Extended-Spectrum β-Lactamase - Producing Klebsiella pneumoniae 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 sulphamethoxazole 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


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 clavulamic 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 clavulamic 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.

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 amikacin 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%
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.

**References**


