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Extended-Spectrum β-Lactamase - Producing *Klebsiella pneumonia* and *Escherichia coli* from Blood Cultures of Hospitalized Patients in Abakaliki Metropolis

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Abstract The incidence of antibiotic resistance in extended-spectrum β-lactamase (ESBL)–producing *Escherichia coli* and *Klebsiella pneumoniae* has obviously increased in recent era. Twelve strains of Gram-negative bacteria comprising of 6 *Escherichia coli* and 6 *Klebsiella pneumoniae* were isolated from blood samples of hospitalized patients in Federal Teaching Hospital Abakaliki I (FETHA I). The extended spectrum β-lactamases detection was ascertained using double disc diffusion methods. Identification of organisms was done using appropriate microbiological technique. Antibiotics susceptibility test was carried out on Mueller-Hinton agar using the disc diffusion method. Ofloxacin and cefoxitin were 83.3% active against *E. coli*, followed by sulphamathroxazole with 66.7% activity. While ofloxacin was 100% active against *K. pneumoniae*, followed by cefoxitin and tetracycline with 83.3% activity. Amikacin and ciprofloxacin showed the highest resistance against *E. coli* and *K. pneumoniae*. This resistance is associated with extended-spectrum β-lactamases (ESBL) production which was detected in *K. pneumoniae* and *E. coli*. ESBL production was observed in 80% of Gram negative bacilli. ESBL-producing organisms have significant impact on several important clinical outcomes and hence clinical microbiology laboratories should take into account the varying epidemiology of ESBL producers in order to improve treatment strategies and expand therapeutic options.

Keywords: *ESBL, antibiotic resistance, blood cultures, hospitalized patients, gram-negative bacteria*

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1. Introduction

ESBLs are a rapidly evolving group of β -lactamases which share the ability to hydrolyse third-generation cephalosporins and aztreonam, yet are inhibited by clavulanic acid [1]. ESBL mediate resistance to all three generations of cephalosporins, including monobactams (e.g. aztreonam) [2]. The β-lactamase-mediated carbapenem resistance among *K. pneumoniae* isolates and others is an emerging problem [3]. This resistance has spread to strains of *Escherichia coli* and to other Gram-negative bacteria as well [4].

Most ESBL are encoded on a large plasmid that can be horizontally transferred to different genera of bacteria, which may be involved with both prevention and treatment aspects of nosocomial infections, particularly with septicemic patients [2,5]. Rapid detection of ESBL is important, not only for treatment guidelines but also to facilitate improved prevention of nosocomial infections [6]. ESBL can be detected using a standard screening test showing reduced susceptibility to five antibiotics, such as ceftazidime, ceftriazone, cefotaxime, aztreonam and cefpodoxime, as detected by standard disk diffusion and minimal inhibition concentration (MIC) [7].

Bloodstream infections (BSIs) caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-KP) isolates are a major concern for clinicians, since they markedly increase the rates of treatment failure and death particularly in intensive care units and amongst pediatric patients and also in medical and surgical wards [8]. Subsequently, the role of routine surveillance cultures as a means of screening for ESBL-E colonization among hospitalized patients is unclear. Rectal surveillance cultures, together with isolation precautions and antibiotic-restriction measures, have been instrumental in ESBL outbreak management [9,10,11], but routine surveillance is costly and may not be effective in predicting clinical disease [12,13].

2. Materials and Methods

2.1. Sample Collection

Two hundred blood samples were recovered over a period of two months (October 2012 to November 2012) from hospitalized patients attending Federal Teaching Hospital Abakaliki I (FUTHA I) were immediately transported to the Department of Applied Microbiology Laboratory for culture, isolation and identification. The isolates were identified on the basis of conventional microbiological procedures [14].

2.2. Antimicrobial Susceptibility Test

Antibiotic sensitivity of the isolates to various antibiotics: amikacin, cefoxitin, ciprofloxacin, ofloxacin, sulphamathroxazole and tetracycline were determined by the disc diffusion methods [15]. The results were interpreted as per National Committee for clinical laboratory standards (NCCLS) recommendations [14]. Isolates which were resistance or intermediate susceptibility by NCCLS criteria to any of third generation cephalosporins were selected for ESBL detection/ screening phenotypically.

2.3. ESBL Detection by NCCLs Phenotypic Method

The NCCLs ESBL phenotypic confirmatory test with ceftazidime (CAZ) and clavulamc acid (CA) were used for all the Gram negative isolates by the disc diffusion method [16]. Muller-Hinton agar plates and disks containing of ceftazidime with 10µg of clavulamc acid (CA) were used.

Susceptibility test results were interpreted according to the NCCLs = 5 mm enhanced in the zone diameter of CAZ and CA was considered indicative of ESBL production. However resistance to the third generation cephalosporins is highly suggestive of the presence of ESBLs in *E. coli* and *K. pneumoniae [17]*.

3. Results

The various results for the test and analysis carried out are shown below:

Two bacteria isolates from blood of hospitalized patients were suspected in this work as indicated in Table 1.

Table 1. Morphological and biochemical test result of bacterial isolates from blood of hospitalized patients

Morphological characteristics								Sugar fermentation test			Isolated organisms
Colour	Consistency/ Texture	Gram staining	Catalase test	Oxidase test	Indole test	Voges praskaur test	Motility test	Glucose	Lactose	Fructose	
Greenish	Rough surface	-ve	+	-	+	-	-	+	+	-	*Escherichia coli*
Large grey-white	Slightly raised	-ve	+	+	-	-	-	+	+	-	*Klebsiella pneumoniae*

Table 2. Inhibition zone diameter (mm) of the antimicrobial agents on *E. coli* isolates

Antibiotics tested	Isolate code						Resistance No. (%)	Susceptible No. (%)
	2a	3c	2d	4d	3b	3c		
AK	09	07	08	08	09		5 (83.3)	1 (16.7)
CIP	09	11	14	12	09		5 (83.3)	1 (16.7)
FOX	24	17	25	29	23		1 (16.7)	5 (83.3)
OFX	19	21	17	20	22		1 (16.7)	5 (83.3)
SXT	15	09	19	15	12		2 (33.3)	4 (66.7)
TE	09	11	15	17	25		4 (66.7)	2 (33.3)

Key: OFX = Ofloxacin, TE = Tetracycline, FOX = Cefoxitin, AK = Amikacin, SXT = Sulphamathroxazole and CIP = Ciprofloxacin

Table 3. Inhibition zone diameter (mm) of the antimicrobial agents on *K. pneamoniae* isolates

Antibiotics tested	Isolate code						Resistance No. (%)	Susceptible No. (%)
	4a	2c	3b	2b	4c	3a		
AK	09	14	12	09	11	12	4 (66.7)	2 (33.3)
CIP	08	07	13	18	15	16	4 (66.7)	2 (33.3)
FOX	22	30	28	08	16	21	1 (16.7)	5 (83.3)
OFX	21	27	34	30	19	24	0 (00.0)	6 (100.0)
SXT	09	19	10	11	15	13	2 (33.3)	4 (66.7)
TE	22	10	18	29	22	15	1 (16.7)	5 (83.3)

4. Discussion

There is currently a great need for reliable and efficient tests to detect ESBLs in clinical isolates of Enterobacteriaceae. Conventional susceptibility testing methods, on their own, fail to offer reliable susceptibility results for β-lactam antibiotics when testing those species that harbour ESBLs [18]. Past attempts to identify risk factors for infection due to ESBL-producing organisms have come to very different conclusions [19]. Hospital acquired due to ESBL producing organisms have been known to cause high mortality [20]. ESBL production by *K. pneumoniae* was reported in bacteremic patients [2]. Although *E. coli* strains have been isolated in the highest numbers in bacteremic patients, the highest percentage of ESBL production was found in *K. pneumoniae* [21,22,23].

Screening disk diffusion has proven to be a useful method for the detection of ESBL production, particularly in for *E. coli* and *K. pneumoniae*.

Ciprofloxacin and amikicin has been reported to be used in sensitive screening indicator for ESBL production [24,25]. It is observed in this work that ciprofloxacin and amikacin showed 83.3% resistance to the *E. coli* isolates with 16.7% activity as shown in Table 1. This work is in line with what was reported by Ben-Ami et al. [24], 65%

of healthcare associated strain of Enterobactericeae isolated from blood samples were resistant to ciprofloxacin. Tetracycline showed 66.7% resistance to *E. coli* with 33.3% activity. All the cases of ciprofloxacin, amikacin and tetracycline resistance to *E. coli* isolated from blood cultures were suspected to be due to ESBL production. The results alert the microbiologist to perform the confirmation test with the suspected organism. However, it is important that screening antibiotic disks are included in the screening program. The NCCLS guideline has been shown to work very well [3]. Cefoxitin and ofloxacin showed 83.3% sensitivity to the *E. coli* isolates followed by sulphamathroxazole with 66.7% activity. This is an indication that these antibiotics can be used in the treatment of infection associated with the organism.

Table 4. Detection of ESBL production

Isolate	Combination disk (CAZ/CA)
Escherichia coli	-
Escherichia coli	+
Escherichia coli	+
Escherichia coli	+
Escherichia coli	+
Escherichia coli	-
Klebsiella pneumoniae	+
Klebsiella pneumoniae	+
Klebsiella pneumoniae	+
Klebsiella pneumoniae	+
Klebsiella pneumoniae	+
Klebsiella pneumoniae	-

In this work, 66.7% of the *K. pneumoniae* were resistant ciprofloxacin and amikacin with 33.3% sensitivity. This is in disagreement with the work of Iroha et al. [26] who revealed that 31.2% of *K. pneumoniae* were resistant to ciprofloxacin. The reason for high resistant of ciprofloxacin in this work might be associated with wide widespread and indiscriminate use in our environment. ESBL production was observed in *E.* coli (51.8%) and *K. pneumoniae* (22.5%). This conforms to the work of Narayanaswamy and Mallika [27] who reported 54.43% to *E. coli* producing ESBL. Ofloxacin and clindamycin were shown to the most sensitive to *K. pneumoniae* with 100% activity, followed by cefoxitin with 83.3%. This is an indication that these antibiotics can be used the treatment of infection associated with the organism. In our study, there were two strain of *E. coli* and one strain of *Klebsiella pneumoniae* that had an inhibition zone diameter of above 5mm (Table 4), indicating negative in ESBL production. This strain could be misread as sensitive to the combination disc (CAZ and CA) if investigating microbiologist did not follow carefully the NCCLS guidelines with the confirmation tests to validate ESBL production. However, some false negatives have also been reported, particularly with strains that produce AmpClike β-lactamase [28]. The loss of an outer membrane protein combined with co-existing TEM-1 and SHV-1 β- lactamases has been reported to give a false identification of ESBL producing K. pneumoniae [29]. These findings have important implications with regard to possible interventions aimed at curbing such outbreaks. Efforts should emphasize limiting contact transmission of resistant isolates as well as controlling antibiotic use.

In conclusion, this study has shown the significance of regular antibiotic susceptibility testing of blood culture isolates in various environments. Cautious attention to barrier precautions to prevent the nosocomial spread of ESBL-producing *E. coli* or *K. pneumoniae* infections must be stressed. And also clinical microbiology laboratories should take into account the changing epidemiology of ESBL producers in order to establish a proper treatment procedure, improving treatment strategies and expanding therapeutic options.

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