



Prevalence, Distribution and Antibiotic Susceptibilities of Nosocomial Infections at a Tertiary Hospital in Port Harcourt, Nigeria

Catherine N. Stanley^{1*} and Inimuvie Ekada¹

¹*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author CNS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IK managed the data collection and analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Many infections could previously be treated effectively based on the clinicians past clinical experience. The development of resistance to essentially all of the antimicrobial agents currently in use in clinical practice has made this scenario more of the exception than the norm. Selecting an appropriate antimicrobial agent has become increasingly more challenging as the clinician has to navigate through the variety of available agents in the face of increasing antimicrobial resistance. The diagnostic laboratory plays very important role in clinical practice. To ensure safe and effective empirical treatment, a surveillance study of the susceptibility pattern of common pathogens and appropriate use of antibiotics is imperative. This current study reports on the prevalence, distribution and antibiotic susceptibility patterns of nosocomial pathogens isolated at the University of Port Harcourt Teaching Hospital (UPTH) and the effectiveness of the antibiotics commonly prescribed at the hospital in treating these infections.

Methods: A retrospective cross-sectional study of specimens received at the Microbiology Laboratory was conducted over a six-month period, from October 2015 to March 2016 using urine,

*Corresponding author: E-mail: catherine.stanley@uniport.edu.ng;

blood and semen specimens respectively. A total of 5,160 samples received and analyzed at the laboratory within the study period were assessed.

Results: Out of the 5160 specimens analyzed, 881(17.07%) were positive for bacteria out of which 691(78.43%), 86(9.76%), 104 (11. 81%) were from urine, blood and semen respectively. *Escherichia coli* (35.74%), *Klebsiella pneumoniae* (52.33%) and *Staphylococcus aureus* (65.4%) were the most frequently isolated pathogens from urine, blood and semen respectively. Wide spread multiple-drug resistance was observed among the organisms. *Klebsiella pneumoniae*, *S. aureus*, and *E. coli* isolated from urine were resistant to amoxicillin/clavulanate, cefuroxime, ceftazidime, ciprofloxacin, ampicillin, gentamycin and ceftriaxone. A review of the pattern of prescribing antibiotics revealed that in the Accidents and Emergency unit, ceftriaxone (34.09%) and metronidazole (30.09%) were most frequently prescribed while in the General Out-Patient Department, metronidazole (19.09%), amoxicillin (16.61%), amoxicillin/clavulanate (9.39%) and ofloxacin (9.39%) were often prescribed. *S. aureus* was susceptible to only ceftriaxone while *K. pneumoniae* and *E. coli* were susceptible only to ofloxacin.

Conclusion: Most of the isolated pathogens were not susceptible to the frequently prescribed antibiotics. Empirical prescribing of antibiotics without current epidemiological data of pathogens in the hospital can only further exacerbate the problem of antimicrobial resistance. The need for epidemiological surveillance and rational use of antibiotics in the Hospital is therefore strongly recommended.

Keywords: Antibiotics; susceptibility pattern; surveillance; pathogens.

1. INTRODUCTION

Microbial pathogens have accompanied humanity for centuries and are among the significant cause of morbidity and mortality worldwide [1,2]. Virtually all groups of bacteria have some members that are pathogenic. Previously, many infections could be treated effectively based on the clinicians past clinical experience. Presently, however, this has become more of the exception than the norm as resistance has been observed to essentially all of the antimicrobial agents currently in use in clinical practice [3,4]. Selecting an appropriate antimicrobial agent has become increasingly more challenging as the clinician has to navigate through the variety of available agents in the face of increasing antimicrobial resistance. The situation has brought to the fore the importance of the diagnostic laboratory in clinical practice as the clinician now depends more than ever before on data from in vitro - antimicrobial susceptibility testing [5]. Susceptibility is also an important first step in providing surveillance data for use in local and international aggregate data bases [6-8]. This problem is further compounded by the increasing trends in antibiotic resistance even in the commonly isolated organisms all over the world [9].

The discovery of antibiotics revolutionized medical practice [10,11]. However, their initial dramatic effectiveness has resulted to irrational and inappropriate use leading to

emergence of resistance to these life-saving drugs [12].

Empirical treatment with ineffective antibiotics prescribed by physicians and poor patient adherence to antibiotic regimens could eventually lead to mutation and drug resistance [13]. The worldwide increase in resistant bacteria is affecting the effective treatment of patients, stressing on the need for continued surveillance, appropriate antibiotic prescribing, effective infection control, and new treatment alternatives [14].

Epidemiological surveillance of antimicrobial resistance is essential for empirical treatment of infections, implementing resistance control measures, and preventing the spread of antimicrobial-resistant microorganisms [15].

The successful treatment of serious infections requires timely administration of effective antimicrobial agents. Hence, clinical decisions about empirical treatment require knowledge of likely pathogens and the likely susceptibility of these pathogens to antibiotics. Such knowledge is gained by clinical experience over time, but more objectively and robustly through surveillance [16].

This retrospective study, therefore, reports on the commonly isolated organisms and describes their susceptibilities to the commonly prescribed antibiotics at the University of Port Harcourt Teaching Hospital. The findings from this study

can serve as a guide in ensuring the safety of empiric therapy as well as control antibiotic resistance.

2. MATERIALS

2.1 Culture Media

Mannitol Salt Agar, MacConkey Agar, Mueller Hinton Agar, Nutrient Agar, and Simon's Citrate Agar were used.

2.2 Reagents

3% Hydrogen Peroxide, Distilled water, N, N, N, N-Tetramethyl-p-phenylene diamine dihydrochloride, Safranin red, Crystal violet, and Lugol's iodine were used for this study.

2.3 Antimicrobial Agents

2.3.1 Gram-positive antimicrobial discs

Ofloxacin 5 μ g , Ceftriaxone 30 μ g , Amoxicillin/clavulanate 20/10 μ g , Amoxicillin 25 μ g,

2.3.2 Gram-negative antimicrobial discs

Ofloxacin 5 μ g , Amoxicillin/clavulanate 20/10 μ g, Metronidazole 25 μ g.

3. METHODS

3.1 Study Design and Setting

This retrospective cross-sectional study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), a tertiary health care facility in Rivers state, Nigeria, to determine the pattern and prevalence of microbial pathogen isolation and susceptibility to commonly prescribed antibiotics, within a six (6) month period, from October 2015 to March 2016.

3.2 Data Collection

The results of all requests for laboratory investigation from different wards and units received at the Medical Microbiology Laboratory between the periods of October 2015 to March 2016 were systematically examined retrospectively. The results for urine, blood and semen specimens were recorded in the data collection sheet. Other data collected included the age of the patient, sex, ward, type of organism isolated and their antimicrobial susceptibility.

4. LABORATORY PROCEDURE

4.1 Collection and Identification of Test Organisms

Clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* which were observed to be the most frequently isolated organisms from the records, were obtained from the Microbiology and Parasitology department of the hospital and identified using standard microbiological and biochemical methods. The test organisms were sub cultured on their selective media; *Staphylococcus aureus* on Mannitol Salt Agar (MSA), *Escherichia coli* and *Klebsiella pneumoniae* on MacConkey Agar. The morphological characteristics of their colonies were observed. Gram staining and other biochemical tests were carried out to confirm their identities.

4.2 Antibiotic Susceptibility Testing

A speck of each test organism was inoculated in 0.1% peptone water for 24 hours, after which the turbidity was adjusted to correspond with the turbidity of 0.5 MacFarland's standard. The antibiotic susceptibility test was performed by Kirby-Bauer disk diffusion method according to the standards of Clinical and Laboratory Standards Institute [17-18]. The diameter of inhibition zones around the antibiotic discs were measured and interpreted as sensitive or resistant according to CLSI guidelines [17].

4.3 Review of Antibiotic Prescribing Pattern

A review of antibiotic prescribing pattern was conducted at the Accident and Emergency Unit and General Out Patient Department (GOPD) to determine if the frequently isolated organisms were susceptible to the commonly prescribed antibiotics to justify the empirical prescribing of antibiotics seen often in these units.

4.4 Data Analysis

The data obtained were tabulated and analysed using the Statistical Package for Social Sciences (SPSS) version 10. *Chi*-square test was used to compare the proportion of bacterial isolates between sex and age. *P*-value of less than 0.05 was considered to indicate statistically significant difference.

5. RESULTS

5.1 Distribution of Pathogens by Clinical Samples

Fig. 1 below shows the percentage distribution of clinical isolates from samples analyzed in the Hospital's Microbiology and Parasitology department. A total of 881 (17.07%) samples out of the 5160 samples received by the Microbiology and Parasitology department showed presence of bacterial pathogen. A breakdown of the bacterial pathogens isolated showed that urine 691(78.43%), semen 104 (11.81%) and blood 86(9.76%) harboured pathogens in decreasing order of prevalence.

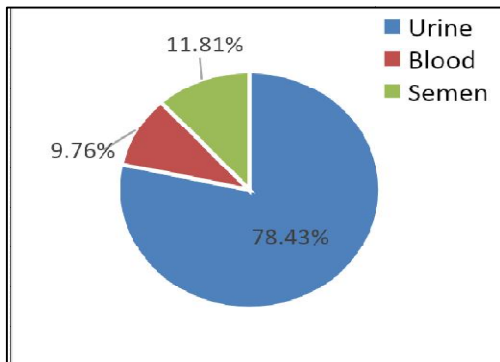


Fig. 1. Distribution of Pathogens by Clinical Samples

Gram negative bacteria were more (64.13%) in comparison to Gram Positive bacteria (35.87%), as shown in Fig. 2a below. However, while Gram-negative isolates were more prevalent in urine and blood samples, Gram -positive isolates predominated in semen culture as shown in Fig. 2b below.

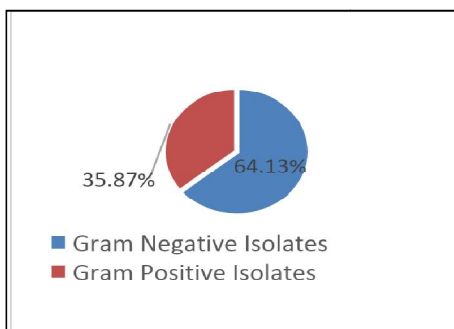


Fig. 2a. Distribution of clinical isolates according to Gram-negative and Gram-positive reaction

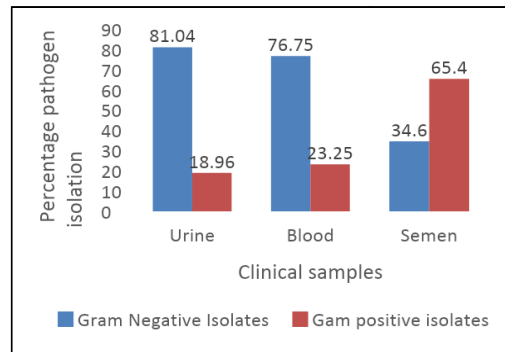


Fig. 2b. Distribution of isolates from different clinical specimens according to Gram-negative and Gram-positive reaction

5.2 Distribution of Clinical Samples in the Various Clinical Wards and the Frequently Isolated Bacterial Pathogens

The frequency of isolation of pathogens from urine, semen and blood specimens from the various clinical wards at the UPTH and the frequently isolated organisms among the clinical samples are shown below in Fig. 3a and 3b. The results showed that Special Care Baby Unit (SCBU), General Outpatient Department (GOPD), Accident and Emergency (A&E) and Obstetrics and Gynaecology (O&G) clinic accounted for majority of the pathogens isolated. The frequently isolated pathogens from these samples were *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. The predominantly isolated organism in semen samples was *S. aureus* (65.4%) while *K. pneumoniae* (52.33%) and *E. coli* (35.75%) predominated in blood and urine respectively.

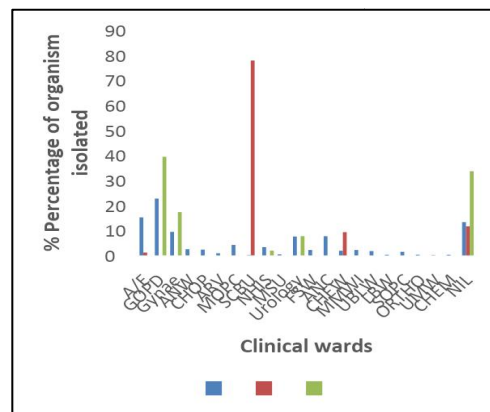


Fig. 3a. The Distribution of the Clinical isolates across the Clinical wards

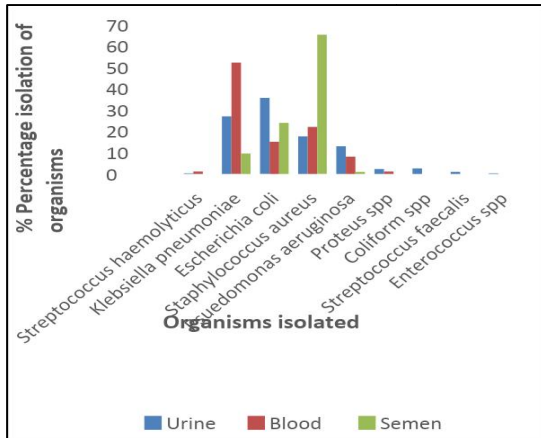


Fig. 3b. Frequently isolated bacterial pathogen in urine, blood and semen samples

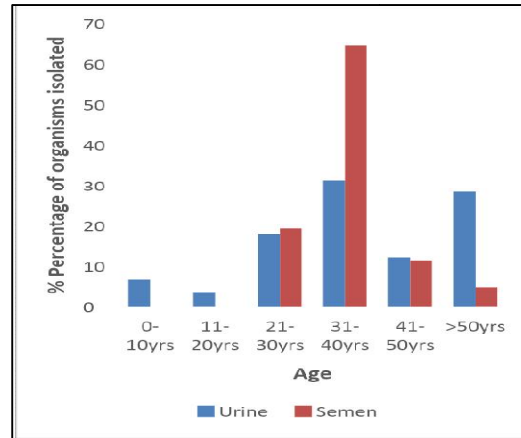


Fig. 4b. The Distribution of Organisms isolated from semen and urine in the different Age groups

A&E- Accidents and Emergency department, GOPD- General Out-Patient Department, Gynae-Gynaecology clinic, ANW- Ante-natal Ward, CHOP-Children Out-Patient Department, ARV-Antiretroviral Clinic, MOPC-Medical Out-Patient Clinic, SCBU-Special Care Baby Unit, NHIS-National Health Insurance Scheme, MSW-Male Surgical Ward, FSW-Female Surgical Ward, ANC-Antenatal Clinic, CHEW-Children Emergency Ward, MMWI-Male Medical Ward I, UBLW-Un-booked Labour Ward, LBW-Labour Ward, SOPC-Special Out-Patient Clinic, Ortho-Orthopaedic, UMW-Unbook Male Ward, CHEW-Children Emergency.

The distribution of isolates from urine, blood and semen samples across sex and different age groups was also done and the results are as shown in Fig. 4a, 4b and 4c respectively below.

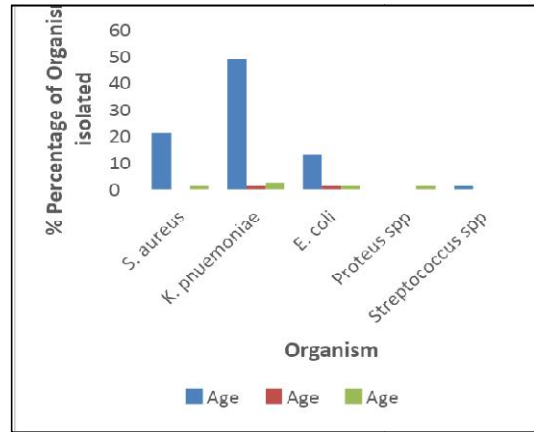


Fig. 4c. Distribution of organism isolated according to age in blood sample

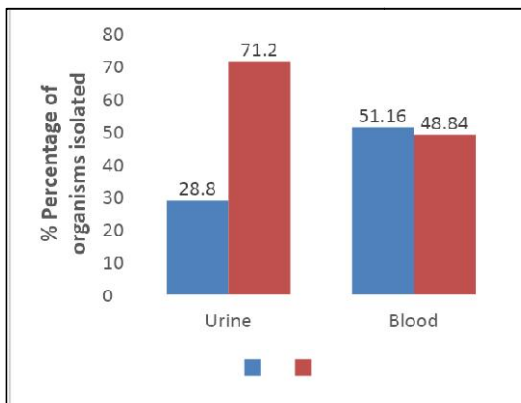


Fig. 4a. Distribution of organisms isolated from urine and blood across the different sexes

5.3 Antimicrobial Susceptibility Pattern of the Commonly Isolated Organisms in Urine and Blood

Fig. 5 shows the susceptibility pattern of the commonly isolated organisms in urine samples at the University of Port Harcourt Teaching Hospital.

Table 1 shows analysis of prescriptions using the WHO prescribing indicators. It shows 47.14% and 34.16% of prescriptions in the A&E and GOPD pharmacies respectively had an antibiotic in them with an average number of 1.52 and 1.42 antibiotic prescribed per encounter respectively. A 22.58% and 12.93% of the total number of drugs prescribed in the A&E and GOPD pharmacy respectively were antibiotics.

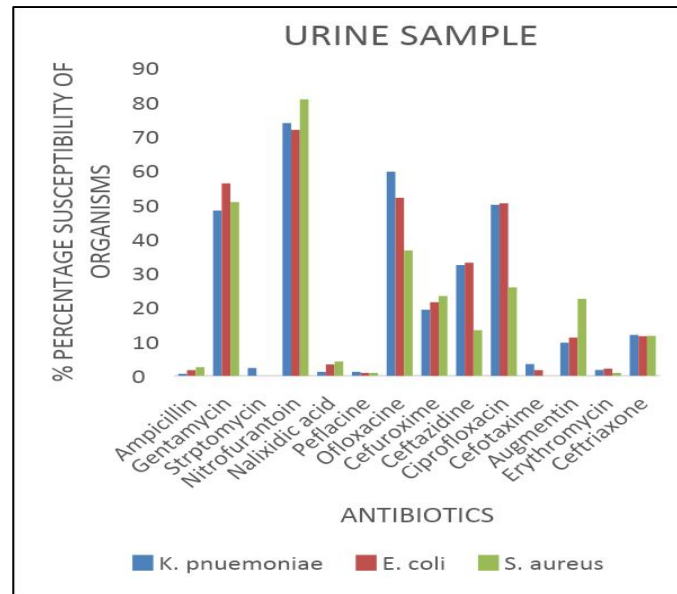


Fig. 5. Susceptibility pattern of commonly isolated organisms in urine to tested antibiotics

Table 1. Prescribing indicators

Prescribing Indicators	A&E Pharmacy No. (%)	GOPD Pharmacy No. (%)	Optimal Values No. (%)
Total number of prescriptions analysed	630	1906	
Total number of drugs prescribed	2002	7167	
Number of antibiotics in relation to number of all medicines prescribed	452(22.58)	927(12.93)	
Average number of antibiotics per prescription	1.52	1.42	
Average number of drugs per encounter*	3.18	3.76	(1.6-1.8)
Drugs prescribed by generic name*	1353(67.58)	2156(30.08)	(100)
Drugs prescribed from the essential medicine list (Rivers)*	401(20.03)	787(10.98)	(100)
Total number of prescriptions with an antibiotic*	297(47.14)	651(34.16)	(20-26.8)
Total number of prescriptions with an injection*	453(71.90)	6(0.31)	(13.4-24.1).

*WHO Prescribing indicators

GOPD- General Outpatient Department

Fig. 6a illustrates the different types and frequencies of antibiotics prescribed in the General Out-patient Department of the Hospital. The most frequently prescribed antibiotics were Metronidazole (19.09%), Amoxicillin (16.61%), Amoxicillin/clavulanate (9.39%) and Ofloxacin (9.39%).

The Fig. 6b illustrates the different types and frequencies of antibiotics prescribed in the accidents and emergency (A &E) department of the Hospital. The most frequently prescribed antibiotics in this unit were Ceftriaxone (34.07%), Metronidazole (30.09%),

Ciprofloxacin (7.3%), and Amoxicillin/clavulanate (7.08%).

6. DISCUSSION

Records from the Microbiology and Parasitology department showed that a total of 5,160 samples were received within the study period. The samples comprised of 4,432 (85.89%) urine, 474 (9.19%) blood and 254 (4.92%) semen samples. Out of the 5,160 samples received, 881 (17.07%) were positive for bacterial pathogens which included 691 (78.43%) from urine, 86 (9.76%) from blood and 104 (11.81%) from semen (Fig. 1).

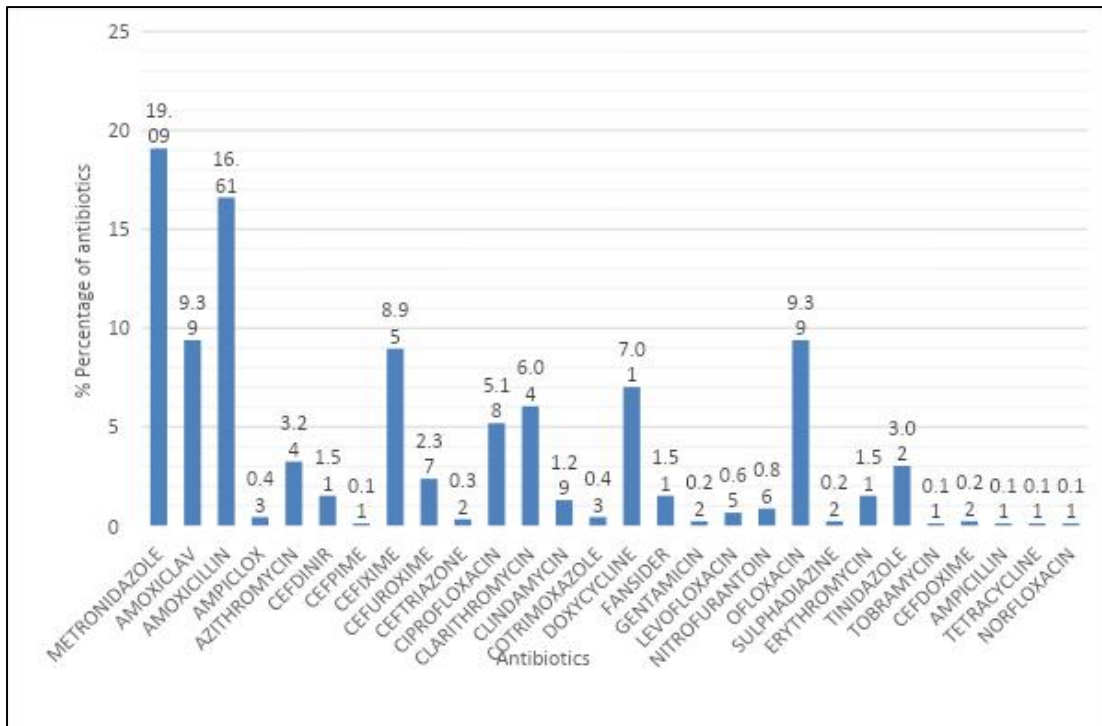


Fig. 6a. Types and frequency of antibiotics prescribed in the GOPD pharmacy

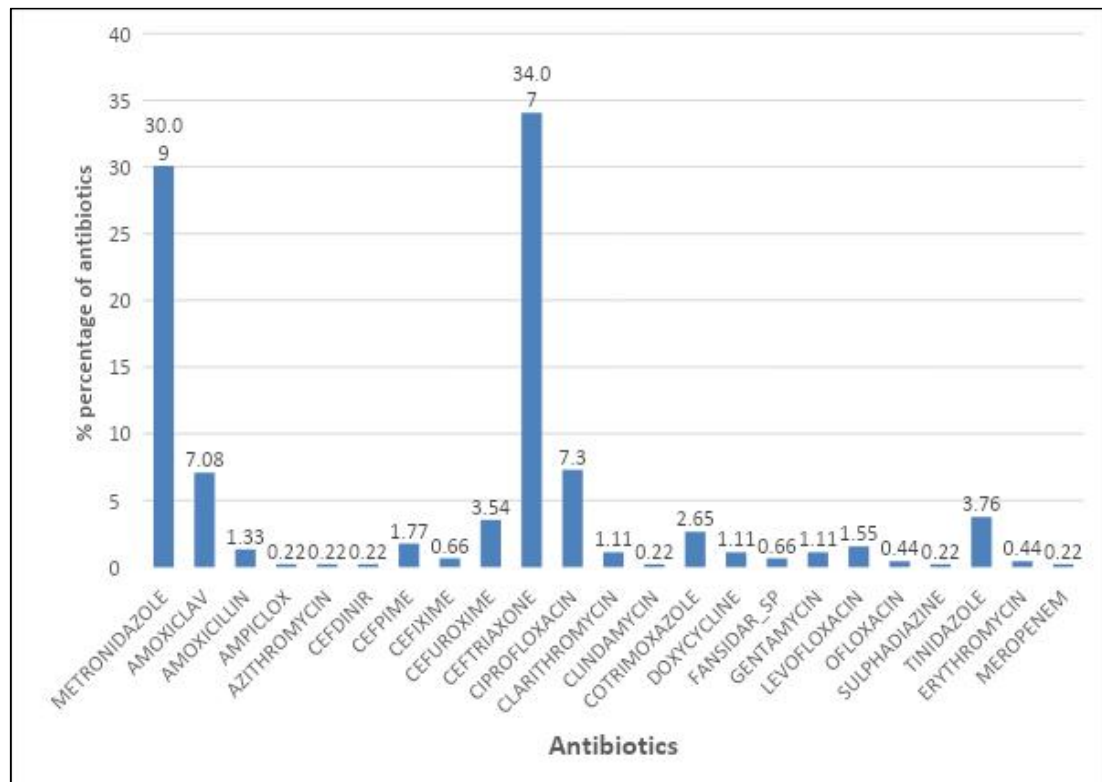


Fig. 6b. Types and frequency of antibiotics prescribed in the A&E pharmacy

From our study, urine samples were the most frequently received samples. This is in agreement with the findings of an earlier study done in Dhaka [19]. Urinary tract infections have also long been established as a common cause of infectious disease in humans regardless of age and gender [20,21].

Gram-negative bacteria were the predominantly isolated organisms from most of the samples (64.13%) while Gram-positives were only 35.87% (Fig. 2a). This is not entirely surprising as Gram negative organisms are increasingly being implicated in nosocomial infections as seen in other studies elsewhere that found a predominance of Gram –negative bacteria [22].

Gram-negative bacterial pathogens were more prevalent in urine and blood samples while Gram-positive organisms were more in semen specimen (Fig. 2b). The predominant bacteria isolated were *E. coli*, followed by *K. pneumoniae* and *S. aureus* (Fig. 3b). The prevalence of *E. coli* as the predominant organism isolated in urine is in harmony with findings from other studies [23-25].

The fact of urinary tract infections being more prevalent in females than males has long been established and is in agreement with findings of this study and supported by other studies [24,26-28]. The high prevalence seen in the age group of 31 to 40 years (31.26%) could be due to increased sexual activity when compared to the age group of 11 to 20 years (6.6%) (Fig. 4a, 4b). The anatomical structure of the female urogenital tract, sexual activity and the use of some form of contraceptives like diaphragms and spermicides in females are thought to account for the higher prevalence of UTI in females [29].

The frequently isolated organisms in this study, *E. coli*, *K. pneumoniae* and *S. aureus* exhibited high level of resistance to many of the antibiotics including amoxicillin/clavulanate, cefuroxime and ampicillin, pefloxacin, nalidixic acid, streptomycin, erythromycin, cefotaxime, ceftriaxone and ceftazidime (Fig. 5). Our study revealed that *E. coli* was resistant to amoxicillin /clavulanic acid which is in agreement with the findings of Rajani and Banerjee in their study [22]. In contrast to our finding, however, a study had reported *S. aureus* to be susceptible to amoxicillin/clavulanate although this may have changed over time [24,29].

The three frequently isolated organisms were very susceptible to nitrofurantoin, gentamycin, ofloxacin, and ciprofloxacin respectively in decreasing order (Fig. 5a). This is in harmony with findings from other studies [24,30]. The high susceptibility of organisms in urine to nitrofurantoin could be as a result of its local effect as a urinary antiseptic concentrated more in the urine as well as the fact that it is prescribed sparingly. This may also explain the reduced susceptibility of organisms isolated from blood to nitrofurantoin.

The organisms isolated from blood samples were *Klebsiella pneumoniae* (52.33%) and *Escherichia coli* (15.12%), both Gram- negative, and *Staphylococcus aureus* (22.09%) and *Streptococcus haemolyticus* (1.16%) (Fig. 3b). *K. pneumoniae* bacteraemia has been reported as a relatively rare but serious infection that occurs in young children with predisposing underlying conditions and it is associated with a significant mortality rate [31]. Studies in Nigeria and elsewhere have reported that Gram-negative organisms were the most common etiological agent of bacteraemia but with *Escherichia coli* as the predominant bacterium [32,33]. Contrary findings have been reported by some Nigerian authors reporting from northern and western Nigeria where Gram-positive organisms were the predominant organisms isolated in blood samples with *Staphylococcus aureus* as the most frequently isolated [34]. This is considerable as different etiologic agents can be related to different geographic areas and within a given area [35]. The rate of bacterial pathogen isolation in blood was observed more in males (51.16%) than in females (48.84%) within the age group of <180 days (20.93%) which is similar to a 25.7% isolation rate reported in a study done in Kano, Nigeria [32]. The prevalence of bacteraemia in neonates may be due to immature immune system.

The records indicated high levels of antibiotic resistance in the frequently isolated micro-organisms in blood. Fifty percent (50%) of *S. aureus* were resistant to gentamycin and nitrofurantoin, 40% of *E. coli* were resistant to cefuroxime (Fig. 5). A wide spread resistance of the organisms to most of the antibiotics used for the susceptibility test was observed. Gram-negative organisms (76.75%) were predominant though *K. pneumoniae* showed a relatively low resistance to ofloxacin (2.78%). This is contrary to other studies that reported the susceptibility of *E. coli* and *S. aureus* to gentamycin, Ciprofloxacin and amoxicillin/clavulanate [32,33].

The antibiotic susceptibility tests of the organisms in blood indicated a high level of susceptibility of *K. pneumoniae* and *E. coli* to ofloxacin, followed by cefuroxime. Although ofloxacin showed a high level of sensitivity, its use is generally contraindicated in children below eighteen years except where the benefits far outweigh the risks [36].

The predominant bacterial pathogens isolated from semen were Gram-positive bacteria which included only *Staphylococcus aureus* (65.4%), while gram negative bacteria (34.6%) consisted of *Escherichia coli* (24%) and *Klebsiella pneumoniae* (9.6%) (Fig. 3.a and 3b). Similar findings were also recorded in a work done in Benin, Nigeria [37]. A high rate of bacterial isolation was recorded within the age group of 31 to 40 years probably due to heightened sexual activity (Fig. 4b).

The samples received by the Microbiology and Parasitology department were mainly from the Accidents and emergency department (15.2% of isolates from urine), General Out-Patient Department (22.72% and 39.42% isolates from urine and semen respectively) and the special care baby unit (77.19% from blood culture) (Fig. 3a).

A review of the pattern of antibiotics prescribing from the accidents and emergency, and general out-patient department was conducted. A total of 2536 prescriptions were analysed with 630 prescriptions from the accidents and emergency department and 1906 prescriptions from the general outpatient department (Table 1).

From this study, (297) 47.14% of prescriptions from the accidents and emergency department contained an antibiotic (Table 1). As high as 78.84% of prescriptions with antibiotic had been reported in an accident and emergency department. Similar findings of a high level of antibiotic prescribing in the accidents and emergency department had also been reported. This reported high level of antibiotic prescribing in the accidents and emergency department could be due to the over exaggeration of infection as most cases in this department are emergency situations and require empiric therapy.

A total of 651 (34.16%) of prescriptions from the general out-patient department contained an antibiotic. Similar findings have been recorded in other out-patient departments [38] Table 1). Our figure of 47.14% (A&E) and 34.16% (GOPD) of

prescriptions having an antibiotic is higher than those specified in WHO (optimal value of 22-26.8%) [39]. Appropriate use of antibiotics is necessary to prevent emergence of drug resistant bacteria.

The commonly prescribed antibiotics in the A&E department were ceftriaxone (34.09%) and metronidazole (30.09%) (Fig. 6b). Similar findings have been reported where ceftriaxone was the commonly prescribed drug in the accidents and emergency department while in GOPD metronidazole (19.09%), amoxicillin (16.61%), amoxicillin/clavulanate (9.39%) and ofloxacin (9.39%) were the frequently prescribed (Fig. 6a). Another study in Port Harcourt, Nigeria also recorded metronidazole, amoxicillin/clavulanate and amoxicillin as the most commonly prescribed antibiotic [40]. From this and all other studies reviewed, amoxicillin, and metronidazole occurred as the most commonly prescribed antibiotics.

These frequently prescribed antibiotics were tested for their effectiveness on the commonly isolated organisms; *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. *Staphylococcus aureus* was susceptible only to ceftriaxone (30 μ g) with an average zone of inhibition of 23mm, and resistant to amoxicillin (50 μ g), ofloxacin (5 μ g) and amoxicillin/clavulanate (20/10 μ g) [41]. *Escherichia coli* and *Klebsiella pneumoniae* were only susceptible to ofloxacin with a zone of inhibition of 20mm and 25mm respectively [41].

From the antimicrobial susceptibility test conducted on the frequently isolated organism with the commonly prescribed antibiotics, *Staphylococcus aureus* was susceptible only to ceftriaxone but resistant to amoxicillin, amoxicillin/clavulanate and metronidazole. In this study, it was observed that amoxicillin/clavulanate was unable to inhibit the growth of the three frequently isolated organisms. *Escherichia coli* and *Klebsiella pneumoniae* were susceptible to ofloxacin and resistant to ceftriaxone, amoxicillin, amoxicillin/clavulanate and metronidazole. The resistance observed with metronidazole could be as a result of its effects being more on obligate anaerobes.

7. CONCLUSION

The frequently isolated organisms from urine, blood and semen at the University of Port

Harcourt Teaching Hospital were *E. coli*, *K. pneumoniae* and *S. aureus*. Multidrug resistance was observed in these organisms. The frequency of antibiotics prescribing in the A&E and GOP Departments fell short of the WHO recommendation and out of the five frequently prescribed antibiotics (ofloxacin, ceftriaxone, metronidazole, amoxicillin/clavulanate and amoxicillin), *S. aureus* was susceptible only to ceftriaxone while *K. pneumoniae* and *E. coli* were susceptible only to ofloxacin. In view of these findings, empirical prescribing of antibiotics in the hospital in the absence of current epidemiological and surveillance data is counterproductive. An urgent review of the hospital formulary, if any, and antibiotic stewardship programme based on local epidemiologic data is strongly recommended to ensure the rational use of antibiotics in the hospital.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Research and Ethics Committee of University of Port Harcourt Teaching Hospital (UPTH). The names of the patients were not recorded and the patients' identities were not included anywhere in the data collection sheets to maintain confidentiality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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