Extended-spectrum β-Lactamase producers among multiple drug resistant *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract

A toral of 127 multiple drug resistant *Escherichia coli* (42) and *Klebsiella pneumoniae* (85) were collected from hospitals in Malaysia. These strains were tested against imipenen, corrimoxazole, tetracycline, chloramphenicol, and other commonly used antibiotics in the group of β-lactam (penicillin and cephalosporins), quinolone and aminoglycosides. Susceptibilities were tested by disc diffusion according to the guidelines of the National Committee for Clinical Laborarory Standards (NCCLS). These strains were also tested for susceptibilities to ceftazidime, cefotaxime and aztreonam. These strains were further tested for extended-spectrum β-lactamase (ESBL) producers wirh the Etesr ESBL strip (AB Biodisk, Solna, Sweden). This Etest ESBL is based on the recognition of a reduction in the ceftazidime MIC in the presence of clavulanic acid. Nineteen percent of isolated *E. coli* and 27.0% of *K. pneumoniae* were detected as ESBL producers. With standard augmentin and unasyn discs, rhe resistance percentage for *E. coli* and *K. pneumoniae* isolates was 40 to 60%. This study suggests rhat for multiple drug resistant *E. coli* and *K. pneumoniae*, the disc diffusion testing with ceftazidime, cefotaxime and aztreonam appears to be useful for detecting hyper-producers of β-lactamases, while the Etest ESBL screen test is a simple technique for validating ESBL producers.

Key Words: multiple drug resistance; extended-spectrum β-lactamases (ESBL); disc testing; Etest ESBL strip

Introduction

Bacteria have acquired a variety of mechanisms to resist the action of antibiotics. Production of the β-lactamases is the most common mechanism among the infecting Gram-negative bacteria (Philippon *et al.*, 1989). The early β-lactams have further evolved into so-called extended-spectrum β-lactamase producers (ESBLs) which are either plasmid or chromosomal-mediated enzymes. These enzymes confer resistance to monocyclic β-lacram and third generation cephalosporins such as ceftazidime and cefotaxime (Jacoby & Mederios, 1991; Payne & Amyes, 1991). They are found predominantly in *Klebsiella* species, especially *K. pneumoniae* and occasionally in *K. oxytoca*, *E. coli* and also *Enterobacter aerogenes* (Ramadan *et al.*, 1995). The activity of imipenen, cotrimoxazole, tetracycline, chloramphenicol and other commonly used antibiotics in the group of β-lactams (penicillin and cephalosporins), quinolone and aminoglycosides were tested against these multiple drug resistant strains. These strains were tested with third generation cephalosporins (cefotaxime and ceftazidime) and monobactam (aztreonam) discs to detect for hyper-producers of β-lactamases. To document the ESBL producers, isolates of *E. coli* and *K. pneumoniae* were tested using Erest ESBL strips.

Materials and Methods

Bacterial isolates

A toral of 127 clinical isolates of Gram-negative bacteria were obtained from rhe Bacteriology Division, Institute for Medical Research, and 11 participating hospirals in Malaysia. These isolates were from various types of clinical specimens such as blood, pus, sputum, cerebrospinal fluid, nasal/throat swab, tracheal nasopharynx aspirate, ear swab and eye swab. They were identified and tested against a list of drugs.

Susceptibility tests

Disc susceptibility tests were performed and interpreted according to the National Commirree for Clinical Laboratory Standards (NCCLS, 1993). The discs tested were of ampicillin, carbenicillin, piperacillin, cephalorhin, cefuroxime, cefoperazone, ceftazidime, cefotaxime, cefrriaxone, norfloxacin, perfloxacin, ciprofloxacin, ofloxacin, kanamycin, gentamicin, nerilmicin, tobramycin, amikacin, imipenen, cotrimoxazole, tetracycline, chloramphenicol, aztreonam, augmentin and unasyn. These isolates were tested for extended-spectrum β-lacramases (ESBL) producers using the Etest ESBL strip (AB Biodisk, Solna, Sweden). Etesr ESBL strip uses stable gradient technology to evaluate the MIC

of ceftazidime alone compared with the MIC of ceftazidime with clavulanic acid (2 μg/ml) to facilitare the recognition of strains expressing inhibiting enzymes.

Results

A toral of 127 clinical strains of *E. coli* (42) and *K. pneumoniae* (85) *were* isolated. These strains were isolated from various specimens of patients such as patients in intensive care, bed-ridden, diabetic, premature babies, children, elderly and cancer patients. These strains were resistant to a range of antibiotics (Table 1). The *E. coli* isolates were highly resistant ro ampicillin (100.0%), carbenicillin (96.0%), cotrimoxazole (96.0%), tetracycline (93.0%) and cephalothin (86.0%). The *K. pneumoniae* were highly resistant to cephalotbin (95.0%), carbenicillin (90.0%), piperacillin (88.0%) and ampicillin (88.0%). Although these isolates were multiple drug resistant strains, they were susceptible to imipenem; the resistance percentage for *E. coli* and *K. pneumoniae* was 2.4% and 5.9% respectively.

Third generation cephalosporins (ceftazidime and cefotaxime) and monobacram (aztreonam) were evaluated to detect hyper-producers of β-lactamases. From Table 2, the resistance percentage for *E. coli* isolates was 61.9% for ceftazidime, 52.0% for aztreonam and 42.9% for cefotaxime. As for *K. pneumoniae* isolates, the comparative figures were 84.7% for ceftazidime, 72.9% for aztreonam and 55.3% for cefotaxime. Etest ESBL strip indicated that 19.0% (8 of 42) *E. coli* and 27.0% (23 of 85) *K. pneumoniae* were ESBL producers (Table 2).

Table 2 shows the resistance percentage for *E. coli* and *K. pneumoniae* against augmentin (amoxicillin/clavulanic acid) and unasyn (ampicillin/sulbactam). The resistance percentage for *E. coli* was 40.5% and 59.6% for augmentin and unasyn respectively, while that for *K. pneumoniae* was 46.7% and 59.0% respectively.

Table 1. Antibiotic susceptibility pattern for *Escherichia coli* and *Klebsiella pneumoniae*

Antibiotics	% resistant	
	E. coli	*K. pneumoniae*
Ampicillin	100.0	88.0
Carbenicillin	96.0	90.0
Piperacillin	79.0	88.0
Cephalothin	86.0	95.0
Cefuroxime	58.0	76.0
Cefoperazone	74.0	32.0
Cetriaxone	33.3	41.2
Norfloxacin	27.0	20.0
Perfloxacin	31.0	23.0
Ciprofloxacin	24.0	16.0
Ofloxacin	27.0	25.0
Kanamycin	75.0	72.0
Gentamicin	68.0	24.0
Netilmicin	37.0	67.0
Tobramycin	79.0	74.0
Amikacin	34.0	32.0
Cotrimoxazole	96.0	72.0
Tetracycline	93.0	41.0
Chloramphenicol	79.0	69.0
Imipenem	2.4	5.9

Discussion

In clinical laboratories, problems of multiple drug resistant isolates bave been characterized by hyper- or extended-specrrum β-lactamases. The isolated *E. coli* and *K. pneumoniae* were highly resistant to various β-lacrams and other classes of antibiotics.

Detection of ESBL producers is very method dependent. Although NCCLS disc diffusion method will miss some ESBL producers, there is another aspect to consider. This is usually the finding of resistance to third generation cephalosporins (ceftazidime and cefotaxime) and monobactam (aztreonam). Among *E. coli* and *K. pneumoniae* isolates conferring resistance to ceftazidime, ceforaxime and aztreonam, 19.0% of *E. coli* and 27.0% of *K. pneumoniae* were detected as ESBL producers.

During this study, rhese strains were nored for susceptibility to the third generation cephalosporins and monobactam discs tested. Ceftazidime and aztreonam discs were able to differentiate ESBL producers (Bush & Singer, 1989; Jacoby & Han, 1996). The isolated *E. coli* and *K. pneumoniae* are of plasmid-mediated β-lactamase producers since they are active against ceftazidime, cefotaxime and aztreonam (Cormican *et al.*, 1996). Such finding and others (French *et al.*, 1996),

Table 2. Distribution of *Escherichia coli* and *Klebsiella pneumoniae* by disc diffusion and Etest ESBL screen

Species (Total)	% resistance by disc diffusion			Etest ESBL screen (%)	% resistance by disc diffusion	
	Aztreonam	Cefotaxime	Ceftazidime		Augmentin	Unasyn
E. coli (42)	52.0	42.9	81.9	19	40.5	59.6
K. pneumoniae (85)	72.9	55.3	84.7	27	46.7	59.0

increased information on the types of resistance due to hyper-production of β-lactamases and ESBLs.

Another finding in this study was the high percentage of resistance to augmentin (amoxicillin/clavulanic acid) and unasyn (ampicillin/sulbactam) among the isolated *E. coli* and *K. pneumoniae* (40-60%). The major inhibiting activity of β-lactamase inhibitors is directed against plasmid-mediated enzymes (Williams, 1997) but evolved plasmid-mediated β-lactamases can have reduced affinities for β-lactamase inhibitors (Bush *et al.*, 1995). Blazquez *et al.* (1993) characterized plasmid-mediated β-lactamases from *E. coli* that was resistant to clavulanate, sulbactam and tazobactam. For *K. pneumoniae*, it was shown that the altered porin channel reduced the access of sulbactam, clavulanic acid or other β-lactamase inhibitors to the enzyme (Martinez-Martinez *et al.*, 1996).

Although these clinical isolates were resistant to β-lactamase inhibitor combinations, they were susceptible to imipenem. Carbapenems are highly resistant to hydrolysis by plasmid-mediated β-lactamases (Sirot, 1995).

The isolates in this study showed resistance to cefotaxime, ceftazidime and aztreonam discs, β-lactamase inhibitor combinations discs, and susceptibility to imipenem. These characteristics suggest that the isolated *E. coli* and *K. pneumoniae* to be of plasmid-mediated β-lactamase producers. This study also suggests that the disc diffusion testing with ceftazidime, cefotaxime and aztreonam appears to be useful for detecting hyper-producers of β-lactamases, while the Etest ESBL screen test is useful for validating the ESBL producers.

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