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Prevalence of extended spectrum beta-lactamase in *Klebsiella pneumoniae*

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ABSTRACT

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Increased irrational use of antibiotics promotes bacterial resistance to these drugs. Among the resistance mechanisms developed by bacteria is the production of â-lactamase which can destroy the â-lactam ring and cause resistance to the other â-lactam antibiotics, such as amoxycillin. Extended spectrum â-lactamase (ESBL), an enzyme found in bacterial plasmids, is capable of hydrolyzing third-generation cephalosporins, namely cefotaxime, ceftazidime, ceftriaxone, and the monobactams. ESBL is predominantly found in *Klebsiellas spp.*, *Escherichia coli* and other bacteria of the *Enterobacteriaceae* family. This study was conducted to determine the prevalence of *Klebsiella* resistant to cephalosporins and the prevalence of those producing ESBL. A total of 65 clinical isolates of *Klebsiella* were tested by the disk diffusion method according to Kirby-Bauer to determine their antibiotic susceptiblity and by the double-disk synergy method to detect the presence of ESBL. The results show that 18.5% of *Klebsiella* isolates tested were resistant to ceftazidime and cefixime, 13.9% to ceftriaxone, and 23.1% to aztreonam. Testing for ESBL revealed that the prevalence of ESBL producers in clinical *Klebsiella* isolates ranged from 10.8% to 12.3%. The presence of ESBL, plus the potential for plasmid-mediated quinolone and carbapenem resistance, undoubtedly will create significant therapeutic problems in the future.

Keywords: Extended spectrum â-lactamase, *Klebsiella*, antibiotics

INTRODUCTION

Antibiotics have long been used for managing infectious disease and lately have tended to be used excessively and irrationally. This tendency has increased the prevalence of antibiotic resistance in previously sensitive bacteria. Bacteria synthesize the enzyme β-lactamase that is capable of opening the β-lactam ring and inducing resistance to β-lactam antibiotics such as amoxicillin. The emergence of extended spectrum β-lactamase in these bacteria subsequently has resulted in their resistance to third-generation cephalosporins.

This situation may complicate antibiotic therapy in patients with infections caused by bacteria of the family *Enterobacteriaceae,* especially *Klebsiella* spp. and *E. coli,* as has been reported in many countries.[1-5]

Extended spectrum β-lactamase (ESBL) is a group of enzymes found in plasmids that are capable of hydrolyzing third-generation cephalosporins, such as cefotaxime, ceftazidime, ceftriaxone, and the monobactam group of antibiotics, such as aztreonam, thus causing resistance to these antibiotics in ESBL-producing bacteria. ESBL is predominantly found in *Klebsiella* spp.[4] and *Escherichia coli*,[5] and also in other members of the *Enterobacteriaceae*.[6] Most of these ESBL-producing organisms are still sensitive to carbapenem, whilst their action against ciprofloxacin, cefepim, and combinations of beta-lactam antibiotics with a beta-lactamase inhibitor, is variable.

The prevalence in microorganisms of beta-lactamase induced resistance to beta-lactam antibiotics is steadily increasing. Bacterial resistance due to beta-lactamase was first encountered in *Staphylococcus aureus*, and has subsequently spread to *Neisseria gonorrhoeae* and *Haemophilus influenzae,* and later also to *Enterococcus faecalis*. Extension of the spectrum of beta-lactamases produced by various bacterial hosts has not been accompanied by a change of enzyme substrate spectrum. Thus the bacteria have readily become resistant to beta-lactam antibiotics, such as the penicillins and cephalosporins, which at the time of emergence of extended-spectrum beta-lactamases possessed only a small number of enzyme substrates. A number of pathogenic bacteria have become resistant through mutation, making them capable of inactivating the substrate.

Isolates of *Klebsiella* may become resistant at cephalosporins through acquisition of plasmid-mediated resistance.[3] There are more than 30 ESBL plasmids, based on substrate profile, response to inhibitors, and isoelectric point. In Indonesia there have been only a small number of reports on ESBL-producing bacteria, but this problem will clearly complicate antibiotic therapy, particularly in patients with infections caused by members of the *Enterobacteriaceae*, especially *Klebsiella,* as has been reported in many countries.[4-6] Studies conducted in several countries have demonstrated the presence of *Klebsiella pneumoniae* in cases of severe sepsis, 38% of which were fatal. The aim of the present study was to determine the prevalence of *Klebsiella* isolates resistant to third-generation cephalosporins and the prevalence of ESBL-induced resistance in these isolates.

MATERIALS AND METHODS

Bacteria

The bacteria used were clinical isolates of *Klebsiella pneumoniae*, which had been stored in *tryptic soy broth* +15% *glycerol* at a temperature of -70°C.

Antibiotics

Antibiotic disks were obtained from BBL (Becton Dickinson, Sparks, MD), comprising ceftriaxone (CRO; 30 µg), ceftazidime (CAZ; 30 µg), aztreonam (ATM; 30 µg), cefixime (CFM; 30 µg), and amoxicillin-clavulanic acid (AMC; 30 µg and 15 µg).

Antibiotic susceptibility assay by disk diffusion method (Kirby-Bauer)

The test isolate was suspended in brain-heart infusion broth (BHI), the suspension was incubated at 37^0C, and subsequently diluted with saline to a concentration equivalent to McFarland standard 0.5. The broth culture was taken with a cotton swab and plated on Mueller-Hinton II agar (MH-II). The antibiotic disks (CRO, CAZ, ATM, CFM, and AMC) were then

placed on the inoculated agar surface and the agar plate incubated at 37^{0}C for 20-24 hours.

Interpretation of disk diffusion assay results

The results of the Kirby-Bauer disk diffusion antibiotic susceptibility test was interpreted according to the criteria defined by the National Committee for Clinical Laboratory Standards (NCCLS).[7-8] For ceftriaxone (CRO), ceftazidime (CAZ), and cefixime (CFM), the bacteria were classified as resistant if the zone of inhibition was ≤16 mm and as susceptible (sensitive) if the zone of inhibition was ≥20 mm. For aztreonam (ATM) the bacteria were classified as resistant if the zone of inhibition was ≤19 mm and sensitive if the zone of inhibition was ≥23 mm.

Double-disk synergy ESBL assay

An MH-II agar plate was inoculated with the isolate to be tested as in the disk diffusion assay and a CRO disk placed at the center. Then an AMC disk was placed on the agar surface at a distance of 20 mm from the CRO test disk and the agar plate was incubated for 20-24 hours at 37^{0}C. The procedure was repeated for CAZ, ATM, and CFM, using a separate MH-II plate for each antibiotic.[2]

Interpretation of double-disk synergy ESBL assay results

An extension of the inhibition zone of the tested antibiotic towards the AMC disk containing clavulanic acid signifies a positive result, indicating that the test organism is an ESBL producer.

Data analysis

Percentage analysis was performed for demonstrating the prevalence of *Klebsiella* strains resistant to the test antibiotics and the prevalence of ESBL producing microorganisms.

RESULTS

Isolation of bacteria

From a total of 78 *Klebsiella pneumoniae* isolates recovered from the blood of febrile patients, thirteen did not grow on repeat culture. Thus only 65 *Klebsiella pneumoniae* isolates were available for testing by the Kirby-Bauer and ESBL assays.

The results of the disk diffusion assay according to Kirby-Bauer on 65 isolates of *Klebsiella pneumoniae* revealed that bacterial resistance was highest against aztreonam with a bacterial resistance pattern of 15 (23.1%) resistant and 50 (76.9%) sensitive isolates. The patterns of resistance to ceftazidime and cefixime were similar, namely 53 (81.5%) vs. 52 (80%) sensitive and 12 (18.5%) vs. 13 (20%) resistant isolates (including 1 or 1.5% of intermediate resistance). Diminished susceptiblity was demonstrated in the Kirby-Bauer results for ceftriaxone, namely 55 (84.6%) sensitive isolates, 1 (1.5%) isolate of intermediate resistance and 9 (13.9%) completely resistant isolates (Table 1, Figure 1).

Table 1. Kirby-Bauer assay on 65 isolates of *Klebsiella pneumoniae*

Antibiotic	Sensitivity test result		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Ceftazidime (CAZ)	53 (81.5)	0 (0)	12 (18.5)
Cefixime (CFM)	52 (80)	1 (1.5)	12 (18.5)
Ceftriaxone (CRO)	55 (84.6)	1 (1.5)	9 (13.9)
Aztreonam (ATM)	50 (76.9)	0 (0)	15 (23.1)

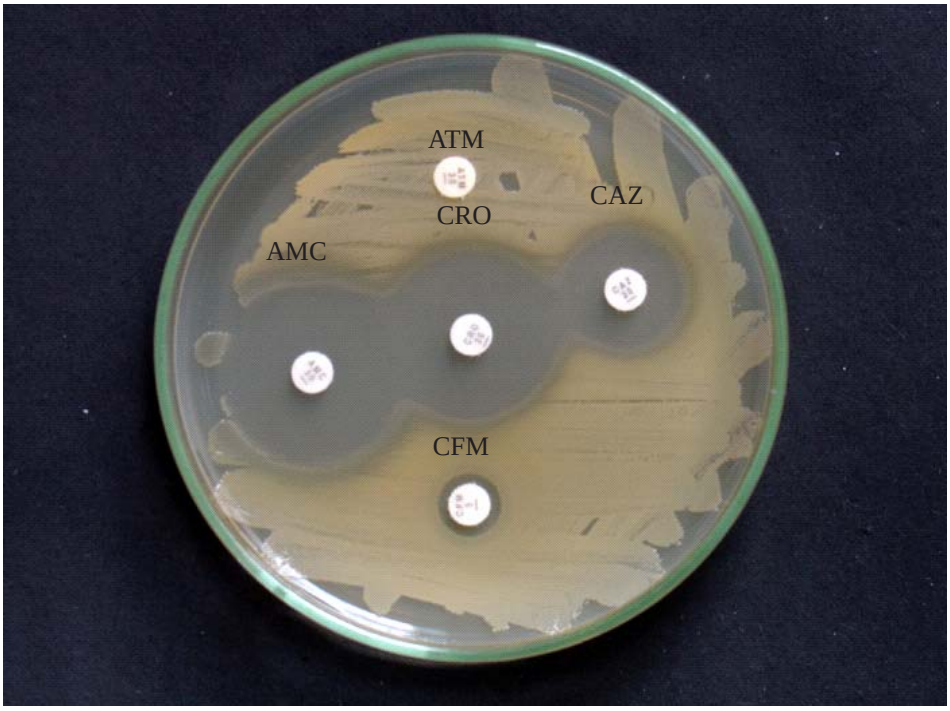

Figure 1. Kirby Bauer test results

The test bacterium shows sensitivity to amoxicillin+clavulanic acid (AMC) *[left]*, ceftriaxone (CRO) *[center]*, and ceftazidim (CAZ) *[right]*, as indicated by the substantially wide zones of inhibition around the disks. The bacterium is resistant to aztreonam (ATM) *[top]* and ceftazidime (CFM) *[bottom]* as there is no zone of inhibition around the respective disks

Isolates yielding Kirby-Bauer results of intermediate resistance and complete resistance were subsequently tested by the ESBL assay. The results of the ESBL assay showed that among the 65 *Klebsiella pneumoniae* isolates tested, 8 (12.3%) produced ESBL against ceftazidime, cefixime, and aztreonam. Similar results were found for ceftriaxone, where 7 isolates (10.8%) were ESBL positive (Table2, Figure 2).

Table 2. ESBL assay on isolates of *Klebsiella pneumoniae* resistant to 4 cephalosporins

Antibiotic	**n**	**ESBL assay (n/%)**	
		ESBL +	**ESBL -**
Ceftazidime (CAZ)	65	8 (12.3%)	57 (87.3)
Cefixime (CFM)	65	8 (12.3%)	57 (87.3)
Ceftriaxone (CRO)	65	7 (10.8%)	58 (89.2)
Aztreonam (ATM)	65	8 (12.3%)	57 (87.3)

Figure 2. ESBL positive results by the double disc synergy test

The disk at center contains ceftriaxone and the disk at left amoxicillin + clavulanic acid. The zone of inhibition around the ceftriaxone disk has enlarged in the direction of the disk containing clavulanic acid

DISCUSSION

In the present study, ESBL-mediated resistance was found in 10.8% to 12.3% of our isolates. This prevalence rate is much higher than those obtained in India[9] and the United States,[10] which were reported to be 6.6% and 5%, respectively, but lower than those in France (14%) and the United Kingdom (16%).[11] Studies performed in Malaysia in 2001 reported that ESBL production by *Klebsiella* spp. in the Asia-Pacific region amounted to 27-38% and in Indonesia was 33.3%.[12] Another study conducted in 2005 at Dr. Soetomo Hospital in Surabaya, reported that out of 85 *Klebsiella pneumoniae* isolates recovered from urine and blood, 73 (86%) were ESBL-positive.[13]

Since the discovery of the â-lactam antibiotics, inactivation of the drugs by â-lactamase has become a principal feature of the resistance mechanisms of gram-negative bacteria. In the *Enterobacteriaceae*, ESBL production has become an important resistance mechanism to â-lactam antibiotics, affecting 50% of antibiotics widely used against infectious disease.[14] ESBL is capable of hydrolyzing a large number of â-lactam drugs, including the newer cephalosporins, but is inactive against cephamycin and carbapenem. In the family *Enterobacteriaceae*, ESBL is found particularly in *Klebsiella* spp. and *E. coli*, but to a lesser degree also in other members of the family.[4-6]

In general, this resistance is coded for by a plasmid-borne gene that is readily transmissible among the *Enterobacteriaceae*, particularly in *Klebsiella* spp. and *E. coli*. Increased broad-spectrum cephalosporin usage has been

associated with nosocomial infections in both hospitalized and ambulatory patients.[4-6]

ESBL, first discovered in Germany, was subsequently widely reported in many European countries, and has now been found in nearly all parts of the world.[2-5] Infections caused by ESBL-producing microorganisms have resulted in serious antibiotic resistance problems. Several investigators reported that ESBL produced by bacteria have frequently induced bacterial resistance to a number of cephalosporin derivatives, such as ceftazidime, cefotaxime, ceftriaxone, and aztreonam. A Brooklyn survey has revealed that ESBL is produced by 17.2% of *K. pneumoniae*, *E. coli*, and *P. mirabilis*.[15] However, a larger study conducted in France found an ESBL prevalence of only 3.2% in *Enterobacteriaceae*.[16] Several recent studies in the United Staes and Europe have reported that ESBL producing strains in the family *Enterobacteriaceae* have increased dramatically and that this trend is having a significant impact on mortality rate and hospitalization costs.[17-20]

In many hospitals, ESBL-producing organisms are already endemic. Is there any hope of controling ESBL producers in such a setting? A number of authors have shown that ceftazidime restriction alone is insufficient to control continued infections with ESBL-producing organisms.[21,22] Rahal et al.[21] were forced to withdraw cephalosporins as an entire class in order to exact control over endemic ESBL producers. Some authors have suggested that use of lactam/lactamase inhibitor combinations, rather than cephalosporins, as workhorse empirical therapy for infections suspected as being due to gram-negative bacilli, may facilitate control of ESBL producers.[23,24]

The mechanism by which these drugs may reduce infections with ESBL producers is not certain. It should be noted, however, that many organisms now produce multiple-lactamases, which may reduce the effectiveness of lactam/lactamase inhibitor combinations.[25,26] Apparently the problem of resistance in *Klebsiella* spp. due to ESBL-production by these bacteria has become increasingly common, such that it may become a serious problem in the future management of infectious diseases.

CONCLUSIONS

Klebsiella organisms still susceptible to ceftazidime, cefixime, ceftriaxone, and aztreonam show a sensitivity rate of 80% or more. Testing for ESBL revealed that the prevalence of ESBL producers in clinical *Klebsiella* isolates ranged from 10.8% to 12.3%.

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