COMPARATIVE IN-VITRO ACTIVITY OF FLUOROQUINOLONES AGAINST CLINICAL BACTERIAL PATHOGENS ISOLATED FROM CENTRAL HOSPITAL WARRI

ISIBOR, C.N.[1]* AND AHONKAHI, I.[2]

[1]Department of Biological Sciences, University of Delta, Agbor, Delta State, Nigeria
[2]Department of Pharmaceutical Microbiology, University of Benin, Benin City, Nigeria

ABSTRACT

Local sensitivity and resistance are important guides to help clinicians to make good choices in a given situation that demands the use of antimicrobial agents. Antibiotic susceptibility testing was carried on bacterial isolates from clinical specimens and ascertain their susceptibility to fluoroquinolones and other antimicrobial agents. A total of 260 clinical samples of urine, Sputum, cerebrospinal fluid, semen, aspirates, and swabs from wound, virginal, ear, and nose consecutively at the Central Hospital, Warri was examined using standard bacteriological techniques. Antibiotic susceptibility tests were performed using the standard disc diffusion method. A total of 118 bacterial isolates were recovered. The significant pathogens included *Staphylococcus aureus* (31.35%), *Klebsiella aerogenes* (27.97%), *Escherichia coli* (22.03%), *Pseudomonas aeruginosa* (9.30%), *Proteus mirabilis.* (8.5%) and *Streptococci sp.* (0.85%). Overall sensitivity was shown to be pefloxacin 100% while ofloxacin, ceftazidime and cefuroxime were 83.05% and ciprofloxacin 80.51%, gentamicin 77.12%, streptomycin 34.75%, penicillin 7.6% and ampicillin 2.5% of strains tested. The sensitivity results showed the continued increase in the prevalence of antibiotic resistance in older penicillin-based B-lactams, penicillin and ampicillin. Also, given the prominent role of susceptibility in health matters, there is a need to keep physicians current on organisms' sensitivity and resistance possibilities, as demonstrated by this work.

Keywords: Anti-Bacterial Agents, Bacterial Infections, Bacteriological Techniques, Fluoroquinolones, Nigeria, Pefloxacin, Urinary Tract Infections.
***Correspondence**: clement.isibor@unidel.edu.ng, + 2348037221650,

INTRODUCTION

Bacterial infections remain a significant cause of mortality and morbidity in tropical countries, accounting for the highest hospital admissions in developed and developing countries. In general, the human body is extraordinarily well equipped to fight bacterial invasion. The skin is very efficient at preventing the entry of organisms under both its physical properties and its ability to produce unsaturated fatty acids, which have antibacterial action. The mucus membranes and their secretions also efficiently prevent the entry of microorganisms [1]. Once invasion has taken place, the bacterium is confronted with a well-orchestrated set of responses, which are innate and acquired immune responses. The innate immune responses are in the form of an inflammatory response, Cellular migration, phagocytosis and Intracellular killing mechanisms. The acquired immune response is manifested as antibody production, complement systems, among others. The overall effect of the host defence in bacterial death. Hence most infections do not require therapy, and they are taken care of by the body's defence mechanisms. But when the body defence is overwhelmed, infections then set in. Antimicrobial agents are used for the treatment of established infections [2]. General practitioners are confronted with varying types of infections daily. They offer their services based on astute clinical judgment. Their scope of experience, facilities, and in-depth knowledge of the *in-vitro* activity and the pharmacokinetic properties of antimicrobial agents obtained from results of specific laboratory tests [3].

In many patients with infections, the diagnosis involves clinical suspicion and laboratory evaluation that includes culture [4]. Most often, patients seek the help of clinicians because of acute infections, most of which are upper respiratory tract infections, lower respiratory tract infection, abdominal infections, urinary tract infections, sexually transmitted infections, gynaecological infections [5]. Other clinical presentations include bone and joint infections, skin and soft tissue infection, and ear and central nervous system. In the modern hospital environment, familiarity with the local epidemiological features of infectious agents and their patterns of resistance to antimicrobial agents become extremely important [6]. The goal of *in-vitro* antibiotic sensitivity testing in clinical laboratories is either to assist the clinicians in choosing an appropriate therapeutic or prophylactic antibiotic individuals or groups of patients or to help them account for failures of response to empirically selected agents [7].

The antimicrobial susceptibility test is the most critical function of the microbiology laboratory. Even failure to isolate a pathogen at least leaves the treating clinicians able to rely on the experience of others, but reporting an isolate as susceptible to a drug to which it is resistant is anathema [8]. As antimicrobial are continually being introduced, it

becomes difficult for clinicians to make a good choice in a given situation that demands antimicrobial agents. Hence comparative study becomes essential to provide some guidance. Although there are considerable studies on older individual antibiotics or those of similar groups, but there is paucity of information in our study area. It was, therefore, to study the susceptibilities of freshly isolated pathogens to some commonly available antimicrobial agents with especially emphasis on some quinolones. Therefore, the aim of this study is to isolate and identify pathogens from clinical specimens received at the Central Hospital Warri and ascertain their susceptibility to the quinolone groups.

MATERIALS AND METHODS

Ethical consideration

Ethical approval was obtained from the Institutional Ethics Committee of Central Hospital Warri. The objective of the study was explained to all subjects who gave informed consent.

Sample collection

Microbiological studies were carried out on two hundred and sixty (260) clinical samples submitted to the Microbiology laboratory of the Central Hospital, Warri, over fourteen months. These consisted of one hundred and fourteen (114) of which One hundred and ten (110) are mid-stream urine samples and four (4) urine samples from catheterised patients. Other samples included high vaginal swabs (50), wound swabs (26), urethral swab- (17), ear swabs (16), nasal swabs (2), sputum (2), aspirates (3), seminal fluids (15) and CSF (15) for the isolation of bacteria. Appropriate samples were cultured in blood agar, Chocolate Agar, and Cystine Electrolyte Deficient (CLED) agar using standard bacteriological techniques. The chocolate agar was incubated under micro-aerophilic environment in a carbon dioxide extinction jar at 37^{o}C. All isolates were characterised and identified by techniques described by Cowan & Steel, (1994). Antimicrobial susceptibility testing was performed on each isolate using the disc diffusion technique [10]. The results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) [11].

RESULTS

A total of 260 clinical samples were microbiologically assessed, from which a total of 118 isolates were identified as pathogens. The most frequently received and screened specimen was urine 39.8%, followed by wound swabs 23.7%, high vaginal swabs 11.9%, ear swabs 9.3%, urethral swabs 6.0%, semen, and aspirates recorded 2.5%, respectively. In comparison, nasal swabs and cerebrospinal fluid accounted for 1.7% of the total isolates from all the examined. The least specimen examined was sputum, whose isolates accounted for 0.9% of the specimens. Table I. shows the frequency of specimen and positive bacterial recovery.

Table 1: Number of Specimen and Positive Bacterial Recovery.

Specimens	**Recovery of Organisms**		
	Number	**Positive**	**Percentage Recovery**
Urine	114	47	32.63
High Vaginal Swabs	50	14	28
Wound swab	26	26	100
Urethral Swab	17	7	41
Ear swab	16	11	68.75
Nasal swab	2	2	100
Cerebrospinal Swab	15	2	13.33
Semen	15	3	20
Aspirates	3	3	100
Sputum	2	1	50

Table 2: Positive Results from Screening 91 Males and 169 Females.

	Males	**Females**	**Total**
Number of Positive cases	51	67	118

Chi -square with Yate's correction
X^2=5.77, P>0.01

Table 2 shows the results from the screening of 91 males and 169 females. In order to determine whether there were any variations among sexes in positive cases statistical analysis was done using Chi square with Yate's correction. There was no significant variation among genders in this study ($p>0.01$). More females than males had bacterial infections, but there was no consistency in microbial variation. Of the 118 bacterial isolates recovered, females isolate represented 57.8% while those from males accounted for the remaining 43.2%.

Table 3 shows the distribution of isolates of a total of 118 isolates, *Staphylococcus aureus* was the most commonly seen species in this study, accounting for 37 (31.35%). There was only one positive case of *Streptococci* sp 0.85%, *Klebsiella aerogenes* was 33 (27.97%), followed by *Escherichia coli* 26 (22.03%) with *Pseudomonas aeruginosa* and *Proteus mirabilis* 11 (9.30%) and 10 (8.5%) of the isolates respectively. Table 4 shows the antibacterial susceptibility patterns of the isolates to antibacterial agents using disc diffusion techniques. Nine different agents were used. All the isolates had 100% sensitivity to pefloxacin, while ofloxacin, ceftazidime and cefuroxime had a sensitivity of 83.95%, respectively. Ciprofloxacin 80.51%, gentamicin 77.12%, streptomycin 34.75%, penicillin 7.6% and ampicillin 2.5%. As would be seen, less than 10% of the significant pathogen were sensitive to ampicillin and penicillin.

Table 3: Cumulative bacteria isolates and their sources of isolation

Source of the specimen (Number examined	**Total Isolates recovered**	**Organisms recovered**						
		***Staphylococcus aureus* (%)**	***Streptococci* spp (%)**	***Escherichia coli* (%)**	***Klebsiella aerogenes* (%)**	***Proteus mirabilis* (%)**	***Pseudomonas aeruginosa* (%)**	**Without growth (%)**
Urine (114)	47	10 (21.28)	-	21 (44.68)	13 (27.66)	1 (2.13)	2 (4.25)	63 (57.30)
HVS (50)	14	11 (78.57)	-	1 (7.14)	2 (14.29)	-	-	36 (72)
Wound Swabs (50)	28	3 (11.0)	-	2 (7.10)	11 (39.3)	8 (28.6)	4 (14.0)	-
Urethral Swab (17)	7	4 (57)	-	-	3 (43)	-	-	10 (42.4)
Ear Swabs (16)	11	1 (9.1)	-	2 (18.2)	2 (18.2)	1 (9.1)	5 (45.4)	5 (31.30)
Semen (15)	3	3 (100)	-	-	-	-	-	12 (80)
CSF (15)	2	2 (100)	-	-	-	-	-	13 (87)
Aspirate	3	3 (100)	-	-	-	-	-	-
Nasal Swabs (2)	2	-	-	-	2 (100)	-	-	-
Sputum (2)	1	-	1	-	-	-	-	1 (50)
Total (260)	118	37 (31.5)	1 (0.85)	26 (22.03)	33 (27.97)	10 (8.50)	11 (9.30)	140 (54.6

Table 4: Antimicrobial Percentage Susceptibility Patterns of Bacterial Isolates

Antibiotics (Disc content)	***Overall (%)***	**Isolates**					
		Staphylococcus aureus (37)	***Streptococci* spp *(1)***	***Escherichia coli (26)***	***Klebsiella aerogenes (33)***	***Proteus mirabilis (10)***	***Pseudomonas aeruginosa (11)***
Pefloxacin (5μg)	100	100	100	100	100	100	100
Ofloxacin (5μg)	83.05	86.84	100	88.5	81.8	70	81.8
Ceftazidime (30μg)	83.05	83.78	100	88.8	87.9	100	100
Cefuroxime (30μg)	83.05	83.78	100	80.8	75.8	70	72.7
Ciprofloxacin (5μg)	83.05	81.08	100	80.8	84.8	80	72.7
Gentamicin (10μg)	77.12	86.48	100	65.4	72.7	70	100
Streptomycin (10μg)	34.75	37.33	100	19.2	24.2	60	63.6
Penicillin (15μg)	7.6	10.8	NT	7.7	NT	NT	NT
Ampicillin (10μg)	2.5	8.82	NT	3.9	NT	NT	NT

Key
NT =Not Tested

DISCUSSION

Bacterial infections remain a significant cause of mortality and morbidity in tropical countries; hence prompt response to such infections through correct diagnosis cannot be underscored. The present study, therefore, has the threefold objectives, namely: isolation and identification of the prevailing pathogens from different clinical specimens and their current status of antimicrobial agents that are commonly prescribed against such bacterial isolates at the Central Hospital Warri.

Urine was the most frequently specimen examined in this study. This may be due to the fact that microscopical and clinical examination of urine often provides helpful information in many abnormal conditions. Infections of the kidney, bladder and urethra may result in pus cells, red cells and microorganisms being present in the urine. When the kidney becomes inflamed, reducing substances such as ketones, bile and proteins can be found in various pathological conditions [12].

Staphylococci are among the most important aetiological agents of human infections, aetiological agents of human infections. *Staphylococcus aureus* continues to be the most persistent pathogen being the most frequently encountered single bacterial agent in human infection caused by Gram-positive organisms. Our findings agree with earlier reports [12, 13].

Escherichia coli was the most commonly incriminated organism in suspected cases of urinary tract infections. It is important to note that twenty-five of the 26 isolates were from females, which explains the suggestion that the gut flora could be the source of bacteria that invade the urinary tract. Personal hygiene may also be considered one of the many predisposing factors to the high rate of urinary infection due to gut flora in females. Improper toilet habits may also cause the higher incidence of urinary tract infections in females due to the tendency of females to wipe the perineum from back to front after passing feaces [15]. *Escherichia coli* was also incriminated in wounds, high virginal swabs ear and swabs specimens. This corroborates the findings [16, 17].

Over the last decades, Klebsiella has become an important opportunistic pathogen. They are often found in the human bowel and in a variety of environmental situations such as soil, vegetation and water [18]. Among the Gram-negative organisms, *Klebsiella* spp. have been recognised as a significant contributor to human infection. *Klebsiella aerogenes* was the highest Gram-negative organism in this study; it accounted for 27.97% of the isolates recovered. The highest number was from urinary isolates followed by wounds. This finding of *Klebsiella aerogenes* as the predominant organism from urine samples does not agree with the reports which reported *Pseudomonas aeruginosa* as the predominant organism from wounds in [19, 20]. The status of *Klebsiella aerogenes* as an increasing source of human infections perhaps deserves more attention.

Pseudomonas aeruginosa, a ubiquitous organism, is an opportunistic pathogen that is responsible for a variety of life-threatening infections. *Pseudomonas aeruginosa* presents a particular problem in hospitals as it is multi-resistant to drugs [21]. In this study, *Pseudomonas aeruginosa* accounted for the highest isolates recorded from ear swabs in suspected cases of otitis media which is the inflammation of the middle ear. *Pseudomonas aeruginosa* is still the primary causative agent in purulent ear discharge [22]. *Pseudomonas aeruginosa* accounted for 14% of the isolates from wounds, ranking third after *Proteus sp*, which was the second most isolated organism in our study. These findings agree with those of [17], who identified *Pseudomonas aeruginosa* as the second most isolated organism in their study of wound infection in Benin City.

Proteus spp accounted for 8.5% of the overall isolates found in this study. It ranked second as wound isolates, accounting for 14% of all wound isolates. This finding is in agreement with that of Fair & Tor [23] reported *Proteus* spp. as the second most common isolate in wound infections.

Bacterial resistance to antimicrobials is natural though the unfortunate result of their use, both in hospital and outpatients setting. This phenomenon of antimicrobial resistance is not a new problem. It has been with us almost since the beginning of the antibiotic era but has worsened dramatically in the last decades, posing a significant public health concern and limiting the effectiveness of antimicrobials [24]. In general, resistance to antimicrobials is increasing globally, with the highest prevalence of resistant strains in developing countries [25].

In this study, *Staphylococcus aureus* resistance was 91.8% for ampicillin and 89.12% for penicillin. This is in agreement with those of earlier authors [26, 27]. Montefiore and colleagues [27] reported the incidence of penicillin to be 90-98% and 86% resistant to ampicillin [29]. Penicillin resistance was observed in 92.3% of strains and 96.15% for ampicillin. This is similar to 60-90% found in Lagos in the study of Montefiore *et al.* [28]. These values obtained are higher than values 8% - 16% in a survey of European countries [30]. Patterns of use of antibiotics may account for these observed differences. Hence the penicillin and

ampicillin could no longer be relied upon for imperial treatment in our environment.

This study shows high susceptibility to the second generation cephalosporins – cefuroxime and ceftazidime ranging from 70 – 100%. Cephalosporins have the benefit of being effective in a variety of microbial strains [24]. This study also demonstrates that cephalosporins have good activity against Pseudomonas infections, making this class of medications vital in this area. When sensitivities of all pathogens were considered, the majority of which were resistant to ampicillin were found to be sensitive to cephalosporins.

Over 80% of the *Escherichia coli* isolates were highly resistant to streptomycin and, to a lesser extent gentamicin (65.4%). *Proteus mirabilis* showed a 30% resistance rate to gentamicin and 40% to streptomycin. No gentamicin-resistant *Pseudomonas aeruginosa* was isolated in this study; however, most of the published reports of the resistance to aminoglycoside are observed as being used in the university teaching hospitals. Most susceptible bacteria remain uniformly sensitive to these agents. This finding agrees with an earlier report [31] on a 2-monthly survey at Scranton P.A Community Hospital, which demonstrated no resistance of *Pseudomonas aeruginosa* to gentamicin.

For the fluoroquinolones, all the isolates were sensitive to pefloxacin. The resistance rate of 15-27.3 percent for ciprofloxacin contradicts the 100% susceptibility in studies [19]. These values corroborate the findings that ciprofloxacin-resistant organisms are now present in our environment [32, 33]. The resistance rate for ofloxacin was higher than those of earlier reports by [34, 35] reported a 3% incidence for ofloxacin. Some studies have indicated that there is a high prevalence of de-novo high-level fluoroquinolone resistance among isolates from patients who have never been treated with the drug [36]

The result of this study appears to provide reasonable guidelines on the common pathogens found in Warri and their sensitivities to antibiotics tested. The study has endorsed the excellent sensitivity patterns of fluoroquinolones in our environment and also confirms that pefloxacin has a much broader activity than other quinolones. Our findings are similar to those of Gonzaiez and Henwood [37].

There is strong evidence that the primary cause of the current crisis in antimicrobial resistance is the uncontrolled self-medication and inappropriate use of antibiotic drugs both in developing and industrialised countries [38, 39]. In developing countries, the problem is compounded by the ready availability of antibiotic drugs across the counter. This allows patients to treat themselves with the wrong medicine or in quantities too small to be effective. Importation of fake and substandard drugs, which lack adequate amounts of active ingredients, further compounds the resistance of bacteria. Unavailability of accurate and affordable diagnostic tests to identify the cause of infection has been identified as a significant reason for the overuse of antibiotics [25, 40] This problem combined with inadequate government regulation on sales of antibiotics, a complete absence of antibiotic prescribing policies, and incomplete information on patterns of bacterial resistance may contribute to the emergence in bacterial resistance.

Effective control of antibiotic usage is therefore advocated since older and cheaper antibiotics face such high resistance as observed in this study and numerous reports and their use may no longer be warranted. The low resistance recorded for the cephalosporins and quinolones may be attributed to their high cost since most patients cannot afford the drugs across the counter and, as a result, making them relevant and appropriate in the treatment of many community-acquired infections.

New drugs such as fluorinated quinolones appear to have a place in the treatment of commonly acquired infections that are resistant to previously established agents. Although fluoroquinolones have proved effective in various types of infection, they should seldom be the drug of choice [41]. Hence when treatment with a fluoroquinolone is considered for specific infections, certain guidelines should be followed. First, a quinolone should be used when alternative antibiotics are more toxic or less efficacious for specific infections. Second, the use of quinolone can be considered when a patient has a history of a severe allergy or adverse effect to one of the usually indicated antibiotics. Third, a quinolone should be chosen when an infection is caused by multiple resistant bacteria and usually necessitates treatment with two or more antibiotics. Fourth, a quinolone can be selected when the use of a parenterally administered agent treatment of resistant bacteria can be avoided. Fifth, changing treatment of a quinolone can be considered for the completion of parental therapy as an outpatient. Finally, a quinolone should be prescribed when it is clearly the preferred drug of choice for a particular infection. The use of these drugs on a much more regular basis would significantly increase the selective pressure for the rise in resistance compared to a situation where they are used as agents of second or third choice.

In conclusion, this study has shown the continued increase in the prevalence of antibiotic resistance in older penicillin-based antibiotics. Newer agents such as fluoroquinolones offer a viable alternative to order agents such as penicillin

and ampicillin in the treatment of infections, provided the earlier stated guidelines are followed.

COMPETING INTERESTS

There are competing interests.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

ACKNOWLEDGMENT

Our debt of gratitude goes to the respondents who voluntarily consented to participate in this study, and to the Medical Laboratory Staff of Central Hospital Warri for their technical support.

REFERENCES

1. JANEWAY, C.A., TRAVERS, P. & WALPORT, M. (2001) *Immunobiology: The Immune System in Health and Disease*. 5th ed. New York: Garland Science, https://www.ncbi.nlm.nih.gov/books/NBK27169.
2. CHAPLIN, D.D. (2010). Overview of the immune response. *The Journal of Allergy and Clinical Immunology*, **125:** S3-23.
3. LEVISON, M.E. & LEVISON, J.H. (2009). Pharmacokinetics and pharmacodynamics of antibacterial agents. *Infectious Disease Clinics of North America*, **23**: 791–815.
4. ROSBENBLATT, J.E. (1991). Laboratory Tests Used to Guide Antimicrobial Therapy. *Mayo Clinic Proceedings*, **66**: 942–948.
5. BRITISH SOCIETY FOR ANTIMICROBIAL CHEMOTHERAPY (1991). A guide to sensitivity testing. Report of the Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy. *The Journal of Antimicrobial Chemotherapy*, **27:** Suppl D: 1–50.
6. RIGHTER, J. (1990). Agar Dilution: Standards and science, the cart before the bone. *Journal of Antimicrobial Chemotherapy*, **26**: 609–611.
7. ROTH, A.R. & BASELLO G.M. (2003). Approach to the Adult Patient with Fever of Unknown Origin. *American Family Physician*, **68:** 2223–2228.
8. SWEET, R.L. (1975) Anaerobic infections of the female genital tract. *American Journal of Obstetrics and Gynaecology*, **122**(7): P891-901. DOI: 10.1016/0002-9378(75)90736-X.
9. COWAN, S.T. & STEEL, K.J. (1994). Cowan and Steel's Manual for the Identification of Medical Bacteria. (2nd Edition) Cambridge University Press, London, pp 67-83.
10. BAUER, A.W., KIRBY, W.M., SHERRIS, J.C. & TURCK, M. (1966). Antibiotic susceptibility testing by a standardised single disk method. *American Journal of Clinical Pathology*, **45**(4): 493–496.
11. GINOCCHIO, C.C. (2002). Role of NCCLS in antimicrobial susceptibility testing and monitoring. *American Journal of Health-System Pharmacy*, **59**: S7–S11.
12. BAKER, F.J. & SILVERTON, R.E. (2001). Introduction to Medical Laboratory Technology. 7th Ed, Butterworths, London.
13. TONG, S.Y.C., DAVIS, J.S., EICHENBERGER, E., HOLLAND, T. L. & FOWLER, V.G. (2015). *Staphylococcus aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. *Clinical Microbiology Reviews*, **28**(3): 603–661.
14. GNANAMANI, A., HARIHARAN, P. & PAUL-SATYASEELA, M. (2017). *Staphylococcus aureus:* Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach. In: Shymaa Enany Laura E. Crotty Alexander (ed) Frontiers in *Staphylococcus aureus*. In Tech, DOI: 10.5772/63039
15. PERSAD, S., WATERMEYER, S., GRIFFITHS, A., CHERIAN, B. & EVANS, J. (2006). Association between urinary tract infection and postmicturition wiping habit. *Acta Obstetricia et Gynecologica Scandinavica*, **85**(11): 1395–1396.
16. OPHORI, E., ISIBOR, C. & OMONIGHO, S.E. (1998). Aerobic bacteria associated with otitis media in Warri, Nigeria. *Nigerian Journal of Microbiology*, **12**: 18–20.
17. ENABULELE, O.I., OGBIMI, A.O. & OBUEKWE, C. (1996). Aerobic bacteria in infected wounds. *Journal of Medical Laboratory Sciences*, **5**: 177–182.
18. EDMONDSON, A.S., COOKE, E.M., WILCOCK, A.P.D. & SHINEBAUM, R. (1980). A Comparison of The Properties of Klebsiella Strains Isolated from different Sources. *Journal of Medical Microbiology*, **13**(4): 541–550.

19. ROTOWA, M.A., MONTEFIORE, O. & ADEYEMI-DORO, F.A.B. (1989). An in-vitro study on ciprofloxacin and other antimicrobials against gram-negative bacteria Isolated from patients in Ibadan. *African Journal of Medicine and Medical Science*, **18**: 63–66.
20. GARBA, I., LUSA, Y.H., BAWA, E., TIJJANI, M.B., ALIYU, M.S., ZANGO, U.U., & RAJI, M.I.O. (2012). Antibiotics Susceptibility Pattern of *Pseudomonas aeruginosa* Isolated from Wounds in Patients Attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Nigerian Journal of Applied Sciences*, **20**(1): 11–14.
21. RAMAN, G., AVENDANO, E.E., CHAN, J., MERCHANT, S. & PUZNIAK, L. (2018). Risk factors for hospitalized patients with resistant or multidrug-resistant *Pseudomonas aeruginosa* infections: a systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control*, **7**(1): 79 - 93.
22. RVEEN, S., NAQVI, S.B. & FATIMA, A. (2013). Antimicrobial susceptibility pattern of clinical isolates from cases of ear infection using amoxicillin and cefepime. *Springer Plus*, **2**: 288- 295.
23. OGUACHUBA, H.N. (1985). Hospital infection in orthopedic traumatological department of the Jos University Teaching Hospital. *Nigerian Medical Practitioner*, **9**: 99–101.
24. FAIR, R.J. & TOR, Y. (2014). Antibiotics and bacterial resistance in the 21st century. P*erspectives in Medicinal Chemistry*, **6**: 25–64.
25. AYUKEKBONG, J.A., NTEMGWA, M. & ATABE, A.N. (2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance and Infection Control*, **6**: 47-54.
26. KAREIVIENE, V., PAVILONIS, A., SINKUTE, G., LIEGIŪTE, S. & GAILIENE, G. (2006). *Staphylococcus aureus* resistance to antibiotics and spread of phage types. *Medicina* (Kaunas, Lithuania), **42**(4): 332–339.
27. AGGARWAL, S., JENA, S., PANDA, S., SHARMA, S., DHAWAN, B., NATH, G., SINGH, N.P., NAYAK, K.C. & SINGH, D.V. (2019). Antibiotic Susceptibility, Virulence Pattern, and Typing of *Staphylococcus aureus* Strains Isolated from Variety of Infections in India. *Frontiers in Microbiology*, **10**: 2763.
28. MONTEFIORE, D., ROTIMI, V.O., & ADEYEMI-DORO, F.A.B. (1989). The problem of bacterial resistance to antibiotics among strains isolated from hospital patients in Lagos and Ibadan, Nigeria. *Journal of Antimicrobial Chemotherapy*, **23**: 641–651.
29. EKE, P.I. & ROTIMI, V.O. (1987). *In Vitro* Antimicrobial Susceptibility of Clinical Isolates of Pathogenic bacteria to Ten Antibiotics Including Phosphomylin. *African Journal of Medical Sciences*, **16:** 18–22.
30. EUROPEAN STUDY GROUP ON ANTIBIOTIC RESISTANCE. (1987). *In vitro* susceptibility to aminoglycoside antibiotics in blood and urine isolates consecutively collected in twenty-nine European laboratories. *European Journal of Clinical Microbiology*, **6**: 378–385.
31. SHARMA, D., PATEL, R.P., ZAIDI, S.T.R., SARKER, MD. M.R., LEAN, Q.Y. & MING, L.C. (2017). Interplay of the Quality of Ciprofloxacin and Antibiotic Resistance in Developing Countries. *Frontiers in Pharmacology*, **8:** 546-549.
32. GAMERO DELGADO, M.C., GARCÍA-MAYORGAS, A.D., RODRÍGUEZ, F., IBARRA, A. & CASAL, M. (2007). Susceptibility and resistance of *Pseudomonas aeruginosa* to antimicrobial agents. *Revista espanola de quimioterapia publicacion oficial de la Sociedad Espanola de Quimioterapia* 2007, **20**: 230–233.
33. AHONKHAI, I. & MONYE, A.C. (1996). In-vitro effect of ciprofloxacin on methicillin-resistant isolates of Staphylococcus aureus. *Journal of Medical Laboratory Science*, **5**: 69–72.
34. GUIMARAES, M.A. & NOONE, P. (1986). The comparative in-vitro activity of norfloxacin, ciprofloxacin, enoxacin and nalidixic acid against 423 strains of Gram-negative rods and staphylococci isolated from infected hospitalised patients. Journal of Antimicrobial Chemotherapy, **17**: 63–67.
35. EGWARI, L.O. & NWACHUKWU, A.E. (1994). Distribution and susceptibility of methicillin-resistant staphylococci to quinolones and four other antibiotics. *Journal of Medical Laboratory Science*, **14**: 81–86.
36. KAATZ, G.W., SEO, S.M. & RUBLE, C.A. (1991). Mechanisms of fluoroquinolone resistance in *Staphylococcus aureus. Journal of Infectious Diseases*, **163**: 1080–1086.

37. GONZALEZ, J.P. & HENWOOD, J.M. (1989). Pefloxacin: A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs*, **37**: 628–668.
38. OBASEIKI-EBOR, E.E., AKERELE, J.O. & EBEA, P.O. (1987). A survey of antibiotic outpatient prescribing and antibiotic self-medication. *Journal of Antimicrobial Chemotherapy***,** **20**: 759–763.
39. RATHER, I.A., KIM, B.-C., BAJPAI, V.K. & PARK, Y.H. (2017). Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. Saudi *Journal of Biological Sciences*, **24**(4): 808–812.
40. KELESIDIS, T. & FALAGAS, M.E. (2015) Substandard/counterfeit antimicrobial drugs. *Clinical Microbiology Review*, **28**: 443–64.
41. PETERSON, E. & KAUR, P. (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Frontiers in Microbiology*, **9**: 2928. DOI: 10.3389/fmicb.2018.02928.