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Antibiogram and Molecular Characterization of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* Isolates in General Hospital, Ilorin

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Abstract
The use of antibiotic agents in the treatment of infectious diseases has greatly contributed to the associated reduction of morbidity and mortality. However, these great advances in treatments are being undermined by the rapid evolution of antibiotic resistance among pathogenic micro-organisms and have become a huge burden to global health. Extended Spectrum Beta-Lactamases are enzymes that hydrolyze beta lactam antibiotics but not caphamycins and carbapenems. The aim of this study was to determine the antibiogram and molecular characterization of ESBL producing *Escherichia coli* isolates in General Hospital, Ilorin. The study was conducted in General Hospital, Ilorin, located in North Central Nigeria. *Escherichia coli* were isolated using standard microbiological methods and antimicrobial susceptibility testing was performed by modified Kirby Bauer's method. Polymerase Chain Reaction was used for the Characterization of extended Spectrum Beta-lactamase genes. Out of 375 samples tested, 11.2% showed growth of *Escherichia coli* where 23.8% were confirmed to be ESBL producers. The organism was tested against Augumentin 33.3%, Ceftriaxone 52.4%, Ceftazidime 16.7%, Gentamicin 26.2%, Imipenem 95.2%, Ciprofloxacin 42.9% and Nitrofurantoin 45.2%. This study revealed a high prevalence of bla_{TEM} 100% where bla_{CTX-M} and bla_{SHV} were not detected in the isolates. This present study established the presence of ESBL genes among *Escherichia coli* isolates in this locality.

Keywords: Antibiogram, Molecular Characterization , Extended Spectrum Beta-Lactamase Producing Escherichia coli, General Hospital Ilorin

Introduction

Antimicrobial Resistance (AMR) occurs when microorganisms including bacteria, viruses, fungi, and parasites become able to adapt and grow in the presence of medications that once impacted them (Fonou *et al.,* 2017). AMR is considered a significant threat to the public health systems not just in developing countries but throughout the world. (Prestinaci *et al.,* 2015).

Extended-spectrum β-lactamases (ESBL)-producing *Enterobacterales* are a widely distributed source of antimicrobial resistance for animals and humans (Mughini-Grass *et al.,* 2019).

Antimicrobial resistance (AMR) is one of the most serious global public health threats in this century, which is especially urgent regarding antibiotic resistance in bacteria (Prestinati *et al.,* 2015), particularly in *Enterobacterales* (Dembélé *et al.,* 2021). This phenomenon has arisen globally in both nosocomial and community settings as a consequence of widespread antibiotics' consumption (Ashley *et al.,* 2011). *Enterobacterales* are a large order of different types of bacteria including *Escherichia coli* that commonly cause infections both in healthcare settings and in communities (CDC, 2019). To survive the effects of antibiotics, some *Enterobacterales* can produce enzymes called extended-spectrum β-lactamases (ESBLs) that

breakdown and destroy some commonly used antibiotics, including penicillins and cephalosporins, and make these drugs ineffective for treating infections (CDC, 2019). Over the last decade, many studies have reported the presence of extended-spectrum β-lactamases (ESBL)-mediated resistance in Gram-negative bacteria causing infections in patients (Rai *et al.,* 2017). Infections that can be caused by ESBL-producing bacteria include urinary tract infection (UTI), diarrhoea, skin infections, and pneumonia (Jewell and Biggers 2017). Possible medications used to treat ESBL infection include carbapenems, which are useful against infections caused by *Escherichia coli*or *Klebsiella pneumoniae* bacteria, fosfomycin, β-lactamase inhibitors, non-β-lactam antibiotics, and colistin when other medications have failed to stop the ESBL infection (Jewell and Biggers, 2017). Unfortunately, the excessive use of antibiotics, in particular β-lactams, leads to the selection of ESBL-producing strains. (Chang *et al.,* 2017) Because of the emergence and distribution of multidrug-resistant (MDR) *Escherichia coli* is complicating the treatment of various serious infections (Mariappan *et al.,* 2018) the World Health Organization (WHO) has long recognized the need for an improved and coordinated global effort to contain AMR (Prestinaci *et al.,* 2015). The burden of AMR, including MDR, varies between the regions; however, low- and middle-income countries share a disproportionate burden due to multitude of factors embedded in the characteristics of the health system, policy, and practice (Pokharel *et al.,* 2019). In European countries, particularly in *Escherichia coli* AMRs are increasing (Van Pujin *et al.,* 2019). AMR is a worldwide threat, with an approximately 25,000 deaths occurring in Europe and 23,000 in the United States each year (Li and Webster, 2018).

Methodology

Study Area

This study was carried out General Hospital, Ilorin which is located in Ilorin and provides quality health care services to the state's masses. General Hospital Ilorin has bed space of about four hundred and fifty (450) and admission rate of about twelve thousand (12,000) patients per annum. Ilorin is the capital city of Kwara State.

Study Population

The study population covered some randomly selected patients attending the general outpatient department and admitted patients of General Hospital Ilorin, Nigeria from May to September 2022. Random sampling technique was employed for the selection of patients that met the inclusion criteria.

Ethical Approval

Ethical approval was sought and obtained from the Ethical Review Committee of the Kwara State Ministry of Health, Ilorin, Kwara-State with approval number of MOH/KS/EU/823/636

Sample Size

The sample size was determined using the sample size formula for estimating single proportion. Lislie and Wiley (2007)

Sample Size (S) = $Z2PQ/D^2$

Where, Z= Standard Normal Deviztion usually set at 1.96 which corresponds to 95% confidence level.

P= The best estimated prevalence of target population; 42.3% (Eze and Ayo-Loto, 2021).

Q=1 – Prevalence of the Target Population i.e (1-P)

D = Degree of accuracy (0.05)

Hence S = $1.962x\ 0.423x\ 0.577/0.05^2$

S = 0.9376231536/0.0025

S = 375

The minimum sample size collected was 375.

Specimen Collection

Urine, wound swab, blood, sputum and throat swab specimens were collected from the patients. The urine and sputum samples were collected by giving the patient a sterile, wide neck, screw cap and leaked proof container. The patients with Urinary Tract Infection were instructed to produce early morning mid-stream urine into the container while the patients with pneumonia were also instructed to produce early morning sputum before eating or mouth rinsing into the container. The Blood samples of patients with sepsis were collected asceptically using needle and syringe into a Tryptic Soy Broth (TSB). The throat swab samples were collected by using a swab stick to swab the affected area with the supervision of Medical Laboratory Scientist and were returned into its container. The wound swab samples were transported immediately to the Laboratory for analysis Extended Spectrum Beta-Lactamase (ESBL) Detection.

Phenotypic ESBLs screening and confirmation was carried out on all 43 *E. coli* samples as recommended by CLSI. The screening was carried out using Ceftazidime 30 µg disc (*Oxoid*, *UK*): a zone of inhibition ≤ 22mm was considered suggestive of ESBLs production and positive isolates were further investigated using the double disc synergy test with a combination of three antibiotic discs (ceftriaxone, amoxicillin-clavulanic acid and ceftazidime). A ≥ 5 mm increase in the inhibition zone for either antibiotic towards the amoxicillin-clavulanic acid with a dumbbell shape was considered indicative of an ESBLs phenotype. Results were recorded as susceptible, intermediate and resistant according to the reference zone of inhibition of each antibiotic according to CLSI. Isolates expressing ESBLs phenotype were preserved in Tryptic soy broth (TSB) (*Biomerieux France*) supplemented with 20% glycerol at -80˚C until further testing.

Antibiotics Susceptibility Testing

Antibiotic Susceptibility Testing: This was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines. In-vitro antibiotic susceptibility testing of established biofilm producing UPEC isolated was carried out by modified Kirby-Bauer disc diffusion method on Muller-Hinton (MH) agar plates. The following OxoidTM antibiotic susceptibility disks (ThermoFisher™, UK) were used; Augumentin (10ug), Ceftriaxone (30ug), Ceftazidime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Nitrofurantoin (300 µg), and Imipenem (10ug).

Results

Table 4.1: Distribution of bacterial isolates in clinical samples by gender

GENDER	NUMBER OF BACTERIA CULTURES (N=375)							
	No Of Isolates	E. Coli	S. Aureus	Kleb. Spp.	Pseudo Spp.	Strept. Spp	Proteus	N.G
Male	80(42.2)	16(38.1)	18(47.4)	14(46.7)	10(62.5)	14(50.0)	4(21.1)	91(45.0)
Female	93(53.8)	26(61.9)	20(52.6)	16(53.3)	6(37.5)	14(50.0)	15(78.9)	111(55.0)
TOTAL	173(46.1)	42(11.2)	38(10.1)	30(8.0)	16(4.3)	28(7.5)	19(5.1)	202(53.9)

Note: "N.G" means No Growth

Table 4.2: Frequency of bacterial isolates in clinical specimens with respect to age

Age	Number of Bacteria Cultures (N=375)							
	No of Isolates	E. Coli	S. aureus	Klebsiella spp	Pseudomonas aeroginosa	Streptococcus Spp.	Proteus	NG
<10	17(9.8)	2(4.8)	5(13.1)	10(33.3)	0(0.0)	0(0.0)	0(0.0)	4(2.0)
11-20	17(9.8)	0(0.0)	1(2.6)	5(16.7)	3(18.75)	4(14.3)	4(21.0)	14(6.9)
21-30	14(8.1)	2(4.8)	3(7.9)	0(0.0)	3(18.75)	6(21.4)	0(0.0)	30(14.9)
31-40	10(5.8)	5(11.9)	2(5.3)	2(6.7)	0(0.0)	1(3.6)	0(0.0)	41(20.3)
41-50	26(15.0)	4(9.5)	5(13.2)	5(16.7)	1(6.2)	4(14.3)	7(36.8)	20(9.9)
51-60	37(21.4)	16(38.1)	9(23.7)	2(6.7)	3(18.75)	4(14.3)	3(15.9)	42(20.8)
>60	52(30.1)	13(30.9)	13(34.2)	6(20)	6(37.0)	9(32.1)	5(26.3)	51(25.2)
Total	173(46.1)	42(11.2)	38(10.1)	30(8.0)	16(4.3)	28(7.5)	19(5.1)	202(53.9)

Key: "N.G" means No Growth

Table 4.3: Prevalence of Extended Spectrum Beta-Lactamase producing *Escherichia coli* in Clinical specimens with respect to age

Age Groups	No of Sample Examined	E. Coli Isolates	ESBL E. Coli	Negative ESBL
<10	26	2(4.8)	0(0.0)	2
11-20	29	0(0.0)	0(0.0)	0
21-30	45	2(4.8)	1(10.0)	1
31-40	54	5(11.9)	1(10.0)	4
41-50	61	4(9.5)	1(10.0)	3
51-60	76	16(38.1)	3(30.0)	13
>60	84	13(30.9)	4(40.0)	9
Total	375	42(11.2)	10(23.8)	32

Table 4.4: Distribution of Extended Spectrum Beta-Lactamase producing *Escherichia coli* with respect to gender.

Gender	No of Samples Examined	E. Coli Isolates	ESBL E. Coli	Negative ESBL
Male	184(49.1)	16(38.1)	4(40)	12(37.5)
Female	191(50.9)	26(61.9)	6(60)	20(62.5)
Total	375	42	10	32

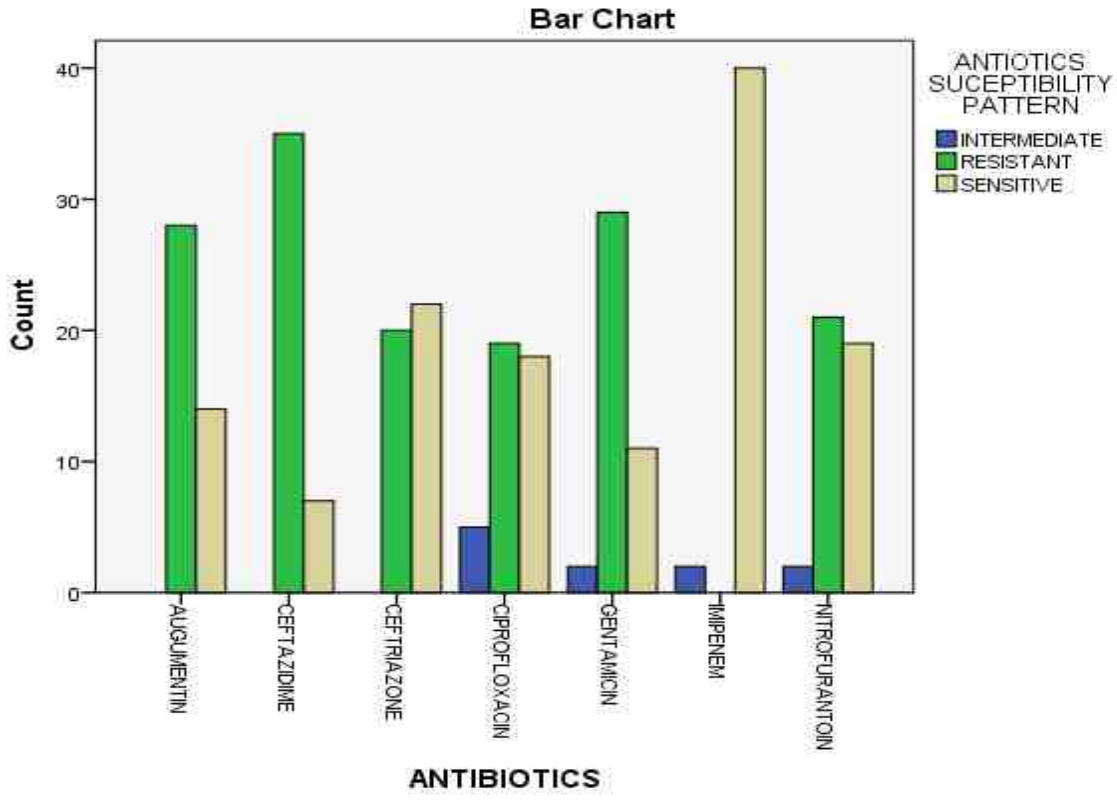

Figure 1.0: A bar chart showing the antibiotics susceptibility pattern of the Escherichia coli isolates in the study area.

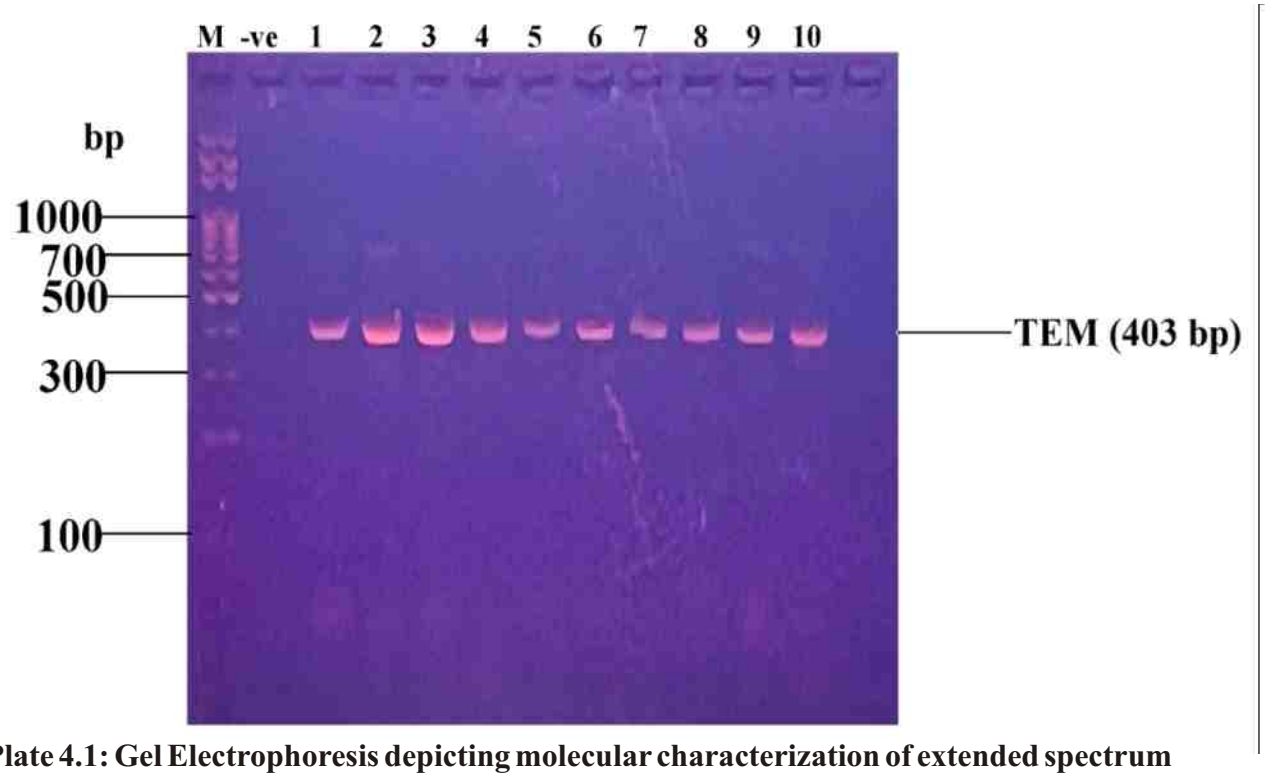

Plate 4.1: Gel Electrophoresis depicting molecular characterization of extended spectrum beta-lactamase producing *Escherichia coli.*

Discussion

This study was conducted to determine the antibiogram and molecular characterization of Extended Spectrum Beta Lactamase *Escherichia coli* isolates in the clinical specimens. *Escherichia coli* is one of the most common bacteria that cause intestinal and extra-intestinal infection (Robins-Browne *et al.,* 2016). Different clinical specimens were used for this study which includes: Urine, Sputum, Throat Swab, Blood and Wound swab samples. The total specimens collected were 375 where 46.1% yielded growth. The prevalence of *Escherichia coli* in this study was 11.2% which is similar to the work carried out in India which shows a prevalence of 15.2% (Nipa-Singh *et al.,* 2016). This is contrast to the prevalence of study that was carried in Enugu, Nigeria which shows a relatively high prevalence of 35% (Nwafia *et al.,* 2019). The high rate of *Escherichia coli* infection being globally reported is a major concern not only to healthcare providers but to the population at large. The results show that *Escherichia coli* is an important pathogen in community acquired bacterial infections especially in urinary tract infections, it's prevalence in urine samples was 47.8%. This agreed with earlier findings that females had higher chances of coming down with UTI than males (Inyang-Etoh *et al.,* 2009). Close proximity of the female urethral meatus to the anus, shorter urethra, and sexual intercourse, incontinence, bad toilet habits have all been reported as factors that influence the higher prevalence in females (Ochei and Kolhatkar, 2007). Prevalence of *Escherichia coli* in throat swab samples was 21.7%, its prevalence in sputum was 17.3%, its prevalence in wound swab sample was 8.7% and it's prevalence in blood samples is 4.3%. Age has been reported as a major factor associated with the acquisition of *Escherichia coli* infection. This study reported a high prevalence of *Escherichia coli* infection among patients above the age of 60 years with prevalence of 30.9%. This agrees with the report of a study that was carried out in Ilorin by Amadu *et al. (*2019) which shows a high prevalence of *Escherichia coli* in patients above 60 years to be 47.8%, then patients within the age bracket of 51-60 years having a prevalence of 38.1% followed by the patients within the age group of 31-40 having a prevalence of 11.9%. The patients within the age bracket of 41-50 were also reported to have a prevalence of 9.5% and the patients below 10 years were reported to have

a prevalence of 4.8%. The prevalence of other organisms isolated were *Staphylococcus aureus*, which shows a prevalence of 10.1%, the prevalence is similar to the work that was carried out in North Central with a prevalence of 10.6%. *Klebsiella spp*. also shows a prevalence of 8.0% this is in concordance with the study that was done in Nepal which shows a prevalence of *Klebsiella spp* to be 9.4% (Khanal *et al.,* 2022), *Pseudomonas aeroginosa* 4.3% which is similar to the study conducted in Iran that shows a prevalence of 3.68% (Mohsen-Rajabnia *et al.,* 2019), *Streptococcus spp.* 7.5% and *Proteus spp.* 5.1%.

Conclusion

The present study established the presence of ESBL genes among *Escherichia coli* isolated from various samples in general Hospital Ilorin from our data and generate molecular epidemiological data that can be used for effective control plan against antimicrobial resistant in this locality.

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