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INCIDENCE OF EXTENDED SPECTRUM ßETA-LACTAMASES (ESBL) PRODUCING STRAINS OF E.Coli FROM PATIENT'S URINE IN POST COVID-19 ERA

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Abstract

The study is designed to detect and evaluate with molecular screening the Extended Spectrum beta-lactamases (ESβL) producing strains of Escherichia coli from patients urine, and aimed as to finding solutions to the spread of ESβL producing bacteria which is a matter of great concern in treating infections in hospitals in covid-19 era. A total of 250 isolates from urine samples were studied phenotypically with antimicrobial susceptibility testing that was determined by, kirby bauer disk diffusion and double disk diffusion synergy test as recommended by clinical Laboratory Standard Institute (CLSI) The results showed, out of 250 isolates from urine sample , 110 isolates were E coli 30(37%) isolates positive On the antibiotics used the isolates showed (100%) resistance to ampicillin (10ug) and augumentine (30µg); followed by ofloxacin(5ug); cefuroxime(30µg); ciprofloxacin (ug); ceftazidime (30µg) and gentamicine(5ug). The result of ESβL production with double disk diffusion test (DDDT) with ceftazidime and cefotaxime with and without clavulanic acid at a distant of 30mm and a zone clearing difference greater than $\geq$ 5mm showed 30(45%) positive with isolates of E coli. Presence of acquired ESßL-encoding gene by PCR using 16S rRNA region sequencing analysis by standard identity yielded positive. The detection of extended spectrum ßeta-lactamase production by Escherichia coli and their confirmation with molecular screening requires adequate infection control with antibiotic management to avoid risks of treatment failures.

Keywords: ESßL, Producing strains, *E,coli*, clinical specimens, ßeta-lactam, antibiotics.

Introduction

β -lactams are one of the most widely used antibiotics in human medicines. But because of the inappropriate and massive use by patients, resistance to these β eta-lactams have increased tremendously as a result of extended spectrum β -lactamases (ESβL) production. These antibiotics act by binding to the components that build the wall and inactivate them. ESβL are found in many types of bacteria and infections caused by them are numerous. Generally, individuals with infections caused by bacteria carrying ESβLs have a higher mortality rate and require longer hospital stay thereby causing an increase in cost of patient care. (Livermore, and Woodford, 2006).

The risk factors associated with ESβL related infections extrapolated from these studies include patients with indwelling medical devices such as urinary catheters, breathing devices and intravenous lines. (Ben, et al., 2009). ESβL are found on an independent element of DNA called the plasmid. These plasmids carry many different genes on them and also have the ability to transfer a replica of themselves to other bacteria. These could be very serious such that these other genes could include-genes that confer resistance to other classes of antibiotics. These could make the recipient bacteria resistant to multiple antibiotics as termed "super bud" (Walsh., 2003).

The spread of ESβL among pathogenic bacteria varies geographically and also in different hospital setting and is rapidly changing over time. The frequency is higher in most community and they are prevalent worldwide (Ben et al., 2009). Though the identification of ESβL producing organisms in clinical laboratories can be challenging and are likely underestimated by all. β -lactamases as enzymes that hydrolyze beta-lactam antibiotics and are the most common mode of action for beta-lactam resistance in gram-negative bacteria. They act by breaking up the nitrogen-carbonyl bond in the beta-lactam ring (Walsh, 2003). Their classification is complicated but are based on similarities at the amino acid level (Bush and Jacoby, 2010). The ESβL classification in this thesis is as found in the Bush and Jacoby functional group 2be, and belong to Amber group A. They hydrolyses penicillins, early cephalosporins and at least one of the oxyimino beta-lactams and also exhibit invitro inhibition by clavulanic acid (Bush and Jacoby 2010).

Not all ESβLs are typical; some enzymes are with extended cephalosporinase activity which belong to Amber group D and others that are not inhibited by clavulanic acid. Though in Bush and Jacoby system these universal enzymes can be found in groups 2ber, 2ce, 2de and 2e, where an "e" in the classification indicates "extended-spectrum" and an "r" indicate inhibitor resistance (Bush and Jacoby 2010). The types and detection of extended-spectrum beta-lactamases as well as treatment of organisms that produce them and also the clinical features and diagnosis of infections that ESβL-producing strains of *E.coli* were the objectives of the study.

Materials and Methods

Methods of Collection

Clinical samples of urine were collected by the assistance of medical lab technologists in the hospital. During collection patients were advised orally on proper procedures and were ensured that collections were before commencement of therapy. The study spanned from September 2020 to January 2021.

Sample Size

A total of two hundred and fifty (250) isolates were collected of which one hundred and ten (110) isolates were positive from *E coli*.

Preparation of Culture Media

All the media used were prepared according to manufacturer's instruction (Oxoid). Nutrient agar was prepared and autoclaved at 121^0c for 15minutes was cooled to 50^0c, mixed well and dispensed aseptically in 20ml amount in petri dishes and 5ml in bijou bottle for agar slope and was allowed to cool and solidify. The sterilized nutrient agar was cooled at 70^0c and fresh whole blood was added and mixed, heated to form chocolate agar. It was dispensed in petri dishes 20ml amount was allowed to cool and solidify. Also MacConkey Agar was dispensed in same 20ml amount was allowed to cool and solidify before it was refridegerated.

Isolation and Preservation

Isolates were directly streaked into different media plates using oxoid sterile nichrome wire calibrated loop. These were inoculated directly onto Nutrient Agar, chocolate Agar and MacConkey Agar. Each was streaked unto small area of the plates. Then the sterile loop was used for cross-streaking to spread the inoculums over the surface of the plates to obtain single colonies after incubation. Each single significant growth and non viable isolates were included in this study. Though culture plates that yielded more than two organisms per specimens were regarded as contaminant and excluded from the study.

Kirby Bauer, Disk Diffusion Technique

Antimicrobial susceptibility was determined by Kirby Bauer disk diffusion method (CLSL recommendation, 2004). Antimicrobial disks used were-ampicillin (10ug), augumentin (30ug), ceftazidin (30ug), ciprofloxacillin (5ug), cefuroxim (30ug), ofloxacin (5ug), nitrofurantion (30ug), gentamicine (5ug), and cefriaxone (30ug), by (Rapid labs, UK). Muller Hinton Agar plates were prepared and sterilized at 121^0c for 15minutes and the cultures were swabbed with sterile swab on the plates. The plates were left at room temperature to remove excess moisture. With sterile forceps, different antibiotic disks were placed carefully on the plates and kept at refrigerator for 30minutes for pre-diffusion of the disk. After, the plates were incubated at 37^0c for 24hours. Following incubation, zone of inhibition in diameter was noted and results interpreted using standard chart (Wayne, P.A).

Double Disk Diffusion Synergy Test (DDST)

Muller-Hinton agar plates were prepared and sterilized at 121^0c for 15minutes and the culture were swabbed on the plates with sterile swabs. With sterile forceps augumentin (amoxicillin/clavulanate) was placed at the centre of the plate. Single disks containing ceftazidime and cefotaxime was placed at a distance of 30mm from the center of the plates, they were incubated at 37^0c for 24hours.

Confirmation by Double Disk Diffusion Test (DDDT)

Using DDDT four (4) disks containing ceftaxidime, cefotaxime with and without clavulanic acid was placed at 30mm distance from each other on the plate. The plates were incubated at 37^0c for 24hours.

Positive

Zone clearing around the test antibiotic disk increased towards augumentine (amoxicillin/clavulanate). This increase occurs because of inactivation of extended spectrum β-lactamases produced by the test organism.

Molecular characterization

Molecular, characterization were screened using 16s rDNA region sequencing analysis for the presence of acquired ESβL encoding genes. By method of (macrogen Inc. Europe). The PCR reaction was performed using 20g of genomic DNA as the template in a 30ul reaction mixture using a EF-Tag(SOIGEMT, Korea). 95% activation of tag polymerase for 2minutes, 35cycles of 95^0c for 1minute (55^0c and 72^0c each for 1minute were performed and finished with 10minutes step at 72^0c.

The amplification products were purified with multi-screen filter plate (Millipore Corp Bedford MA. USA). Sequencing reaction was performed using a prism Bigdye terminator V3.1 cycle sequence kct. The DNA samples containing the extension products were added to HI-DI formamide (applied Biosystems, Foster city CA). The mixture was incubated at 95^0c for 5minutes followed by 5minutes on ice and was analyzed by ABI prism 373 OXL DNA analyzer.

Results

A total of 250 isolates from urine were collected and tested, (110) isolates were positive table 1.

Table 1: incidence of isolates from urine sample

Sample specimen	No of isolates	Percentage
Urine	110	44

Table 2

: Sensitivity and resistant pattern of the study samples

Sample	Total isolates	Growth
Urine	110	82

Antimicrobial susceptibility test by agar disk diffusion method on study samples of urine,is shown on bar chart fig i ,

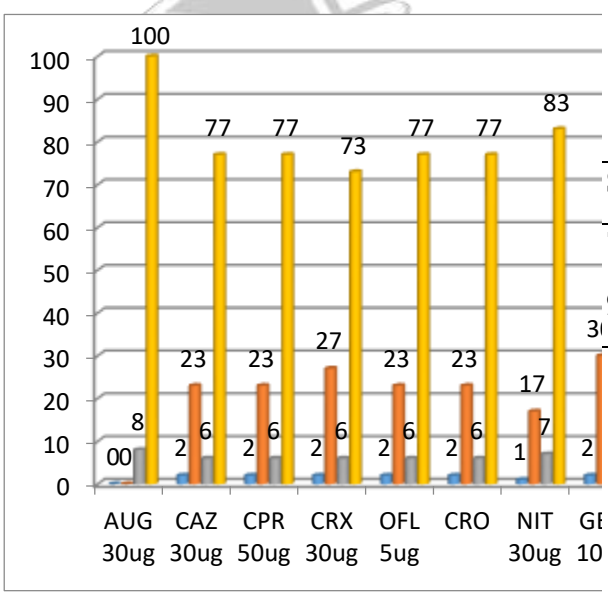

Figure 1: Antimicrobial sensitivity and resistant pattern of urine sample (30)

Keys

AUG = Augumentine CAZ = Ceftazidime CPR = Ciprofloxacin
CRX = Cefuroxime OFL = Ofloxacin
CRO = Cefriaxone
NIT = Nitrofurantoin GEN = Gentamicin AMP = Ampicillin

βeta –lactamases enzymes production by study samples of bacteria isolates using double disk diffusion synergy Test were used for detection of ESβL. DDDT were used for confirmation see 3

Table 3:βeta-lactamases enzyme production by urine positive (30) samples with zone clearance > 5mm = 19

Clinical sample	No	Positive isolates	%	Sensitive Enzymes produced		Resistant Enzyme not produced	
				No	%	No	%
Urine	30		37	19	63	11	33

The result of molecular characterization with screened positive bacteria isolate of *E.coli* is shown in table 4 and figure 2

Table 4: 16S rRNA region sequencing analysis primer information

Sequencing primer name primer sequences	PCR p
785F5’ (GGA TTA GAT ACC CTG GTA) 3’	27F 5’
907R 5’ (CCG TCA ATT CMT TTR AGT TT) 3	1492R

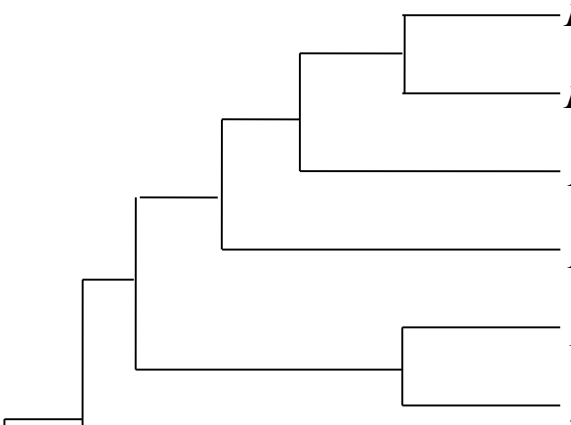

Figure 2: Standard ID 16S rRNA Service Report

Discussion

The study examines the incidence of Extended Spectrum beta-lactamases (ESβL) producing strains of *Escherichia coli* from patients clinical specimens and screened, the collaboration with the molecular characterization to confirm the presence of the bacterial isolate. The presence of *E.coli* (37%) in urine, in this study findings is similar to the 28.7% reported in Tanzania by (Ndugulile et al 2015) But low compared to the figure reported in a study in Khartoum state by (Mekki et al, 2010) which recorded 53%. And much lower than 60.9% observed in Egypt and 78% in Pakistan by (Hussain et al., 2011) and (Mohamed et al., 2006). In all, these findings indicate that the incidence of bacteria producing ESβL varies worldwide.

ESßL detection is not routinely examined in many clinical microbiology laboratories because they can be difficult to detect and have varying activities against various antimicrobial agents and some of them were not easy to procure from the conventional market. Therefore the emergency of the ESβL-producing strains should create a need for their detection among bacteria pathogens. (Brandford, 2001). The present study detected and confirmed ESβL by DDST and DDDT phenotypically. The tests yielded equal accuracy in the determination of ESβL production. They had been previously documented as effective test for ESβL-detection by (Brandford, 2001). High resistant of 63% were recorded in this study by some antimicrobial agent probably because ESßLs are transmissible to resistant gene elements for other antibacterial from one organisms to another (Nathisuwan et al., 2 .

In conclusion, the detection of ESβL-producing strains of *Escherichia coli* and their confirmation with molecular screening requires adequate infection control and antimicrobial management to avoid risk of treatment failures. And also to ensure proper routine laboratory detection of ESβL-producing strains to reduce their spread.

Recommendation

Ensure good hand hygiene by patients and hospital staff and avoidance of indiscriminate use of antibiotics and prompt visit to doctor or nursing staff before taken antibiotic will help to curb the incidence of ESβL producing strain of *E.coli.* Also, embrace all covid-19 protocols to avoid spreading the organism that can be resistance to antibiotics.

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