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Assessment of multi-drug-resistant *E. coli* and *S. aureus* from patients attending selected health facilities in Makurdi, Benue state.

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Abstract
The present study was conducted from January 2023 to July 2023 with an aim to determine antibiotic susceptibility pattern of Staphylococcus aureus and Eschericia coli identified from the urine and stool samples among hospitalized patients in Benue State University Teaching Hospital and General Hospital North bank, Makurdi. The total of 81 non duplicate S. aureus and 97 non duplicate isolates of E. coli were processed, isolated and identified using standard microbiological procedure and biochemical test. Antibiotic susceptibility test was carried out using Modified Kirby Bauer's Disc Diffusion Method. Out of total samples, 64% of the S. aureus were determined MDR and 63% of the E. coli where determined MDR isolates. From the 10 single disc antibiotics that were used in the study imipenem was found to have the highest activity toward both MDR S. aureus and MDR E. coli. Isolates of S. aureus was also found to be sensitive to ciprofloxacin at 68%. Unfortunately they were found to be completed resistant against amoxicillin. Augmentin was determined to have activity against MDR E. coli. Sex is not a notable factor in the carriage of MDR E. coli and S. aureus from this study.

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I. BACKGROUND

At the moment, the entire world has witnessed uncontrolled increase in the development of resistance mostly by bacterial pathogens against known powerful and broad-spectrum antibiotics. This development renders a wide array of effective antimicrobial agents useless so quickly that public health disaster is imminent. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become increasingly difficult or impossible to treat (WHO, 2020). Especially alarming is the rapid global spread of multi- and pan-resistant bacteria (also known as "superbugs") that cause infections that are not treatable with existing antimicrobial medicines such as antibiotics (WHO, 2020).

Antimicrobial resistance occurs naturally over time, usually through genetic changes. Antimicrobial resistant organisms are found in people, animals, food, plants and the environment (in water, soil and air). They can spread from person to person or between people and animals, including from food of animal origin. The main drivers of antimicrobial resistance include the misuse and overuse of antimicrobials; lack of access to clean water, sanitation and hygiene for both humans and animals; poor infection and disease prevention and control in health-care facilities and farms; poor access to quality, affordable medicines, vaccines and diagnostics; lack of awareness and knowledge.

Escherichia coli is implicated in many infections with increasing reports of resistance to commonly used antibiotics (Medugu *et al.,* 2018). *E. coli* is one of the most common causes of urinary tract infections (UTI), sepsis and diarrhea mostly in children and we noted the high rates of resistance among this group of isolates. Infections with *E. coli* have been reported to be associated with increased length of hospital stay, higher cost of care, drain on limited resources, and high rates of morbidity and mortality (Naylor *et al.,* 2019). Some factors that can enhance antimicrobial resistance in our environment are increase or inappropriate use antibiotics for prophylaxis in human and animals for growth promotion or treatment (Manyi-Loh *et al.,* 2018). In the developing countries like ours, where the antimicrobial resistance burden is already reported as high (Williams *et al.,* 2018), inadequate environmental hygiene, poverty, poor healthcare systems, antibiotic-laden animal feeds, fake/substandard antimicrobials, with a background of expensive second‐line treatments, potentially create conditions for AMR pathogens to emerge and thrive (Kariuki and Dougan, 2014). This problem, in addition to the high burden of infectious diseases, fewer antibiotics in the market, and poor laboratory diagnostics, makes for a "perfect storm" for the emergence and spread of resistant bacterial strains.

Staphylococcus aureus is a coagulase positive, facultative anaerobic bacterium and can be microscopically characterized as either single, pairs or clusters of Gram-positive cocci (Deresinki, 2005). *S. aureus* is a non-motile, non-sporing,coagulase and catalase positive bacterium which can be differentiated from *Streptococci* and other Gram-positive bacterium due to the production of catalase (Kloos and Schleifer, 2016). *S. aureus* bacteria ferment glucose to produce lactic acid (Waldvogel, 2000). The cocci commonly form irregular clusters with a grape like appearance under the microscope. However *S. aureus* cocci can appear as single cells or in pairs or short chains (Waldvogel, 2000). The individual coccus size is approximately 0.5 to 1.5 µm in diameter (Wilkinson, 2013). The cell wall of *S. aureus* is composed of a thick peptidoglycan layer which contributes to the virulence of the bacterium (Lowy, 1998). The peptidoglycan stimulates the production of cytokines by macrophage resulting in complement system activation and platelet aggregation (Lowy, 1998).
Infections caused by *S. aureus* can occur in two stages;
(i) *S. aureus* cells enter the body through damaged endovascular points of the host where platelet- fibrin-thrombi complex have formed and attached via microbial surface component that recognizes adhesive matrix molecules (MSCRAMM) mediated mechanism and
(ii) The bacterial cells may attach to endothelial cells via adhesion- receptor interactions or by bridging ligands, including serum components such as fibrinogen (Todar, 2005).
Upon entry into the host tissue, immune cells phagocytose *S. aureus* cells, which promotes the production of proteolytic enzymes and toxins that facilitate the spread to adjoining tissues and the release of the Staphylococci into the bloodstream resulting in bacteraemia (Timbury *et al.*, 2002). The infected endothelial cells produce tissue necrosis factors as part of the immune response to infection which results in necrosis and abscess formation (Timbury *et al*., 2002).
Staphylococcal diseases are usually a result of the production of a toxin or through the invasion of tissue (Murray *et al*., 2005). Diseases that arise from exclusively *Staphyloccal* toxins include Staphylococcal scalded skin syndrome (SSSS), *Staphylococcal* food poisoning and toxic shock syndrome [TSS] (Murray *et al.*, 2005). Other *Staphylocoocal* diseases include suppurative infections, wound infections and catheter related infections (Murray *et al.*, 2005).

II. Materials and Methods

Culture Media

Mannitol Salt Agar (MSA), Nutrient Agar (NA), Nutrient Broth (NB), Muller Hinton Agar, Cysteine Lactose Electrolyte Difficient Agar (CLED), Eosin Methylene Blue Agar (EMBA) medium, Triple Sugar Iron (TSI), Simmon Citrate Agar (SCA) all from Oxoid, UK.

Antibiotics

The following antibiotic disc from Oxoid, UK were used; Gentamicin (10 µg), Ciprofloxacin (5 µg), cotrimoxazole (trimethoprim-sulfamethoxazole 1.25/23.75) (25 µg), Azetromycin (15 µg), Amoxicillin (10 µg) Amoxicillin clavulanic acid (Augmentin) (30 µg) (containing amoxicillin/clavulinic acid 20/10 µg) Imipenem (10 µg), Cefoxitin (30 µg), Tetracycline (30 µg), cefotaxime (30 µg) Ceftazidine (CAZ 30 µg), and Ceftriazone (CRO 30 µg).

Ethical Clearance

Ethical approval for this study was obtained from the Health Research Ethics Committee of the BSUTH and the Hospital Management Board (HMB) Makurdi Benue State with the ref. numbers BSUTH/CMAC/HREC/101/V.III/19 and HMB/OFF/215/VOL.II/488 respectively before the commencement of all laboratory analyses. Permission was also obtained from the Chief/Principal Medical Officer (PMO) of the respective health facilities**.**

Methods

Eighty one (81) non- duplicate isolates of *Staphylococcus aureus* and ninety seven (97) non duplicate isolates of *Eschericia coli* were obtained from urine and stool samples submitted to medical microbiology laboratory of selected health facilities within Makurdi town, Benue state within the period of six months. The probable isolates of *E. coli* and *S. aureus* were subcultured on Eosin Methylene Blue Agar (EMBA) and manitol salt agar plates respectively and incubated for 24 hours at 37^{o}C. Characteristic *Staphylococcus aureus* colonies were further identified by gram stain, catalase and coagulase testing according to standard bacteriological procedures (Cheesbrough, 2006). Characteristics *Eschericia coli* colonies were further identified by indole test, motility test, triple sugar iron test coagulase test, oxidase test and Gram staining reaction. A suspension of each confirmed *Staphylococcus aureus* and *Eschericia coli* isolates were prepared in peptone water to match 0.5 Mcfarland turbidity standards and subjected to antimicrobial susceptibility test (AST) using the disc agar diffusion method. The isolates were inoculated onto the surfaces of Mueller-Hinton agar plates and the

antibiotic discs (from Oxoid, UK) were asceptically placed on the inoculated plates. Incubation was done at 37^oC for 18- 24 hours.
The Zones of inhibition were measured and compared with national committee for clinical laboratory standards (NCCLS) interpretative chart for antimicrobial sensitivity testing guidelines. The results were classified as resistant, intermediate and sensitive.

III. Results

The result from this study shows that the total of three hundred and eighty two clinical samples of urine and stool was obtained from Benue state teaching hospital and General hospital North bank all situated in Makurdi, Benue state. The cultural characteristic of the bacterial isolates were determined and the result is shown in **Table 1.** The biochemical characteristics were also determined as shown on **Table 2**.

Table 1: Cultural Characteristics of the Isolates

Code	CLED	EMBA	MSA	Suspected isolate
A	Deep yellow	No growth	Yellow	*Staphylococcus aureus*
B	Yellow	Greenish metallic sheen	No growth	*Escherichia coli*

Key: CLED = Cystine lactose electrolyte deficiency agar.

EMBA = Eosin methylene blue agar,
MSA = Manitol salt agar

Table 2: Biochemical Characteristics of the Isolates

Code	Gr	Mot	Ind	Coa	Cat	Cit	Ur	Lac	Suc	Glu	Gas	H_2S	Slope	Butt	Isolates identified
A	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	Y	Y	*Staphylococcus aureus*
B	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	Y	Y	*Escherichia coli*

Key: Gr = Gram reaction, Mot = motility, Ind = indole, Coa = coagulase, Cat = catalase, Cit = citrate, , Ur = urease, Lac = lactose, Suc = sucrose, **:**, Glu = glucose, H_2S = hydrogen sulphide, Y = acid reaction, , - =negative, , + = positive

From this study, the distribution of *Escherichia coli* by the different clinical samples were determined, 58.8% was isolated from stool samples, meanwhile 41.2 % was isolated from urine, as shown on **table 3**. The distribution of *Staphylococcus aureus* by the different clinical samples in this study shows that 92.6% was recovered from urine while only 7.4% was recovered from stool samples. The result is shown on **table 4:**

Table 3: Distribution of *Eschericia coli* species from clinical samples

Clinical Sample	No of samples	No of Culture Positive Samples
Stool	190	57 (58.8 %)
Urine	192	40 (41.2 %)
Total	**382**	**97 (100 %)**

Table 4: Distribution of *Staphylococcus aureus* species from clinical samples

Clinical samples	no of samples	No of culture positive
Stool	190	6 (7.4 %)
Urine	192	75 (92.6 %)
Total	**382**	**81(100 %)**

The zones of growth inhibition obtained were classified based on the CLSI Interpretative Chart for Antimicrobial Sensitivity Testing as seen on **Table 5** below. Antibiotics susceptibility pattern of *S. aureus* using the disc agar diffusion (DAD) test shows that the isolates were only sensitive to imipenem with 74.3 %, followed by gentamicin with 68.4 %. Meanwhile amoxicillin had no diameter of zone of growth inhibition with 0 %. From the results in this study, **Figure 1** is a barchart that represent the level of sensitivity of *S. aureus* to the antibiotics single disc which were used.

Table 6 shows the antibiotics susceptibility pattern of *E. coli. E. coli* sensitivity to imipenem recorded the highest percentage with 68.3 %, followed by augmentin with 31.4 % while the least diameter of zone of growth inhibition was from tetracycline (30 µg) and amoxicillin (10 µg) with 8 % and 0 % respectively. **Figure 2** is a barchart representing the level of sensitivity of *E. coli* to the ten antibiotics single discs that were used in this study.

Table 5: Antibiotic susceptibility pattern of *Staphylococcus aureus* clinical isolates

Antibiotics	Disc potency	Diameter of zone of growth inhibition (mm)		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Gentamicin	10 µg	19.3	12.3	68.4
Ciprofloxacin	5 µg	20.5	11.7	67.8
Erythromycin	15 µg	55.6	19.9	24.6
Amoxicillin	10 µg	91.1	8.9	0
Azethromycin	15 µg	72.5	15.8	11.7
Cotrimoxazole	20 µg	74.3	8.2	17.5
Augmentin	30 µg	59.1	9.4	31.6
Imipenem	10 µg	17.5	8.2	74.3
Cefoxitin	30 µg	44.5	26.4	29.2

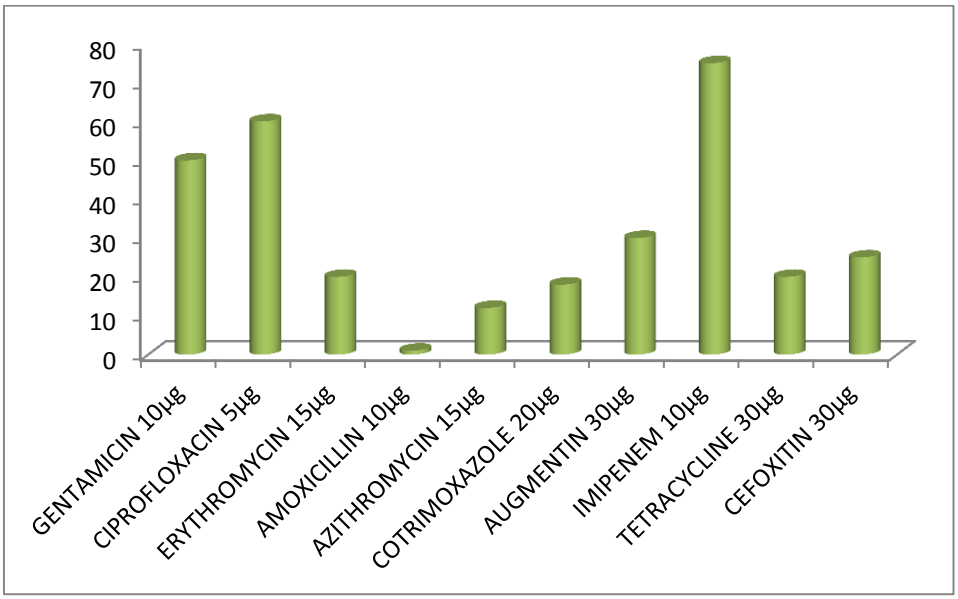

FIG 1: Antibiotic sensitivity profile of *S.aureus* clinical isolates

Table 6: Antibiotic susceptibility pattern of *E. coli* clinical isolates

Antibiotics	Disc potency	Diameter of zone of growth inhibition (mm)		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Gentamicin	10 µg	92. 8	0	9.6
Ciprofloxacin	5 µg	82.8	14.3	7.2
Erythromycin	15 µg	59. 6	19.9	20.6
Amoxicillin	10 µg	100	0	0
Azethromycin	15 µg	78.6	14.3	28.6
Cotrimoxazole	20 µg	74.3	8.2	17.5
Augmentin	30 µg	59.1	9.4	31.6
Imipenem	10 µg	19.3	12.3	68.4
Cefoxitin	30 µg	82	0	18
Tetracycline	30 µg	76	16	8

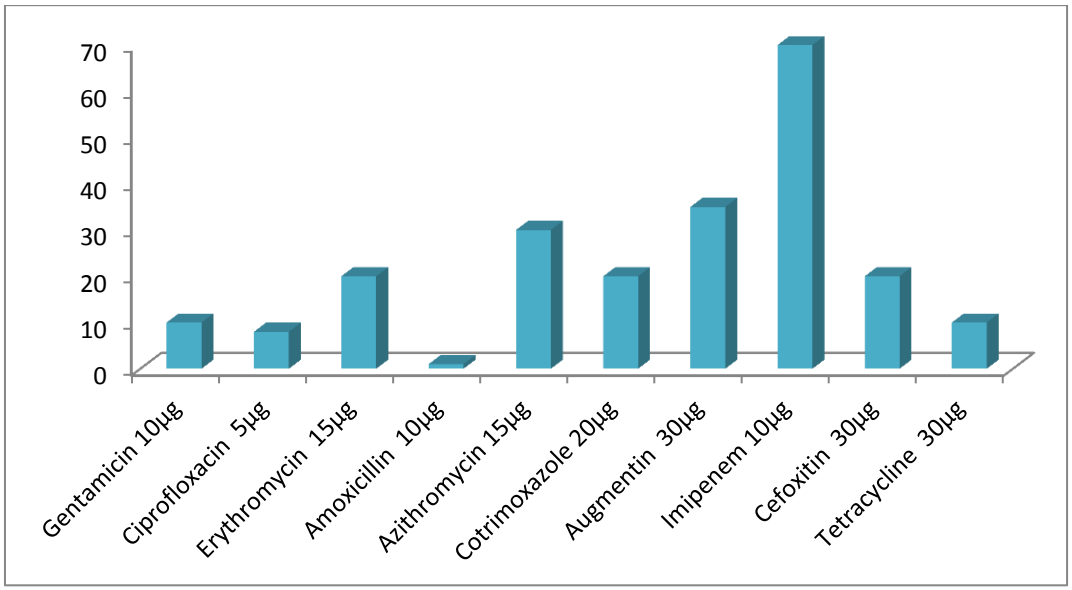

Figure 2: Antibiotic sensitivity profile of *E. coli* clinical isolates

Table 7: Distribution of MDR *Escherichia coli* with Respect to Gender

S/N	Gender	No of MDR Strain	Total No of Isolates
1	Male	29 (47.5 %)	39 (40.2 %)
2	Female	32 (52.5 %)	58(59.8 %)
	Total	**61**	**97**

Table 8: Distribution of MDR *Staphylococcus aureus* with Respect to Gender

S/N	Gender	No of MDR Strain	Total No of Isolates
1	Male	24 (46.2 %)	45 (55.6 %)
2	Female	28 (53.8 %)	36 (44.4 %)
	Total	**52**	**81**

Table 7 shows the distribution of multi-drug- resistant *E. coli* clinical isolates in this study with respect to gender. From the total of 61/97 isolates that were determined to be multi-drug- resistant *E. coli* species, 32 (52.5 %) were isolated from female patients while 29 (47.5 %) were isolated from male patients. Furthermore, Table 8 shows the distribution of MDR *Staphylococcus aureus* with respect to Gender. From the total of 52/81 (64.2 %) that were determined to be MDR *Staphylococcus aureus* clinical isolates, 28 (53.8 %) were isolated from female and 24 (46. 2 %) were isolated from male patients.

IV. Discussion

The recovery rate of multi-drug resistant *S. aureus* in this study is 64.2 %. This is higher than the result from a similar research by Bidhya *et al.*, (2021) who had 50 % level of multi-drug resistance. MDR cases may be due to accumulation of multiple genes, expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs, mutational alteration of the target protein, enzymatic inactivation of drugs, etc. (Nishanthini *et al.*, 2014).

In our study, most *S. aureus* strains (68%) were isolated from urine specimen. This is consistent with a previous study done at Kenya National Hospital (Kanaga *et al.*, 2014). In a study done to determine the antimicrobial susceptibility pattern of *S. aureus* strains isolated from hospitalized

patients in Iran, most of the isolates were from blood specimens (29%) (Soltani *et al.*, 2016) Another study done on prevalence and antibiotic susceptibility pattern of *S. aureus* from clinical isolates in Nigeria showed a majority of the isolates were from urine specimens (76%) Obiazi *et al.*, 2007)

The high number of *S. aureus* isolated in urine may be attributed to the wrong use of antibiotics for certain clinical conditions or the use of antibiotics in improper dosage and poor hygiene.

The present study showed the prevalence of multi-drug-resistant strains of *E. coli* is lower in comparison with previous studies carried out in Sokoto (92.85 %) by Salem (2010) and Kibret (2011). Another similar work carried out in in National Hospital Abuja between March 2019 and September 2020 found a very high proportion (72.2 %) of *E. coli* isolated from patients was MDR**.** The high and increasing rates of antibiotic use in our locality may be the primary driver of antimicrobial resistance (AMR) due to selective pressure (Ravindran *et al.,* 2019).

Susceptibility testing of the Staphylococcal isolates in this study against commonly available antibiotics showed that the isolates were generally resistant to β–lactam (Amoxicillin), while being generally sensitive to gentamicin, an aminoglycoside and ciprofloxacin (a fluoroquinolone) antibacterial agents. *Staphylococcus aureus* isolates showed high sensitivity to imipenem. This is consistent with a similar study done in Iran (Arianpoor *et al.,* 2015).

Research done on antibiotics currently used in the treatment of infections caused by *S. aureus* in Australia indicates imipenem and linezolid have good antistaphylococcal activity but are very expensive (Rayner *et al* 2015). The susceptibility of clinical isolates (including *S. aureus*) to the quinolones have gradually decreased over the years in this environment. Ehinmidu, (2013) reported a 97.06 % sensitivity level in *Staphylococcus aureus* isolated from urine; Onanuga *et al,* (2015) reported a 96.7 % sensitivity level. The susceptibility level to ciprofloxacin in this study is lower than the 99.7 % reported by Akerele *et al*., (2002). This development may be connected with the increasing availability of the cheaper generics of the fluoroquinolones in this environment leading to increased use and probably misuse. The exposure to quinolones may have selected for spontaneous mutants that are present in large bacterial populations and which contain chromosomal mutations that alter the target protein or increase the level of efflux pump expression (Hooper, 2012; Rogues *et al.,* 2017). This resistance among clinical isolates is reportedly greatest in *Staphylococcus aureus* (Hooper, 2012).

Gentamicin was effective on 68.7 % of the isolates in this study with only 19.3 % of the isolates being resistant to the antibiotics. The susceptibility level is lower than 91.18 % reported by Ehinmidu (2013) and the 100 % susceptibility reported by Umolu *et al*, (2012) for *Staphylococcus aureus* isolates from different human specimens.

In the present study, apart from the 19.3 % *Staphylococcus aureus* resistant to gentamicin, 12.3 % of the isolates are intermediate phenotypes which may result in treatment failure with the antibiotic in clinical settings. The highest resistance was with azithromycin and amoxicillin followed by cotrimoxazole. Least resistance was with imipenem. Which implies that the highest susceptibility was with imipenem.

The high level of resistance in the isolates to the β-lactam drugs is not surprising. This is consistent with the observation that clinical Staphylococcal isolates are resistant to a large number of commonly prescribed antimicrobial agents (Olukoya *et al.*, 2015) and the β-lactams in particular. It is believed that more than 80% of Staphylococcal isolates now produce penicillinase regardless of the clinical setting (Lowy 2003, Pantosti *et al*., 2017).

The results obtained from this study indicate that MDR *E.coli* isolate were highly resistant to most of the antibiotics which were used. Here, we found that ciprofloxacin, gentamicin and amoxicillin were some of most frequently used antibiotics and not surprisingly, they had very high resistance rate that may have contributed to high resistance observed in our study. High ciprofloxacin and gentamicin use in hospitals and its increasing resistance rates have been reported in similar clinical settings in Africa and other developing countries with use reported as 51% in Tanzania and 59% in Ethiopia (Ayele *et al.,* 2018, and Sonda *et al*, 2019). These results are comparable to findings in other studies (Bartoloni *et al*., 2006, Sahaquillo-Arce *et al.,* 2011). In this study, it is observed that the *E. coli* isolates showed very high resistance to ciprofloxacin and is in support of reports of (Drago *et al.,* 2010) whose findings advocated the appropriate use of fluoroquinolones in humans. Resistance to fluoroquinolones varies geographically and is an emerging problem in both developed and developing countries (Boyd *et al.,* 2008, Namboodiri *et al.,* 2011).

Similarly high resistance rate to augmentin was found in a study on (urinary tract infection) UTI in the southern part of Nigeria which reported 86 %, (Abiodun *et al*., 2014). Amoxicillin had an absolute resistance rate in this study, has thus been rendered almost impractical in this population although lower rates were found in a study targeting community infections in 2015 (Adenipekun *et al*, 2015). The high level resistance to most of the antibiotics used in this study could be associated with earlier exposure of these drugs to isolates which may have enhanced development of resistance. There is high level antibiotic abuse in this environment arising from self-medication which is often associated with inadequate dosage and failure to comply to treatment (Uwazuoke *et al.,* 2014) and availability of antibiotics to consumers across the counters with or without prescription.

Following good susceptibility profile of imipenem found in this study, further real life investigation into its potential usefulness in this locality is warranted as it has proven to be a good resource on other settings.

Azethromycin displayed a relatively good profile with some of the isolates being susceptible. This is similar to reports from studies outside of the region (Kibret and Abera, 2011) and contrasts with other studies which reported higher resistance to azethromycin in Nigeria.

Despite known side effects, erythromycin preserves some functionality as shown in this study and warrants consideration in our locality because it is relatively cheaper and more available than imipenem. In this study for instance, imipenem was the only susceptible antibiotic to MDR *E. coli* isolates. Unfortunately, the carbapenems are either unavailable or unaffordable for most of the Nigerian population.

V. Conclusions

Indiscriminate use of antibiotics or drug abuse should be discouraged by the health professional and efforts to control procurement and use of antibiotics officially in this locality will probably help to limit the increasing rate of drug resistance in the pathogens. Also, it is imperative for proper patient care and constant evaluation of antibiotic sensitivity pattern of pathogens for commonly used antimicrobial agents in this environment under study.

REFERENCE

[1]. Abiodun, A. O., Olufunke, O. A., Dunah, F. C. & Oladiran, F. (2014). Phenotypic identification and phylogenetic characterization of uropathogenic Escherichia coli in symptomatic pregnant women with urinary tract infections in South-Western Nigeria. Internal Journal of Biology **6**, page 145-147

[2]. Adenipekun, E. O. (2015). Prevalence and antimicrobial resistance in Escherichia coli from food animals in Lagos, Nigeria. Microbial Drug Resistance. Vol **7** Pages 1-4 222

[3]. Akerele J, Abdulimen P, Okonfua F (2002) Prevalence of asymptomatic genital infection among pregnant women in Benin City, Nigeria. Africa Journal of Reproductive Health **6**(3): 93-7

[4]. Arianpoor A, Estaji F, Naderinasab M, Askari E, (2015). Antimicrobial susceptibility pattern of Staphylococcus aureus Isolates against newly marketed antibiotics. Journal of Ayub Medical College Abbottabad. 3:3–6.

[5]. Ayele, A. A. (2018) Prospective evaluation of Ceftriaxone use in medical and emergency wards of Gondar university referral hospital, Ethiopia. Pharmacological Research Perspective. 6, 383.

[6]. Bartoloni A, Pallecchi L, Benedetti M (2006) Multidrug-resistant commensal E. coli in children, basis of resistance to fusidic acid in Staphylococci. Antimicrobial Agents Chemotherapy. 51: 1737-1740.

[7]. Bidhya M, Shovana T, Roshani M., (2021) Department of Pathology, International Friendship biofilms. Journal of bacteriology 186: 1838-1850

[8]. Boyd L, Atmar R, Randall G, Hamill R, Steffen D, (2008) Increased fluoroquinolone resistance with time in E. coli from >17,000 patients at a large country hospital as function of Culture Site, Age, Sex and Location. BMC Infectious Diseases 8: 4.

[9]. Deresinki S. (2005) Review of MRSA Clinical infectious Diseases 40: 562-573

[10]. Drago L, Nicola L, Mattina R, Vecchi E (2010) In vitro selection of resistance in Escherichia coli and Klebsiella spp at in vivo fluoroquinolone concentrations. BMC Microbiology 10: 119

[11]. Ehinmidu J (2013) Antibiotic susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Tropical Journal of Pharmaceutical Research 2(2):223-228

[12]. Hooper DC (2012) Fluroquinolone resistance among Gram-positive cocci. Lancet Infectious Diseases 2(9):530-8

[13]. Kariuki, S. & Dougan, G. (2014). Antibacterial resistance in sub-Saharan Africa: An underestimated emergency. Annual National Youth Academy Science.https:// doi. org/ 10. 1111/ nyas. 12380

[14]. Kanaga EL. (2014). Antimicrobial susceptibility of bacteria that cause wound sepsis in the paediatric surgical patients at Kenyatta national hospital. /11295 /95412 .

[15]. Kibret M, Abera B (2011) Antimicrobial susceptibility patterns of E. coli from clinical sources in north east Ethiopia. African Health Science 11: S40-S45.

[16]. Kloos W.E and Schleifer K.H (2016) The genus Staphylococcus. In :Sneath, PHA, Mair, NS and sharpe, ME (ed) Bergey's manual of systematic Bacteriology. Baltimore: Williams and Wilkins, USA 1013-1035

[17]. Lowy F. D. (1998). Antimicrobial resistance: The example of Staphylococcus aureus. The Journal of Clinical Investigation.; 111(9):1265-1273. DOI: 10.1172/JCI18535

[18]. Manyi-Loh, C., Mamphweli, S., Meyer, E. & Okoh, A. (2018) Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. Molecules 23, 78).

[19]. Medugu, N., Iregbu, K., Tam, P.-Y.I. & Obaro, S. (2018). Aetiology of neonatal sepsis in Nigeria, and relevance of Group b streptococcus: A systematic review. PLoS ONE 13, 7

[20]. Murray P.R., Rosenthal K.S and Pfalter M.A (2005) Staphylococcus and related organisms. In: Mandell G.I, Bennett J.E and Polin R, Medical microbiology (5th edition). Elsevier mosby, Edinburg , united kingdom: 221-236

[21]. Namboodiri S, Opintan J, Lijek R, Newman M, Okeke N (2011) Quinolone resistance in E. coli from Accra Ghana. BMC Microbiology11: 44.

[22]. Naylor, N. R. Manyi-Loh, C., Mamphweli, S., (2019). The health and cost burden of antibiotic resistant and susceptible Escherichia coli bacteraemia in the English hospital setting: A national retrospective cohort study. PLoS ONE 14, e0221994

[23]. Nishanthini A, Mohan VR, Jeeva S (2014) Phytochemical and GC-MS analysis of stem and leaf of Tiliacora acuminata (lan.) hook f and Thomas (menispermaceae). International Journal of Pharmaceutical Science Res **5(9):**3977–398

[24]. Obiazi HAK, Ekundayo AO, Ukwandu NCD (2007). Prevalence and antibiotic susceptibility pattern of Staphylococcus aureus from clinical isolates grown at 37 and 44 ℃ from Irrua, Nigeria. African Journal of Microbiology Research. 1:57–60.

[25]. Olukoya K, Asielue J, Olasupo N, Ikea J, (2015). Plasmid profiles and antibiotic resistance patterns of Staphylococcus aureus isolates from Nigeria. Africa Journal Mediicion Medical Science. 24:135-39.

[26]. Onanuga A, Olayinka BO, Oyi AR, Onaolapo JA (2015) Prevalence of community-associated methicillin-resistant Staphylococcus aureus (MRSA) isolates among women in Federal Capital Territory (Abuja), Nigeria Journal College of Medicine 11(1): 47-52

[27]. Pantosti A, Sanchini A and Monaco M (2017) Mechanisms of antibiotic resistance in Staphylococcus aureus. Future Microbiology 2:323-334

[28]. Rayner C, Munckhof J (2015). Antibiotics currently used in the treatment of infections caused by Staphylococcus aureus. International Medical Journal.;35(Suppl2):3–16.
[29]. Rogues A, Dumartin C, Amadeo B, Venier AG, Marty N, Parneix P, Gadne JP (2007) Relationship between rates of antimicrobial consumption and the incidence of antimicrobial resistance in Staphylococcus aureus and Pseudomonas aeruginosa isolates from 47 French hospitals. Infectious Control Hospital Epidemiology. 28(12): 1389-95
[30]. Sahaquillo-Arce JM, Selva M, Perpina'n H (2011) Antimicrobial resistance in more than 100,000 E.coli isolates according to Culture Sites and Patient, Age and Gender and Location. Antimicrobial Agents Chemotherapy 55: 1222-1228
[31]. Salem M, Muharram M, Alhosiny I (2010) Distribution of classes and 2 integrons among Multi-Drug Resistant E. coli Isolated from Hospital Patients with Urinary tract infection in Cairo, Egypt.Australian Journal of Basic and Applied Sciences 4: 398-407
[32]. Soltani R, Khalili H, Rasoolinejad M, Abdollahi A. (2016) Antimicrobial susceptibility pattern of Staphylococcus aureus strains isolated from hospitalized patients in Tehran, Iran. Iran Journal of Pharmaceutical Science; 6:125–32.
[33]. Umolu P, Okoli E, Izomoh I., (2012) Antibiogram and beta-lactamase production of Staphylococcus aureus isolates in Edo State, Nigeria. West Africa. Journal of Medicine 21(2)124-7.
[34]. Waldvogel F.A (2000) Staphylococcus aureus (including toxic shock syndrome). In: Mandell,
[35]. Wilkinson B.J (2013) Staphylococcal capsules and slime. In : Easmon, csf and Adlam, C (ed)
[36]. Williams, P. C. M., Isaacs, D. & Berkley, J. A. (2018). Antimicrobial resistance among children in sub-Saharan Africa. Lancet Infectious. Disease.18, e33–e44