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Presence of extended spectrum β-lactamase (ESBL) *E. coli and K. pneumoniae* isolated from blood cultures of hospitalized patients

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ABSTRACT

Ten strains of Gram-negative bacteria comprising of 5 Escherichia coli and 5 Klebsiella pneumoniae were isolated from blood samples of hospitalized patients in Ebonyi State University Teaching Hospital (EBSUTH), Abakaliki. Detection of extended spectrum β-lactamases was carried out by double disc diffusion test methods. Identification of organisms was done using appropriate microbiological technique. Antibiotics susceptibility test was carried out Mueller-Hinton agar using the disc diffusion method. Ofloxacin and cefoxitin were 100% active against E. coli, followed by sulphamathroxazole with 80% activity. While ofloxacin was 100% active against K. pneumoniae, followed by cefoxitin and tetracycline with 80% 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. This present study suggested that clinical microbiology laboratories should take into account the changing epidemiology of ESBL producers in order to establish a proper treatment procedure.

Key words: ESBL, antibiotics, blood sample, Gram-negative bacteria.

INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are a variant of the beta-lactamase enzyme. The enzyme has one position mutation in the gene at the active site that is believed to be the cause of high beta-lactamase activity. ESBL mediate resistance to all three generations of cephalosporins, including monobactams (e.g. aztreonam) (1, 2). ESBL are mostly reported in *Klebsiella pneumoniae* and *Escherichia coli*. However, they have also been found in other species of Enterobacteriaceae, including other genera of gram-negative bacteria (2).

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 (1, 2). In addition, ESBL-producing *Escherichia coli* have been reported in a community-acquired bacteremic infection (3).

There is an increased need to detect ESBL-producing gram-negative bacteria in routine microbiological work. Rapid detection of ESBL is important, not only for treatment guidelines but also to facilitate improved prevention of nosocomial infections (4). 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)(5).

The consequences of infection due to ESBL-producing Enterobacteriaceae (ESBL-E) are well known. Bloodstream infections due to ESBL-E (ESBL-BI) have led to increased length of hospital stay (6, 7), increased hospital costs (7), improper antibiotic use (8), and most notably, increased mortality (7, 8 and 9). Not surprisingly, prior colonization with an ESBL-E is a risk factor for ESBL infection (10, 11). However, 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, 12), but routine surveillance is costly and may not be effective in predicting clinical disease (13, 14).

MATERIALS AND METHODS

A total of 210 blood samples, recovered over a period of four months (July 2011to October 2011) from hospitalized patients attending Ebonyi State University teaching Hospital (EBSUTH) 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 (15).The identified isolates were subjected to Antimicrobial susceptibility Testing by Disc Diffusion Method.

Antimicrobial Susceptibility Test

Sensitivity of the isolates to various antibiotics: ofloxacin, tetracycline, cefoxitin, amikacin, sulphamathroxazole and ciprofloxacin were determined by the disc diffusion methods (16). The results were interpreted as per National Committee for clinical laboratory standards (NCCLS) recommendations (5). Isolates which were resistance or intermediate susceptibility by NCCLS criteria to any of third generation cephalosporins were selected for ESBL detection/ screening phenotypically.

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 (17).

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* (18).

RESULTS

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

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

Morphological characterization								Sugar Fermentation Test			Suspected Organisms
Colour	Consistency/ Texture	Gram staining	Catalase Test	Oxidase Test	Indole Test	Voges Proskauer	Motility Test	Glucose	Lactose	Fructose	
Greenish	Rough surface	-ve	+	-	+	-	-	+	+	-	*Escherichia coli*
Large grey-white	Slightly raised	-ve	+	+	-	-	-	+	+	-	*Klebsiella pneumoniae*

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

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

Antibiotic Tested	Isolate Code 131	127	130	48	43	Resistance No. (%)	Susceptible No. (%)
OFX	17	19	23	35	23	0 (0.0)	5 (100.0)
TE	08	12	17	06	27	3 (60.0)	2 (40.0)
FOX	20	28	19	31	18	0 (0.0)	5 (100.0)
AK	06	06	06	08	06	5 (100.0)	0 (0.0)
SXT	17	10	12	18	09	1 (20.0)	4 (0.0)
CIP	08	10	12	07	13	5 (100.0)	0 (0.0)

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 *Klebsiella pneumoniae* isolates

Antibiotic Tested	Isolate Code 22	56	60	167b	76	Resistance No. (%)	Susceptible No. (%)
OFX	19	20	24	29	36	0 (0.0)	5 (100.0)
TE	20	10	16	27	30	1 (20.0)	4 (80.0)
FOX	34	19	06	29	22	1 (20.0)	4 (80.0)
AK	08	12	06	15	06	3 (60.0)	2 (40.0)
SXT	10	29	08	20	07	2 (40.0)	3 (60.0)
CIP	06	08	06	12	19	4 (80.0)	1 (20.0)

Table 4: Result for detection of ESBL production

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

DISCUSSION

The increasing prevalence of ESBL among high-risk patients mirrors a national increase in ESBL production among Enterobacteriaceae (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 (6). 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, 24). Screening disk diffusion has proven to be a useful method for the detection of ESBL production, particularly in *E. coli* and *K. pneumoniae*.

However, ciprofloxacin and amikicin has been reported to be used in sensitive screening indicator for ESBL production (25, 26). In this work, ciprofloxacin and amikacin showed 100% resistance to the *E. coli* isolates with no activity as shown in Table 1.This work is inline with what was reported by Ben-Ami et al., (25), 65% of healthcare-associated strain of Enterobactericeae isolated from blood samples were resistant to ciprofloxacin. Tetracycline showed 60% resistance to *E. coli* with 40% 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 (27). Cefoxitin showed 100 % sensitivity to the *E. coli* isolates followed by sulphamathroxazole with 80% activity. Is an indication that these antibiotics can be used the treatment of infection associated with the organism.

In this work, 80% of the *K. pneumoniae* were resistant ciprofloxacin with 20% sensitivity, indicating the most resistant antibiotic to *K. pneumoniae*. This is in disagreement with the work of Iroha et al. (28) 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. Amikacin also revealed 60%

resistant each to *K. pneumoniae* in this work. ESBL production was observed in 74.3 % of Gram-negative bacilli, *K. pneumoniae* (22.5%) and *E.* coli (51.8%). This conforms to the work of Narayanaswamy and Mallika (29) 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 80%. Is an indication that these antibiotics can be used the treatment of infection associated with the organism. In our study, there was one 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 AmpC-like β-lactamase (30). 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 (31).

CONCLUSION

This study has demonstrated how important significance of regular antibiotic susceptibility testing of blood culture isolates in various communities, particularly with *E. coli* and *K. pneumoniae* in other to control, prevent and reduce their spread. And also clinical microbiology laboratories should take into account the changing epidemiology of ESBL producers in order to establish a proper treatment procedure.

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