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Detection of Extended-Spectrum Beta-Lactamases in Gram- Negative Bacteria from Urinary Tract Infections

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Abstract

This study investigated 150 urine samples from patients presented with urinary tract infections for the occurrence of extended spectrum beta- lactamases in gram- negative isolates. Out of the 150 urine samples tested only 90 samples were positive with bacterial isolates namely *E. coli* 51*(*57%), *Pseudomonas* sp 30(33%), *Klebsiella* sp 6(7%) and *Proteus* sp 3(3%) .The antibiogram of the isolates were performed using Kirby-Bauer technique and the extended-spectrum beta- lactamase determined using the double disc synergy test. Out of the 90(60%) positive urine samples 37(41%) were Extended-Spectrum Beta Lactamase ESBL producers and all extended-spectrum beta lactamase (ESBL) producers were resistant to ceftazidime, ceftriaxone, cefotaxime, cefpodoxime and aztreonam antibiotics used. All isolates were sensitive to imipenem. This study detected ESBL producers in gram- negative bacteria from urine samples of patients with urinary tract infections.

Keywords: Extended-spectrum beta lactamase, gram negative bacteria, Urinary tract infections, cephalosporins, imipenem, double disc synergy tests.

1.0 Introduction

Extended-spectrum beta-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients (Mehrgan and Rahbar,2008). Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis. Extended-spectrum beta-Lactamases are able to hydrolyze Oxyimino-cephalosporins e.g. cefotaxime, ceftazidime and ceftriaxone and monobactams like aztreonam (Paterson and Bonomo 2005) but do not affect 2nd generation cephalosporins such as cephamycin. Enterobacteriaceae, especially, *Klebsiella* sp. producing ESBLs such as Sulphydryl variable (SHV) and Temoniera (TEM) types have been established since the 1980s as a major cause of hospital acquired infections. However, during the late 1990s, several community –acquired pathogens that commonly cause urinary tract infections and diarrhea have been found to be ESBL producers (Afazal *et al.,* 2001). The ESBL producing bacteria are increasingly causing urinary tract infections (UTIs) both in hospitalized and out-patients and this is making treatment of urinary tract infections difficult and promoting greater use of expensive broad spectrum antibiotics. The failure of treatment of both complicated and uncomplicated UTIs is continually increasing morbidity and mortality among UTI patients (Mehrgan and Rahbar, 2008). This study was carried out to determine the ESBL producing gram-negative isolates from urinary tract infections, using the double disc diffusion method for early correct detection of ESBLs.

2.0 Materials and Methods

2.1 Sample Collection

The clean catch mid-stream was employed to collect 150 urine samples from patients attending clinical laboratories in Owerri municipality, Imo State, Nigeria. Our case patients represented only the most visible part of the population harbouring these organisms and demographic prevalence was not investigated in the study. Urine collection and transportation were done according to standard methods (Cheesbrough, 2000).

2.2 Urine Microscopy

Urine was transferred into a clean and dry 15ml

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centrifuge tube and was centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and one drop of sediment was taken into a clean glass slide and a cover slip was placed over it and then examined under low and high power magnifications for gram –negative bacilli.

Each urine sample was inoculated into blood agar and Mac Conkey agar media by calibrated loop technique (Colle *et al.*, 1996). They were incubated at 37°C for 24h. Patients with positive urine cultures (pure culture of a single microorganism with a colony count > 10^5 CFU/ml) were considered to have UTIs.

2.3 Isolation and Bacterial Identification

Urine samples were plated using spread plate method and were identified by their colony morphology, staining character, pigments production, haemolysis, motility and other biochemical tests according to standard method (Cheesbrough, 2000; Colle *et al.*, 1996).

2.4 Antimicrobial Susceptibility Tests

The Kirby-Bauer technique using Mueller-Hinton agar plates was used for the antimicrobial susceptibility testing of the isolates (Patel *et al.*, 2012). .Commercially available antimicrobial discs (Oxford England) were used including: Imipenem (15mg), Aztreonam (30mg), Amikacin (30mg), Cefpodoxime (30mg), Ceftazidime (30mg), Ciprofloxacine (15mg), Amoxicillin/Clavulanate (20/10mg).

2.5 Standardization of Inoculum

Using a sterile wire loop, three isolated bacterial colonies were transferred to screw-capped tube containing 4ml of sterile normal saline and standardized with 0.5 MacFarland standards by adding more organisms or more saline. The solution approximately corresponds to 1.5 x10^8 organisms/ml (Cheesbrough, 2000).

2.6 Inoculation on Mueller-Hinton Agar Plates

After standardization of inoculum, a sterile cotton swab was immersed into the bacterial suspension and excess broth removed by pressing the swab against the inner side of the tube. The swab was then streaked evenly on the surface of Mueller-Hinton agar plate. The inoculum was allowed to dry for 10-15 minutes at room temperature keeping the lid closed. Using sterile disposable needles, the discs were then placed on the inoculum surface 15mm away from the edge of the petridish and having20-25mm gap between the discs. The plates were then incubated for 37°C for 24 hours. After incubation each plate was examined and complete zones of inhibition measured with a meter rule.

2.7 Detection of ESBL Producers

Double disc synergy tests (DDST) was used to detect ESBL producers. Isolates showing resistance to one or more of the third generation cephalosporins were identified as potential ESBL producers. Mueller-Hinton agar plates were prepared and inoculated with standardized inoculum (compared with0.5 MacFarland standard) by using sterile cotton swab. Augmentin disc(20mg amoxicillin +10 mg clavulanic acid) was placed in the centre of the plate and 3rdgeneration cephalosporins (Ceftazidime, Ceftriaxone, Cefotaxime, Cefpodoxime and Aztreonam discs) were placed 20-30mm apart (centre to centre) from the augmentin disc. The plate was observed after overnight incubation at 37°C (Linscott and Brown, 2005).Inhibition around the 3rd generation cephalosporins or aztreonam disc were increased towards the augmentin disc. Bacterial growth was inhibited where two antibiotics diffuse together and interpreted as ESBL positive (Shuka *et al.*, 2004). *Klebsiella pneumonia* were used as positive control and *E. coli* were used as negative control.

3.0 Result

Only 60% of the case patients had bacterial growth. It could be that some of them had recurrent UTIs, underlying diseases and are on antimicrobial treatment which are the major risk factors in UTIs.

Table 1: Bacteria growth from urine samples

Colonies	Frequency	Percentage
Growth	90	60
No growth	60	40
Total	150	100.0

Out of 90(%) positive urine samples *E.coli* occurred most frequently (57%) followed by *Pseudomonas (33%), Klebsiella sp (7%) and Proteus sp (3%).*

Table 2: Bacterial isolates from urine samples

Isolates	Frequency	Percentage occurrence
E.coli	51	57
Klebsiella sp	6	7
Pseudomonas aeruginosa	30	33
Proteus sp	3	3
Total	90	100

The highest ESBL producer in this study was *E. coli* (51%) and the least ESBL producer was *Proteus* species (3%).

Table 3: Percentage occurrence of ESBL producers

Isolates	ESBL Producers		Total
	Positive	Negative	
Escherichia coli	19 (51%)	32	51
Klebsiella sp	2 (5%)	4	6
Pseudomonas aeruginosa	15 (41%)	15	30
Proteus sp	1 (3%)	2	3

4.0 Discussion

The continued emergence of ESBL producers present diagnostic challenges to the clinical microbiology laboratories. In this study (41%) of *Pseudomonas sp* isolates were ESBL producers. Ali *et al*. (2004), reported ESBL production in *Pseudomonas sp* to be 36% and *Proteus sp* to be 50% which is higher than the percentage (3%) reported in the present study. The common isolates from the urine samples *were E.coli, Klebsiella* sp, *Pseudomonas* sp and *Proteus* sp. The reason for the differences observed in other studies may be due to the different methods used in screening for ESBL producers. *E. coli* was the predominant organism isolated from the urine samples (57%) and the highest ESBL producers (51%).Similar result was reported by Mansour *et al*. (2009) who stated that *E.coli* is still the most common isolated bacteria from urine samples of patients presented with UTI. Mostaqim (2007) found 56.7% of *E.coli* and among them 34.1% were ESBL producers. Rahaman *et al*. (2004) reported that *E.coli* was the predominant organism in urine and among them majority were ESBL producers. In the present study ESBL producing isolates were found to be multi drug resistant to commonly used cephalosporins namely aztreonam, ceftazidime, Ceftriaxone, Cefotaxime, Cefpodoxime which are important drugs in UTI treatment but sensitive to imipenem .This finding agrees with the recent findings by Shanthi and Sekar (2010), who showed the ESBL producing isolates displayed co-resistance patterns against most of the antibiotics tested in his study. In this study, 41% of *Pseudomonas* sp. were ESBL producers. Ali *et al.* (2004) reported ESBL production in *Pseudomonasaeruginosa* to be 36.36% and Proteus vulgaris to be 50%.On the contrary Hemachandren *et al*. (2011) reported frequencies that were lower in *E.coli (43%) and Pseudomonas (25%) but higher in Klebsiella sp (31%) and* reported no ESBL among *Proteus* isolates. All isolates were 100% sensitive to imipenem. This result agrees with that of Saiful *et al.* (2014). The frequency of ESBL producing isolates varies according to countries regions or even hospitals and is rapidly changing over time (Kader and Kumar, 2005). Imipenem can be used for the treatment of infections caused by ESBL producing organisms because they showed high levels of sensitivity (100%) to Imipenem. The study identified ESBLs and their resistance to multiple cephalosporins. Therefore ESBL producing organisms should be promptly identified for appropriate antibiotic prescription and proper implementation of infection

Table 4: Antimicrobial susceptibility patterns of ESBL producers.

Antimicrobial Agent	*E.coli*	*Klebsiella* sp	*Pseudomonas sp*	*Proteus* sp
	Resistance pattern			
Imipenem	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Aztreonam	19 (100%)	2 (100%)	15 (100%)	1 (100%)
Ceftazidime	19 (100%)	2 (100%)	15 (100%)	1 (100%)
Ceftriaxone	19 (100%)	2 (100%)	15 (100%)	1 (100%)
Cefotaxime	19 (100%)	2 (100%)	15 (100%)	1 (100%)
Cefpodoxime	19 (100%)	2 (100%)	15 (100%)	1 (100%)

All isolates were (100%) sensitive to *Imipenem*

control measures like appropriate selection of antimicrobial treatment.

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