobials particularly carbapenems (11,18,24). Our study revealed that longer duration of ICU stay (21.5±6.1 with IRAB compared to 16.1±5.6 with ISAB infection, P=0.005), hospital stay before ICU admission (P=0.04), exposure to emergent surgery (P=0.000) and exposure to CVC (P= 0.04) were significantly associated with IRAB acquisition. Our results are supported by previous studies that demonstrated the same results (11,29,30). Prior exposure to antimicrobials has been recognized as being, by far, the most important risk factor for developing of multi-drug resistance in *A. baumannii* (31). This was evident with IRAB where exposure to broad-spectrum antibiotics particularly third generation cephalosporins (32), fluoroquinolones (33) and carbapenems (11) were all significant risk factors for IRAB acquisition. Our result comes to confirm these observations where previous carbapenem use was significantly associated with IRAB infection (P=0.02). This highlights the importance of implementing strict antimicrobial policies in health care facilities. Further studies that assess the level of hygiene and the compliance to infection control measures as other risk factors are warranted.

CONCLUSION

In conclusion, the spread of antibiotic resistance among clinically important Gram-negative bacilli seems to be an unstoppable problem. This study demonstrated that the incidence of IRAB in ICUs of our hospital is relatively high. Longer ICU stay, hospital stay prior to ICU admission, usage of CVC, exposure to emergent surgery and prior use of carbapenems were all significant risk factors for IRAB infection. Restricted use of carbapenems in the ICUs of our hospital should be considered. Implementation of infection control measures may help to control the danger of this challenging organism.

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EVALUATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE IN MALAGASY VETERINARY STUDENTS

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RUNNING TITLE: MRSA NASAL CARRIAGE INMALAGASY VETERINARY STUDENTS

ABSTRACT

Purpose: Populations that are frequently in contact with animals such as veterinary students have been demonstrated to be at risk of MRSA carriage.Thus, it is relevant to generate baseline data in MRSA nasal carriage and multidrug resistance among Malagasy veterinary students (Madagascar).**Method:**A cross-sectional study was carried out among veterinary students coming for laboratory training. After their wise consent, nasal swabs of the anterior nares were carried out; and *S. aureus*was isolated by selective chromogenic culture. They were then assessed for antimicrobial susceptibility.

Results: Nasal swabs of 155 Malagasy veterinary students (Sex-ratio M/F: 0.91), enabled to isolate 30 (19, 35%) *S. aureus*strains, among which 14 (46, 66 %) were méthicillin-resistant (MRSA). Risk factors analysis revealed that history of hospitalization, recent antibiotic intake and frequent contact with animals and livestock workers/veterinarians increase the risk of MRSA nasal carriage. Among MRSA nasal isolates, a high rate of multidrug resistance and particularly an intriguing resistance to gentamycin (20%) and vancomycin (7.14%) were observed.

Conclusion: These results suggest that MRSA is spreading in Malagasy community requiringa strategic policy againstmultidrug resistant strains.

Key-words: Madagascar, MRSA, Risk factors, Veterinary

EVALUATION DU PORTAGE NASAL DE *STAPHYLOCOCCUS AUREUS* RESISTANT A LA METHICILLINE CHEZ LES ETUDIANTS VETERINAIRES MALGACHES

Résumé: Objectifs: Les populations qui sont souvent en contact avec des animaux telles que les étudiants vétérinaires ont été démontrées comme étant à risque de portage de *Staphylococcus aureus* résistant à la méticilline(SARM). Ainsi, il est pertinent de générer des données de référence dans le portage nasal SARM chez les étudiants vétérinaires malgaches (Madagascar).**Méthodes:**Une étude transversale a été menée auprès des étudiants en médecine vétérinaire. Après leur consentement éclairé, des écouvillons nasaux des narines antérieures ont été réalisées et ayant permisd'isolerdes *S. aureus* sur milieu culturechromogènique sélectif et la réalisation de tests de sensibilité aux antimicrobiens.

Résultats: Les écouvillons nasaux de 155 étudiants malgaches vétérinaires (sex-ratio H / F: 0,91), a permis d'isoler 30 (19, 35%) souches de *S. aureus* parmi lesquels 14 (46, 66%) étaient résistantes à la méticilline (SARM). L'analyse des facteurs de risque a révélé que l'histoire de l'hospitalisation, la récente prise d'antibiotiques et les fréquents contacts avec les animaux et les travailleurs d'élevage/vétérinaires augmentent le risque de SARM portage nasal. Parmi les isolats de SARM, un taux élevé de multirésistance et en particulier une résistance intéressante à la gentamycine (20%) et à la vancomycine (7,14%) ont été observés.

Conclusion: Ces résultats suggèrent que le SARM se répand dans la communauté malgache nécessitant une politique stratégique contre les souches multirésistantes.

Mots-clé: Madagascar-MRSA-Facteurs de risque-Vétérinaire

INTRODUCTION

*Staphylococcus aureus*and methicillin-resistant *S. aureus* (MRSA) are known as an invasive human pathogen responsible of serious infections in both hospitals and community. Moreover, it has been-established that nasal carriage of MRSA represents a

major risk factor for subsequent infection and transmission of this pathogen [1, 2]. Likewise, several studies have confirmed asymptomatic colonization of animals at veterinary clinics worldwide and veterinarian has been identified as high-risk group for asymptomatic MRSA carriage, likely because of their close animal contact [3]. However, MRSA nasal carriage rate vary widely among countries. For instance, prevalence of 4.6% MRSA nasal carriage was documented among Dutch veterinary doctors and students with history of contact with livestock [4]. MRSA carriage was 12.3% in UK veterinarians attending MRSA-infected animals [5]. An international study reported that 12.5% (34/272) of veterinarians from nine country carried MRSA in the nares or their throat[6]. In a study of Brazilian university students, the percentage nasal carriage of *Staphylococcus aureus*was 40.8% with 5.8% of MRSA strains where all of them were susceptible to most of the antimicrobial agents tested [7].

Although, there is a worldwide increase in the number of infections caused by MRSA, the Pasteur institute of Madagascar reported that the prevalence of MRSA in *S. aureus* infection in Malagasy community still very low (5.8 %)[8]. In parallel, cross-sectional studies of unexposed Malagasy community reported 38.16 % of *S. aureus*nasal carriage with 14.8 % of MRSA strains[9]. However, to the best of our knowledge, no data concerning the frequency of MRSA nasal carriage among potentially exposed Malagasy community is available yet. Determining the prevalence of nasal carriage among exposed population is important in public health in so far as it strongly contributes to the update of the susceptibility of *S. aureus*to various drugs largely used in our community. Veterinarian students are considered as exposed population due to frequent contact with animal during their training. Thus, we assessed the *S .aureus*nasal carriage state of the veterinarian students coming for training in the Laboratory of Training and Research in Medical Biology of Madagascar, in order toestimate MRSA colonization in particular group of Malagasy community which is progressively in contact with domesticated animals and to identify some colonization risk factors.

MATERIALS AND METHODS

Sampling procedures

After a wise consent of veterinarian students coming for lab training, nasal swabs of the anterior nares were carried out by qualified technician according to the French C-CLIN (Centrede Coordination de la Luttecontre les Infections Nosocomiales) recommendation procedure[10]. Voluntary participants in the five academic level years (University of Antananarivo, Madagascar) completed anonymously, a very brief questionnaire designed to identify status and potential risk factors for staphylococcal colonization, including age, gender, previous hospitalization and antimicrobial use, frequent contact with animals or healthcare workers or veterinarians/livestock workers. Approval of the appropriate ethical committee had been obtained

Bacterial identification and antimicrobial susceptibility

Single swab from each volunteer was immediately inoculated in Columbia blood Agar 5 % and incubated for 24h at 37°C. Plates were read at 24 h and *Staphylococcus aureus* isolates were identified according to their colony morphology, Gram-positive stain, positive catalase reaction, positive tube coagulase assay and Slidex Staph Kit ® (Biomerieux ®, France). Then *Staphylococcus aureus* isolates were inoculated onto selective chromogenic MRSA agar supplemented with 4 µg/mL of cefoxitin from CONLAB® for isolation of methicillin resistant *S. aureus*. Methicillin resistance *S. aureus* was confirmed by demonstration of blue colony growth on selective chromogenic MRSA agar [11]. Finally, susceptibility of MRSA to eight antibiotics (oxacillin, penicillin, erythromycin, vancomycin, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, gentamicin, clavulanicacid+amoxicillin) was assessed by disc diffusion technique following the guidelines of the Antibiogram Committee of French Society for Microbiology (CASFM) [12]. Briefly, an inoculum of 10^6 CFU/ml was prepared and seeded in a Mueller-Hinton square plate. After an incubation of 24h at 37°C, the inhibition zone around antibiotic disks (Biorad®) was measured. For susceptibility to oxacillin, an inoculum of 10^7 CFU/ml was prepared and the plate was incubated at 37°C for 24 hours on Mueller-Hinton agar + 2% NaCl. The breakpoints for resistance were those recommended by the CASFM [12]. Reference *S. aureus* ATCC® 25923 strains have been used as a quality control.

Multidrug resistance was defined as resistance to penicillin and oxacillin plus two or more antibiotics listed previously.

Statistical analysis

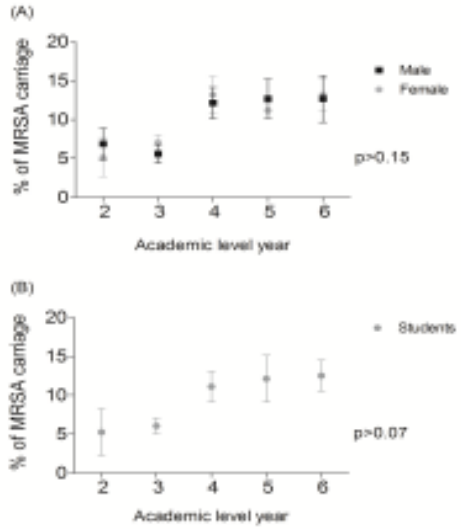
Prevalence and 95% confidence intervals CIs were calculated for overall *S. aureus*, MRSA and MSSA colonization. Categorical comparisons were performed using $\chi 2$ analyses. Logistic regression was used to estimate the association between age, sex and colonization. $P<0.05$ was considered significant for all comparisons. Risk factors for *S. aureus*colonization were also evaluated and variables achieving a $P<0.05$ level were considered significant; odds ratios (ORs) with 95% CIs were

calculated by using the Graphpad prism5® software.

RESULTS

In two months, our lab received 210 veterinarian students for training during academic year 2014. Finally, nasal swabs were collected from 155 (73.80%) consenting students, sex-ratio M/F: 0.91with a mean ± SD age of 23 ± 5.55 years. *S. aureus* was isolated from 30 of 155 (19.35%) of students (Table 1).

Figure 1: Frequency of MRSA colonization depending on gender and academic level year. A Frequency of MRSA colonization according to gender; B Frequency of MRSA Colonization According To Academic Level Year.



Sixteen (53.34%) individuals were colonized with MSSA and fourteen (46.66%) were colonized with MRSA, for an overall estimate of MRSA colonization prevalence of 9.04%.

There was no significant association between sex and MRSA colonization (Fig A, B) nor between academic level year and MRSA colonization (P=0.06, P=0.75).

Recent antibiotic use (within one month)and history of hospitalization (OR, 5.13, 95% CI, 1.64–16.03; P=0.002; OR, 5.38, 95% CI, 1.72–16.77; P=0.002, respectively) were identified as being associated with MRSA colonization. Moreover, frequent contact with animals and livestock workers/ veterinarians (OR 5.28, 95% CI 1.14 to 24.47; P=0.02; OR 3.70, 95% CI 1.20 to 11.37; P=0.02, respectively) were identified as being associated with MRSA colonization. Most of the MRSA strains (14/30) expressed heterogeneous character according to the present of isolated colony close to the oxacillin disc inhibition zone. All MSSA strains were resistant to penicillin and resistance rate of MRSA to the other antibiotics tested are shown in Table 2.TeenMRSA strains were multidrug resistant among which eight (26.66%) MRSA isolates were resistant for eight antibiotics except vancomycin while three (10%) strains were resistant for all antibiotics.

DISCUSSION

This present study is the first document of the prevalence of *S. aureus*and MRSA nasal colonization among veterinary students in Malagasy community. Our result, 9.04 % (n=14)of MRSA nasal carriage is higher than those documented in Netherland and Brazil afore mentioned [4, 7]. Likewise, MRSA nasal carriage of Danish veterinary practitioners (means professionally exposed to animals) was 3.9% [13] which is similar to the MRSA carriage rate in the general population (healthy individuals and outside the healthcare environment) estimated to be less than 4 %[14-15].However, this recorded rate seems to be lower compared to those reported among veterinarians from international study (12.5%)[6], from UK veterinarians (12.3%)[5] and intriguingly among unexposed Malagasy population (14.8%)[9]. In one hand, a lower rate could be comprehensive as students are considered as healthy population, with limited risk till they do frequently in touch with high risk factor area such as health and veterinarian care unit. In the other hand, a progressive increase of nasal carriage rate is predictable as students are progressively exposed to health-care facility without an effective hospitalization which may contribute to explain this intermediate rate. However, our results show no significant nasal carriage risk according to academic level year. Likewise, occidental studies yield a predominance of sex male in *S. aureus* carriers [16-17]. Herein, lack of association between *S. aureus* carriage and gender may be attributed to our lack of male (sex-ratio M/F: 0.91).However, it may suggest that there is no host influence at all. In that, influence of host habits and environment like hygiene habits may be more interesting than the only host status and should be well-explored.

It's particularly difficult to suppose a community-acquired MRSA (CA-MRSA) nasal carriage for those carriers even with lack of hospitalization history (n=119). Indeed, all of them, except the first academic year are frequently in contact with healthcare unit (medical lab and veterinary lab). Moreover, we didn't ask for an eventual ambulatory hospitalization or a recurrent hospital visits (as a visitor) which could represent risk factors for hospital-acquired MRSA colonization. Likewise, due to the impossibility to know the hospitalization history of MRSA nasal carriers, we couldn't identify a nosocomial nasal carriage. Those points are desirable in the way to distinguish the susceptibility of CA-MRSA strains to hospital-acquired one. One can assume that all multidrug resistant MRSA nasal

carriers (n=16) presented are prior to hospitalization.

TABLE 1: CHARACTERISTICS OF STUDENTS COLONIZED BY *STAPHYLOCOCCUS AUREUS* AND METHICILLIN-RESISTANT *S. AUREUS* (MRSA)

Characteristic	All participants (n=155)	With *S. aureus* result test n (%)			With MRSA result test n (%)		
		Negative=125	Positive =30	*p*	Negative =141	Positive =14	*p*
Age, mean ± SD, years	23.47 ± 7.55	22.5 ± 6.5	24.75 ± 8.75		25 ± 6.5	26 ± 7.2	
Male	74 (47.74)	58(46.40)	16 (53.33)		64 (45.39)	10 (71.42)	
Female	81 (52.26)	67(53.60)	14 (46.67)		77 (54.61)	4 (28.58)	
Previous[a] antimicrobial use	30 (19.35)	16 (53.33)	14 (46.67)		23 (76.66)	7 (23.34)	.002
Previous hospitalization	36 (23.22)	17 (42.22)	19 (47.78)		28 (77.77)	8 (22.23)	.001
Frequent contact with healthcare workers[b]	36 (23.22)	19 (47.78)	17 (42.22)		33 (91.66)	3 (8.34)	
Frequent contact with animals[b]	87(56.13)	40(45.97)	47(54.03)		75 (86.20)	12(13.80)	.02
Frequent contact with animal workers[b]	37(23.87)	19(51.35)	18(48.65)		30 (81.08)	7(18.92)	.02

[a] within one months; [b] more than once a week

TABLE 2: ANTIBIOTIC RESISTANCE PROFILES OF 30 *S. AUREUS* (SA) AND 14 MRSA NASAL ISOLATES AS DETERMINED BY DISK DIFFUSION

Antibiotics	SA (n=30) No (%)	MRSA(n=14) No (%)
Penicillin	30 (100)	14 (100)
Oxacillin	00 (100)	14 (100)
Gentamycin	06 (20)	06 (42.85)
Erythromycin	18 (60)	09 (64.28)
AMC	16 (53.33)	06 (42.85)
Tétracyclin	21 (70)	11 (78.57)
Ofloxacin	18 (60)	08 (57.14)
Trimethoprim-sulfamethoxazole	23 (76.67)	10 (71.42)
Vancomycin	01 (07.14)	01 (07.14)

In comparison with MRSA isolated from potentially ill Malagasy community reported in our previous study[9], MRSA nasal strains from veterinary students present similar rates of resistance, particularly for trimethoprim-sulfamethoxazole (68.89 % versus 71.42 %),erythromycin (66.67 % versus 64.28 %), tetracycline (71.11 % versus 78.57 %) and ofloxacin (53.33 % versus 57.14 %). Drug resistance of MRSA concern essentially the most antimicrobial used in our community. As a matter of fact, these antimicrobials are accessible to anyone without any medical prescription and very used even in non-bacterial infection. Intriguingly, resistances to gentamycin (20%) and vancomycin (7.14%) are unusual. As students are familiar to hospital and healthcare unit they can easily obtain hospital antibiotics compared to non-student one although we could not evidence real use of these antibiotics. However, these facts suggest the influence of antibiotics consumption habit in our population which can increase the acquisition of drug resistance by adaptive mutation [18].

In our study, identified risk factors for MRSA colonization were consistent with those yielded in other studies [16, 19]. Indeed, history of antibiotics use and hospitalization as well as frequent contact with animals and livestock

workers/veterinariansincreases MRSA colonization (Table 1).Although, we couldn't establish a molecular characterization (*mec*A, *fem*A) of our MRSA strains to confirm MRSA identification, we establish baseline information of nasal carriage of MRSA in Malagasy veterinary student and confirm the place of prior hospitalization and antimicrobial use as high-risk factors of MRSA carriage. However, our samples are represented by a restricted population that comes to a particular health-care facility so that any extrapolation is hazardous. Furthermore, we were not able to distinguish neither their locality origin (townsman or peasant), nor their activities and social conditions that may have an influence in the carriage rate.

In our country, future studies should be addressed to *S. aureus* colonization in healthy population and really inpatients ones. Identification of specific risk factors is strategically important for preventive activities against MRSA spread. In this aspect, systematic screening at hospital admission should be debated as well as improved individual hygiene, decontamination of colonized individuals [20].Besides, longitudinal studies should be led to evaluate the progression of carriers and non-carriers through years of university study. Such studies could address the matter of persistent and intermittent carriers. Finally, identification of relevant pets contact and screening of MRSA colonization in frequently cared animals should contribute to evaluate reservoirs and transmission origins. However, the presence of high rate of MRSA nasal carriage and the increase of their resistance to other drugs in our community are disquieting. Without waiting for a nationwide survey results, it's highly recommended to establish a strategic policy in order to slow down the spread of these strains by different preventive measure such as control of antibiotic use.

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OCCURRENCE OF UNUSUAL NON-FERMENTATIVE GRAM NEGATIVE BACILLI IN INTENSIVE CARE UNITS OF A UNIVERSITY HOSPITAL, EGYPT

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RUNNING TITLE: UNUSUAL NON-FERMENTATIVE GRAM NEGATIVE BACILLI IN AN EGYPTIAN HOSPITAL

ABSTRACT

Non-fermentative Gram-negative bacilli (NFGNB) other than *Pseudomonas* and *Acinetobacter* species have emerged as nosocomial pathogens. No much data is currently available concerning the occurrence of these types of bacteria in Zagazig University Hospitals (ZUHs). In this study, the occurrence as well as the antimicrobial susceptibility pattern of unusual NFGNB obtained from clinical samples collected from intensive care units (ICUs) of ZUHs was assessed. Additionally, the genetic relatedness among the most prevalent unusual NFGNB species was studied. Results: Out of 516 non-repeated clinical sample, 97 NFGNB (18.7%) were isolated. Among them, 17 unusual NFGNB were identified by API 20NE, accounting for 17.5% of NFGNB and 3.3% of all tested samples. Within the unusual NFGNB, *Burkholderiacepacia*complex (*Bcc*) was the most prevalent species accounting for 94.1% of NFGNB and 3.1% of total samples. This was followed by *Burkholderia pseudomallei* (*B. pseudomallei*) which accounted for 5.9% of NFGNB and 0.2% of all obtained specimens. Tigecycline antibiotic was the most effective antibiotic against *Bcc* isolates (68.8% susceptibility) in disc diffusion method. After random amplified polymorphic DNA (RAPD) testing, the obtained *Bcc* isolates were found to be genetically diverse. This highlights *Bcc* as an emerging nosocomial pathogen in ICUs of ZUHs. Continuous monitoring of the occurrence of *Bcc* in ICU as well as in other hospital wards is warranted.

Key words: "Unusual", "Non-fermentative", "gram-negative bacilli", "intensive care unit"

Abbreviations: *Bcc*; *Burkholderia cepacia* complex, CF; cystic fibrosis, ICUs; intensive care units, MIC; minimal inhibitory concentration, NF; non-fermenters, NFGNB; non-fermentative gram-negative bacilli, RAPD; random amplified polymorphic DNA, ZUHs; Zagazig University Hospitals

OCCURRENCE D'INHABITUEL NON – FERMENTAIRE BACILLES GRAM EN UNITES DES SOINS INTENSIFS D'UN HOPITAL UNIVERSITAIRE, EGYPTE

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TITRE COURANT : NON – FERMENTAIRE BACILLES GRAM NEGATIF DANS UN HÔPITAL EGYPTIAN

RESUME

L'espèce non – fermentaire bacilles Gram négatif (NFGNB) autre que *Pseudomonas* et *Acinetobacter*ont émergé comme agents pathogènes nosocomiaux. Il n'y a pas beaucoup de données actuellement disponibles concernant la présence de ces types de bactéries aux hôpitaux universitaire de Zagazig (ZUHs). Dans cette étude, l'occurrence ainsi que le motif de la sensibilité aux antimicrobiens des NFGNB inhabituelle obtenue d'échantillons cliniques prélevésd'unités de soins intensifs de ZHUs ont été évalués. Par ailleurs, la parenté génétique entre lesespèces les répandues de NFGNB inhabituelles a été étudiée.

Résultats : Sur 516 échantillons cliniques non – répétés, 97 NFGNB (18,7%) étaient isolés. Parmi eux, 17 NFGNB inhabituelle ont été identifié par API 20NE, représentant 17,5% de NFGNB et 3,3% de tous les échantillons testés. Au sein de NFGBN, le

complexe *Burkholderiacepacia* était l'espèce le plus répandureprésentant 94,1% de NFGNB et 3,1% des échantillonstotal. Ceci a été suivi par *Burkholderiapseudomallei (B. pseudomallei)* lequel représentait 5,9% de NFGNB et 0,2% de tous spécimens obtenus. Tigecycline antibiotique était l'antibiotique le plus efficace contre les isolats *Cci* (68,8% susceptibilité) dans la méthode de diffusion sur disque. Suite a des tests aléatoires d'ADN polymorphique amplifie (RAPD), les isolats *Cci*obtenus se sont trouvés d'êtregénétiquement divers. Ceci souligne *Cci* comme un pathogènes nosocomiaux émergents en USI de ZUHs. La surveillance continue de l'occurrence de *Cci* en USI, ainsi que dans d'autres services hospitaliers est justifiée.

Mots clés : Inhabituelle, Non fermentaire, bacille gram négative, unité des soins intensifs.
Abréviations : Cci :le complexe Burkholderiacepacia ; CF : fibrose kystique ; USI : Unités des soins intensifs ; MIC : concentration minimal inhibitrice ; NF : non fermenteurs ; NFGNB : non fermentaire bacilles gram négative ;RAPD ; ZUHs : Hôpitaux universitaire de Zagazig.

INTRODUCTION

Non-fermentative gram-negative bacilli (NFGNB) are a diverse group of aerobic non spore-forming bacilli. They usually present as saprophytes in the environment, particularly, in soil and water. Although being saprophytic in nature, NFGNB has emerged as important healthcare-associated pathogens. Their resistance to disinfectants, in addition to their potential to spread from patient to patient via fomites, or the hands of medical personnel have made them of great concern in hospital settings [1] [2].

The majority of earlier studies have only focused on *Pseudomonas* spp. and *Acinetobacter* spp. being the most frequently isolated NFGNB, considering the unusual group as having a minor clinical significance. Nevertheless, serious infections due to NFGNB other than *Pseudomonas* spp. and *Acinetobacter* spp. are currently being reported with increasing frequency forming a significant contribution to in-hospital mortality, particularly, in immunocompromised patients[3] [4].

Unfortunately, human infections caused by these bacteria are underestimated. This is partly due to their complex identification along with their frequent misidentification by phenotypic methods commonly used in clinical laboratories. Furthermore, the results obtained for some of these organisms by disc diffusion method do not correlate with successful clinical outcome nor with those obtained by the more accurate minimal inhibitory concentration (MIC) methods [5] [6].

As no much data is currently available concerning the occurrence of these types of bacteria in Zagazig University Hospitals (ZUHs), this study aimed to assess the prevalence as well as the antimicrobial susceptibility pattern of unusual NFGNB isolated from intensive care unit (ICU) patients in ZUHs. A further aim was to assess the genetic relatedness between the isolates of the most prevalent species among this group, considering this a primary step for further epidemiologic studies.

MATERIALS AND METHODS

Patient selection and collection of samples

A cross-sectional study was conducted in the Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, during the period from December 2014 to December 2015. One hundred and eighty patients (141 male and 39 female), who developed different infections at least 48 h after admission in either surgical or emergency ICU, were enrolled in this study. Their age ranged from one to 80 years(mean 33.4±15.6). A written informed consent was obtained from each patient or from their guardians before obtaining the samples. Demographic and clinical data of each patient was obtained through a worksheet filled for each case. Different non-repeated clinical samples (urine, endotracheal tube aspirate (ETA), pus and blood) were collected from patients according to the site of infection, using standard microbiologic methods. This study has been approved by the Institutional Review Board (IRB) of ZUHs.

Isolation and Identification

All samples were cultured on 5% blood agar and McConkey agar (Oxoid, England). Culture plates were incubated aerobically at 37° C for 48-72 h. Identification of non-fermenting colonies (NF) was primarily made by their reaction on triple sugar iron (TSI) medium (Oxoid, England), gram staining, oxidase test and was confirmed by API 20 NE (Bio-Mérieux, Marcy L´Etoile, France).

Antibiotic Susceptibility Tests

Isolates that were identified as being NFGNB, with the exclusion of *Pseudomonas* and *Acinetobacter* spp., were tested for their susceptibility to 14 different antimicrobials by disc diffusion method. Antibiotic discs included cefepime 30 µg, amikacin 30 µg, aztreonam 10 µg, tigecycline 15 µg, piperacillin/tazobactam (100/10) µg, meropenem 10 µg, colistin 10 µg, ceftazidime 30 µg, trimethoprim/sulphamethoxazole (1.25/23.75) µg, piperacillin 100 µg, imipenem 10 µg,

cefoperazone/sulbactam (75/30) µg, ciprofloxacin 5 µg and gentamycin 10 µg. All discs were purchased from Bioanalyse (TibbiMalzemelerSanayiveTicaret Ltd. Sti., Turkey) except tigecycline was from Oxoid, England. In addition, the MICs of ceftazidime, meropenem and levofloxacin (Sigma-Aldrich, St. Louis, USA) were determined by agar dilution method according to EUCAST[7] and CLSI guidelines [8].

Interpretation of the diameter of inhibition zones for meropenem, ceftazidime and TMP-SMX was done according to Kirby-Bauer zone diameter interpretative standardsas documented in CLSI**.** Those of cefoperazone-sulbactamwere interpreted according to the interpretative standards of *Enterobacteriaceae*. The results of other antibiotics were interpreted according to that of *Pseudomonas aeruginosa* (*P. aeruginosa*), as no interpretative standards for unusual NFGNB in concern to these antibiotics are yet available**.** Tigecycline inhibition zone diameters were interpreted according to FDA [9]. Interpretation of agar dilution tests was done according to CLSI guidelines. $MIC_{50}$and MIC_{90} values were calculated according to Schwartz et al. [10]. In all antibiotic susceptibility tests, *P. aeruginosa* ATCC® 27853 (Global Bioresource, Center of American Type Culture Collection KWIK-STIK ™) served as a quality control strain.

Random Amplified Polymorphic DNA (RAPD) Genotyping

The isolates belonging to the most prevalent species (*Burkholderiacepacia*complex [*Bcc*]), were genotyped by RAPD technique. *Bcc* isolates that were identified with accuracy of less than 99% by API 20NE were subjected to PCR reaction using 16S rDNA primers as described previously [11] to ensure their identity. RAPD genotyping was achieved by extracting DNA from the obtained *Bcc* isolates using QIAamp DNA Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions, followed by RAPD PCR fingerprinting using the primer RAPD-270 (5′-TGC GCG CGG G-3′) and the cycling conditions described previously [12]. Reactions were performed in 20 µl reaction mixtures using Maxime PCR PreMix Kit (*i*-Taq) PCR beads (iNtRON Biotechnology, Korea). For each reaction, twoµl of extracted DNA and 40 µM of the mentioned primer (LGC, Biosearch Technologies, USA) were added. Amplification reactions were carried out using Veriti 96-well thermal cycler (Applied Biosystems, Singapore). PCR products were examined after electrophoresis in 1% agarose gel and visualized under UV light. Product sizes were determined using 50 bp DNA ladder (GeneOn, Germany). Interpretation of the fragments resulting from RAPD reaction was carried out by calculating the similarity index [13]. Then the similarity matrix data was subjected to cluster analysis with PAST (paleontological statistics) software [14] using unweighed pair group method for arithmetic average (UPGMA) to generate a dendrogram.

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. Chi-square (χ^2) test and Student t-test were used to compare two qualitative and quantitative groups, respectively. Kruskal-Wallis test, a nonparametric test, was used to compare more than two groups.Kappa agreement was used to test the agreement level between two tests. P value of < 0.05 was considered significant.

RESULTS

Out of 516 cultivated clinical samples, 97 isolates (18.7%) were identified as being NFGNB. Among them, *P. aeruginosa* (44/97) had the highest isolation frequency (45.4%) followed by *Acinetobacterbaumannii* (36/97, 37.1%) then *Bcc* (16/97, 16.5%) and finally *Burkholderia pseudomallei* (*B. pseudomallei*) which had the lowest isolation frequency (only one isolate, 1.03%). After the exclusion of both *Pseudomonas* spp. and *Acinetobacter* spp., it was shown that unusual NFGNB were isolated from 3.3% (17/516) of all tested specimens, accounting for 17.5% (17/97) of all isolated NFGNB where *Bcc* isolates were the most prevalent (n=16) accounting for 94.1%, followed by *B. pseudomallei*(n=1) accounting for 5.9%.

Out of the 17 unusual NFGNB isolates, 11 isolates (64.7%) were obtained from urine specimens taken from catheterized patients. The remaining isolates (35.3%) were obtained from ETA specimens. No isolate was obtained from either blood or pus samples. The only isolate of *B. pseudomallei* was obtained from urine sample, while 62.5% of *Bcc* isolates were from urine samples and 37.5% were from ETA.

Nearly half of the patients (9/17 or 52.9%) who yielded positive culture for unusual NFGNB were below 20 years old (mean 23.4±13.3), while no isolates were obtained from patients between 60 and 80 years old. Most of unusual NFGNB isolates (15/17 or 88.2%) were obtained from male patients (88.2%) **(Table1)**, though this was insignificant statistically when analyzed using Chi-square test (P= 0.29). Regarding *Bcc* isolates, 93.75% were obtained from

male patients, the mean duration of ICU stay was 7.4±3.8 and prior antibiotic administration was observed in 62.5% of cases (10/16) **(Table 1)**.

TABLE (1): DEMOGRAPHIC AND CLINICAL DATA OF ICU PATIENTS FROM WHOM UNUSUAL NFGNB ISOLATES WERE OBTAINED

	Age (years)	Gender	Type of infection	Ward	Length of hospitalization (days)	Urinary catheter	Ventilator	Antibiotic use
1	22	Male	Urinary	Emergency ICU	5	√	√	√
2	15	Male	Respiratory	Emergency ICU	4	√	√	-
3	32	Male	Urinary	Emergency ICU	6	√	√	-
4	13	Male	Urinary	Emergency ICU	11	√	√	√
5	6	Male	Urinary	Emergency ICU	4	√	√	√
6	50	Male	Urinary	Surgery ICU	3	√	√	√
7	17	Male	Respiratory	Surgery ICU	15	√	√	√
8	47	Male	Urinary	Surgery ICU	5	√	√	√
9	16	Male	Urinary	Emergency ICU	3	√	√	√
10	32	Male	Urinary	Emergency ICU	7	√	√	-
11	37	Female	Respiratory	Surgery ICU	7	√	√	-
12	5	Male	Urinary	Emergency ICU	6	√	√	√
13	30	Male	Respiratory	Emergency ICU	4	√	√	-
14	12	Male	Respiratory	Emergency ICU	5	√	√	-
15	15	Male	Urinary	Emergency ICU	10	√	√	√
16	18	Male	Respiratory	Surgery ICU	16	√	√	√
17	30	Female	Urinary	Surgery ICU	3	√	√	-

Isolates 1-16 represent *Bcc* isolates, isolate 17 represents *B. pseudomallei*

Unfortunately, the only isolate of *B. pseudomallei* was lost during preservation. For this reason, only *Bcc* isolates were subjected to antibiotic susceptibility tests. The highest susceptibility ratio in disc diffusion method (68.8%) was recorded to tigecycline followed by ciprofloxacin (62.5%). On the other hand, no isolates were sensitive to ceftazidime, cefepime or piperacillin **(Figure 1)**.

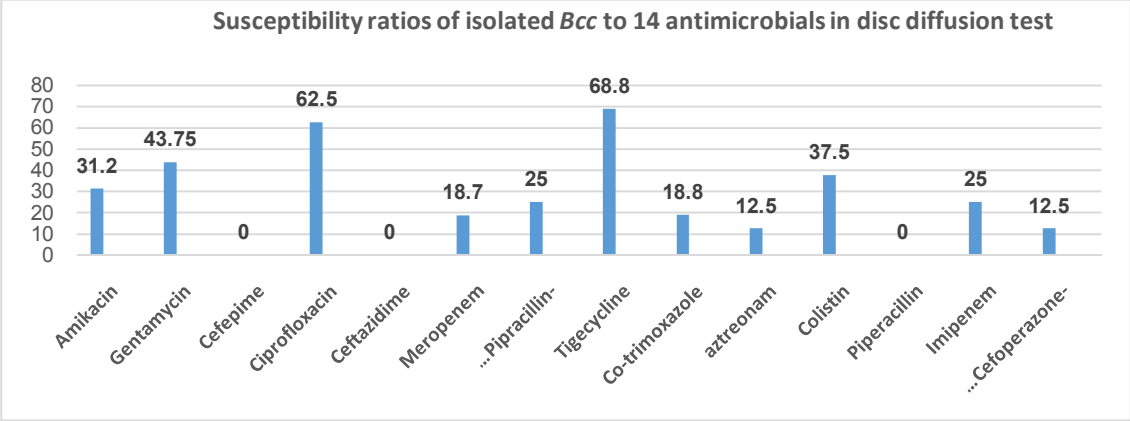

FIGURE (1): ANTIBIOTIC SUSCEPTIBILITY OF *BCC* ISOLATES BY DISC DIFFUSION METHOD. ISOLATES THAT HAD INTERMEDIATE SUSCEPTIBILITY WERE CONSIDERED RESISTANT IN THIS CHART. THE HIGHEST SUSCEPTIBILITY WAS RECORDED WITH TIGECYCLINE. NO ISOLATES WERE SUSCEPTIBLE TO CEFEPIME, CEFTAZIDIME OR PIPERACILLIN.

The MIC values of ceftazidime, levofloxacin and meropenem are presented in **Table 2**. According to CLSI 2015 guidelines, it was found that 62.5% of *Bcc* isolates were susceptible to ceftazidime with 6.25% of strains having intermediate susceptibility, 75% were susceptible to levofloxacin, and 50% were susceptible

to meropenem. When the MIC_{50} and MIC_{90} values for the three antimicrobials were calculated, those of ceftazidime were found to be 8 µg/ml and 512 µg/ml, respectively. Regarding levofloxacin, the values were 1 µg/ml and 32µg/ml, respectively. Finally, the values recorded for meropenem were 4 µg/ml and 128 µg/ml, respectively.

TABLE (2): CEFTAZIDIME, LEVOFLOXACIN AND MEROPENEM MIC VALUES (µg/ml) FOR *BCC* ISOLATES (N=16) BY AGAR DILUTION METHOD

Antibiotic	**Ceftazidime**			**Levofloxacin**			**Meropenem**		
***Bcc* isolates**	**S ≤8µg/ml**	**I =16µg/ml**	**R ≥32µg/ml**	**S ≤2µg/ml**	**I =4µg/ml**	**R ≥8µg/ml**	**S ≤4µg/ml**	**I =8µg/ml**	**R ≥16µg/ml**
1		16		2			0.015		
2	0.25			0.03			0.5		
3	8			0.25			0.125		
4	8			0.5					128
5	4			1					128
6			32	2			2		
7			512			32			128
8			512			8			32
9	8			1			4		
10	8			0.25			0.125		
11	4			2			2		
12	4			1					128
13			64			32			32
14	0.25			0.03			0.5		
15	8			1					128
16			512			32			128
P. aeruginosa ATCC ® 27853	1			4			0.25		
Median (range)	8 (0.25-512)			1 (0.03-32)			18 (0.015-128)		
Kruskal Wallis	6.45								
P	0.04*								

S; susceptible, I; intermediate, R; resistant,*significant.

Considering agar dilution as the reference method, good agreement (Kappa 0.5, P 0.001) was found between disc diffusion and agar dilution concerning the results of meropenem antibiotic. For ceftazidime antibiotic, the level of agreement could not be analyzed, as all *Bcc* isolates were resistant to it in the disc diffusion method **(Table 3)**. Levofloxacin antibiotic was not tested by disc diffusion method. For this reason, it was not included in this comparison.

TABLE (3): LEVEL OF AGREEMENT BETWEEN DISC DIFFUSION AND MIC RESULTS BY AGAR DILUTION (MIC) OF *BCC* ISOLATES (N=16) FOR CEFTAZIDIME AND MEROPENEM ANTIMICROBIALS

Antibiotic	**Ceftazidime**		**Meropenem**	
Isolate	**Disc**	**MIC**	**Disc**	**MIC**
1	R	I	R	S
2	R	S	S	S
3	R	S	I	S
4	R	S	R	R
5	R	S	R	R
6	R	R	I	S
7	R	R	R	R
8	R	R	R	R
9	R	S	S	S
10	R	S	I	S
11	R	S	I	S
12	R	S	R	R
13	R	R	R	R
14	R	S	S	S
15	R	S	R	R
16	R	R	R	R
X^2	NA		12.44	
P	-------		**0.002***	
Kappa agreement	NA*		0.50	
P	-------		**0.001****	
Disc sensitivity for detection of sensitive	0.0%		37.0%	
Disc specificity for detection of resistant	100.0%		100.0%	

R; RESISTANT, S; SUSCEPTIBLE, I; INTERMEDIATE SUSCEPTIBILITY, NA; NOT APPLICABLE, * SIGNIFICANT, **HIGHLY SIGNIFICANT

Following RAPD analysis of the obtained *Bcc* isolates **(Figure 2)**, the similarity indices ranged from zero to 0.66 **(Table 4)**. The resulting dendrogram demonstrated the presence of two different genetic clusters with different subclusters. It also demonstrated that the clusters are placed far from each other indicating the genetic diversity among the tested strains **(Figure 3)**.

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

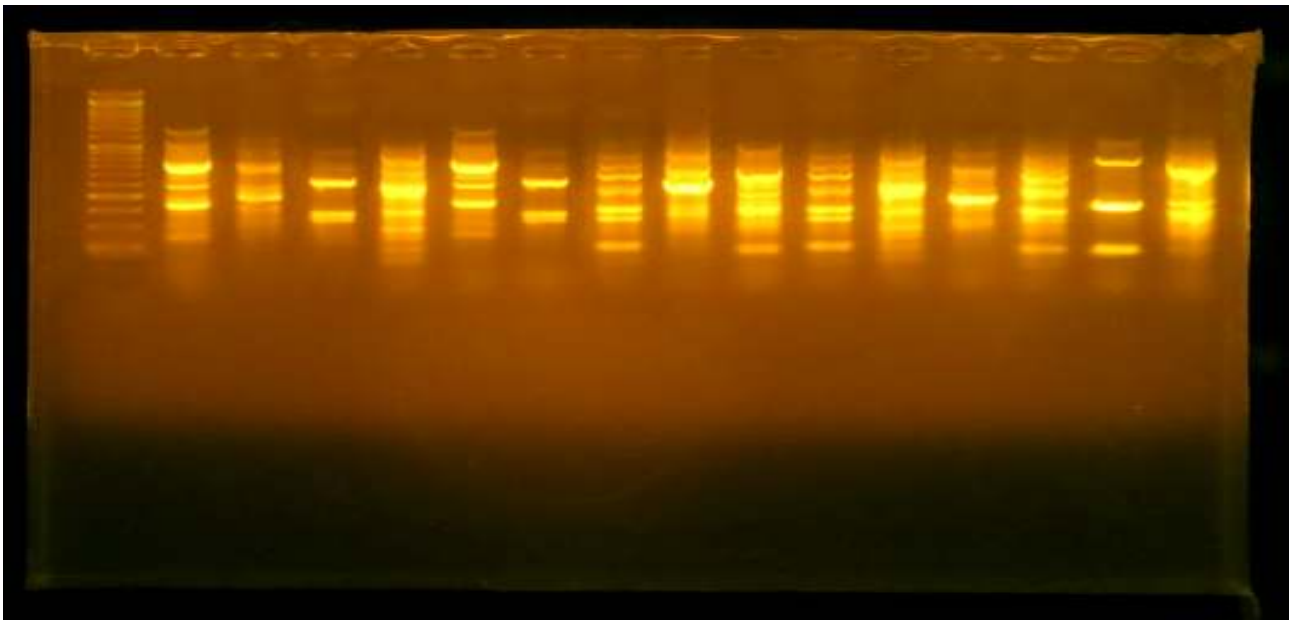

FIGURE (2): ELECTROPHORESIS GEL PHOTO DEMONSTRATING RAPD ANALYSIS RESULTS OF 15 *BCC* ISOLATES. LANE 1 (L) HAS 50-BP LADDER AND LANES 2-16 DEMONSTRATE RAPD RESULTS OF 15 *BCC* ISOLATES ARRANGED FROM 1 TO 15, THE SIXTEENTH ISOLATE IS NOT INCLUDED IN THIS PHOTO.

TABLE (4): JACCARD'S SIMILARITY COEFFICIENT (SIMILARITY INDEX) OF THE OBTAINED *BCC* ISOLATES BY RAPD ANALYSIS

	*Bcc*1	*Bcc*2	*Bcc*3	*Bcc*4	*Bcc*5	*Bcc*6	*Bcc*7	*Bcc*8	*Bcc*9	*Bcc*10	*Bcc*11	*Bcc*12	*Bcc*13	*Bcc*14	*Bcc*15	*Bcc*16
*Bcc*1	1															
*Bcc*2	0	1														
*Bcc*3	0	0	1													
*Bcc*4	0	0	0	1												
*Bcc*5	0	0.28	0	0.4	1											
*Bcc*6	0	0	**0.66**	0	0	1										
*Bcc*7	0.18	0.2	0	0.18	0	0.22	1									
*Bcc*8	0	0.28	0	0	0.25	0.29	0.2	1								
*Bcc*9	0	0	0	0.2	0	0.29	**0.6**	0.25	1							
*Bcc*10	0	0.2	0	0.18	0.2	0	0.33	0	0.2	1						
*Bcc*11	0	0	0.25	**0.6**	0.4	0	0	0	0	0.54	1					
*Bcc*12	0	0	0.25	0	0	0	0	0.2	0	0.36	0.4	1				

*Bcc*13	0	0	0.36	0.15	0	0.18	0.29	0.16	0.5	**0.14**	0.15	0.31	1			
*Bcc*14	0.22	0	0	0.25	0.57	0	0	0	0	0	0.25	0	0	1		
*Bcc*15	0.2	0	0	0.2	0	0	0	0.25	0	0.2	0.2	0.4	0.17	0.29	1	
*Bcc*16	0.2	0	0.29	0	0	0	0	0	0	0.2	0.2	0.2	0.17	0	0	1

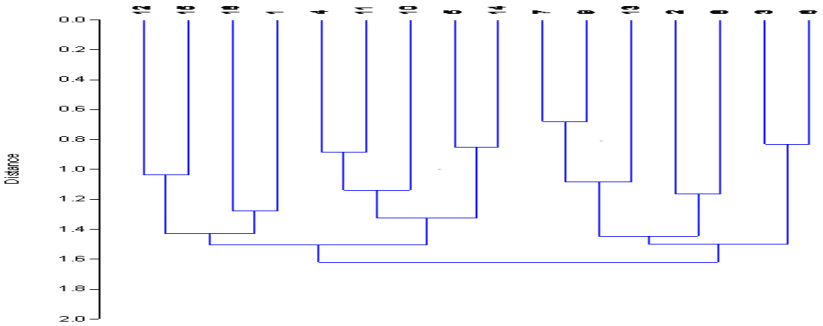

FIGURE (3):DENDROGRAM OF THE OBTAINED *BCC* ISOLATES AFTER ANALYSIS USING PAIRED GROUP METHOD. NO SINGLE ROOT IS DETECTED. IT ENDS IN TWO MAJOR CLUSTERS WITH SUBCLUSTERS THAT ARE PLACED FAR FROM EACH OTHER.

DISCUSSION

The isolation rate of NFGNB (18.7%) recorded in our study comes in agreement witha previous Indian study carried out in a tertiary care hospital, where it was 16.18% [15].Whereas, it comes higher than that recorded in another Indian tertiary care hospital where it was 9.32% [16].

The unusual NFGNB accounted for 3.3% isolation frequency among all tested specimens that comes much similar to what was reported previously among respiratory tract infection in an Indian tertiary care hospital (4%) [17]. *Bcc* was the most prevalent species accounting for16.5% of the total NFGNB. This comes much higher than what was reported previously where *Bcc* accounted for 4.66% of all isolated non-fermenters in an Iranian study [18]. Lower ratios were also reported in other countries(4.58% and 12.1%) [15] [17]. On the other hand, our result is lower than that recorded in Latin America, where it accounted for 47.15% among all NFGNB (83/176) in a surveillance study that lasted from 1997 to 2002 [19]. While *Bcc* represented the main unusual NFGNB (16/17, 94.1%) in the current study, *Stenotrophomonas maltophilia* (*S. maltophilia*) was the most frequently isolated species among unusual NFGNB in India (45.5%) [17] as well as in Saudi Arabia (20.8%) [6]. The different identification system used in the previous two studies (Vitek 2 system) compared to API 20NE used in the current study may have contributed to this difference.

Only one isolate of *B. pseudomallei*was obtained accounting for 5.9% of the unusual NFGNB and 1.03% of the total NFGNB. Similar results were reported in a previous Indian study where two isolates among 33 unusual NFGNB were *B. pseudomallei* accounting for 6.1% of unusual NFGNB isolated from patients with nosocomial pneumonia [17].

Urine was the main source of isolation of *Bcc* (62.5%) followed by ETA (37.5%). This comes in contrast to the general belief that *Bcc* is a cause of chronic respiratory infection or colonization particularly in cystic fibrosis (CF) patients [20]. In a Turkish study, ETA was the main source for *Bcc*isolation(58.9%) [21]. However, 62.7% of *Bcc* were isolated from blood specimens in another study [19] and in a previous Egyptian study, the highest percent (85.7%) of *Bcc* isolates were from pus specimens [22]. This difference could be attributed to the difference in the clinical conditions of patients along with different hospital wards selected in each study.

The relatively short ICU stay for patients from whom *Bcc* isolates were isolated (mean 7.4 ± 3.8 days), in our study, was an unusual finding. In a previous Turkish study, the researchers reported that the mean duration of hospitalization for *Bcc* infected patients was 15.2 ± 9.9 days [21]. This may, in some way, point to lower compliance to infection control measures that may result in a more rapid infection.

Our study revealed that prior antibiotic use was observed in 62.5% of patients positive for *Bcc* isolates.

Prior antibiotic administration in 38.5% of *Bcc* infected patients was previously reported [21]. This difference may be explained by the different treatment policies concerning antimicrobials in different hospitals. Besides, the researchers of the previous study conducted their work in different hospital wards, whereas, we focused on ICU patients where excessive use of antimicrobials is confronted.

*B. cepacia*complex bacteria are well known for their multidrug resistance. Both intrinsic and acquired mechanisms contribute to this phenomenon [23] [24]. In the current study, the antimicrobial susceptibility pattern of the sixteen *Bcc* isolates was tested by disc diffusion and agar dilution methods. In disc diffusion, the highest susceptibility rate was observed for tigecycline (68.8%) and ciprofloxacin (62.5%). This was followed by gentamycin (43.75%), colistin (37.5%), amikacin (31.2%), then piperacillin-tazobactam and imipenem (25% each), co-trimoxazole (18.8%), meropenem (18.7%) then both cefoperazone-sulbactam and aztreonam (12.5% each). On the other hand, all isolates were resistant to ceftazidime and piperacillin.Different patterns were recorded in previous studies [15] [21] [22] [25]but the striking point, in our results, is the high level of ceftazidime resistance (100%) which was not recorded previously, as far as we know. This could be attributed to the excess use of 3rd generation cephalosporins in our hospital. This clearly highlights the importance of local susceptibility tests that should guide treatment policies.

The agar dilution method demonstrated that 62.5%, 75% and 50% of *Bcc* isolates were susceptible to ceftazidime, levofloxacin and meropenem, respectively. Varying results were obtained in previous studies [19] [21] [26] [27]. All of them recorded *Bcc* as one of the highly resistant microbes encountered. In spite of this, a more recent study reported that all *Bcc* isolated from CF children (*B. cepacia*, *B. cenocepacia*, and *B. multivorans*) were susceptible to levofloxacin, ceftazidime, and meropenem[28]. This indeed confirms the different behavior of *Bcc* isolates in susceptibility tests and points to the unpredictable nature of their results.

Our study recorded higher MIC_{90} values for the tested three antimicrobials, compared to previous works [19] [26]. This reflects the higher number of resistant *Bcc* isolates obtained from our hospital compared to the other studies. A significant agreement (P 0.001) between disc diffusion and agar dilution methods for meropenem was found in our study. As all isolates were resistant to ceftazidime in disc diffusion, the level of agreement with agar dilution could not be assessed, but this made the test 100% specific in detecting resistant isolates with no false susceptibility detected.

Being the first time to record the isolation of *Bcc* in our hospital,we tried further to assess the genetic relatedness of the obtained isolates to be a primary step towards more understanding of the spread of this organism. Our results demonstrated that *Bcc* isolates obtained from patients in surgery and emergency ICUs, belonged to two genetic clusters which have further subclusters and which are genetically distinct from each other. To date, most of the previous studies performed genotyping for *Bcc* obtained from CF patients with their results being contradicting [29] [30].

CONCLUSION

In conclusion, this study demonstrated that *Bcc* constituted an emerging nosocomial pathogen in ICUs of ZUHs with high resistance to different antimicrobials. So far, the problem is not great. However, this necessitates further studies that continuously monitor its occurrence and that assess possible sources of infection among ICU patients as well as other hospital units.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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A SURVEY OF MALARIA PREVALENCE AND ANTIMALARIAL PREVENTIVE MEASURES AMONGST STUDENTS OF UNIVERSITY OF LAGOS, NIGERIA

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ABSTRACT

The prevalence of malaria parasite and antimalarial preventive measures among students of University of Lagos, Nigeria was carried out between November 2014 and February 2015. Blood samples were collected from 400 students (with age ranging from 15-46year) by finger pricking and analyzed microscopically and by Rapid Diagnostic Test kit. Semi-structured questionnaire probing into their knowledge of the cause of malaria, ability to recognize signs and symptoms, treatment seeking behaviour was distributed to the respondents. The prevalence of *Plasmodium falciparum* by microscopy was significantly lower (p=0.0000042). More than three quarters of the respondents (88.5%) had a good knowledge of the cause of malaria and were able to identify signs and symptoms of malaria which included headache (51.5%), fever (26%), dizziness (9.5%). Also more than half of the students (202) visit hospital when they recognize such signs while others visit Pharmaceutical outlets (130), drug hawkers (37) or use herbal remedy (31).Malaria preventive measures employed by students ranges from the use of Long-Lasting Insecticide Treated Bednets (74), topical mosquito repellant cream (65) to cleaning the environment (54). Furthermore, half of the students (50%) reported having malaria bouts twice per year, 35% once annually while 15% could not remember how often they have it. These bouts of various frequencies resulted in 360 (90%) of the students being absent from school from less than 10 days to more than 20 days. Proactively placing preventive measures would negate cascades of effects amongst the students. Continuous studies should be carried out to assess the prevalence of malaria in different foci of Nigeria and the level of awareness on the benefits of use of preventive measures should be emphasized among community members if Nigeria and Africa as a whole will meet the elimination target by 2030.

UNE ENQUETE SUR LA PREVALENCE DU PALUDISME ET DES MESURES PREVENTIVES ANTIPALUDIQUES PARMI LES ETUDIENTS D'UNIVERSITE DE LAGOS, NIGERIA

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RESUME

La prévalence du parasite du paludisme et des mesures préventives contre le paludisme parmi les étudiants d'université de Lagos, Nigeria, a été réalisée entre novembre 2014 et février 2015. Echantillons de sang ont été recueillis de 400 étudiants (avec l'âge de 15 à 40 ans)par piqure au doigt en utilisant un kit de test de diagnostic rapide microscopique. Une semi – structuré questionnaire cherchant leur connaissance de la cause du paludisme, la capacité à reconnaitre signes et symptômes, le traitement, comportement de recherche a été distribué. La prévalence du paludisme à la suite d'une infection par *Plasmodium falciparum* était significativement plus faible (0,0000042) par microscopie. Plus de trois quarts de répondants (88,5%) avaient une bonne connaissance de la cause du paludisme et pouvaient identifier les signes et symptômes du paludisme qui inclut le mal de tête (51,5%), la fièvre (26%), le vertige (9,5%). Egalement, plus de lamoitié des étudiants (202) visitent l'hôpital quand ils reconnaissent tels signes tandis que d'autres visitent points de vente pharmaceutiques (130), les colporteurs de médicaments(37) ou utilisent une tisane(31). Mesures préventivescontre le paludisme employées par les étudiants varient de l'utilisation de long terme moustiquaires traitées (74), crème anti – moustique topique(65), au nettoyage de l'environnement(54). En outre, la moitié des étudiants (50%) ont déclaré d'avoir des épisodes de paludisme deux fois par an, 35% une fois par an, tandis que 15% ne pouvaient pas se rappeler combien de fois ils l'ont. Ces épisodes de différentesfréquences ont donné lieu à 360 des étudiants**étant absent de l'école de moins de 10 jours à plus de 20 jours. De manière proactive, plaçant des mesures préventives**

annulerait cascades d'effets parmi les étudiants. Des études continues devraient êtremenées pour évaluerles différent foyers du Nigeria et le niveau de sensibilisation de membres de la communauté devrait être augmenté afin de prioriser des stratégies de contrôler et de répondre à l'objectif d'élimination avant 2030.

INTRODUCTION

Malaria is the world most widely spread disease, caused by infection with single celled parasites of the genus *Plasmodium*. It is usually transmitted through the bite of female *Anopheles* mosquitoes (1).

It is the most prevalent tropical disease resulting in high morbidity with its consequent economic and social loss. Malaria accounted for an estimated 207 million cases in 2012 with 627 000 deaths occurring in same year, 90% of these deaths occurred in sub-Saharan African (2). Malaria is the single most important cause of ill-health and poverty in sub-Sahara Africa (3).

Malaria is widespread in many part of the world mainly in tropical and subtropical regions but extending into some of the temperate areas. Most malaria endemic regions are characterize by warm temperature and rainfall both of which are suitable for mosquito breeding.

Symptoms of malaria include fever, headache, vomiting and loss of appetite (4, 5). Malaria can also result to anaemia, cerebral malaria and acidotic condition (6). *Falciparum* malaria is responsible for most of the serious or even lethal forms of the disease which in most cases result to anaemia which is seen in the rupturing of 5-10% of the red blood cell by schizont, disturbance of the central nervous system (resulting to coma and confusion), or respiratory disease, muscle pain, fatigue, and elevated temperature of 104 -106°F. Malaria infection impairs physical and mental development in children and as a result of this, it is a major cause of death due to anaemia in young children. More so, malaria is known to have a negative impact on learning in children resulting in absenteeism and ultimately leading to poor academic performance (7, 8).

In areas of high and stable transmission, people tend to develop immunity following repeated infections, so that the population at greater risk of malaria are young children who had not yet had multiple exposures. In those areas such as Nigeria, infection during pregnancy is estimated to cause as many as 10,000 maternal deaths each year, 8-14% of all low birth weight babies and 3-8% of all infant deaths. (9, 10, 11, 12).

Evidence from Nigeria shows that malaria incidence throughout the country had been on the increase over the years ranging between 1.12 million at the beginning of 1990,2.25 million by the turn of the millennium 2000 and 2.61 million in 2003 (13).

Four major problems are associated with the management of malaria, the most important is that the parasite are resistance to the most widely, affordable and safest drugs which were used as the first line treatment such as chloroquine and fansidar (sulphadoxine-Pyrimethamine) and even the most recently introduce artemisinin combination therapy (3, 14). Secondly, the overall control of the mosquitoes which transmit malaria is made difficult by their resistance to wide range of insecticides. The third is a new and rapidly developing problem which is the widespread production of fake antimalarial drugs (15). Lastly many countries in Africa lack the infrastructures and resources to manage and control malaria (10). Effective control of malaria entails a detailed assessment of the prevalence in order to allow priority distribution and allocation of control tools to most deserving area as well as analysis of various methods that have proved effective in formally holoendemic areas.

This study was therefore designed to determine the status of malaria among the students of University of Lagos, Nigeria as well as assess the preventive measures employed by the students.

MATERIALS AND METHODOLOGY

Study area

The study was carried out at the University of Lagos (UNILAG), Lagos Akoka, Medical centre, Yaba, Lagos state. The school presently has three campuses in Yaba and Surulere, with two of its campuses located in Yaba (the main campus in Akoka and the recently created campus at the former school of Radiography). Its College of Medicines is located in Idi-Araba, Surulere.The main campus is largely surrounded by the scenic view of the Lagos lagoon on 802 acres of land in Akoka, Lagos. The University is located within the latitude and longitude $6^0$31'0"N3^0 23'10"E/6.51667^{0}N 3.38611^{0}E southwestern part of Nigeria (16).

Sample and data Collection

Blood samples were collected from students by finger prick with the application of little pressure. The first drop of blood was cleaned off with cotton wool soaked in 70% ethanol and subsequent drops of blood were collected on clean microscopic slide for the preparation of thin and thick blood films and also on the rapid diagnostic test kit (SD Bioline, South Korea; Batch number: 0823521). Semi-structured questionnaire was administered to the students probing into their knowledge of the cause of malaria, ability to recognize malaria symptoms, treatment seeking behaviour and preventive measures used.

Smear Preparation

Thick and thin blood films were prepared according to the technique outlined by the World Health Organization (17). A drop of blood was spread on a grease free microscopic slide to a moderate thickness and allowed to air dry and stained with 10% Giemsa stain. The stain was allowed to stay for 10 minutes before washing off with clean water. The slide was then placed vertically and allowed to dry, after which a drop of immersion oil was placed on the slide and examined under the microscope with ×100 magnification.

Thin film was prepared by immediately placing the smooth edge of a spreader slide on a drop of blood, adjusting the spreader to an angle of 45^0 and then smearing the blood swiftly and steadily along the surface. The film was then allowed to air dry, fixed with 70% ethanol and stained with 10% Giemsa for 10 minutes before washing off with clean water. The slide is then allowed to air dry in a vertical position and examined under oil immersion microscope (18, 17).

Presence of ring forms of *Plasmodium falciparum* trophozoites indicates positive results. A blood smear is considered negative if no parasite is seen after 10 minutes of examination or examination of 100 fields.

All slides were read and the parasite density was estimated by counting the number of asexual parasites per 200 leukocytes in the thick blood films and assuming white blood cells (WBC) count of 8,000 parasite/µl (17, 19).

Rapid Diagnostic Tests (RDT)

All kits components were acclimatized to room temperature prior to testing. The flat surface used was swabbed with cotton wool dipped into 70% of ethanol in order to sterilize it. Test cassettes were removed from foil pouch, placed on a flat, dry surface. Blood (0.1ml) was pipetted into the sample hole of the RDT cassette. About 2 – 3 drops of diluents buffer was added and left for 15 minutes before reading the result of the slide.

Data Analysis

Prevalence of *Plasmodium falciparum* was calculated as the proportion of sampled persons with positive blood smear divided by the number of persons who provided blood. Descriptive statistics (Mean ±SD; chi-square) with SPSS package 2007 was used to analyze the data obtained, P- value < 0.05 was taken as statistical variable of relevance.

Ethical Consideration

Approval for this study was sought from Head of the Department of Medical laboratory of the University of Lagos Medical Centre. All participants gave their inform consent before enrolling for the study.

RESULTS

Socio-demographic background of the respondents

The study recruited 400 students which include male and female from the University of Lagos, Akoka Yaba Lagos. Three hundred and forty of the respondents (85%) were undergraduate students while 60 (15%) were postgraduate students. Females were significantly (p=0.0013) higher (232) than males (168). More than three-quarters of the students (342) were single at the time the study was conducted while 58 were married.

Malaria prevalence

The prevalence of malaria by microscopy (2%) and Rapid Diagnostic Test kit (3%) was significantly lower (p=0.0000042) (Figure 1 A and B) and the species of Plasmodium implicated in this study as revealed by microscopy is *Plasmodium falciparum*.

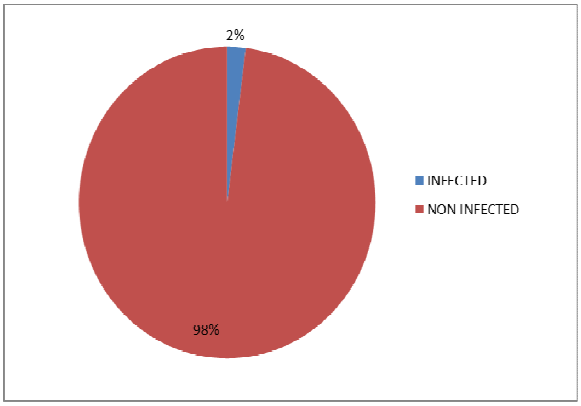

FIGURE 1 A: MALARIA PREVALENCE BY MICROSCOPY

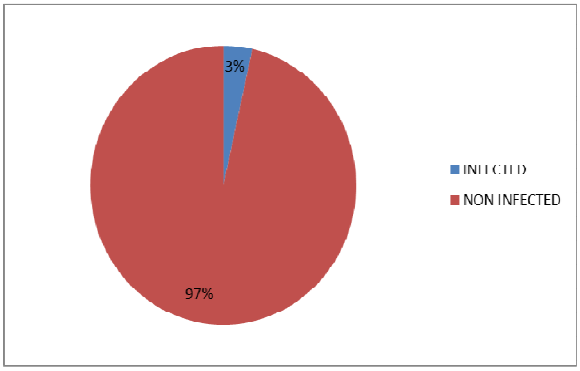

FIGURE 1 B: PREVALENCE OF MALARIA BY RDT

Knowledge of malaria

Majority of the respondents 354(88.5%) ascribed the causes of malaria to receiving bite from a female *Anopheles* mosquito, about 20(5%) said it is caused by extended duration under the sun while others mentioned poor hygiene 12(3%), dirty environment 6(1.5%) and poor diet 8(2%).

Probing further on their ability to recognize signs and symptoms revealed that headache 206(51.5%) was the most recognized signs followed by fever 104(26%), dizziness 38(9.5%) and vomiting 24(6%) (Table 2).

TABLE 1: SOCIO-DEMOGRAPHIC BACKGROUND OF RESPONDENTS

	CATEGORY	FREQUENCY
ACADEMIC LEVEL		
	Undergraduate	340(85%)
	Postgraduate	60(15%)
GENDER		
	Male	168(42%)
	Female	232(58%)
AGE		
	15-20	80(20%)
	21-25	140(35%)
	26-30	80(20%)
	31-35	62(15.5%)
	36-40	30(7.5%)
	41-46	8(2%)
ETHNICITY	Yoruba	220(55%)
	Igbo	87(21.8%)
	Northerners	23(5.7%)
	South-South	70(17.5%)
MARITAL STATUS	Single	342(85.5%)
	Married	58(14.5%)

TABLE 2: IDENTIFICATION OF THE CAUSE OF MALARIA AND ITS SYMPTOMS

Variables	Responses	Frequency
Cause of malaria		
	Mosquito bite	354(88.5%)
	Poor hygiene	12(3%)
	Dirty environment	6(1.5%)
	Sun	20(5%)
	Poor diet	8(2%)
Signs and symptoms of malaria		
	Headache	206(51.5%)
	Fever	104(26%)
	Dizziness	38(9.5%)
	Vomiting	24(6%)
	Body Pain	20(5%)
	Loss of appetite	8(2%)

Treatment seeking behaviour

More than half of the respondents (202) visit the hospital when any sign of malaria is suspected while 130 respondents patronize pharmaceutical outlets, drug hawkers (37) or use traditional remedy (31) Figure 2.

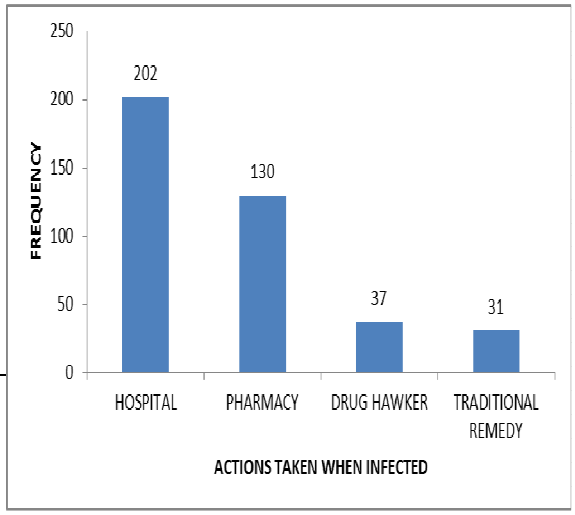

FIGURE 2: TREATMENT SEEKING BEHAVIOUR OF RESPONDENTS

Malaria Preventive measures employed by respondents

More than half (239) of the respondents claimed to possess Long Lasting Insecticide Treated Bed Nets (LLIN), sixty-five (16.2%) use various kinds of topical mosquito repellants, while 54 (13.5%) try to keep their various rooms clean as a way of preventing the breeding of mosquito. Of the total respondents that possess LLIN, only 74 use it consistently, the others either use it occasionally (62) or do not use it at all (103) Table 3.

Burden of malaria amongst the respondents

Very few of the respondents (4) reported to have had malaria in the last three days preceding the survey. Probing into the frequency of malaria bouts amongst the respondents revealed that, half (50%) usually have malaria twice every year, 35% have it once every year while 15% could not remember how often they do have it. More than three-quarters (360) said they have been absent from school at one point in time or the other as a result of malaria. Of the total number of respondents absent from school, more than half (240) were absent for less than 10 days while 80 were absent for more than 10 days.

Only 140 (38.9%) of the respondents have been hospitalized as a result of malaria and 15% (60) of the respondents were aware of malaria related deaths in the school community (Table 4).

TABLE 3: MALARIA PREVENTIVE MEASURES USE BY RESPONDENTS

Variables	Responses	Frequency (%)
Type of preventive measure employed		
	Long Lasting Insecticide Bed Net(LLIN)	**239(59.8%)**
	Insecticide	**42(10.5%)**
	Removal of dirty plates from the room	**54(16.2%)**
	Use of mosquito skin repellant	**65(16.2%)**
Consistency of LLIN use		
	Always	**74(31%)**
	Occasionally	**62(25.9%)**
	Never	**103(43.1%)**

TABLE 4: BURDEN OF MALARIA AMONGST THE RESPONDENTS

	RESPONSE	FREQUENCY (%)
Have you had malaria in the last three days		
	Yes	4(1%)
	No	396(99%)
How often do you have malaria		
	Once a year	140(35%)
	Twice a year	200(50%)
	Do not remember	60(15%)
Ever absent from school due to malaria		
	Yes	360(90%)
	No	40(10%)
Number of days absent		
	Less than 10 days	240(66.7%)
	More than 10 days	80(22.2%)
	Above 20 days	40(11.1%)
Were you hospitalized		
	Yes	140(38.9%)
	No	160(44.4%)
	No response	60(16.7%)
How long were you hospitalized		
	Less than 10 days	95(67.9%)
	More than 10 days	35(25%)
	Above 20 days	10(7.1%)
Any knowledge of malaria related death in this community		
	Yes	60(15%)
	No	340(85%)

DISCUSSION

Approximately half of those countries with ongoing malaria transmission are on track to meet the World Health Organization's target of reaching the elimination stage by year 2030 (20). In order to achieve this target in Nigeria, there is need to evaluate the occurrence of malaria in different foci vis-à-vis preventive measures and treatment options.

This present study recruited more undergraduates than the post-graduates. In like manner, majority were females with few males and were in their early twenties and it is understandable that more than half of the respondents were Yorubas, perhaps due to the geographical location of the school.

The prevalence of malaria in this study area is very low as detected by both techniques used. This could be due to a number of reasons such as time of survey (carried out in the dry season), regular fumigation of the school hostels to destroy mosquito breeding habitants amongst others. The findings of this study is in contrast to that reported in other studies carried out in Universities in South eastern (7, 21) and western parts of the country (22) where more than half and almost half of the students were found infected with falciparum malaria respectively. This disparity in prevalence could be due to difference in effort put towards clearing the vegetation around the hostels or the period of the survey.

It is interesting to know that the students in this survey have a good knowledge about the cause of malaria, ascribing it to mosquito bite, though very few of them still mentioned sun and poor hygiene as probable cause of malaria. It is very important that awareness be carried out so as to enlighten everyone about the transmission and cause of malaria as this would make more of the populace utilize more malaria preventive options. This good knowledge was also demonstrated in their ability to identify signs and symptoms of malaria as more than half of them mentioned headache, others were able to associate fever, dizziness and vomiting to malaria. Although fever is a symptom of malaria, it is very important to emphasize that it could also result from other non-malarial illnesses, therefore the importance of malaria diagnosis should be emphasized in order to prevent non-specific drug use.

Probing further to know what action is taken as soon as they suspect malaria revealed that, majority of the students quickly visit the University Medical Centre, while others prefer to go to pharmaceutical outlets, drug hawkers or use traditional remedy. Visiting the pharmaceutical outlet should not be a cause for alarm if the cause of infection has been prior ascertained or perhaps there is a resident Pharmacist to prescribe the drug not just by indiscreet buying of drugs which could contribute to the problem of drug resistance.

Furthermore, the study revealed that more than half of the students claim to possess LLIN, however not up to half of these students regularly sleep under the treated net. This is another area calling for sensitization as many possessor of LLIN keep it unused in their homes. This is one of the preventive strategies the World Health Organization has outlined, other measure such as Indoor Residual Spraying of the hostel environment and use of chemoprophylaxis amongst others could be successfully implemented in such academic setting where the populace is small compare to larger communities (20, 23).

Very few students reported to have had malaria episodes in the three days preceding survey, although it was not made known if the episode was self-ascertained or laboratory-determined. Half of the students reported having two malaria bouts in a year, while more than one-quarter reported one bout per year and others could not remember the frequency. These repeated malaria bouts resulted in absenteeism by more than three quarters of the students leading to valuable loss of time in academic work and even performance as majority of them spent less than 10->20 days in hospital, an appreciable amount of time that could have been spent on their academics. Therefore, proactively placing preventive measures would have negated these cascades of effects amongst the students. Continuous studies should be carried out to assess the prevalence of malaria in different foci of Nigeria and the level of awareness of the foci community members should be assessed in order to prioritize control strategies and meet the elimination target by 2030.

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REVIEW ARTICLE

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HAEMATOLOGICAL PROFILE OF HIV SEROPOSITIVE HAART NAIVE CLIENTS IN KOGI STATE UNIVERSITY TEACHING HOSPITAL, ANYIGBA, KOGI STATE. NIGERIA

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ABSTRACT:

Examination of haematological profile is useful for baseline assessment, treatment monitoring and prognostic evaluation in Hiv/AIDS management. The objectives of the study was to assess the haematological profile of HIV seropositive HAART naive patients who attended Kogi State University Teaching Hospital, Anyigba, Kogi State, Nigeria between January 1, 2014 and December 31, 2014. The study was prospective experimental research. Ethical clearance was obtained from ethical committee of Kogi State University Teaching Hospital, Anyigba. Written and verbal informed consent was taken from all patients. A total of 404 HIV seropositive HAART naive patients comprising 147 (36.4%) males and 257(63.6%)females were examined. The overall mean age of patients was 33.0 ±12.7 years and female-male ratio was 1.7: 1. Half of respondents 200(50.4%) accessed HIV care and treatment for the time in stage three HIV disease. Patients had overall mean CD4 cells count of 381.8 ± 240.8 cells/mm^3 , white blood cells count of 5.8 ± 3.6 × 10^9/L, lymphocyte count 2.3 ±1.3, granulocyte count 0.8 ± 0.8 and platelet count of 260.0 ± 109.1 × 10^9/L. The mean packed cell volume was 34.1± 5.9 %, haemoglobin 11.3 ± 2.1 g/dl and mean corpuscular haemoglobin concentration was 31.5 ± 14.3 g/dl. The packed cells volume (p=0.0001, T test= 4.0259) and haemoglobin (p=0.0001, T test= 4.1534) profiles of HIV clients were respectively statistically significance with gender, while CD4 cells count (p=0.004, F= 4.523) and platelet count (p= 0.008, F = 3.974) were significance respectively with HIV disease staging. The study recommended the need for awareness programs, nutritional education and micronutrients supplementation including multivitamins for HIV clients.

Keywords: Haematological, HIV, HAART, patients, KSUTH, Nigeria.

PROFIL HEMATOLOGIQUE VIH SEROPOSITIVE HAART CLIENTS NAIFS A L'HOPITAL UNIVERSITAIRE D'ETAT DE KOGI,ANYIGBA, ETAT DE KOGI, NIGERIA.

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RESUME

L'examen du profil hématologique pour l'évaluation de base, le contrôle du traitement et de l'évaluationpronostique dans la gestion du VIH/SIDA. Le but de l'étude était d'évaluer le profil hématologiquedes HAART séropositifs au VIH chez les patients naïfs qui fréquentaient l'hôpital universitaire d'état de Kogi, Anyigba, état de Kogi, Nigeria entre 1er janvier, 2014 et 31emedécembre, 2014. L'étude était la recherche expérimentale prospective. L'approbation éthique a été obtenue du comité de l'hôpital universitaire d'état de Kogi, Anyigba. Le consentement informé verbal et écrit a été prise de tous les patients. Un total de 404 VIH séropositifs HAART patients naïfs comprenant 147 (36,4%) hommes et 257 (63,6%) femmes ont été examinés. L'âge moyenglobale des patients était de 33,0 ±12,7 ans et le ratio femme – homme était 1,7 : 1. La moitié des répondants 200 (50,4%) ont accédé les soins et le traitement du VIH pour le moment à la troisième phase de la maladie de VIH.Les patients avaient la numération moyenne globale des cellules CD4 de 381,8 ± 240,8 cellules/mm^3, numération des globules blancs de 5,8 ± 3,6 x 10^9/L, nombre de lymphocyte 2,3 ± 1,3, nombre de granulocyte 0,8 ± 0,8 et numération plaquettaire de 260,0 ± 109,1 x 10^9/L. Le

volume cellulaire moyen emballé était 34,1 ± 5,9%, hémoglobine 11,3 ± 2,1 g/dl et la concentration corpusculaire moyenne en hémoglobine était 31,5 ± 14,3 g/dl. Le volume des cellules emballés (p=0,0001, T test = 4,0259) et les profilsd'hémoglobines (p=0,0001, T test = 4,1534) des clients VIH étaient respectivement statistiquement significative avec le sexe tandis que la numération des cellules CD4 (p=0,004, F= 4,523) et la numération plaquettaire (p= 0,008, F= 3,974) étaient respectivement signification avec le stade de la maladie VIH. L'étude a recommandé la nécessité pour les programmes de sensibilisation, l'éducation nutritionnelle et micronutriments supplémentation y compris des multi vitamines pour les clients du VIH.

Mots clés : Hématologique, VIH, HAART, KSUTH, Nigeria.

INTRODUCTION

The World Health Organization (WHO) and United Nations Agency for International Development (UNAID) in 2013 reported that about 35 million people worldwide and 3.4 million Nigerian were living with human immune deficiency virus (HIV). HIV has a great prediction for the CD4 glycoprotein receptor on the surface of immune cells such as the T helper cells, monocytes, macrophages and dentritic cells[4-7]. Examination of haematological profile especially packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpsular Haemoglobin concentration (MCHC), CD_4 Count, white blood cell count (WBC) and platelet count are cardinal as baseline assessment, treatment monitoring and prognostic evaluation in HIV/AIDs management[8-16]. Anaemia is the most common cytopenia in HIV complication and is characterized by packed cells volume (PCV) less than 30%, haemoglobin below 10milligram/deciliter and typically of normochromic and normocytic morphology in 10–20% of patients with HIV infection at diagnosis and the prevalence can range from 66–85% during the course of the disease[8,17,18]. Common causes of normocytic anaemia in HIV patients are chronic diseases, infectious agents such as parvovirus B_{19} or Mycobacterium avium complex (MAC), thrombotic thrombocytopenic purpura (TTP), drug toxicities, autoimmunity and hypersplenism[19-21]. The main mechanism of anaemia of chronic disease is due to haemopoiesis suppression of marrow progenitors by HIV infected T cells[22].

Microcytic anaemia with mean corpuscular volume (MCV) below 80 femto litres are described in HIV patients with iron deficiency anaemia as sequelae of hookworm parasitic infestation, intestinal malabsorption (Giardiasis, Crytosporidosis), lactose intolerance (chronic infection), gastrointestinal bleeding (shigellosis, amoebiasis) and nutritional deficiency[19,23,24]. Megaloblastic or macrocytic anaemia are uncommon except in HIV infected patients on zidovudine, cotrimoxazole and cytotoxic drug therapy[19]. Most patients develop macrocytosis after 2 weeks of zidovudine initiation and have mean corpscular volume (MCV) greater than 110 femoliters[19]. Macrocytosis can be used as a prognostic marker of zidovdine adherent though is dose dependent and correlate with the climax stage of the disease[19]. The mechanism of megaloblastic anaemia in HIV infected patients on cotrimoxazole therapy is attributed to folate antagonism especially in patients with nutritional deficiency[19]. Anaemia and hypochromic red cells morphological pattern were reported in HIV clients among iron deficiency and thalassemic patients, while hyperchromia were described among sickle cell disease and hereditary spherocytosis. Anisocytosis, poikilocytosis and rouleaux formation has also been noted in the peripheral blood films of some HIV patients[25-27]. Granulocytopenia including neutropenia and abnormal granulocyte functions are problems commonly encountered in patients with HIV infection[28-29]. Neutropenia (Neutrophil count below 1.5 x $10^9/_L$ may occur in 10-30% of HIV patients typically with advanced disease. The pathogenesis of granulocytopenia in patients with HIV infections is multi-factorial. The mechanism may involves direct effect of HIV infection in the bone marrow, opportunistic infections (cytomegalovirus, mycobacterium avivum complex, tuberculosis, histoplasmosis and leishmaniasis), auto immune disease, infiltrative disorders (infection, maligancy) and drug toxicity (zidovudine, starvudine, Cotrimoxazole and antituberculous; ironiazid, rifampicin and rifabution)[28-29].

The other morphological findings observed in HIV infection include hypogranular giant meta myelocyte, toxic granulation, dohle bodies and cytoplasmic vacuolation in the peripheral blood smear. The CD_4 T lymphocyte count in HIV infected patients were described to relate or correlate with the degree of immune suppression and the clinical stage of the patients. A normal CD_4 T lymphocyte count in a healthy, HIV negative adult is usually between 500 and 1500 cells per cubic millimeter of blood[30]. In people with untreated HIV infection, the CD_4 cells count decline by approximately 50–80 cells per cubic millimeter per year. The pattern of decline may be slow and steady or the CD_4 cells count may level off for an extended period of time and then decrease[31].

Thrombocytopenia occurs in 15-60% of patients with AIDs and is seen in 10% of patients at early stage of HIV infection. The thrombocytes are usually of normal morphology except where there is immune destruction. The possible aetiologies of thrombocytopenia in HIV infection include immune mediated destruction, thrombocytopenic purpura, impaired haemopoicsis, drug toxicity (heparin,

quinidine), alcoholism, splenomegaly and liver disease[31-32].

The objective of the research was to study the haematological profile of HIV seropositive HAART naïve patients in Kogi State University Teaching Hospital Anyigba between January1, 2014 to December 31, 2014.

MATERIALS AND METHODS

Materials used were Sysmex XP 300 haematology analyzer, XP300 cell pack, stromatolyser cellclean, eightcheck 3WP, JIK solution, 7½ surgical gloves, cottonwool and methylated spirit. Other materials were partec cyflow counter cylometer, partec cleaning, sheat and decontamination fluids. The materials also include partec CD_4 and CD_4% easy count kits, rohren tubes, pipettes, tubes rack, EDTA tubes (5mls), determine HIV Test kits with LOT number 38099k300 and unigold HIV test kits with LOT number 2010143. The research was prospective and experimental study that was conducted between January 1, 2014 to December 31, 2014. 404 HIV seropositive clients who attended Kogi State University Teaching Hospital Anyigba after screening with determine and umgold HIV test kits were studied. Ethical approval for the study was obtained from the Ethical committee of the Kogi State University Teaching Hospital Anyigba Kogi State Nigeria.

TABLE 1: DISTRIBUTION OF RESPONDENTS BY DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

(A) Age distribution of respondents:

Age	Frequency 404(100%)
0-9	22(5.4)
10-19	13(3.2)
20-29	125(30.9)
30-39	140(34.7)
40-49	64(15.8)
50-59	28(6.9)
>60	12(3.0)

(B) Distribution of respondents by gender:

Gender	Frequency 404(100%)
Male	147(36.4)
Female	257(63.6)

(C) Distribution of respondents by HIV disease stage:

Clinical Stage	Frequency 404(100%)
1	72(18.1)
2	121(30.9)
3	200(50.4)
4	11(2.7)

Verbal and written informed consent was obtained from all patients. History and examination was conducted on all patients to obtain biodata, weight, clinical staging and risk of opportunistic infections including pulmonary tuberculosis. Whole blood sample was collected between 8.00am to 10.00am by veno- puncture into 5ml vacutainer tubes containing EDTA anticoagulant and processed within 4hours of collection. Sysmex XP– 300™ automated hematology analyzer was used after calibration to estimate packed cell volume (PCV), haemoglobin (Hb), mean corpscular haemoglobin concentration (MCHC), whole blood cells (WBC), granulocytes, lymphocytes, eosinophils and platelet counts. Flow cytometry using partec cyclow counter flow cylometer was used to determine CD_4 T lymphocyte. Data analysis was done using SPSS version 20 statistical software. The study was conducted at a predetermined $p<0.05$ and 95% confidential interval. Data were generated into cross tabulations, T test and analysis of variance(ANOVA) were conducted to determine statistical significance among the variables.

RESULTS

A total of 404 HIV seropositive HAART naive patients comprising 147 (36.4%) males and 257(63.6%) females were examined. The overall mean age of patients was 33.0 ±12.7 years and female-male ratio was 1.7: 1. Half of respondents 200(50.4%) accessed HIV care and treatment for the time in stage three HIV disease.

TABLE 2: THE BASELINE MEAN HAEMATOLOGICAL PROFILE OF HIV SEROPOSITIVE HAART NAIVE CLIENTS IN KSUTH, ANYIGBA, KOGI STATE

HAEMATOLOGIC PROFILE	DISTRIBUTION OF MEAN AND STANDARD DEVIATION
CD_4	381.8 ± 240.8 Cells /mm^3
WBC	5.8 ± 3.6 x 10^9/L
Lymphocyte	2.3 ± 1.3
Granulocyte	0.8 ± 0.8
Platelet	260.0 ± 109.1 x 10^9/L
PCV	34.1 ± 5.9%
Haemoglobin	11.3 ± 2.1 g/dl
MCHC	31.5 ± 14.3 g/dl

TABLE 3: THE DISTRIBUTION OF HAEMATOLOGICAL PROFILE OF HIV SEROPOSITIVE HAART NAÏVE CLIENT BASED ON CLINICAL DISEASE STAGING

HIV DISEASE STAGING	CD_4	WBC	LYMPHOCYTE	GRANULOCYTE	PLATELET	PCV	HB	MCHC
1(n=72)	464.6±258.0	5.7±2.2	2.4±1.3	0.62±0.5	255.2±109.2	35.0±6.3	11.4±2.2	30.9±1.1
2(n=121)	390.9±219.7	6.4±5.4	2.3±1.3	0.8±0.8	225.3±97.7	34.5±4.8	11.4±1.9	33.3±26.0
3(n=200)	346.0±238.5	5.5±2.5	2.2±1.2	0.8±0.8	263.4±116.4	33.6±6.3	11.1±2.2	30.6±1.3
4(n=11)	390.9±242.9	5.8±3.0	2.9±2.1	0.7±0.4	304.3±104.8	31.7±6.0	10.6±2.0	31.3±2.2
F Test	4.523	1.602	1.344	1.165	3.974	1.836	0.998	0.948
P value	0.004	0.188	0.260	0.323	0.008	0.140	0.394	0.417
Df	403	403	403	403	403	403	403	403

Patients had overall mean CD4 cells count of 381.8 ± 240.8 cells/mm^3 , white blood cells count of 5.8 ± 3.6 × 10^9/L, lymphocyte count 2.3 ±1.3, granulocyte count 0.8 ± 0.8 and platelet count of 260.0 ± 109.1 × 10^9/L. The mean packed cell volume was 34.1± 5.9 %, haemoglobin 11.3 ± 2.1 g/dl and mean corpuscular haemoglobin concentration was 31.5 ± 14.3 g/dl. The packed cells volume (p=0.0001, T test= 4.0259) and haemoglobin (p=0.0001, T test= 4.1534) profiles of HIV clients were respectively statistically significance with gender, while CD4 cells count (p=0.004, F= 4.523) and platelet count (p= 0.008, F = 3.974) were significance respectively with HIV disease staging.

TABLE 4: THE GENDER DISTRIBUTION OF BASELINE HAEMATOLOGICAL PROFILE OF HIV SEROPOSITIVE HAART NAÏVE CLIENT IN KSUTH, ANYIGBA, KOGI STATE

Gender	CD_4 Cells/mm^3	WBC x 10^9/L	Lymphocyte	Granulocyte	Plalelet 10^9/L	PCV (%)	HB g/dl	MCHC g/dl
Male 147(36.4)	**377.2±181.9**	**5.94±3.2**	**2.36±1.6**	**0.8±1.0**	**262.6±131.7**	**35.6±6.5**	**11.8±2.4**	**32.8±23.6**
Female 257(63.6)	**384.4±245.0**	**5.8±3.9**	**2.2±1.1**	**0.7±0.7**	**258.6±94.3**	**33.2±5.3**	**10.9±1.9**	**30.7±1.1**
TOTAL 404(100)	**381.8±240**	**5.8±3.6**	**2.3±1.3**	**0.8±0.8**	**260.0±109.1**	**34.1± 5.9**	**11.3±2.1**	**31.5±14.3**
T Test	**0.3106**	**0.3698**	**1.1866**	**1.1768**	**0.3537**	**4.0259**	**4.1534**	**1.4251**
Df	**402**	**402**	**402**	**402**	**402**	**402**	**402**	**402**
P value	**0.756**	**0.712**	**0.2361**	**0.240**	**0.7238**	**0.0001**	**0.0001**	**0.1549**

DISUSSION

Two third of HIV clients were found in the 20 – 39 years age group categories. This finding was collaborated in many comparative studies[34-44]. Most respondents belonged to the female gender. The female genital anatomy was reported to increase the risk of sexually transmitted infection including AIDs[41]. Access to reproductive health including HIV treatment and care was noticed to be higher in female than males[42]. Half of the HIV client presented at the facility for the first time in HIV disease stage three to access treatment . This observation of client presentation in advance HIV disease when complications have developed was reported in sexually transmitted infection clinic in Ile Ife by **Olayinka[38].** Stigmatization, ignorance, belief and poor health seeking behavior were among many reasons attributed to such behavior. The mean CD_4 cell count was low among HIV clients, while the CD4 profile based on the HIV disease staging was statistically significance (p=0.004) in this study. This observation was also reported by many Researchers [43-44]. CD_4 lymphocyte cells also known as T cells or T helper cells are the primary targets of HIV [45]. The degree of immuno-suppression as assessed by the CD_4 cell counts closely correlates with the HIV disease stage as reported in past studies[43-44]. The mean white blood cells count, granulocyte and platelet counts in this study lied within normal limit. The client's HIV disease staging was statistically significance with platelet count (p=0.008). Some researchers in their separate studies observed leucopenia, granulocytopenia and thrombocytopenia in patients with HIV infection[10,15,46]. Cytopenias are one of the most common complications of HIV. The mechanism of cytopenia in HIV clients is multi-factorial in dimension. The mechanisms were described to relate with direct effect of HIV on the bone marrow, opportunistic infections, autoimmune diseases, infiltrative disorders and dry toxicity [28-29]. However, lymphocyte count in this study was found to be high. This study recorded low mean packed cell

volume and haemoglobin concentration, which also correspond to findings obtained by many researchers [21,47-49]. The profile of packed cells volume and haemoglobin based on gender in this study were statistically significance (p= 0.0001). The main mechanism of anaemia of chronic disease is due to haemopoiesis suppression caused from direct suppression of bone marrow progenitors by HIV infected T cells. In this study the mean corpsular haemoglobin concentration (MCHC) was within normal limit. This finding was however in contrast to low MCHC value recorded by Osunkalu [50].

CONCLUSION

Most clients in this study were youth in the 20- 39 years age category and presented for the first time in HIV disease stage three. The clients have low mean CD4 cells count, packed cells volume and haemoglobin profile. Gender was statistically significance with packed cells volume and haemoglobin, while the client's HIV disease staging was significance with CD4 cells and platelet counts. This study recommended the need for awareness campaigns, nutritional education and micronutrients supplementation including multivitamins for HIV clients.

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REVIEW ARTICLE

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A REVIEW OF LASSA FEVER, AN EMERGING OLD WORLD HAEMORRHAGIC VIRAL DISEASE IN SUB-SAHARAN AFRICA

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ABSTRACT

Lassa fever is an acute immunosuppressive illness of increasing public health concern causing severe morbidity and significant mortality (Case fatality rate (CFR) ≥ 50%) especially in epidemic cases. Although Lassa fever has emerged (following its first detection (1969) in Lassa town, Nigeria) as one of the most prevalent and debilitating viral haemorrhagic fevers endemic in West Africa region (Nigeria inclusive), yet, the control/prevention of the regular outbreak of the disease has become an herculean task in the areas affected; there is inadequate healthcare facility (including Laboratory/diagnostic and care centres), poor socio-economic environment, lack of awareness among the populace and presence of favourable ecologic niche for the survival and propagation of the natural host and reservoir mouse (*Mastomys natalensis*) of Lassa virus . Lassa fever is mainly transmitted by contact with excretions and secretions of infected rats via foods and water as well as exposure to other contaminated items. Lassa virus is a member of an Old World Arenariruses, of family *Arenaviridae*. It is an enveloped, single-stranded (SS) bisegmented RNA virus with ability to replicate very rapidly. It consists of 4 lineages; 3 members are identified as ancenstral strains found in Nigeria, while the fourth is domiciled in other West Africa Countries. Lassa virus infects almost every tissue in human body resulting in multisystemic dysfunction. The incubation period is generally between 6 to 21 days resulting in 3 stages of clinical manifestation viz: Acute phase characterized by flu-like, non-specific illness; haemorrhagic phase accompanied with gastrointestinal symptoms and cardiovascular/neurologic complications. Currently, there is no clinically certified Lassa fever vaccine thus complicating deterrent or preventive measures. Hence, there is need for intensification of educational programs for the populace on the useful control measures against Lassa fever. The stakeholders need to prioritize intervention and support program and also speed up the processes leading to the production of effective vaccine to limit the menace of Lassa fever outbreak and associated morbidity, fatality and high socio-economic cost.

Key words: Lassa fever, endemic, epidemic, reservoir rodent, West Africa.

UN EXAMEN DE LA FIEVRE DE LASSA, UNE MALADIE VIRALE HEMORRAGIQUE EMERGENTE D'ANCIEN MONDE EN AFRIQUE SUB – SAHARIENNE.

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RESUME

La fièvre de Lassa est une maladie immunosuppressive aiguë de plus en plus préoccupant de la sante publique causant plusieurs morbidités et de mortalités (taux de fécondité de cas CFR ≥ 50%) en particulier dans les cas d'épidémie. Bien que la fièvre de Lassa a émergé (après sa premièredétection (1969) dans la ville Lassa, Nigeria) comme l'une des fièvres virales hémorragiques les plusrépandues et débilitantes dans la région d'Afrique de l'Ouest (le Nigeria inclus), mais le contrôle et la prévention de l'épidémierégulière de la maladie est devenue une tache herculéenne dans les zones touchées ; il y a des facilités inadéquates de soins de santé (ycompris les laboratoires /centres de diagnostic et de soins),une mauvaise environnement socio – économique, le manque de sensibilisation de la population et la présence de niche écologique favorable pour la survie et la propagation de l'hôte naturel et le réservoir souris (*Mastomysnatalensis*) de virus de Lassa. La fièvre de Lassa se transmet principalement par contact avec les excrétions et secrétions des rats infectés à travers des aliments et de l'eau ainsi que l'exposition à d'autres objets contaminés. Virus de Lassa est membre de famille Arénavirus*Arenaviridae*d'Ancien Monde. C'est un virus à ARN enveloppé, simple brin (SS), àdeux segmenté avec la capacité de se répliquer rapidement. Il se compose de quatre lignées, 3 membres sont identifiéscomme des souches ancestrales trouvé au Nigeria, tandis que le quatrième est domicilié dans d'autres pays africain. Virus de Lassa infecte presque tous les tissus dans le corps humain entrainant a un dysfonctionnement multi systémique. La période d'incubation est généralement entre 6 à 21 jours résultant en 3 étapes de

manifestations cliniques à savoir : la phase aiguëcaractérisée par la grippal, une maladie non – spécifique, la phase hémorragiqueaccompagnée par des symptômes gastro – intestinaux et les complications cardiovasculaires/neurologiques. Actuellement il n'y a pas de vaccine certifié cliniquement contre la fièvre de Lassa compliquant ainsi de dissuasion et des mesures préventives. Par conséquent, il est nécessaire d'intensifier des programmes éducatifs pour la population sur les mesures de contrôle utiles contre la fièvre de Lassa. Les parties prenantes doivent donner la priorité à l'intervention et le programme de soutien et également d'accélérer les processus menant à la production de vaccine efficace pour limiter la menace de l'épidémie de la fièvre de Lassa et de la morbiditéassociée, la fatalité et le coût socio – économiqueélevé.

Mots clés : La fièvre deLassa, endémique, l'épidémie, réservoirde rongeurs, Afrique de l'Ouest.

INTRODUCTION

Lassa fever is an acute immunosuppressive and multisystemic viral disease characterized by severe morbidity and high mortality especially during epidemic outbreak and among hospitalized patients [1-3]. It is one of the most common viral haemorrhagic fevers endemic in sub-Saharan Africa, particularly West Africa sub-region (Nigeria inclusive). [4-6] Increasing outbreak of Lassa fever in the past decade involving expanded region of endemicity with serious public health and socio-economic implications has become worrisome[2,5].

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Lassa virus (a member of *Arenaviridae* family and Old World Arenaviruses) [7] was first discovered in 1969 at a small town of Lassa in Borno State, Northeast Nigeria [8]. It's reservoir and natural host was later identified as the Natal multimammate (with many beasts) African mouse (*Mastomys natalensis*), commonly found in the forest and Savannah grass land of sub-Saharan Africa [9]. These rodents (with inherent capacity to reproduce at high rate) shuttle between surrounding bushes and human houses in villages, towns and cities where they co-habit human populace in their residences and commercial or business centres [6].

Reports of various investigations [2,8,9] have suggested that Lassa virus is probably transmitted by contact with excretions or secretions (including faeces and urine) of infected rats accessing food items and water inside human residences and other centres with human activities. Other possible routes of transmission of Lassa fever such as broken skin or mucus membrane directly exposed to infectious material have also been suggested by other investigators [5,9]. Epidemics arising from human-to-human transmission have equally been established in healthcare institutions in Africa [10].

Lassa fever virus infects about half a million people in countries where the disease is endemic (including Nigeria, Guinea, Sierra Leone, Liberia as well as Central Africa Republic, (CAR) and recently Senegal and Mali[5] resulting in over 5,000 deaths annually. [6] However, between 70-80% of Lassa virus infection remains asymptomatic, mild or self-limiting and in most cases may pass unnoticed. Nonetheless, about 20-30% of cases progress to severe disease condition and fatality rate may be up to 50 percent or more in such situation [2,11,12]. Increased population (with population explosion in some poor-resourced areas of sub-Saharan Africa), large scale deforestation (by either natural or manmade e.g. for industrial, housing and other social facility thus depriving the rodents of their natural habitat) and poor environmental hygiene are believed to contribute to the increased incidence of Lassa fever in the affected areas of West Africa [6,13].

Lassa fever is endemic in Nigeria. However, the increasing frequency of epidemic outbreak of the disease in the last decade has become worrisome in view of its threat to public health and associated severe morbidity, significant mortality and high socio-economic cost [11]. The national government's efforts geared towards curtailing the regular outbreak of Lassa fever in Nigeria via public enlightenment campaigns especially during epidemic outbreak of the disease have not yielded the desired results. Therefore, the present effort is meant to further sensitize the stakeholders in healthcare system and the populace about the health and socio-economic consequences/effects of the menace of Lassa fever, and the crucial need to adopt effective control/preventive strategies to checkmate the increasing menace of the disease, and thus limit associated morbidity, mortality and high socio-economic cost in this environment.

Epidemiological Trend of Lassa Fever

Previous studies [2,6,7,13] have reaffirmed the initial widespread speculation that Lassa virus probably evolved from the Eastern part of sub-Saharan Africa, and then gradually spread to the West African sub-region. A large area of West Africa is now considered as Lassa fever belt due to its recurrent outbreak in that geographical location [14].

As earlier stated, Lassa fever virus was first detected (1969) and reported (1970) by Frame and his colleagues [8] in Lassa town (from where the virus derived its name) located in the North east geopolitical zone of Nigeria. The first victim of Lassa

virus infection is believed to be an American Missionary working in the area who later died of complications arising from the illness. Two other female Nurses who attended to the index case also contracted the disease; one of them later died while her counterpart survived after she was flown to the United States (U.S.) for medical care. Tissues and blood specimens were collected from index case for analysis through which Lassa virus was identified [8].

Since its historical discovery in Nigeria, Lassa virus has been transported across borders of Central and West Africa affecting between 300,000 – 500,000 people (predominantly) in Nigeria, Guinea, Sierra Leone and Liberia (Table 1) resulting in over 5,000 deaths annually [2,6,15]. In endemic situation, the overall case fatality rate (CFR) of Lassa fever is estimated to be in the range of 1-10 percent. However, during epidemic outbreak, the CFR of Lassa virus may be up to 50 percent while higher rate has been recorded in severe cases [3,9,10,14]

.

In contrast to the above scenario, there is relatively low incidence of Lassa fever in advanced countries of the West including North America and parts of Europe [16]. Hence, the risk of contracting the disease or its possible large scale transmission or spread among the populace is highly limited. Understandably, aside the near absence of the natural reservoir or host animal (*M. natalensis*) of Lassa virus in the aforementioned areas, availability of highly developed environmental hygiene and sanitation, as well as large scale accessibility to advance and well-managed social and healthcare infrastructure appear to greatly influence the low risk status of those industrialized countries of the West [15,17]. Nonetheless, there have been sporadic cases of Lassa fever outbreak among travellers and tourists returning from endemic regions to Europe and America [16,18,19].

It is estimated that between 15-20% of all hospitalized Lassa fever patients are likely to die from the illness if appropriate medical intervention is not instituted early [3,10] On the other hand, epidemiological and immunological studies have shown that immunity developing in those who survived Lassa virus infection is long lasting [20,21].

In Nigeria, Lassa fever outbreak has been a recurrent event over the last two decades. In recent outbreak of the disease (August, 2015-March, 2016), over 200 people were affected across 18 States (out of 36 States and Federal Capital Territory, Abuja) of Nigeria. [22] Initially, there were 92 confirmed cases which resulted in 43 deaths constituting 46.7% CFR. By the end of March, 2016, the number of deaths had risen to 80, making it one of the worst outbreaks of Lassa fever in Nigeria in recent times [22].

TABLE 1: PREVALENCE OF LASSA FEVER IN THE POPULATIONS OF MOSTLY AFFECTED COUNTRIES IN WEST AFRICA

Prevalence of Lassa fever	
Country	**Range in percentage**
Nigeria	**21 – 45**
Guinea	**4 – 55**
Sierra Leone	**8 – 52**
Liberia	**12 – 48**

Sogoba *et al.*, (201)[6]; WHO, (2016)[2]

Mechanism of Transmission of Lassa fever

Lassa fever is a zoonotic disease (ie infectious disease of animal or originating from animal source) transmitted to humans via contact with an infected rodent (*M. natalensis*), or through inhalation of air contaminated with infected rat's excretions or excretions such as faeces, urine or nasal discharges (aerosols) [9,23]. Lassa virus infection can also be acquired through broken skin or mucous membrane directly exposed to infectious material or item [5,9,18]. Nosocomial acquisition of Lassa virus infection is mainly through contact with infected patient, exposed hospital workers or unscreened infected blood [24]. Such blood and its products pose a serious risk to patients receiving them by transfusion in health care institutions. Similarly, direct contact with infected semen, or vaginal fluids including consumption of infected breast milk have been suggested as possible mode of transmission of Lassa fever[24,25].

It has been shown that immunosupression arising from certain underlying communicable or non-communicable diseases, chemotherapy as well as pregnancy (especially if infection occurs during the third trimester) can enhance the acquisition and establishment of Lassa fever, and may aggravate mortality rate pushing it up to about 80 percent [1, 14,15,26]. Infection during pregnancy can lead to fetal death (because the virus has high affinity for placenta and other highly vascularized tissues), abortion, including loss of newborn (in 90% of cases) or maternal death. [3,26,27] Serious congenital defects or abnormalies are common sequelae in children born with Lassa fever infection [27,28].

Virology of Lassa Fever Virus

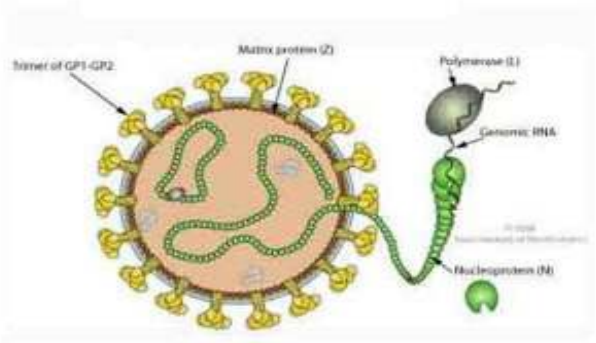
Lassa virus (a member of Arenaviridae family) is categorized under the group known as 'Old World Arenaviruses' on the basis of their antigenic and molecular properties [7,29, 30]. The group consists of Lassa virus and Lymphocytic choriomeningitis virus (LCMV). Lassa virus is characterized by high genetic

variability hence there was initial difficulty regarding the design of primers for Polymerase Chain Reaction (PCR) in molecular studies of the virus. Consequently, some Lassa virus strains were believed to escape, detection by PCR during the early studies [15,21].

Other members of the Old World African Arenaviruses that share similar properties and closely related to Lassa virus include Ippy virus, Mobala and Mopeia (Table 2) [7,13,31,32]. However, these strains of Arenaviruses have not yet been associated with any human disease [7].

Lassa virus is an enveloped, single-stranded (SS) bisegmented RNA virus. It is a rapidly replicating virus but has inherent ability to temporarily control its replication. This attribute preferentially allows the spike proteins component of that virus to be produced last during replication, and therefore, delay the recognition of the virus by the host's immune system. Consequently, the process is believed to aid the virus pathogenicity, pathogenesis and evasion of the host's defense mechanism [33,34].

Studies [35, 36] on Lassa virus genome nucleotide have revealed the existence of lineages of the virus; 3 members of which are found in Nigeria while the fourth was traced to other parts of West Africa including Liberia, Guinea and Sierra Leone [29].



Lassa Fever Virus. Information Nigeria [30]

TABLE 2: PHYLOGENETIC, GEOGRAPHICAL AND PATHOGENIC GROUPING OF ARENAVIRIDAE FAMILY

Group	Arenavirus complex	Geography/virus Species	Family Arenaviridae: Pathogenicity	Associated Disease
1	Old World	Lassa	P	Lassa Fever: Flu-like illness, Gastrointestinal symptoms, Bleeding, Organ failure, Neurological Complications
		Mopeia	NP	NE
		Mobala	NP	NE
		Ippy	NP	NE
		Lymphocytic Choriomeningitis Virus (LCMV)	P	Mild febrile illness, Benign meningitis, Birth defects: Hydrocephalus, Chorioretinitis, Blindness, Deafness, Mental retardation. Lassa fever-like illness in Primates, Calitrichide hepatitis
2	New World	Junin	NA	NE
		Tacaribe	NA	NE
		Pichinde	NA	NE

Bowen *et al.* (1997); [7] Gonzalez *et al.*, (1983); [13] WHO, (2016; [17] Whulff *et al.* (1977); [31] Swanepoel *et al.* (1985) [32].
LCMV- Lymphocytic Choriomeningitis Virus, P- Pathogenic, NP-Non-pathogenic, NE-Not established, NA-Not available.

Pathogenesis of Lassa Fever

The pathogenesis of Lassa fever has not been clearly understood. [20] However, studies [34,37] show that Lassa virus infection leads to immunosuppression while the main targets of Lassa virus once inside the host are the antigen-presenting cells. Lassa virus infects almost every tissue in human body leading to multisystemic dysfunction, and can suppress host's innate interferon (IFN) response by inhibiting the translocation of interferon regulatory factor -3 (IRF-3). In addition, Lassa virus characteristically exhibits exonuclease activity to only double-stranded RNAs (ds RNAs), which often blocks IFN responses. This is achieved through digestion of Pathogen-associated molecular pattern (PAMP), which enables the virus to evade host's immune responses [33,34,37,38].

Clinical Manifestation of Lassa Virus Infection

[3,11,12]. Generally, incubation period ranges from 6 to 21 days [12,15]. The typical case progression can be divided into 3 main stages (Table 3) as shown below.

Stage 1: Prodromal Illness/Acute Stage
At this stage, the onset of the disease mimics malaria or typhoid fever. First, it begins with respiratory flu-like (non-specific illness) symptom characterized by headache, myalgia (general body's weakness) febrile illness (fever ≥ 38^0C, which does not respond to standard treatment for malaria or typhoid; accounts for 10-16% of total cases and about 30% of deaths) cough, pharyngitis (sore throat and back ache). Other signs include tremors chest paid, insommia (restlessness), sometimes rashes coupled with gastrointestinal symptoms including diarrhea and vomiting. [11,24] These early symptoms often appear

Lassa fever is a grave illness of significant fatality (CFR, 40≥ 50%) especially during epidemic outbreak indistinguishable from other bacterial, viral or parasitic infections [24] and can be treated with antiviral drug such as Ribavirin if diagnosed at early stage [39].

Stage 2: Haemorrgagic Stage
This stage involves internal haemorrhage whereby victim bleeds from inside through nostrils, mouth and other orifices resembling that of Ebola. This may lead to organ failure and death [8,21,23].

Stage 3: Neurologic Complications
This constitutes part of the late Stage of the illness manifesting as neurological complications including encephalopathy or encephalitis (Table 3) [28]. The virus can be detected in the urine of infected patient for 3-9 weeks and in semen for up to three months [2].

TABLE 3: STAGES OF CLINICAL MANIFESTATIONS OF LASSA FEVER

Stages/Signs and Symptoms of Lassa Fever:		
Stage 1: Prodromal/Acute Phase	**Stage 2: Haemorrhagic Phase**	**Stage 3: Cardiovascular/Nervous System/ Neurological Complications**
*Flu-like illness characterized by:	* Gastrointestinal Manifestations:	* Hypotension
- Fever (≥38^0C)	- Stomach ache	* Pericarditis
- Headache	- Diarrhoea	* Tachycardia
- Myalgia	- Vomiting	* Hypertension
- Cough	- Dysentery	* Meningitis
- Pharyngitis	- Hepatitis	* Encephalitis
- Chest Pain	- Facial swelling	* Seizures
- Tremors	- Conjunctivitis	
- Back ache	- Muscle fatigue	
- Rashes (in some cases)	* Haemorrhage via:	
- Insomnia	- Mouth, nostrils, skin etc	
	- Bloody vomiting	
	- Bloody diarrhoea	

Frame *et al.,* (1970); [8] Richmond *et al.,* (2003) [11] Emound *et al.,* (1982); [21] WHO, (2015; [23] Bausch *et al.,* (2001); [24] Gunther *et al.,* (2001) [28].

Diagnosis of Lassa Fever
Lassa fever has emerged as one of the most prevalent viral haemorrhagic fevers in West Africa (Nigeria inclusive) [6,40]. However, in most Lassa fever endemic areas of the region, there are serious challenges regarding the laboratory diagnosis and confirmation of the disease due to inadequate facility and low capacity [23, 40]. For instance, in Nigeria (with estimated population of over 170 million), there are only two diagnostic centres (Irua, Edo State in South-South Nigeria, and Lagos, South-West Nigeria) where Lassa virus infection could be confirmed[40].

The currently used Laboratory investigations for the detection of Lassa virus infection include:
*Enzyme-Linked Immunosorbent Assay (ELISA) test for the detection of IgM antibodies in patient's serum. The test gives 88% sensitivity and 90% specificity for the presence of Lassa fever.

- Analysis of cerebrospinal fluid (CSF) to detect the presence of Lassa virus.
*Confirmation of Lassa fever by molecular analysis using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method [41] (Table 4). Notably, due to non-specific nature of the early symptoms of Lassa fever, molecular diagnostic method is very crucial and plays a decisive role in the early diagnosis of Lassa virus infection [36,42-45]. On the other hand, cell foci infected with Lassa virus can equally be detected with Lassa virus NP-Specific monoclonal antibody such as the use of L_2F_1 test [46, 47].

Regardless of the method adopted, Lassa fever will require a Biosafety Level 4 – equivalent containment during Laboratory diagnosis to prevent the acquisition and spread of the disease in the Laboratory and hospital environment [2].

TABLE 4: LABORATORY INVESTIGATIONS FOR DETECTION OF LASSA VIRUS INFECTION

Laboratory Test	Finding Suggestive/Confirmation of Infection
- ELISA	IgMAntibodies;Sensitivity=88%,Specificity=90%
- Lyphopenia	Decrease/low White Blood Cell Count
- Thrombocytopenia	Low Platelet Count
- Blood Aspartate Aminotransferase levels	Elevated
- Detection of Lassa Virus in Cerebrospinal Fluid (CSF)	
- Lassa Virus NP-Specific Monoclonal Antibody (L_2F_1) Test	Positive
- Molecular Analysis by:	
RT-PCR Assay, eg targeting L RNA segment of Lassa virus	Detection of Lassa Virus Infected Cell Foci. - Confirmation of Lassa fever. - Detection of conserved regions in the polymerase domain of the L gene.

Gunther *et al.,* (2001); [28] Demby *et al.,* (1994); [41] Drosten *et al.,* (2002); [44] Vieth *et al., (2007);* [36] Hufert *et al,.* (1989); [46] ter Meulen *et al.,* (1998) [47].

Useful Prevention/Control Measures

Lassa fever transmission is enhanced by cohabitation of *M. natalensis* species of rodent with humans in their residences in the affected areas having access to water and food items in the household. These rats are also prepared and consumed as delicacies by many inhabitants of West African region [9,22]. Therefore, any control/preventive measures to be adopted must take cognizance of routes and mechanism of transmission of Lassa fever. The following measures are imperative in curtailing the regular epidemic outbreak and spread of Lassa fever in sub-Saharan region of Africa. These include:

- Observance of general hygiene including personal and environmental hygiene by the populace.
- Since Lassa fever transmission is associated with infected mouse (*M. natalensis),* therefore, every household needs to device all means geared towards preventing rats from having any contact with foods, water and utensils utilized by the household. This may be achieved by:
  -Covering of foods and water meant for human consumption regularly.
  -Foods should be kept in tightly sealed containers.
  -Ready-to-eat food item (such as gari) should not be spread in the open or by the roadside where rats can have access to it.
- Public enlightenment campaign about Lassa fever should be conducted regularly in areas where the disease is prevalent.
- Every community should be counseled to avoid foods and other items contaminated with rat's excretions and secretions.
- People should be admonished to kill and destroy rats in and around the house, shops or market places.
- Foods and water should be boiled adequately before consumption.
- Encourage members of the community to always attend healthcare centre nearest to them for medical attention when they are sick or have had contact with contaminated environment.
- All persons suspected of Lassa virus infection should be admitted to isolation facilities and promptly attended to with utmost care.
  -Hospital workers should take universal precautions and protective measures when attending to such patients.
  -Every body fluids and excreta produced by such patients should be handled with care and properly disposed of.
- Early detection of the disease and aggressive treatment (such as the use of intravenous ribavirin) [39] is important for the survival of infected patient.
- Healthcare workers should be sensitized about the need to adopt universal preventive measures in their routine hospital procedures to limit the transmission and acquisition of Lassa virus infection and indeed all infectious diseases in hospital setting.
- Governments at all levels (National, State and Local) should demonstrate political will in mobilizing logistics and necessary materials and financial support to aid adequate management and effective control of Lassa fever.
- More diagnostic and treatment centres for Lassa fever should be established at various regions of each country endemic for Lassa fever.
- Development of effective vaccine against Lassa fever (which has reached advanced stage with positive results in animal trials) [49] is crucial in checkmating the spread of Lassa fever.

Conclusion/Recommendations

Lassa fever has emerged as one of the most prevalent, immunosuppressive and highly fatal haemorrhagic

fevers endemic in sub-Saharan Africa particularly West and Central Africa. Transmission of the disease is influenced by cohabitation of reservoir rodent (*M. natalensis*) with human population and poor environmental hygiene common in most parts of the region resulting in regular outbreak of the disease and fatality. Currently, there are no clinically certified vaccines against Lassa fever which limits the scope of control/preventive measures against Lassa fever. Hence, there is need to intensify public educational or enlightenment program in all affected areas on the useful control measures against Lassa fever. The stakeholders need to prioritize the intervention, support and deterrent program and speed up the process leading to production of effective vaccine to checkmate the menace of Lassa fever outbreak and associated morbidity and mortality.

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OCCURRENCE OF MALARIA AND UTILIZATION OF ANTIMALARIA PREVENTIVE MEASURES AMONGST PREGNANT WOMEN ATTENDING AJEROMI- IFELODUN GENERAL HOSPITAL, LAGOS STATE, NIGERIA

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ABSTRACT

Malaria in Pregnancy poses a serious health problem both for the mother and her unborn baby and this can be prevented with the use of Intermittent Preventive Treatment with sulphadoxine pyrimethamine (IPTp-SP), Long Lasting Insecticide-treated Nets (LLIN) and other preventive measures. This cross-sectional survey was carried out among pregnant women attending Ajeromi Ifelodun General Hospital between August 2013 and February 2014. A total of 414 pregnant women (with mean age of 29±8.7) in their first (84), second (124) and third (206) trimesters were recruited for this study. Blood samples for making both thin and thick smears were collected and semi-structured questionnaires administered to the respondents. The questionnaire probed into their knowledge on cause of malaria, the preventive measures taken against mosquito bite, use of IPTp-SP, possession and use of LLIN. The overall prevalence of malaria due to *Plasmodium falciparum* is 24 (5.8%) out of which 13 were primigravid, 5 were secundi-gravid, and 6 were multigravida with no significant difference (P>0.05) amongst them. Two hundred and eighty-six (69.1%) pregnant women had good knowledge of the cause of malaria. 164 (39.6%) of the sampled population had a tertiary education while 182 (44.0%) had secondary education, 124 (30.4%) were traders and 80(19.6%) were civil servants. Preventive measures claimed to be adopted by the respondents in avoiding mosquito bites include the use of LLIN (62.6%), insecticides sprays (36.2%), and locally adopted measures (1.5%). The number of respondents who had not availed themselves of IPTp-SP was significantly higher 258 (62.3%) compared to those who had (P<0.05). It was observed that educational status had no significant effect on the knowledge of the cause of malaria in sample population (p>0.05). The findings of this study reveal that there is a good knowledge on the cause of malaria among pregnant women but low use of IPTp-SP. In order to meet the new target of reaching an elimination stage set by the World Health Organization, factors responsible for the low use of these preventive measures should be investigated and quickly addressed so as to reduce both maternal and child morbidity/mortality resulting from malaria infection.

Keywords: Malaria, Pregnant women, Prevention, Intermitent preventive treatment, Sulphadoxine-pyrimethamine, Long-lasting Insecticide treated net.

L'OCCURRENCE DU PALUDISME ETDE L'UTILISATION DES MESURES PREVENTIVES ANTIPALUDIQUES PARMI LES FEMMES ENCEINTES FREQUENTANT HÔPITAL GENERAL D'AJEROMI – IFELODUN, ETAT DE LAGOS, NIGERIA.

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RESUME

Le paludisme pendant la grossesse pose un grave problème de sante pour la mère et son bébé à naitre et ceci peut êtreéviter avec l'usage d'un traitementpréventif intermittent avec sulfadoxine pyriméthamine (IPTp-SP), des filets traités à l'insecticide de longue durée (LLIN) et d'autres mesures préventives. Cette enquête transversale a été réalisée chez les femmes enceintes fréquentantl'hôpitalgénérald'Ajeromi Ifelodun entre août 2013 et février 2014. Un total de 414 femmes enceintes (avec l'âge moyen de 29±8,7) dans leurs premier (84), deuxième (124), et troisième (206) trimestres ont été recrutées pour cette étude.Deséchantillons de sang pour faire des frottis minces et épais ont été recueillis et des questionnaires semi structurés administrés aux répondantes. Le questionnaire a sondé dans leur connaissance sur la cause du paludisme, la

mesure préventive prise contre les piqures de moustiques, l'utilisation d'IPTp-SP, la possession et LLIN. La prévalence globale du paludisme en raison de *Plasmodium falciparum* est 24 (5,8%) dont 13 étaient primigeste et 5 étaient secundi – gravide et 6 étaientmulti geste d'aucun différence significative (P>0,05) chez elles. Deux cent quatre – vingt-six (69,1%) femmes enceintes avaient une bonne connaissance de la cause du paludisme. 164 (39,6%) de la population échantillonnée avait l'enseignement supérieur tandis que 182 (44,0%) avait l'enseignement secondaire, 124 (30,4%) commerçantes et 80 (19,6%) étaient fonctionnaires. Les mesures préventivesqu'on affirmaitêtreadopté par les répondantes pour éviter les piqures de moustiques comprennent l'utilisation de LLIN (62,6%), pulvérisations d'insecticides (36,2%), et les mesures adoptées localement (1,5%). Le nombre de répondantes qui n'a pas profité d'usage d'IPTp-SP est significativement plus élevé 258(62,3%) par rapport à ceux qui en avaient (P<0,05). Il a été observé que la situation éducative n'a aucun eu effet significatif sur la connaissance de la cause du paludisme dans la population échantillonnée (p>0,05). Les résultats de cette étude ont révélé qu'il y a une bonne connaissance de la cause du paludisme chez les femmes enceintes, mais une faible utilisation d'IPT-SP.Afin de répondre à la nouvelle cible d'atteindre un stade d'élimination fixé par l'Organisation Mondiale de la santé, les facteurs responsables de la faible utilisation de ces mesures préventives devraient êtreétudiés et rapidement traités de manière de réduire la morbidité/ mortalité maternelle et infantile résultant de l'infection du paludisme.

Mots clés : Paludisme, Les femmes enceintes, Prévention, le traitement préventive intermittent, Sulphadixine – pyrimethamine, des filets durables traités à l'insecticide.

INTRODUCTION

Malaria is a life threatening disease that is caused by species of the genus *Plasmodium* and transmitted by the female *Anopheles* mosquito. It is the most prevalent tropical disease resulting in high morbidity with its consequent economic and social loss. Malaria accounted for an estimated 207 million cases in 2012 with 627 000 deaths occurring in same year, 90% of these deaths occurred in sub-Saharan African (1).

Malaria in pregnancy is a serious health problem in sub-Saharan Africa where it affects 24 million pregnant women. In Nigeria, malaria in pregnancy is responsible for 11% maternal mortality (2).

Malaria infection in pregnant women increases the risk of maternal anaemia, delivery of low birth weight baby, spontaneous abortion and neonatal death. The policy for malaria prevention and control during pregnancy in areas of stable transmission, as is the case in Nigeria, as stipulated by the World Health Organization (WHO), emphasizes the use of Intermittent Preventive Treatment in pregnancy (IPTp), the use of Long-lasting insecticide treated net (LLIN) and effective case management of malaria illness and anaemia (3).

Currently, the best available drug use during pregnancy as IPTp is sulphadoxine pyrimethamine (SP) because of its safety. It involves the administration of a curative dose of SP (1500mg/kg sulphadoxine and 75mg/kg pyrimethamine as a single dose) twice during the second and third trimester of pregnancy without reference to the status of the woman with regards to infection and it should be given free of charge using the Directly Observed Therapy (DOT) as advocated by WHO (3, 4). Nigeria adopted IPTp as a national strategy in 2005 replacing the weekly prophylaxis with chloroquine (5).

The current national malaria treatment guideline and policy in Nigeria recommends that SP should be given as the first line drug for IPTp and quinine for treatment of clinical malaria in all trimesters, while artemisinin-based combination therapy (ACT) is considered safe in the second and third trimester and can also be used in the first trimester where there are no suitable alternatives. To further achieve this, the Lagos State Government has taken the initiative to build maternal and child centres in most of its hospitals where attention is paid only to the pregnant

woman, her unborn child and newly delivered infants. IPTp has been shown to reduce anaemia and parasitaemia in pregnant women (6, 7).

A number of studies have been reported on the use of IPTp, knowledge of the cause of malaria, the transmission dynamics, different preventive measures and factors hindering the use of IPTp (6, 7, 8). Given that the new target for malaria is that by the year 2020, more than half of endemic countries should reach pre-elimination stage, there is need to assess prevalence as well as preventive measures employed by this vulnerable group. This study evaluates the prevalence, knowledge of the cause of malaria, level of awareness and the use of malaria preventive measures among pregnant women in Ajeromi Ifelodun General Hospital, Lagos, Lagos State, Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out at the Maternal and Child Centre (MCC) of Ajeromi–Ifelodun General Hospital, located in Ajeromi- Ifelodun Local Government Area of Lagos State, Nigeria (6°45′N; 3°33′E). The MCC is a three – storey 110 bed capacity secondary health facility that is owned by the Lagos State Government.

This centre was constructed to provide quality service for the restoration, improvement and promotion of the health and well-being of women,

infants and children in the locality focusing essentially on obstetrics, gynecology and family planning. Patients visit the clinic on Wednesday for registration and Fridays for booking of appointment.

This Local Government Area is home to 684,105 inhabitants and covers an area of about 12km^2. It is an urban area and one of the four Local Government Areas under the Badagry administrative division of Lagos State. It has only one Local Council Development Area (Ajeromi Ifelodun LCDA) with six primary health centres. Residents in the study area are of different Nigerian ethnic groups and are mainly involved in trading with some of them engaging in corporate activities (www.lagosstate.gov.ng).

It shares boundary with Surulere Local Government Area in the North, Apapa Local Government Area in the South-East and Amuwo-Odofin Local Government Area in the South-West. Settlements in this area are mostly close to each other and rainfall period covers April to September with a peak period in June and a break in August called 'August break'. The presence of stagnant water in different locations in the area enhances mosquito breeding.

Study design

The study was a cross–sectional survey involving 414 pregnant women determined by the WHO formula for calculating sample size (9) in their first and second visit selected randomly from the pool of registered pregnant women from August 2013 to February 2014 at the health facility.

The inclusion criteria were registration at the ante-natal clinic, having no sign of severe malaria, and pregnancy gestational age of at least two months before term. Women who had severe malaria (detected clinically and microscopically) and/or having just a month before delivery were excluded from the study.

Sample and data collection

Blood samples were collected from the respondents by finger prick by applying little pressure. The first drop of blood was cleaned off with cotton wool soaked in 70% ethanol and subsequently drops of blood were collected with a clean microscopic slide for the preparation of thin and thick blood films.

Semi-structured questionnaires were administered to the consenting pregnant women by the principal investigator and captured information on their knowledge on malaria, attitude, malaria control practices and compliance with IPTp-SP.

Smear Preparation

Thick and thin blood films were prepared according to the technique outlined by the World Health Organization (10). Briefly, a drop of blood was spread on a clean, grease free microscopic slide to a moderate thickness and allowed to air dry. The thickness was such that prints could be seen through it when still wet. The air dried film was stained with 10% Giemsa stain. The stain was allowed to stay for 10 minutes before washing off with clean water. The slide was then placed vertically and allowed to dry, after which a drop of immersion oil was placed on the slide and examined under the microscope with ×100 magnification.

Thin film was prepared by immediately placing the smooth edge of a spreader slide on a drop of blood, adjusting the spreader to an angle of 45^0 and then smearing the blood swiftly and steadily along the surface. The film was then allowed to air dry, fixed with 70% ethanol and stained with 10% Giemsa for 10 minutes before washing off with clean water. The slide is then allowed to air dry in a vertical position and examined under oil immersion microscope (10, 11).

Positive specimens were identified on the basis of microscopy using standard methods. Presence of ring forms of *Plasmodium falciparum* trophozoites indicates positive results. A blood smear can be considered negative if no parasite is seen after 10 minutes of examination. The prevalence was calculated as the proportion of those infected in relation to the total number of women examined.

Ethical consideration

Approval was obtained from the Lagos State Ministry of Health and the ethics committee of the hospital. The study was discussed with the Chairman, ethics committee of the hospital as well as the head of the Laboratory Department of the same hospital. Informed verbal consent was also sought from each respondent included in the study.

Data analysis

Data were entered in Epi-Info version 2002 statistical software (Centre for Disease Control and Prevention). Frequencies and proportions were used to compare respondent's knowledge on cause of malaria, preventive measures used against mosquito bite, use of IPTp-SP, gestational age, gravidity and educational status. For all statistical tests in this study, $P < 0.05$ was considered significant.

RESULTS

Socio-Demographic Characteristics and Pregnancy History

The mean age of the 414 pregnant women included in the study is 29 (S.D ±8.7) years with the youngest aged 17 years and the oldest 42 years. Majority of the respondents had received post primary education; tertiary 164 (39.6%) and secondary education 182 (44.0%). The respondents were mainly traders (36.7%), civil servants (19.3%), students (16.4%) and housewives (13.5%); and

majority were of the Yoruba 119 (28.7%) and Igbo 94 (22.7%) ethnic group while others were from the northern 45 (10.9%) and south-southern 61 (14.7%) parts of the country. However, 95(23.0%) did not reveal their ethnicity (Table 1).

TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE RESPONDENTS (N=414)

	Categories	Frequency (%)
Age Group (Mean Age of 29 ±8.7)		
	16-20	28(6.8)
	21-25	82(19.8)
	26-30	195(47.1)
	31-35	66(15.9)
	36-40	35(8.4)
	41-45	4(1.0)
	No response	4(1.0)
Education		
	Tertiary	164(39.6)
	Secondary	182(44.0)
	Primary	22(5.3)
	No formal education	6(1.4)
	Non-response	40(9.7)
Occupation		
	Traders	152(36.7)
	Civil servant	80(19.3)
	Students	68(16.4)
	Housewives	56(13.5)
	Others	30(7.3)
	Artisans	22(5.3)
	No response	6(1.5)
Ethnicity		
	Yoruba	119(28.7)
	Igbo	94(22.7)
	Hausa	45(10.9)
	Others (Delta, Edo, Akwa Ibom)	61(14.7)
	No response	95(23.0)

Their classification by parity revealed that 302(72.9%), 62(15.0%) and 50 (12.1%) were primi-graviida, secundi-graviida and multi-graviida respectively. Almost half of them 206 (49.8%) were in their third trimester (Table 2).

TABLE 2: PREGNANCY HISTORY OF RESPONDENTS

	Categories	Frequency (%)
Gravidity		
	Primi-gravidae	302(72.9)
	Secundi-gravidae	62(15.0)
	Multi-gravidae	50(12.1)
Gestational age of pregnancy		
	First trimester	84(20.3)
	Second trimester	124(30.0)
	Third trimester	206(49.7)

TABLE 3: PREVALENCE OF MALARIA ACROSS VARIOUS CATEGORIES OF THE RESPONDENTS

Categories	Examined	Positive
Primi-gravidae	302	13(4.3%)
Secundi-gravidae	62	5(8.1%)
Multi-gravidae	50	6(12%)
First trimester	84	9(10.7%)
Second trimester	124	7(5.6%)
Third trimester	206	8(3.9%)

Malaria Prevalence

The overall prevalence is 5.8% (24) with the primigravid women (13) being more infected than the secondi-gravid (5) and the multi-gravid (6) women with no significant difference (P>0.05). In like manner, women in their first trimester were the most (9) infected than those in other trimesters (Table 3).

Knowledge of the cause of malaria and preventive measures

Assessing their knowledge of the cause of malaria revealed that majority of the respondents 286 (69.1%) had a good knowledge of the cause of malaria, ascribing it to mosquito bite, while 15.2% attributed it to cold, and tiredness (1.2%) (Table 4).

Thirty-seven percent of the respondents had used IPTp-SP which is significantly lower (P<0.005) than those 258 (62.6%) who had not taken IPTp-SP since pregnancy. Only one hundred and sixteen (45.0%) of the total two hundred and fifty-eight pregnant women who possess LLIN affirm to sleep under it daily Lack of use of LLIN by others 142(55.0%) was attributed to sweating and itching experienced during it use. One hundred and fifty (36.2%) used indoor insecticide spray as a major control option, while a few 6 (1.5%) resort to use of orange peels in preventing mosquito bites (Table 5).

TABLE 4: KNOWLEDGE OF THE CAUSE OF MALARIA (N=414)

Cause of malaria	Frequency (%)
Mosquito bite	286(69.1)
Stress	18(4.3)
Sun	7(1.7)
Tiredness	5(1.2)
Cold	63(15.2)
Dirty water	14(3.4)
Fever	10(2.4)
Do not know	11(2.7)

TABLE 5: MALARIAL PREVENTIVE MEASURES (N=414).

Preventive measures against mosquitoes bite		Frequency (%)
	Insecticide treated nets	116(45.0)
	Local measures (e.g orange peels)	6(1.5)
	Synthetic insecticides (e.g Mosquito coil)	150(36.2)
	Nothing	58(14.0)
	No response	84(20.3)
Use of IPTp-SP during pregnancy		
	Yes	154(37.2)[a]
	No	258(62.3)
	No response	2(0.5)

a: statistical significance between IPTp-SP usage, p=0.015

Impact of education on knowledge and malaria and use of preventive measure

Assessing the impact of education on the respondents knowledge of the cause of malaria revealed a significant ($p<0.05$) difference between those with post-primary education 272 (95.1%) and primary education 10 (45.5%) (Table 6). Pregnant women with tertiary education 86(52.4%) reported to have used IPTp-SP more, followed by those with secondary education 61(33.5%) and only 16.7% of those without formal education reportedly use IPTp-SP (Table 7).

TABLE 6: RELATIONSHIP BETWEEN LEVEL OF EDUCATION AND KNOWLEDGE OF THE CAUSE OF MALARIA

Education	Knowledge of the cause of malaria.	
	Yes	No
Tertiary	134(81.7)	30(18.3)
Secondary	138(75.8)	44(24.2)
Primary	10(45.5)	12(54.5)
No Formal Education	2(33.3)	4(66.7)
Others	2(5.0)	38(95.0)

TABLE 7: LEVEL OF EDUCATION AND USE OF INTERMITTENT PREVENTIVE TREATMENT WITH SULPHADOXINE PYRIMETHAMINE BY THE RESPONDENTS (N=154)

Education	IPTp use	
	Yes	No
Tertiary	86(52.4)	78(47.6)
Secondary	61(33.5)	121(66.5)
Primary	4(18.2)	18(81.8)
No formal education	1(16.7)	5(83.3)
Others	2(5.0)	38(95.0)

DISCUSSION AND CONCLUSION

Malaria in pregnancy poses a serious problem both for the mother and her unborn baby and this can be prevented with the use of IPTp-SP, ITN and other preventive measures.

The overall prevalence of this study is quite low and this could be due to a number of factors which include; the time of collection of sample which was more of dry season than the rainy season, the prevailing environmental condition which does not support the breeding of mosquitoes. Prevalence was gravidity dependent as primigravid women were heavily infected than the other group explaining the acquisition of immunity with repeated pregnancy by both the secundigravid and multigravida women (12, 13).

Most of the respondents of this study were literate, thus reflecting in the large number of the respondents who had good knowledge of the cause of malaria attributing it to mosquito bite. This finding is similar to previous study carried out in Federal Capital Territory of Nigeria (14), Midwestern part of Nigeria (15) and in northern Ethiopia (16).

There are some erroneous beliefs about the cause of malaria as some respondents attributed it to drinking dirty water, staying under the sun, cold and fever. This will have negative implication on malaria control because the respondents may be unwilling to embrace malaria preventive practices. This may also affect the treatment seeking behaviour of the respondents, as those with good knowledge of the infection are likely to seek prompt treatment when infected while those who have wrong view of the cause of malaria may not embrace the interventions.

The result from this study shows that only 45% of the 258 respondents who possess LLIN actually sleep under the net. This is higher than that obtained in previous study in Nigeria (17) but lower than what was observed in Mukono district in Uganda (18) and Southern Tanzania (19). This finding did not meet the target of 80% coverage of antimalarial preventive measures in vulnerable groups which include pregnant women set by the World Health Assembly in 2005, this therefore calls for more sensitization to instill the benefit of ITN use in the populace.

More than a quarter of the respondents had assessed IPTp –SP which is consistent with the result of a study carried out in a rural south-western area in Nigeria (20) and in southern Mozambique (21) but lower than what was obtained in a study carried out in Calabar, (22) and Oyo State, Nigeria where more than half of the respondents reported to have used IPTp–SP (23). Although the literacy level in this study translated into having good knowledge of the cause of malaria, nevertheless it did not result into high use of IPTp –SP. This also falls below the WHO expectation of administration of IPTp to 80% of all pregnant women in areas of high malaria transmission (24).

Furthermore, the study showed that there is a relationship between education and knowledge of malaria as those who have had both tertiary and secondary education had a good knowledge of the cause of malaria. This is quite expected as those with certain level of education are enlightened and knowledgeable and as such will take certain preventive measures against malaria infection. This same association was also observed between education and IPTp–SP, however, the use of SP was frequent among the primigravidae than the secundigravid and multigravidae. This is quite unexpected as women with previous pregnancy are expected to know the importance of IPTp and as such embrace its use more than the primigravidae.

Almost half of the respondents reported to the antenatal clinic (ANC) in their third trimester of pregnancy as reflected in time of their first visit to ANC. The importance of early registration for ANC will enhance high level of IPTp-SP use. Awareness should therefore be created through the use of Information Education and Communication (IEC) materials for early registration.

In order to meet the new target of reaching a pre-elimination stage set by the World Health Organization, factors responsible for the low use of IPTp-SP and LLIN should be investigated, the benefits emphasized so as to reduce both maternal and child morbidity/mortality resulting from malaria infection.

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