Antimicrobial susceptibility pattern of *Escherichia coli* at University of Abuja Teaching Hospital: A Retrospective Study

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Abstract

Escherichia coli (E. coli), a normal gastro-intestinal microflora of humans and animals can be pathogenic causing a wide range of diseases. Rational use of antibiotics is one way of reducing antimicrobial resistance. This study retrospectively assessed the antimicrobial resistance patterns of E. coli from clinical samples at University of Abuja Teaching Hospital. Records of test results of microbial, culture and sensitivity from clinical samples from January 2016 to December 2019 were studied. A proforma was used to collect data from the record on antimicrobial sensitivity pattern of E. coli. Discrete and categorical variables were reported as frequencies and percentages. Requirements for the study were met in 91.7% of the data collected. Female clients 81.8% were more than their male counterparts. Urine samples have the highest number of E. coli 62%. This was followed by samples from palate swab 14%. Eye swab, vaginal swab, ascitic fluid and pleural fluid had one each (0.03%). The penicillin were mostly resisted by the isolates, while most were sensitive to the fluoroquinolones and cephalosporins. Summary of the resistance patterns from 2016 to 2019 showed most isolates were sensitive to the effect of antimicrobials used in 2016 and 2018, while most were resistant in 2017 and 2019. The urinary tract was the most common site of E. coli infection. The pattern of resistance was inconsistent across all antibiotic classes studied.

Keywords: Antimicrobial resistance, Antimicrobials, Clinical samples, E. coli

Introduction

Escherichia coli (*E. coli*) is a key member of the normal intestinal microflora of humans and other mammals which typically colonizes the gastrointestinal tract of newborns a few hours after delivery (Kaper, Nataro, & Mobley, 2004; Khader *et al*., 2020; Braz, Melchior, & Moreira, 2020; Kaper *et al*., 2004). *E. coli* and its human host coexist in good health and with mutual benefits, as the strains rarely cause diseases, and were widely studied as a cloning host in recombinant DNA technology (Kaper *et al*., 2004). Hence, *E. coli* has an amazing ability to adjust very well, replicate and disseminate (Braz *et al*., 2020). Literature on the genetics and physiology of *E. coli* abound. However, there are few works of literature on the mechanism of how *E, coli* depicts symbiotic relationships in the colon (Kaper *et al.,* 2004). To further look into the relationship, an interesting hypothesis suggests that *E. coli* might achieve its ability to use gluconate in the colon more proficiently than other local species, thereby allowing it to occupy a highly explicit metabolic niche (Kaper *et al*., 2004; Sweeney *et al*., 1996).

Pathogenic *E. coli* represents a phenotypically diverse group of pathogens (Sullivan *et al*., 2006). However, the pathogenic forms of *E. coli* can cause a variety of diarrhoeal diseases in hosts due to the presence of specific colonization factors, virulence factors, and pathogenicity-associated genes which are generally not present in other *E. coli*. Of the strains that cause diarrhoeal diseases, six pathotypes are now recognized (Sullivan *et al*., 2006). On the other hand, urinary tract infections (UTIs) which are one of the most common bacterial infections acquired in community and hospital settings are most commonly caused by uropathogenic *Escherichia coli* (UPEC) (Alqasim, Jaffal, & Alyousef, 2018; Cristea *et al*., 2019; Klein & Hultgren, 2020)

Virulence factors comprise mechanisms that allow pathogenic bacteria to cause infections (Nuhu et al., 2020; Schmidt & Hensel, 2004). Bacterial virulence factors are mostly encoded by or are associated with mobile genetic elements, such as plasmids, phages, transposons, and insertion elements. (Schmidt & Hensel, 2004). The acquisition of virulence genes confers an evolutionary pathway to the pathogenicity of microorganisms, and understanding the virulence factors carried by a strain enables the determination of the pathogenic potential of the strain (Chapman *et al*., 2006; Nuhu *et al*., 2020). It has been reported that the carriage of virulence genes essential to the pathogenesis of each pathogenic *E. coli* type and the ability to adapt to different conditions, allow the emergence of hybrid pathogenic *E. coli* (HyPEC) (Braz *et al*., 2020; Rahman *et al*., 2018). However, several highly adapted E. coli clones have acquired specific virulence attributes, which confers an increased ability to adapt to new niches and allows them to cause a wider range of diseases (Braz *et al*., 2020; Kaper *et al*., 2004).

In modern-day medicine, antibiotics have become a necessary medical intervention in the field of surgery, management of cancer, cases of critically ill patients, organ transplantation, and management of immune-compromised patients (Khan, Miller, & Arias, 2018). Bacteria resistant to various classes of antibiotics are related to the complex combination of intrinsic and acquired resistance genes, which may act synergistically (Braz *et al*., 2020; Khan *et al.*, 2018). The complex combination of multidrug-resistant (MDR) bacteria and emerging hybrid bacteria with intrinsic or acquired bacterial virulence factors disseminated by genetic mobility elements, and the intense and inappropriate use of antibiotics has simultaneously favored the

emergence of resistance to various antibiotics (Khan *et al*., 2018).

There is a steady increase in antimicrobial resistance (AMR) among nosocomial pathogens, as well as pathogens in the community (Khan *et al*., 2018). AMR occurs when microorganisms can overcome drugs that target them, resulting in ineffective treatment (Bush, 2018; Limmathurotsakul *et a*l., 2019). The World Health Organization (WHO) and the United Nations have named antibiotic resistance as one of the most important public health threats of the 21st century (Aenishaenslin *et al*., 2019).

Multi-drug resistant (MDR) organisms are associated with increased mortality compared to infections caused by susceptible organisms. Although the economic burden of these effects cannot be ascertained as far as Nigeria's context is concerned, due to the dearth of information on that aspect. This study aimed at assessing the antimicrobial resistance patterns among *E. coli* isolates and their major sources to guide clinicians and minimize the emergence and spread of resistance isolates at a university teaching hospital. Despite numerous studies conducted on antimicrobial sensitivity, the need to gather more information on the patterns of resistance in the study area can never be overemphasized.

Materials and Methods

The study was conducted at the University of Abuja Teaching Hospital (UATH). Records of results of microbial culture and sensitivity from clinical samples (urine, ear swab, pus, wound swab, eye swab, HVS, endometrial swab, urethral swab, endocervical swab, ascitic fluid, and pleural fluid) from January 2016 to December 2019 were analysed.

Inclusion criteria

The antimicrobial susceptibility records considered are those that showed solely *E. coli* as their indicating/infecting organisms between 2016 and 2019. Records showing only *E. coli* positive results with their respective microbial culture and sensitivity test were considered.

Exclusion criteria

The antimicrobial susceptibility records that are not considered are records that showed *E. coli* as the indicating organism before 2016 and after 2019. In addition, records with other organisms asides from *E. coli* are not considered. Records of results showing other organisms were excluded from this study.

A proforma was used to collect data from the record on the antimicrobial sensitivity pattern of *E. coli.* Necessary information such as gender, age, occupation, site of

infection, the clinical sample collected, as well as results showing either sensitivity or resistance.

Data management and analysis

Each section of the proforma was coded using numerical values (0, 1, 2, 3…). These values (Data) extracted from the proforma were manually entered into Microsoft Excel 2010, sorted, and analyzed, while discrete values were expressed as a percentage.

Ethical consideration

The ethical committee of UATH approved the protocol with reference number UATH/HREC/2020/002 alongside approval number UATH/HREC/2020/007. Confidentiality and anonymity of the patients' information were maintained during and after the study.

Results

A total number of 1150 records of microbial culture and sensitivity tests for all sample sources were found, out of which 420 had *E coli presence*. Furthermore, from the 420 sample sources that had the presence of *E.coli*, 385 (91.7%) were found to meet the criteria for the study while 35 (8.3%) sample sources showed cases of *E.coli* co-infected with other strains of micro-organisms or incomplete information.

Table 1 shows the demography of patients' records included in the study.

Table 1: Demographic data of clients used in the study

Variable	Frequency		N = 385
Age (years)	Gender		Total (%)
	Male	Female	
0 – 10	10	21	31 (8.1%)
11 – 20	5	16	21 (5.4%)
21 – 30	11	78	89 (23.1%)
31 – 40	9	103	112 (29.1%)
41 – 50	13	35	48 (12.5%)
51 – 60	16	32	48 (12.5%)
≥ 61	6	30	36 (9.3%)
Total	70	315	385

On the distribution of *E. coli* from clinical samples, *E. coli* was majorly isolated from urine samples (237/385), followed by samples from palate swabs (54/385), while eye swabs, vaginal

swabs, ascitic fluid, and pleural fluid accounted for one each, and are grouped under others. Figure 1 shows the distribution of *E. coil* from the various clinical samples obtained from patients' records.

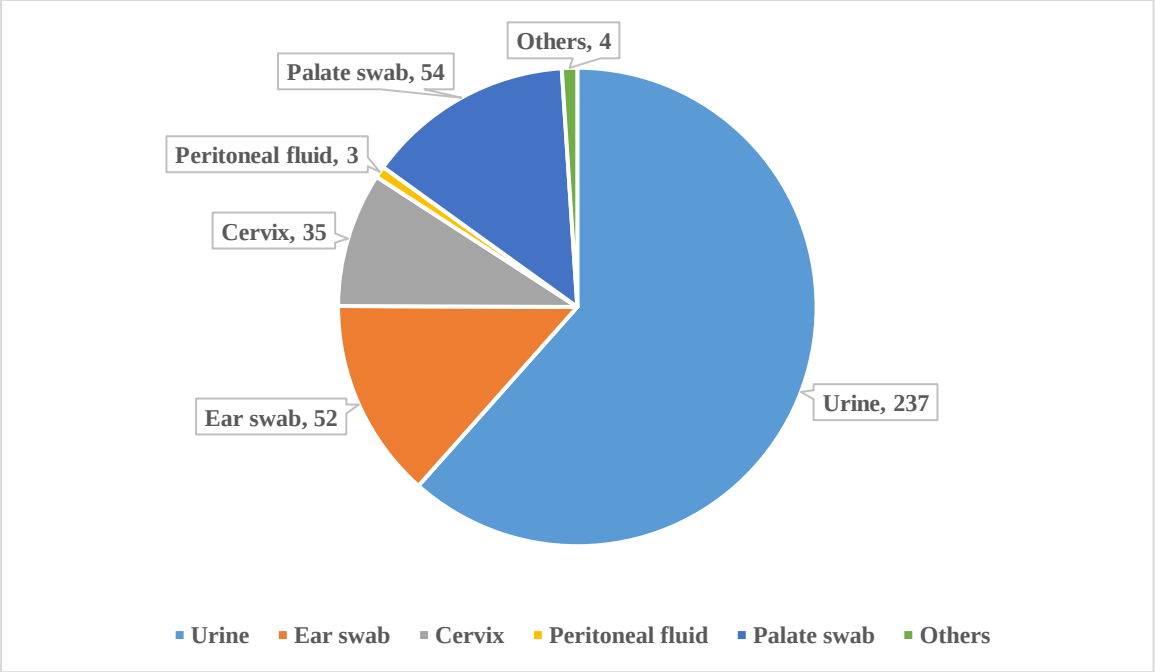


Figure 1: Distribution of *E. coli* in clinical samples

Key: Others = eye swabs, vaginal swabs, ascitic fluid, and pleural fluid.

For this study, antibiotics used are classified into four major groups (penicillin, fluoroquinolones, cephalosporins, and others), and the summary of the antimicrobial sensitivity testing (AST) for the duration of the study is shown in Figure 2.

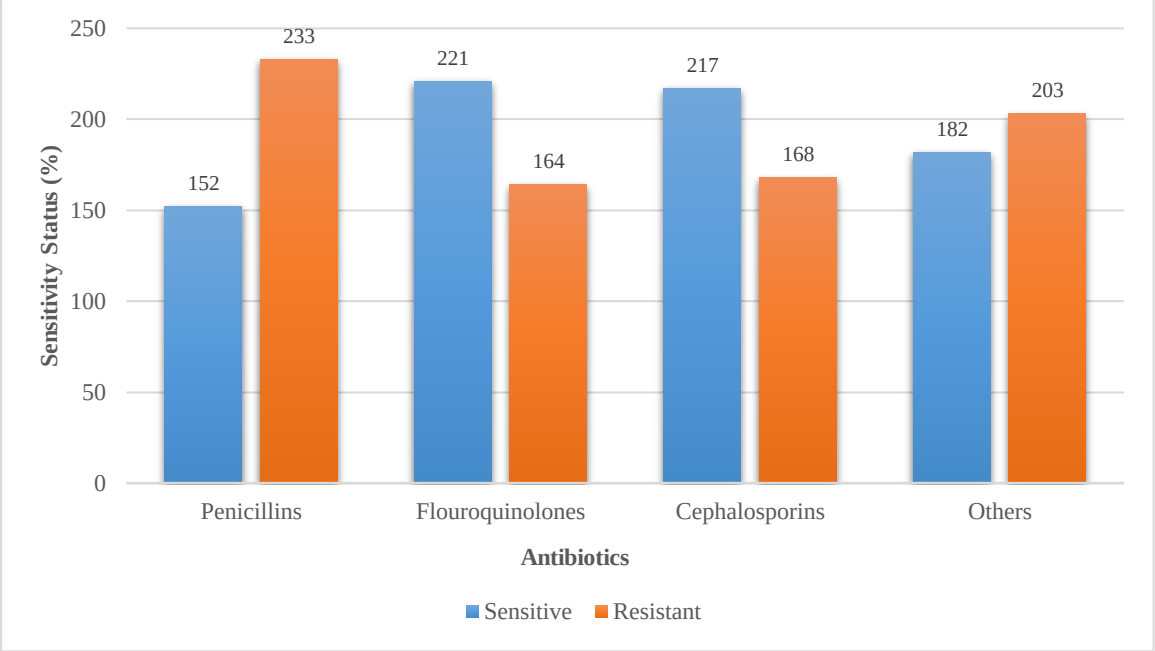


Figure 2: Summary of classes of antibiotics and their AST results used during the period of study

For the penicillins, two antibiotics (cloxacillin and amoxicillin/clavulanic acid) were commonly used for AST as shown in figure 3 and figure 4 for cloxacillin and amoxicillin/clavulanic acid respectively.

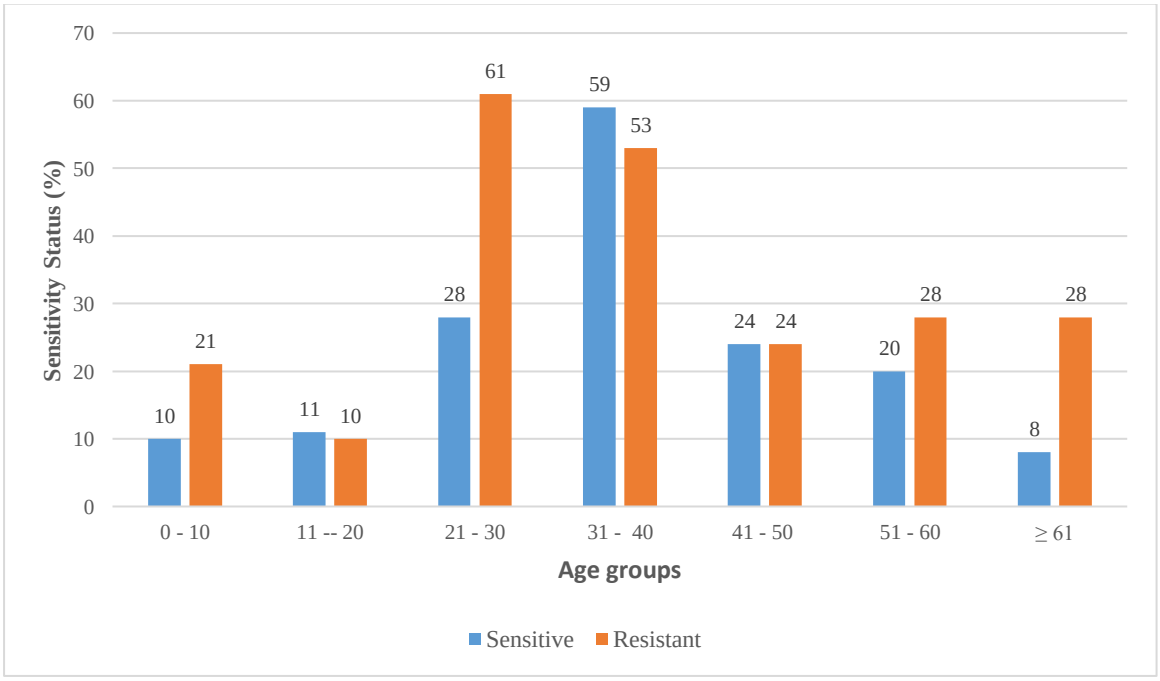


Figure 3: AST of cloxacilllin across age groups

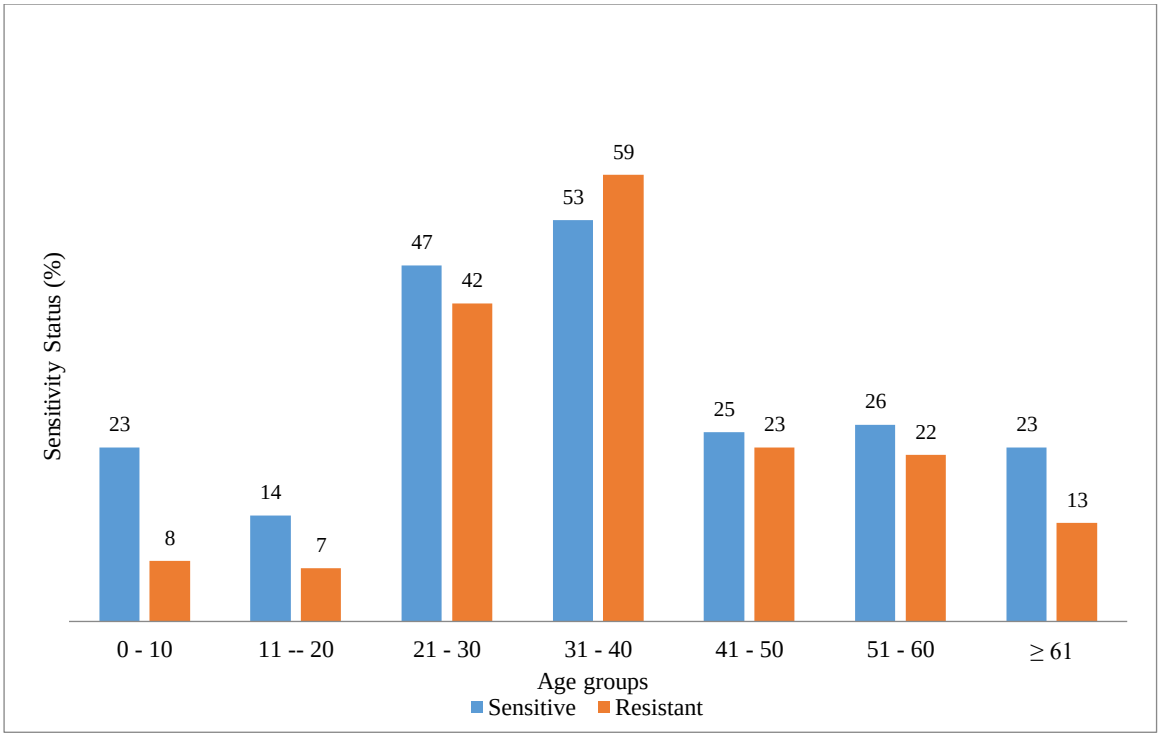


Figure 4: AST for amoxicillin/clavulanic acid across age groups

The cephalosporins (cefuroxime, ceftriaxone, and cephalexin) used for AST as shown in Figures 5, 6, and 7 for cefuroxime, ceftriaxone, and cephalexin respectively.

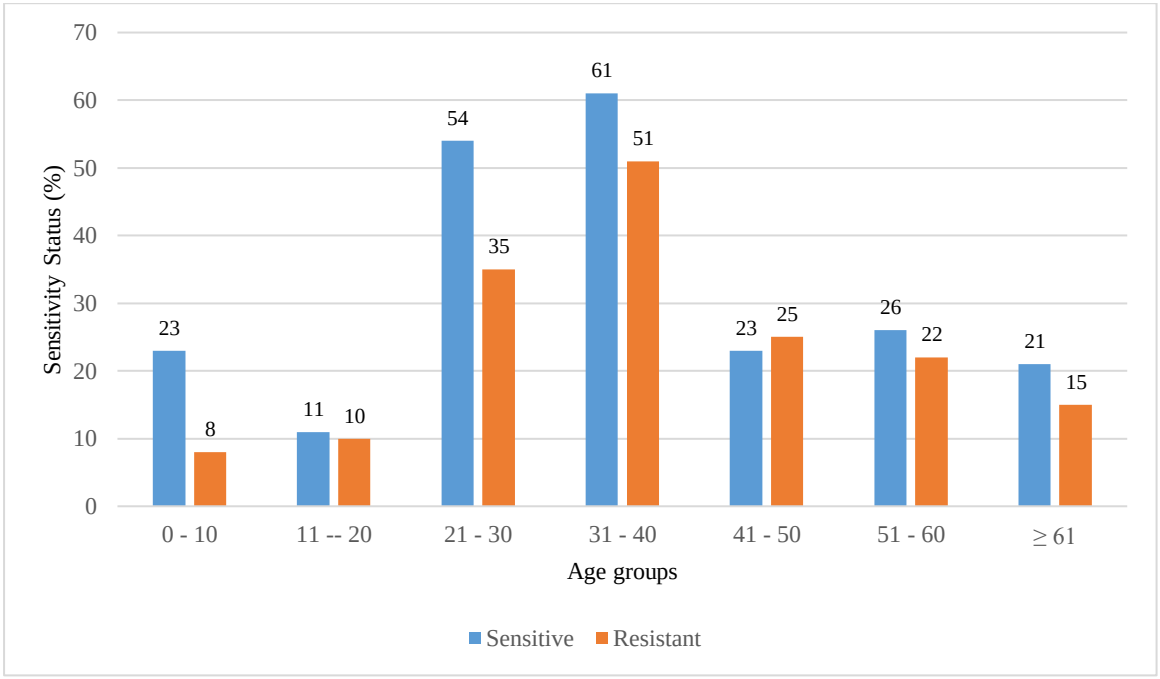


Figure 5: AST for cefuroxime across age grades

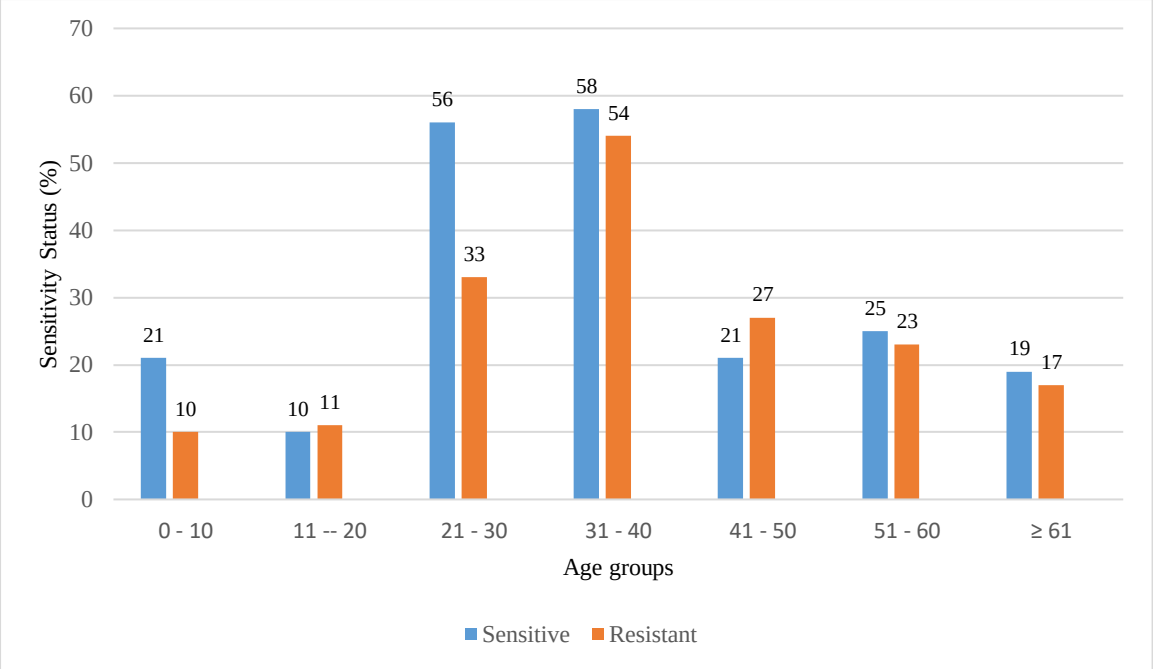


Figure 6: AST for ceftriaxone across age groups

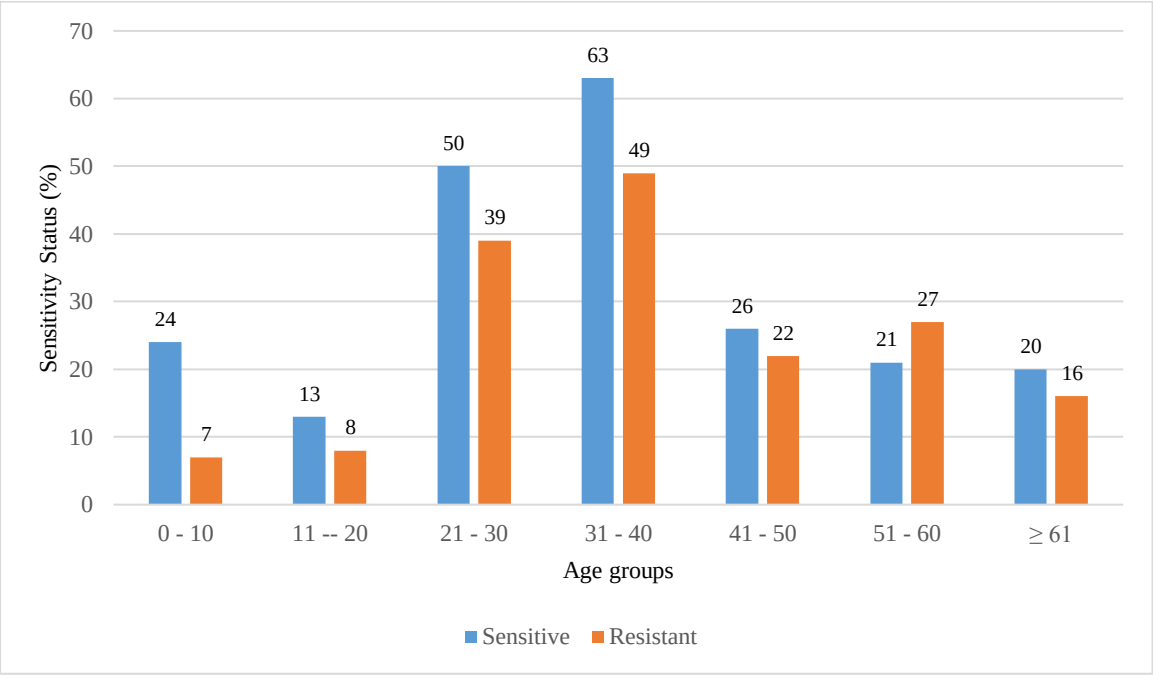


Figure 7: AST for cephalexin across age groups

For the fluoroquinolones (ciprofloxacin, levofloxacin, and ofloxacin), figures 8, 9, and 10 respectively are shown for their AST.

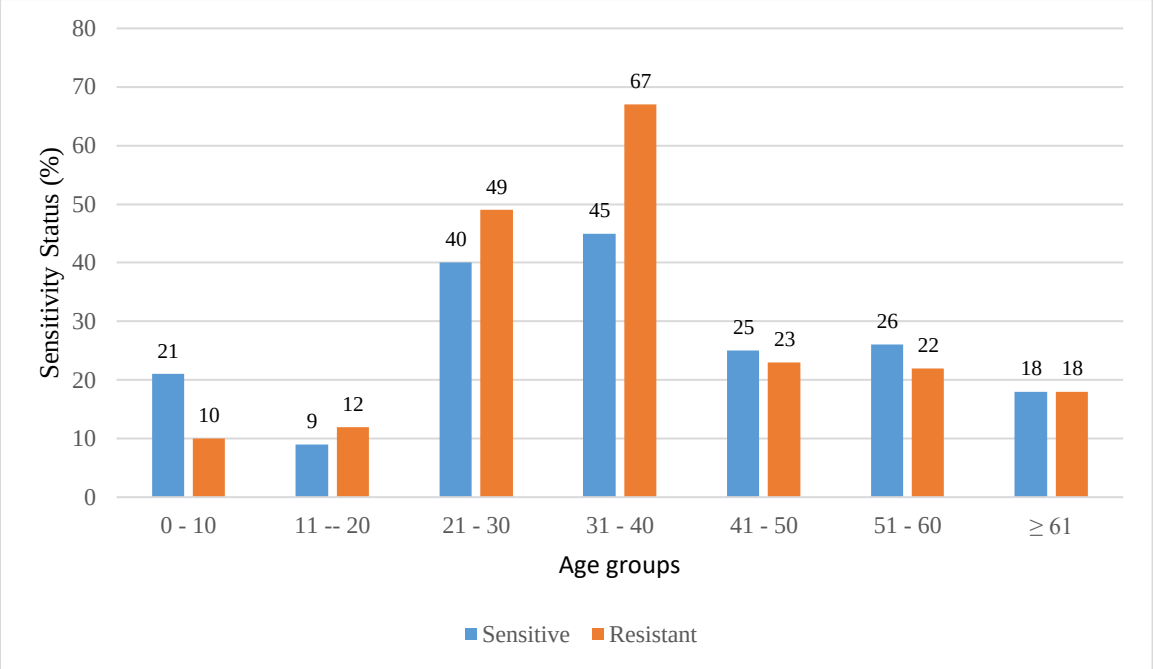


Figure 8: AST for ciprofloxacin across age groups

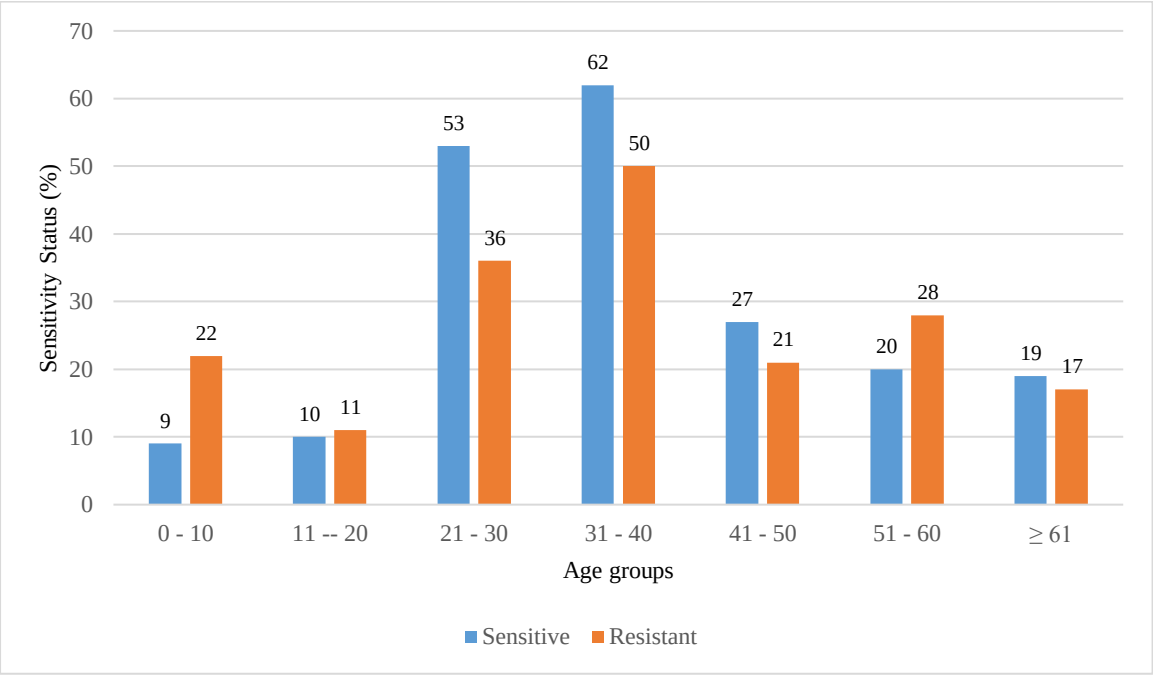


Figure 9: AST for levofloxacin across age groups

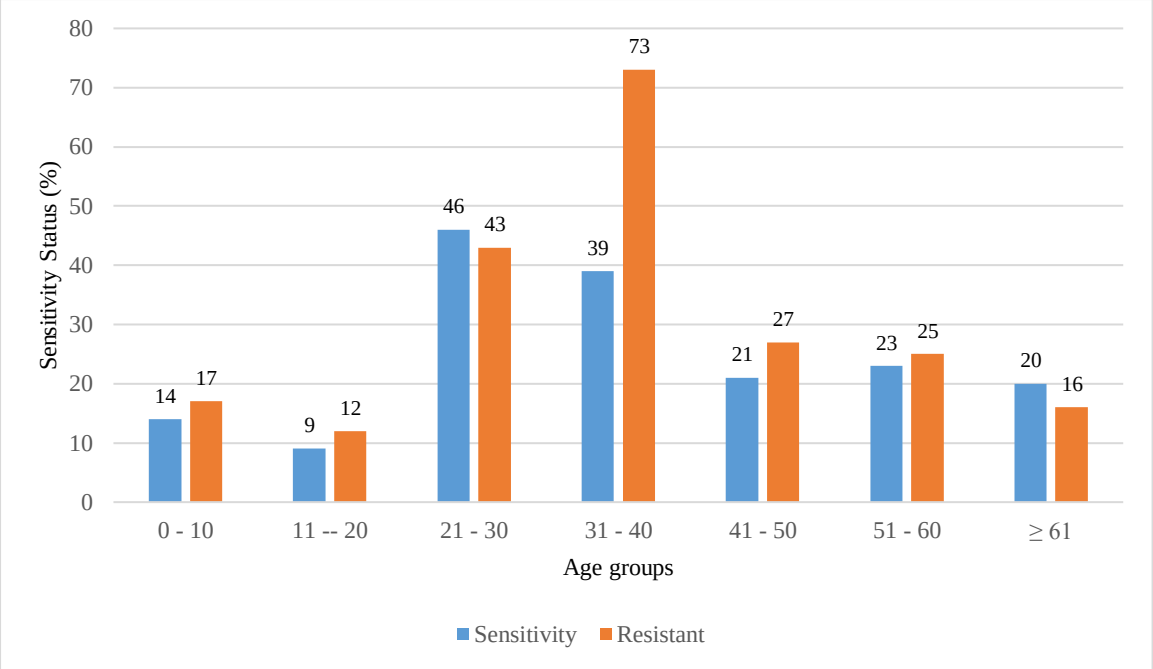


Figure 10: AST for ofloxacin across age groups

Aminoglycosides are also used during that period for AST. Two (gentamicin and neticillin) are used as shown in figures 11 and 12 respectively.

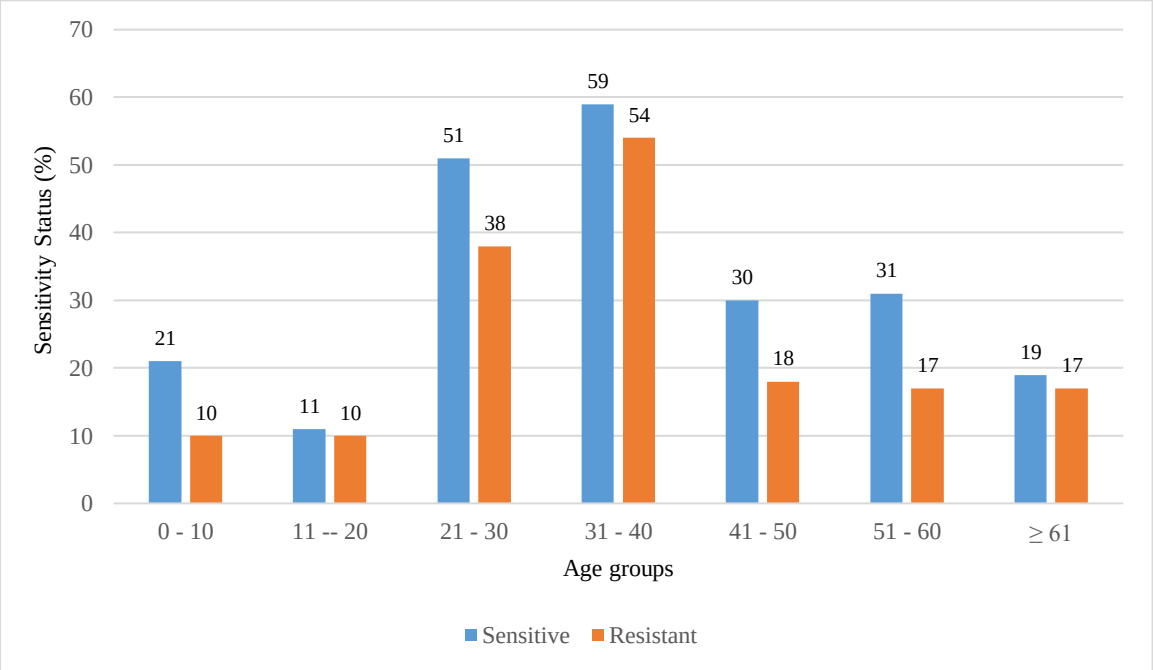


Figure 11: AST for gentamicin

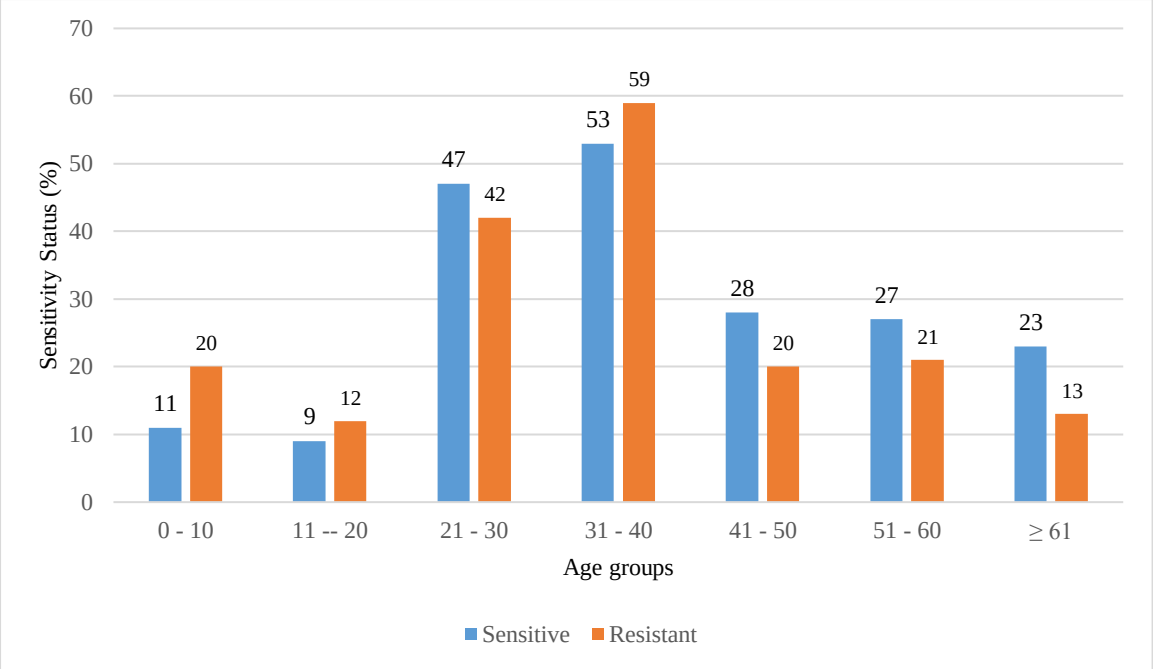


Figure 12: AST for neticillin across age groups

Other antibiotics (sulphamethoxazole/trimethoprim, clindamycin, erythromycin, and tetracycline) were used and their AST is shown in figures 13, 14, 15, and 16.

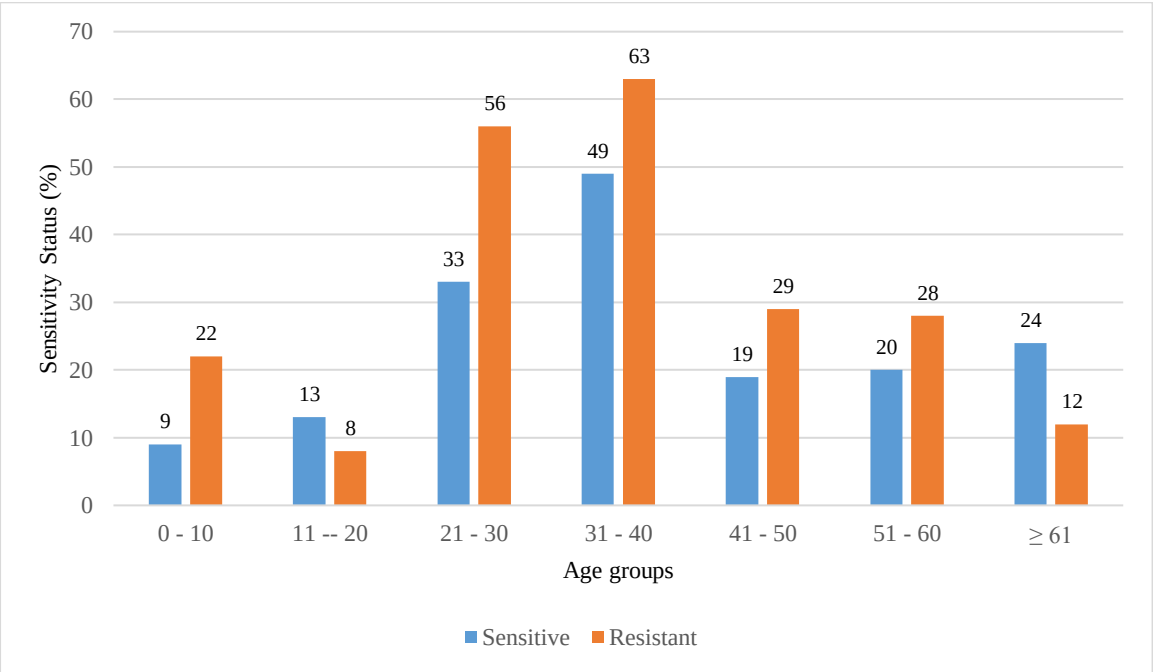


Figure 13: AST for sulphamethoxazole/trimethoprim across age groups

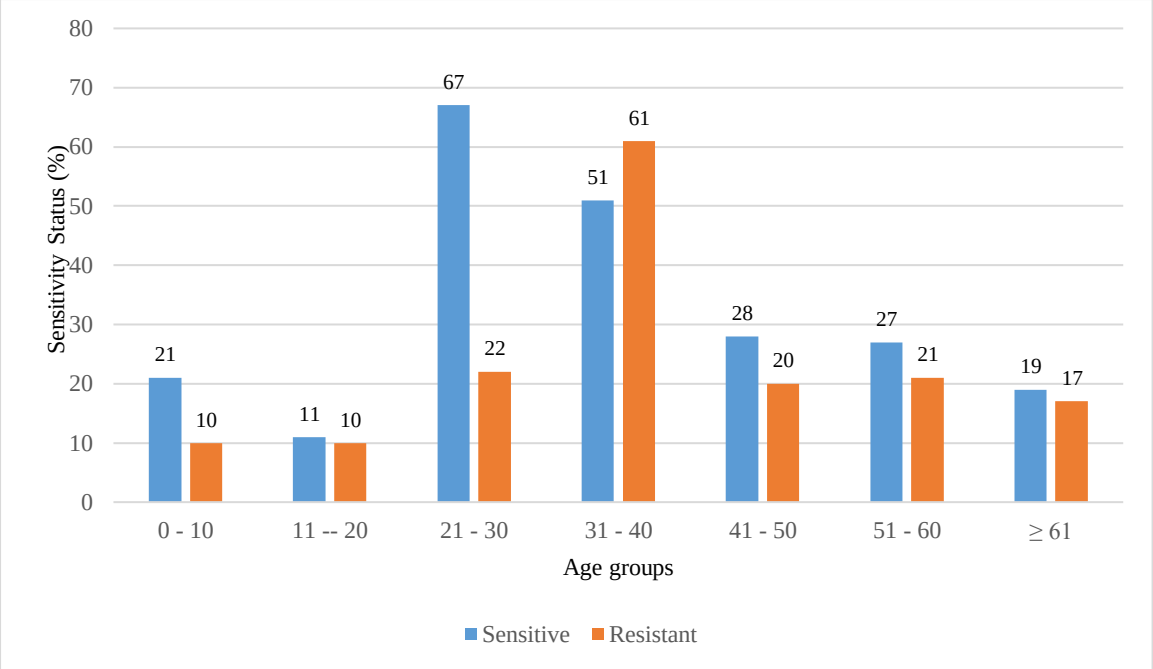


Figure 14: AST for clindamycin across age groups

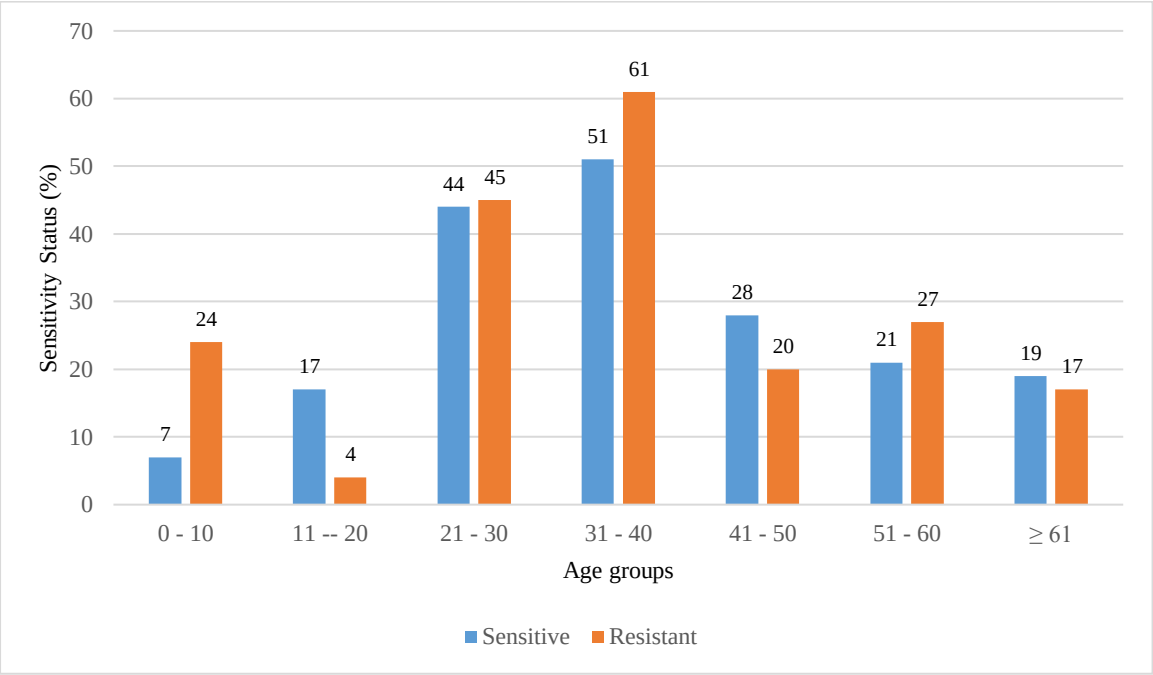


Figure 15: AST for erythromycin across age groups

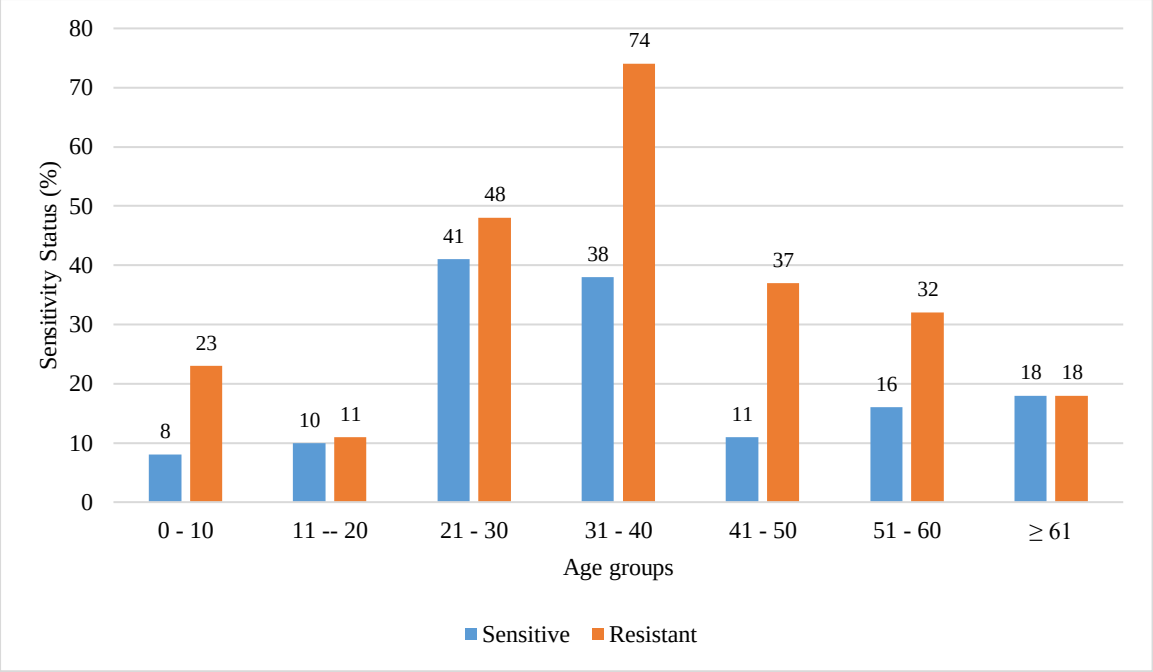


Figure 16: AST for tetracycline across age groups

Discussion

Based on the distribution of gender from the four-year study (2016 - 2019), females are higher in number than their male counterparts. The reason for this variation cannot be explained as data were strictly obtained from records of AST from January 2016 to December 2019. So during the period, it has been shown that the number of females attended to and sent for laboratory investigations is greater than the number of males.

The findings from the records have shown that urine has the highest number of *E. coli* isolates with over 60.0% of the total samples. It is not surprising, because many studies reported urine as the major clinical source of *E. coli* (Abimiku *et al.*, 2019; Adenipekun *et al.*, 2016; Khoshbakht, Salimi, Aski, & Keshavarzi, 2013). In addition to that, *E. coli* has been reported as the predominant facultative anaerobe and the commensal microbiota in the mammalian GIT, thereby playing a key role in hospital and community-acquired infections (Kouadio *et al.*, 2017; Nuhu *et al.*, 2020). Although some reported factors led to the dominance of *E. coli* as urine pathogens. In a study by Khoshbakt and co-workers (2013), they reported that *E. coli* is the most prevalent causative organism of UTI in all age groups and both sexes (Khoshbakht *et al.*, 2013). To further look at the predominance of *E. coli* in urine as reported, certain virulence factors such as

hemolysin production and the presence of fimbriae may be the cause of UTI (Nuhu *et al*., 2020). Virulence genes such as adhesion genes confer the organisms the ability to colonize different niches, with type 1 and type 3 fimbria reported to play a major role in the attachment of Enterobacteriaceae to the host epithelial and endothelial cells (Ghasemian, Mobarez, Peerayeh, & Abadi, 2019; Sarkar, Vagenas, Schembri, & Totsika, 2016). The possession of these virulent genes or factors by these organisms confers an advantage to them because it helps them to colonize different niches, thereby increasing their pathogenicity and may contribute to their ability to overcome different defense mechanisms thereby causing diseases (El-shaer, Abdel-rhman, Barwa, & Hassan, 2018; Dadi *et al*., 2020). Further laboratory investigation like testing for specific characteristics, phenotypic or genotypic features in the *E. coli* isolates is out of the context of this study.

Having an understanding of the scientific basis of antimicrobial resistance is crucial to fighting this public health threat (Holmes *et al*., 2016). It is also important to note that this understanding should cover the resistance mechanisms, that will enable novel approaches to diagnostics and therapeutics which are essential for the development of proper interventional policies (Holmes *et al.*, 2016; Jasovsky Dusan, Littmann, Zorzet, & Cars, 2016). *As mentioned earlier, only records of laboratory results were used for this study. In the penicillin group, most of the E. coli isolates were found to be sensitive to amoxicillin/clavulanic acid, with only the age group 21 – 30 years depicting a high resistance to amoxicillin/clavulanic acid. On the results recorded for cloxacillin, E. coli in all age groups were resistant to the drug but the highest resistance rate was noticed among age group 31-40 years. Additionally, the AST testing result of other antibiotics demonstrate individuals in age grade 31-40years has the highest resistance rate. The reason for this may be associated with the availability and easy access to these antibiotics, which may likely be a reason why patients can decide to opt for self-medication even without doctors' prescriptions. Patients in these age brackets (31 - 40) are the most active in any given human population setting.* The variation in the resistant pattern may be partly through a Darwinian selection process microorganisms have developed various mechanisms to avoid destruction from many toxic substances (Holmes *et al.*, 2016). Another factor responsible for the resistance of the isolates to cloxacillin may be attributed to the irrational use of penicillins in the study area. Even though our study did not look at the history of

antibiotic usage in this populace *but it is without a doubt that the antibiotics consumption rate has increased over the last 50 years, putting selective pressure on susceptible bacteria species and possibly favouring the development and survival of resistant strains. A study by Brain and his associates (2014), reported that there is a positive association between bacterial resistance and increased antibiotic consumption (Brain et al., 2014).*

From the three cephalosporins (cefuroxime, ceftriaxone, and cephalexin) used, their pattern of resistance is almost the same with the majority of the *E. coli* isolates showing susceptibility to the drugs. Some studies show microorganisms are resistant to this class of antibiotics (Dirar, Bilal, Ibrahim, & Hamid, 2020; Oli et al., 2017; Shakya, Shrestha, Maharjan, Sharma, & Paudyal, 2017). The general use of antimicrobials in clinical settings has exposed the human microbiota to high concentrations of these drugs (Holmes et al., 2016), thereby leading to the depletion of the natural microbiota in the human system giving rise to the perpetuation of pathogenic and resistant organisms. Additionally, *E.coli* isolates were highly susceptible to cephalosporins, except for the age group 51-60 years which the isolate showed great resistance to cephalexin, while among the age group 41-50 years, *E.coli* exhibited a high level of resistance to ceftriaxone and cefuroxime.

Although there is inconsistency in the pattern displayed by the fluoroquinolones, *E. coli* isolates were more resistant to ofloxacin, and few cases of resistant to levofloxacin. The observed resistance displayed in ofloxacin may be due to selective pressure from using the same drug over a given period on individuals in the management of infections caused by *E. coli*. As reported by Holmes *et al*., (2016), through a Darwinian selection process, microorganisms have developed robust mechanisms to evade destruction from many toxic substances (Holmes *et al*., 2016). *In addition, this could be due to the chaotic nature of drug distribution, people having unrestricted access to medicines, and clinicians and patients having poor antibiotic stewardship policies. In these situations, the possibility of abusing such drugs can never be ruled out*. Hence, the widespread misuse and overuse of ofloxacin may contribute to its resistance to *E. coli* isolates. *According to Mary et al., (2020), there is a paucity of data to implement good antibiotic stewardship policies, and all included studies demonstrated a reduction in antibiotic resistance with improved antibiotic stewardship intervention.*

In all the age grades, *E. coli* isolates were sensitive to gentamicin, and a similar pattern was also observed in neticillin. But isolates in age-grade 0 – 10 and 11 – 20 years were observed to have a higher resistance to neticillin. Antibiotics under others tested against *E. coli* isolates are mostly resisted. For sulphamethoxazole/trimethoprim tested, only age grades 11 – 20 and ≥ 61 years were found to show the *E. coli* being sensitive to the tested antibiotic. The isolates were susceptible to the effect of clindamycin, while most isolates were highly resistant to erythromycin and tetracycline in all the various age grades. These variations in the pattern may be a result of the earlier assertion made on antibiotic usage related to misuse and overuse which in most cases led to these organisms developing resistance to these drugs.

Limitation of the study

There are other drugs used in carrying out AST at UATH, but due to poor documentation of these antibiotics and in some cases, inconsistency observed, they were not included in the study. Such antibiotics include bacitracin, azithromycin, imipenem, cefixime, and nitrofurantoin. While exploring the sensitivity of individual antimicrobial groups over time would have been interesting, the database containing this information was not consistent. Although the AST record was labeled 2016-2019, the individual dates and sections of each year were not clearly stated. Our study could not ascertain whether there are specific phenotypic resistance tests carried out, such as carbapenemase resistance, extended-spectrum beta-lactamases production, tetracycline resistance, aminoglycoside resistance, colistin resistance, and other resistance phenotypes in the hospital. The study looked at records of clients not minding whether they are in-patient, out-patient, or patients for routine examination.

Conclusion

The main findings were that female clients were more in number than their male counterparts, and the most frequent origin of infection due to *E. coli* was the urinary tract. Also revealed from the records was inconsistency in the pattern of resistance in all classes of antibiotics used. Since antibiotic resistance is a major public health challenge, clinical laboratories are indispensable for prompt recognition of resistant organisms. Hence, for clinicians to best practice infection control due to resistant organisms and reduce their spread, laboratory investigation before treatment can never be overemphasized. This will help in the selection of suitable and appropriate antibiotics. Efforts by the hospital infection unit should be taken to

monitor the spread of *E. coli* most especially in the hospital as well as in the community. This will go a long way in preserving the last resort of antibiotics in the management of serious infections.

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PROFORMA

DEMOGRAPHICS

1. Age
2. Gender: Male (0) Female (1)

PRESENT ILLNESS

3. Indicating Micro-organism: Escherichia coli
4. Site of Infection: Eye(0), Urinary tract(1), wound(2), Ear(3), Vaginal(4), Cervix(5) Teeth(6) Peritonealcavity(7) Pleural cavity(8)
5. Site of sample collection: Eye swab (0) Urine (1) Urethra swab (2) Wound swab (3) Ear swab (4) Endocervical swab (5) High vagina swab (6) Palate swab (7) Ascitary fluid (8) Pleural fluid(9)

RESULTS

ANTIBIOTICS	SENSITIVITY	RESISTANCE

Note: sensitivity I= 1, sensitiviy II= 2 , sensitivity III= 3s

Resistance=0