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INCIDENCE AND DISTRIBUTION OF MULTI-DRUG RESISTANT PATHOGENS FROM CLINICAL SAMPLES IN A TERTIARY HOSPITAL IN SOUTH-SOUTH NIGERIA.

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ABSTRACT

Background

Antibiotics have proven to be a dynamic category of drugs in the fight against infectious bacteria. However, antibiotic resistance is one of the greatest current challenges to the effective treatment of infections and there is every indication that antibiotic resistance will become an even greater challenge in the future.
Methodology
Ten clinical samples with varying frequencies were analyzed for bacterial growth, antibiotic susceptibility testing and multiple antibiotic resistances. The clinical samples includes; urine (42%), wound swab (21.33%), blood (10%), ear swab (9.33%), catheter tip (5.33%), endocervical swab (4.67%), high vaginal swab (HVS) (2.67%), urethral swab (2.67%), pus (1.33%) and antral aspirate (0.67%).
Results
A total of 150 bacterial isolates distributed among these ten (10) clinical samples were identified, of which *Staphylococcus aureus* (30%) was the most predominant, while *Klebsiella oxytoca, Citrobacter* spp. and *Streptococcus* spp. were the least (0.67%). These were tested for sensitivity against 9 antibiotics. The resistance rate observed was as follows; cefuroxime (93%), ceftazidime (87%), gentamycin (79%), augmentin (70%), cloxacillin (67%), ofloxacin (54%), ciprofloxacin (51%), ceftriaxone (38%) and ocefix (34%). One hundred and forty-three (95.3%) of the isolates showed multiple resistance against 3 – 8 antibiotics. None was resistant to as few as 1 – 2 antibiotics.
Conclusion
The high susceptibility to some antibiotics such as ceftriaxone and ocefix could be a welcoming relief, since they can be used to address the problem of resistance in this area. There is need for nationwide surveillance programme to monitor microbial trends and antimicrobial resistance patterns in Nigeria.

Key words: multi-drug resistant, clinical samples, *Staphylococcus aureus*, ocefix.

INCIDENCE ET REPARTITION DES AGENTS PATHOGENES MULT-RESISTANTS AUX ANTIBIOTIQUES ISOLES DES ECHANTILLONS CLINIQUES A L'HOPITAL DU SOIN TERTIAIRE DANS LE SUD DU NIGERIA

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Contexte
Les antibiotiques se sont démontrés être une catégorie dynamique des médicaments pour lutter contre les infections bactériennes. Cependant, la résistance aux antibiotiques est l'un des grands défis actuels pour le traitement efficace des infections et tout porte à croire que la résistance aux antibiotiques devient un défi encore plus grand à l'avenir.
Méthodologie
Dix échantillons cliniques avec leurs grandes fréquences ont été analysés par la culture bactériennes, le teste de sensibilité aux antibiotiques et le teste de la multirésistance aux antibiotiques. Les échantillons cliniques sont constitués de l'urine (42%), de pus de la plaie (21,33%), de sang (10%), de prélèvement d'oreille (9,33%), d'extrémité du cathéter (5,33%), de prélèvement d'endocervical (4,67%), de prélèvement vaginal (2,67%), de prélèvement urétral (2,67%), de pus (1,33%), de ponctionantrale(0,67%).
Résultats
Au total, 150 souches bactériennes réparties parmi les dix (10) échantillons cliniques ont été identifiées, parmi lesquelles *Staphylococcus aureus* (30%) était le plus prédominant, alors que *Klebsiellaoxytoca,Citrobacter*spp et*Streptococcus*spp étaient les moindres (0,67%). Ils ont été testés pour la sensibilité de 9 antibiotiques. Le taux de résistance observé était le

suivant: céfuroxime (93%), ceftazidime (87%), gentamicine (79%), augmentin (70%), cloxacilline (67%), ofloxacine (54%), ciprofloxacine (51%), ceftriaxone (38%) etocefix (34%). Cent-quarante-trois (95,3%) d'isolats ont montré la multi-résistance contre 3 à 8 antibiotiques. Aucune souche n'a été résistante à moins de 1 à 2 antibiotiques.

Conclusion

La forte sensibilité de certains antibiotiques tels que la ceftriaxone et l'ocefix pourraient être un ouf de soulagement, car ils peuvent être utilisés pour résoudre le problème de la résistance dans cette région. Il est nécessaire pour le programme national de surveillance pour suivre les tendances microbiennes et les situations de résistance aux antimicrobiens au Nigeria.

Mots clés: multi-résistance aux antibiotiques, échantillons cliniques, *Staphylococcus aureus*, ocefix.

INTRODUCTION

Antimicrobial resistance is a well known clinical and public health problem [1]. Over the last 60yrs, bacteria in particular, those pathogenic for humans have evolved toward antimicrobial drug resistance. This evolution has 2 key steps; emergence and dissemination of resistance [2]. Antimicrobial resistance in nosocomial infections is increasing with both morbidity and mortality greater than when infection is caused by drug sensitive organisms. These infections are opportunistic and microorganisms of low virulence can cause diseases in hospitalized patients whose immune mechanisms are impaired. The outcome is that many antibiotics can no longer be used for the treatment of infections caused by such organisms and the threat to the usage of other drugs increases [3, 4].

The widespread use of broad- spectrum antibiotics, immunocompromised patients, and exogenous transmission of bacteria, usually by hospital personnel has led to the emergence of nosocomial infections caused by drug resistant microbes [5, 6]. Available therapeutic options for antibiotic resistant organisms are severely limited, as these organisms frequently display a multi-drug resistant phenotype [7, 8, and 6]. Multi-drug resistance (MDR) and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge [9]. However, strategies for addressing antimicrobial drug resistance stress the need for new drug [10] and yet the rate of drug development is in decline [11, 12].

Several investigators revealed that the most frequently reported bacterial pathogens from clinical sources with multi-drug resistance have been *E. coli, S. aureus, P. aeruginosa, K. pneumoniae, Proteus* spp., *Acinetobacter* spp., *Enterococcus* spp., coagulase negative staphylococci (CONS), etc.[13, 14, 15 and 16].

As the proliferation of multi-drug resistance pathogens continue unavoidably in the hospital settings, within and around us, it is imperative that their resistance trend be put under check through intensive research and antibiotic surveillance. Therefore, this current study reports on the incidence and distribution of multi-drug resistant bacteria pathogens from clinical samples in a tertiary hospital in south-south Nigeria.

MATERIAL AND METHODS

Sample collections

One hundred and fifty clinical samples classified into ten groups were obtained from routine section in Medical Microbiology Laboratory of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The sources of the samples were as follows: urine (42%), wound swab (21.33%), blood (10%), ear swab (9.33%), catheter tip (5.33%), endocervical swab (4.67%), high vaginal swab (2.67%), urethral swab (2.67%), pus (1.33%), and antral aspirate (0.67%). The samples were analysed for bacterial growth within 1-2 hrs after collection.

Identification of bacterial isolates

All the samples were plated on Blood agar, MacConkey agar and replicated on Mannitol salt agar (oxoid, England). The inoculated plates were incubated at 37°C for 24h. Identification of bacterial isolates was done on the basis of their cultural and standard biochemical characteristics. [17]. The isolates were sub-cultured on nutrient agar slants periodically to maintain pure culture.

Antibiotic Susceptibility Testing

Isolates were tested for antimicrobial susceptibility testing by the standard disc diffusion method. Standard inoculums adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar and was allowed to soak for 2 to 5 minutes. After that the appropriate antibiotic disc were aseptically placed on the agar surface using sterile forceps and the plates were incubated at 37^0C for 24hrs. Commercially available antimicrobial discs used included: ciprofloxacin (30μg), ceftriaxone (10μg), ofloxacin (25μg), augmentin (25μg), cefuroxime (30μg), gentamycin (10μg), ceftazidime (30μg), cloxacillin (10μg), and ocefix (10μg). The degree of susceptibility of the test isolates to each antibiotic was interpreted as either sensitive (S) or resistant (R) according to National Committee for Clinical Laboratory Standards [18].

Statistical analyses:

The Chi-square goodness of fit test was used to test for significant differences in the data obtained. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non- significance difference was defined when $(p \leq 0.05)$ and $(p \geq 0.05)$ respectively.

RESULTS

Figure 1 showed the sources of 10 clinical samples used in the study. Urine (42%) was the most predominant clinical sample, followed by wound swab (21.3%) and blood (10%); while the least clinical sample was antral aspirate (0.7%), closely followed by pus (1.3%) and HVS (2.7%).

The isolates were confirmed as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*, coagulase negative staphylococci (CoNS), *Citrobacter* spp., *Alcaligenes* spp., *Enterobacter* spp., *Klebsiella oxytoca* and *Streptococcus* spp. Of these isolates, 57(38%) were Gram positive and 93(62%) were Gram negative. *Staphylococcus aureus* was the most common Gram positive isolates (79%), while *Klebsiella pneumoniae* was the most common Gram negative isolates (31%). *Staphylococcus aureus* 45(30%) was the most predominant isolate, followed by *Klebsiella pneumoniae* 28(18.7%), while *Citrobacter* spp., *Klebsiella oxytoca* and *Streptococcus* spp. were the least with 1(0.7%) each (Table I). *Staphylococcus aureus* had their highest occurrence from urine samples. *Pseudomonas aeruginosa* had its highest incidence in wound swab, while *Klebsiella pneumoniae* was the most common organism isolated from catheter tip and blood. The result also showed that *Staphylococcus aureus* and CoNS were the most predominant organisms in endocervical swab, while *S. aureus and P. aeruginosa* were the most occurring isolates from ear swab.

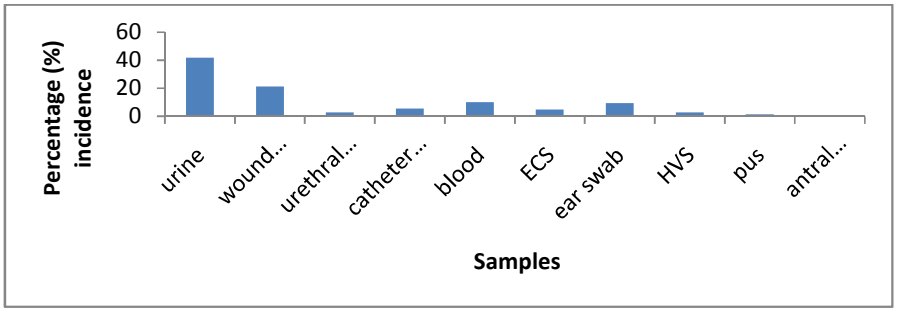

Figure 1: Percentage incidence of clinical samples.

Key: ECS = Endocervical swab, HVS = high vagina swab

TABLE I: PREVALENCE OF BACTERIA ISOLATES FROM CLINICAL SAMPLES (%)

CLINICAL SAMPLES	SA	EC	KP	KO	CONS	PA	PM	CS	AS	ES	SS
Urine	21(46.67)	15(57.69)	12(42.85)	-	4(36.36)	-	4(33.33)	-	6(75)	1(50)	-
WS	6(13.33)	7(26.92)	2(7.14)	-	-	11(73.33)	3(25)	-	2(25)	1(50)	-
US	4(8.89)	-	-	-	-	-	-	-	-	-	-
CT	-	-	6(21.43)	-	-	-	2(16.67)	-	-	-	-
Blood	4(8.89)	2(7.69)	5(17.86)	1(100)	1(9.09)	-	1(8.33)	1(100)	-	-	-
ECS	3(6.67)	-	1(3.57)	-	3(27.27)	-	-	-	-	-	-
Ear swab	4(8.89)	-	2(7.14)	-	2(18.18)	4(26.67)	2(16.67)	-	-	-	-
HVS	1(2.22)	2(7.69)	-	-	-	-	-	-	-	-	1(100)
Pus	2(4.44)	-	-	-	-	-	-	-	-	-	-
AS	-	-	-	-	1(9.09)	-	-	-	-	-	-
Total	45(30)	26(17.33)	28(18.67)	1(0.67)	11(7.33)	15(10)	12(8)	1(0.67)	8(5.33)	2(1.33)	1(0.67)

KEY: SA = *Staphylococcus aureus*, EC: *Escherichia coli*, KP = *Klebsiella pneumoniae*, KO = *Klebsiella oxytoca*, CONS = Coagulase negative Staphylococcus PA = *Pseudomonas aeruginosa*, PM= *Proteus mirabilis*, CS = *Citrobacter* spp., AS = *Alcaligens* spp., ES = *Enterobacter* spp., SS = *Streptococcus* spp., WS = Wound swab, US = Urethral swab, CT = catheter tip, ECS = Endocervical swab, HVS = High vagina swab, AS = Antral aspirate

Table II showed the antibiogram of the isolates. Resistance by all the isolates to cefuroxime was the highest (92.7%), followed by ceftazidime (86.7%), gentamycin (79.3%), augmentin (70%), cloxacillin (66.7%), ofloxacin (54%) and ciprofloxacin (51.3%). The least resistance was to ocefix (34%). Resistance

TABLE II: ANTIBIOGRAM OF ISOLATES (%)

Antibiotics	No.sensitive	No.resistance
Ciprofloxacin	73(48.7)	77(51.3)
Ceftriaxone	93(62)	57(38)
Ofloxacin	69(46)	81(54)
Augmentin	44(29)	106(70)
Cefuroxime	11(7.3)	139(92.7)
Gentamycin	31(20.7)	119(79.3)
Ceftazidime	20(13.3)	130(86.7)
Cloxacillin	50(33.3)	100(66.7)
Ocefix	99(66)	51(34)

against cefuroxime by all the isolates were not significantly different from that of ceftazidime and gentamycin ($p > 0.05$), but were significantly higher than other antibiotics ($p < 0.05$). Similarly, resistance against ocefix by all the isolates were not significantly different from that of ceftriaxone and ciprofloxacin ($p > 0.05$), but were significantly lower than that of other antibiotics ($p < 0.05$). The result further showed significant correlation between cefuroxime and ceftazidime ($p < 0.01$).

TABLE III: NO. OF ORGANISMS WITH MULTIPLE ANTIBIOTIC RESISTANCE (%)

No. of organisms	Percentage of organisms	No. of antibiotics
0	0	1
0	0	2
7	4.7	3
21	14.0	4
29	19.3	5
40	26.7	6
39	26.0	7
14	9.3	8
150	100	

Of the 150 isolates obtained, 143(95.3%) of them were multi-drug resistant. Seven(4.7%) of the isolates were resistant to 3 antibiotics, 21(14%) were resistant to 4 antibiotics, and 29(19.3%) were resistant to 5 antibiotics. While 40(26.7%), 39(26%), and 14(9.3%) of the isolates were resistant to 6, 7, and 8 antibiotics respectively. Ninety three (62%) of the isolates were resistant to at least 6 antibiotics (Table III).

DISCUSSION

The result of this study shows that the most predominant clinical sample was urine. This result was in conformity with the works of some researchers who in their studies also reported that urine was the most common clinical sample encountered [13, 19 and 20].

The observation in this study that *Staphylococcus aureus* was the predominant organism from nosocomial samples examined is in support with reports from other researchers [21, 22, 23, 24 and 25]. It however contradicts reports from some researchers who reported other organisms to be most predominant. Such organisms include *E. coli* [26, 27 and 20], *Klebsiella pneumoniae* [19], *and Staphylococcus epidermidis* [6]. The high incidence of *S. aureus in* this study could be as a result of its minimal growth requirements, ability to survive long in most unfavorable environments and in a susceptible host. It could also be due to the virulent nature of the organism, which gives it the ability to overcome body defense mechanisms and resistance to antibiotics [24]. Urinary tract instrumentation and presence of indwelling catheter increase the risk of *S. aureus* carriage in the urinary tract [28].

The finding in this study that *Pseudomonas aeruginosa* had its highest occurrence in wound swab is contrary to the findings of Mordi and momoh [25] who reported that *S. aureus* was the most predominant organism in wound swabs.

The observation in this work that *K. pneumoniae* was the most predominant organism from catheter tips and blood is contrary to Iffat *et al* [*20*], who reported *S. aureus* to be the most predominant isolate instead. Their report that the most predominant isolate from pus was *E. coli* is also contrary to the observation in this study which revealed *S. aureus* to be the predominant organism from pus

The most predominant Gram negative organism isolated in this study was *K. pneumoniae*. This result disagrees with that of some researchers who reported *E. coli* to be the predominant Gram negative organisms in their samples [20, 26 and 27]. Also, Mordi and Momoh, [25] reported that *Proteus* spp. was the most predominant Gram negative isolate in their study. Variations in the types of organisms isolated could be attributed to factors such as environmental difference, media, culturing method, time and method of sampling. Period of transportation and storage before culturing can also be causative factors.

The result of this study revealed very high resistance of all isolates towards cefuroxime

(92.7%). This result is contrary to the findings of Yah and Eghafona [29], who reported very low resistance of their diarrhoeal isolates towards cefuroxime. It however, agrees with the work of Adeleke *et al*. [30], who reported high resistance towards cefuroxime (81.8%) by Gram negative organisms. Their reported high resistance against ceftriaxone however contradicts the observation in this study in which very low resistance to same antibiotic was observed. Iffat *et al*. [20], in their work reported high resistance rate among Gram negative organisms against all generations of cephalosporin antibiotics as well as β-lactam/β-lactamase inhibitors. This study in contrast observed low resistance rate towards ceftriaxone (38%) and ocefix (34%), both of which are cephalosporins.

The contrasting antibacterial activity of ceftriaxone (38% resistance) and cefuroxime (92.7% resistance) in this study agrees with the previous reports of a better activity for ceftriaxone than cefuroxime [31, 32 and 33]. Nevertheless, the equally contrasting multiple drug resistance against cefuroxime, ceftazidime, ceftriaxone and ocefix (all cephalosporins) confirm the alarm raised by Paul *et al*.[34], on the alarming rate of resistance to cephalosporins by the hospital strains especially Gram negative bacteria and more so with the advent of the extended spectrum β-lactamases producers [35, 36]. This confirms a report that in Nigeria, β-lactams are the most frequently prescribed antibiotics especially in Gram negative infections and selective pressure exerted by the use of these β-lactam drugs have resulted in the strains producing the extended spectrum β-lactamases enzymes [37].

The result of this study further showed that all the isolates were highly resistant against gentamycin (79.3%), augmentin (70%), cloxacillin (66.7%), ofloxacin (54%), and ciprofloxacin (51.3%). Similar resistance patterns were reported by other workers [19, 26]. High resistance toward ciprofloxacin by *Klebsiella pneumoniae* and low resistance against ciprofloxacin by CoNS and *Pseudomonas aeruginosa* as observed in this study had also been reported by Iffat *et al*. [20].

Almost all the pathogens isolated in this study have also been shown to cause different forms of nosocomial infections [7, 8, 38, 39, and 40]. *S. aureus* exhibits remarkable versatility in their behaviour towards antibiotics [41]. Outbreaks of *S. aureus* resistant to β-lactam antibiotics have been frequently associated with devastating nosocomial infections [6, 42 and 43].

Klebsiella pneumoniae (and some related species) is an important opportunistic Gram-negative rod pathogen involved in the outbreaks of nosocomial infections, meningitis, lower respiratory, urinary tract and burn wound infections. The members of this genus have also been linked to epidemics of diarrhea, because some strains appear to have acquired plasmids from *E. coli* (that code for the heat labile and heat stable enterotoxins [44, 45].

Pseudomonas aeruginosa is a ubiquitous organism, an opportunistic pathogen, and can cause a wide range of infections including bacterial meningitis, endocarditis, Otitis media [46] urinary tract infection [47] and osteomyelitis [48]. *Pseudomonas aeruginosa* is notorious for its resistance to antibiotics and is therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistance to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharide (LPS). Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics [49].

Escherichia coli is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics [50, 51]. Intestinal strains of *E. coli* are primary cause of urinary tract infections, septicaemia, diarrhoea, neonatal meningitis and nosocomial infections. Individuals who are debilitated or have other predisposing factors are at much risk for infection than healthy person [52]. Antibiotic resistance was high among *E. coli* strains, which emphasize the need for judicious use of antibiotics. Certain virulence factors like hemolysin production and presence of fimbriae in the strain may be associated with its virulence especially urovirulence [53].

Coagulase negative staphylococci (CoNS) especially *Staphylococcus epidermidis* is a major cause of nosocomial infections because of its ability to form biofilms on the surface of medical devices. Bacterial biofilms are inherently resistant to antibiotics and host defences, and this could explain the reason for the high resistance seen in the strains isolated in this study [6, 54, and 55].

Eight (8) strains of *Alcaligenes* spp., were isolated in this study, and were 100% resistant to cefuroxime, augmentin and ceftazidime. The strains were also highly resistance to ofloxacin, gentamycin and ciprofloxacin. *Alcaligenes* spp. is generally considered non-pathogenic but can occur as an opportunistic pathogen in urinary tract infection [56]. Some species of *Alcaligenes* are potential causes of chronic pulmonary diseases in patients with cystic fibrosis [57].

High rates of drug resistance were found in most of the isolates studied. In developing countries like Nigeria, self medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable

to treat themselves. On admission, the community acquired resistant strains exchange genetic material with nosocomial isolates resulting in the emergence of 'superbugs' that could cause difficult to treat infections [6, 58]. Inappropriate practice like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, counterfeit drugs, inadequate hospital infection control measures can as well promote the development of resistance in clinical isolates [6, 59].

Conclusion

It is apparent from the results of the antibiogram that hospitals could be a potential reservoir of nosocomial antibiotic resistant pathogens. The high incidence of antibiotic resistant strains isolated from clinical samples of patients in University of Benin Teaching Hospital (UBTH) is worrisome, and need to be addressed properly. We highly recommend the development of infection control programmes for the surveillance of nosocomial infections and epidemiologic typing of clinical isolates in hospitals in order to check the emergence and spread of antibiotic resistant infections in patient.

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