

Prevalence and Susceptibility Patterns of Bacteria Producing Extended Spectrum Betalactamases in a University Hospital

Nalinee Aswapokee M.D., M.M.Sc
 Surapee Pruksachatvuthi M.Sc (Microbiol)
 Busaba Charoensook Cert (Microbiol)

Abstract

In 1983, *Klebsiella* species producing a plasmid-mediated betalactamase related to SHV-1 was reported for Federal Republic of Germany. Since then these enzymes were prevail. We studies prevalence and susceptibility pattern of *K.pneumoniae* and *E.coli* producing extended spectrum betalactamases (ESB). The 11 antimicrobial agents tested were cefotaxime, ceftazidime, aztreonam, amikacin, ceftoxitin, cefpirome, amoxicillin-clavulanate, sulbactam-ampicillin and ciprofloxacin. It was found that 37% of *K.pneumoniae* and 12% of *E.coli* produced ESB. These bacteria were resistant to ceftazidime, cefotaxime, aztreonam and amikacin suggested that the enzymes probably were TEM- and SHV-related and were plasmid-mediated. The bacteria were moderately susceptible to ceftoxitin, a cephamycin antibiotic. They were also moderately susceptible to serine-class inhibitor, but interestingly were resistant to ciprofloxacin. The most active agent for these bacteria remained to be a carbapenem class. These results suggested that the enzymes were TEM- and SHV-related and were discussed. The prevalence of ESB producing bacteria in this location may be higher than those in contemporary reports from other countries. (*J Infect Dis Antimicrob Agents* 1994;11:49-53).

Key words : Extended betalactamases

เรื่องย่อ

ความชุกและแบบแผนความไวของเชื้อแบคทีเรียที่ผลิตเอนไซม์เบต้าแลคตามเนสรุ่นดัดแปรที่พบในโรงพยาบาลมหาวิทยาลัยแห่งหนึ่ง

นลินี อัสวโปเก้ พ.บ., สุรณี พฤษชาติวุฒิ วท.ม., บุษบา เจริญสุข ประกาศนียบัตร

ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล บางกอกน้อย กทม. 10700

ในปี พ.ศ. 2528 เริ่มพบเชื้อ *K.pneumoniae* ที่ผลิตเอนไซม์ extended-spectrum betalactamases (ESB). ต่อจากนั้น พบเชื้อที่ผลิตเอนไซม์นี้กระจายทั่วไป และมีชนิดต่าง ๆ ของเอนไซม์เหล่านี้เพิ่มขึ้น. ส่วนใหญ่เป็นชนิด TEM- และ SHV-related. การศึกษานี้ทำเพื่อดูความชุกของเอนไซม์ ESB จากเชื้อ *K.pneumoniae* และ *E.coli* ที่ได้จากผู้ป่วยที่รับไว้ในคณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล และดูแบบแผนความไวของเชื้อต่อยาต้านจุลชีพ 11 ตัว คือ cefotaxime, ceftazidime, aztreonam, amikacin, ceftoxitin, cefpirome, cefepime, amoxicillin-clavulanate, ampicillin-sulbactam, ciprofloxacin และ imipenem. พบว่ามีอุบัติการณ์ของ *K.pneumoniae* และ *E.coli* ที่ผลิต ESB ร้อยละ 37 และ 12 ตามลำดับ. เชื้อทั้ง 2 genera คือต่อยา ceftazidime, cefotaxime, aztreonam และ amikacin ซึ่งเป็นแบบแผนความไวที่พบได้ในเชื้อที่ผลิต ESB ชนิด TEM- หรือ SHV-related. เชื้อไวปานกลางต่อ ceftoxitin ซึ่งเป็น cephamycin เชื้อทั้ง 2 genera คือไวปานกลางต่อ serine class inhibitor และ ciprofloxacin. เชื้อทั้ง 2 ชนิดไวต่อเซฟาโลสปอรินรุ่นที่ 4 และไวมากต่อ carbapenem.

Reprint request : Assoc. Prof. Nalinee Aswapokee, M.D., Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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จากผลการศึกษารูปได้ว่าเชื่อน่าจะผลิตเอ็นซัยม์ชนิด TEM- หรือ SHV-related, อาจมีกลไกการดื้อยามากกว่า 1 ชนิด และอาจมีการกำกับโดยพลาสมิด. ซึ่งได้อธิบายผลการศึกษานี้ไว้. นอกจากนั้น พบว่า ความซุกของการพบเชื้อ *K.pneumoniae* และ *E.coli* ที่ผลิต ESB สูงกว่าที่เคยมีรายงานมา. (วารสารโรคติดเชื้อและบาดานจุลชีพ 2537;11:49-53.)

In 1983, *Klebsiella* species producing a plasmid-mediated betalactamase related to SHV-1 was reported from the Federal Republic of Germany (1). Since then, this enzyme and other related betalactamases became prevailed (2), and more enzymes continually emerged (3,4). It was shown that most enzymes in this group were TEM-and SHV-related and capable of inactivating broadspectrum cephalosporins. Although the most recent classification included the enzymes which hydrolyse cephamycins and carbapenem (5), the most common enzymes remain to be TEM-and SHV-related produced by *Klebsiella pneumoniae* and *E.coli* (2,3). We reported here the prevalence and susceptibility pattern of *K. pneumoniae* and *E.coli* obtained from hospitalized patients in a university hospital, Bangkok, Thailand.

MATERIALS AND METHODS

Bacterial isolates All isolates identified as *K.pneumoniae* and *E.coli* obtained from patients hospitalized at Siriraj Hospital, Mahidol University, Bangkok in the year 1992 were stored in Brain-heart infusion broth with 10% glycerine, then were frozen at -70°C prior to susceptibility testing.

Antimicrobial agents A total of 11 antimicrobial agents were used in susceptibility testing. These included cefotaxime, ceftazidime, cefoxitin, cefpirome, cefepime, aztreonam, imipenem, amoxicillin-clavulanic acid, ampicillin-sulbactam, amikacin and ciprofloxacin. These were standard powders and were supplied as generous gifts from representative manufacturers. The antibiotics were processed as directed by the manufacturers' instructions.

Susceptibility testing Standard agar dilution susceptibility testing as described by the National Committee for Clinical Laboratory Standard was performed(6). Mueller-Hinton agar (BBL^R), pH of 7.1 ± 0.2 , was used. The final inocula contained approximately 10^5 cfu/ml. The inoculation onto agar plates containing serial

dilution of antibiotics was performed by a replicator. Plates were incubated at 35° C for 18 hours.

Test for presence of extended-spectrum beta-lactamases Double-disk synergy test as described by Jarlier et al (7) was used to detect the presence of extended-spectrum betalactamases. Briefly, disk of Augmentin^R (20 µg of amoxicillin and 10 µg of clavulanate) was placed in the middle of agar plate. Disks of cefotaxime, ceftriaxone, ceftazidime and aztreonam were placed 30 mm apart. The presence of extended-spectrum beta-lactamases was shown by a decreased susceptibility to third generation cephalosporins and monobactam (designated as zone size diameter in the range of resistance) combined with synergy between these cephalosporins/monobactam and Augmentin^R, as seen as an extension of the edge of cephalosporins/monobactam inhibition zone toward the disk containing clavulanate (Fig 1).

RESULTS

Prevalence of extended-spectrum betalactamases producers

During January to December 1992, a total of 95 strains of *K.pneumoniae* were obtained. Most strains were from the Department of Medicine. Thirty-five strains (37%) of *K.pneumoniae* produced extended-spectrum betalactamases. In this period, a total of 100 strains of *E.coli* were obtained, and 13 of these strains produced extended-spectrum betalactamases (13%).

Susceptibility patterns

Susceptibility of *K. pneumoniae* The strains of *K.pneumoniae* producing extended-spectrum betalactamases were resistant to ceftazidime, aztreonam, amikacin and ciprofloxacin as judged by the susceptibility break points suggested by the National Committee for Clinical Laboratory Standard. These strains were moderately susceptible to cefotaxime. Cefoxitin, cefpirome, cefepime were active against these organisms. Imipenem was the most active agent. As far as the

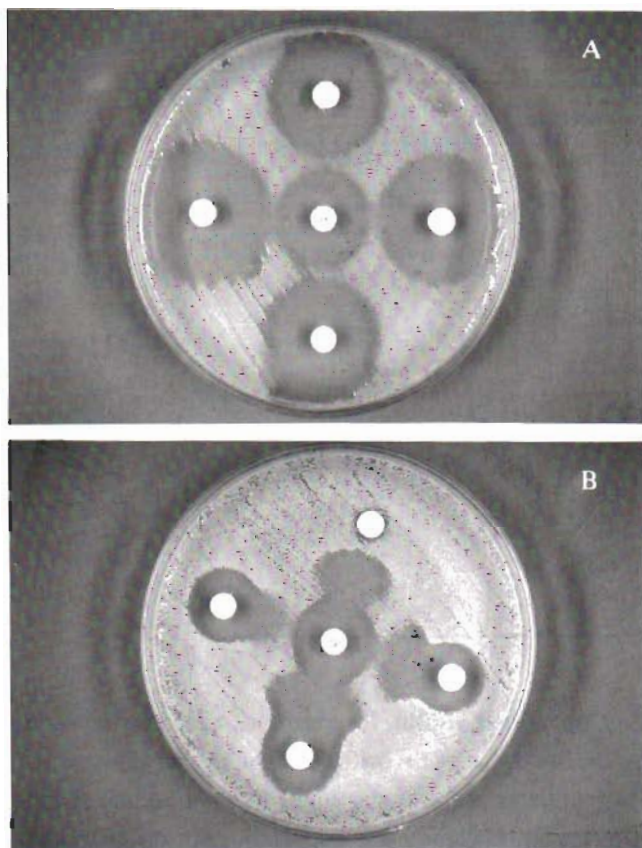


Fig. 1 Double-disk synergy test for presence of extended-spectrum betalactamase. (A) Absence of extended-spectrum betalactamases (B) Presence of extended-spectrum betalactamases.

serine inhibitors concern, these strains were moderately resistant to amoxicillin-clavulanate, but were resistant to ampicillin-sulbactam.

Susceptibility pattern of *E.coli*. Strains producing extended-spectrum betalactamases to 11 antimicrobial agents was similar to that of *K.pneumoniae*. These strains were more susceptible to ampicillin-sulbactam, but were more resistant to ciprofloxacin than *K.pneumoniae*.

Table 1 and 2 show susceptibilities to 11 antimicrobial agents of *K.pneumoniae* and *E.coli*.

DISCUSSION

This study reveals that almost 40% of *K.pneumoniae* and over 10% of *E.coli* in a university hospital in Bangkok, Thailand, produced extended-spectrum betalactamases. We did not characterize these enzymes, but the susceptibility patterns suggested that these enzymes inactivated ceftazidime more than cefotaxime. The probability that these enzymes were TEM- and SHV-related was suggested by the susceptibility of both genera to ceftioxin, a cephamycin antibiotic, since the enzymes of this class did not inactivate cephamycin (2,3,8-10). Although the organisms were susceptible to the fourth generation cephalosporins namely ceftiofime and ceftipime, it is less likely that they produced AmpC-related enzymes because these strains were uncommonly found in clinical isolates at the present(11). Of interest, *K.pneumoniae* was quite resistance to serine betalactamase inhibitors. It is probable that these strains also hyperproduced TEM-1 or SHV-1. There were few reports concerning *Klebsiella* sp. hyperproduced TEM-1 and SHV-1, but the most frequent genus hyperproducing

Table I Susceptibility to 11 antimicrobial agents of *K.pneumoniae* producing extended-spectrum betalactamases

Agents	MIC (mg/L)		
	Range	MIC ₅₀	MIC ₉₀
Cefotaxime	≤0.1-32	16	32
Ceftazidime	≤0.1->128	128	>128
Aztreonam	0.1->128	>128	>128
Amikacin	0.5-64	32	32
Ceftioxin	2-16	4	8
Ceftiofime	0.025-16	2	4
Ceftipime	0.025-8	2	4
Amoxicillin-clavulanate	2-16	8	16
Ampicillin-sulbactam	4-128	16	64
Ciprofloxacin	0.05-16	0.5	4
Imipenem	0.025-0.1	0.1	0.1

Table II Susceptibility to 11 antimicrobial agents of *E.coli* producing extended spectrum betalactamases

Agents	MIC (mg/L)		
	Range	MIC ₅₀	MIC ₉₀
Cefotaxime	≤0.1-32	8	32
Ceftazidime	≤0.1->128	32	>128
Aztreonam	0.25->128	64	>128
Amikacin	2-64	32	32
Cefoxitin	1-8	2	4
Cefpirome	≤0.01-16	0.5	8
Cefepime	≤0.01-4	1	4
Amoxicillin-clavulanate	0.5-16	8	16
Ampicillin-sulbactam	2-64	16	32
Ciprofloxacin	0.025-32	0.25	32
Imipenem	0.025-0.1	0.1	0.1

TEM-1/SHV-1 was reported recently to be *E.coli*(12,13). In this study, *E.coli* also was less susceptible to clavulanate and sulbactam than susceptible strains, but the levels of MICs were still lower than those of *K.pneumoniae*. The susceptibility patterns also suggested that these enzymes might be plasmid-mediated, since these strains were resistant to amikacin(14,15). *E.coli* strains were more resistant to ciprofloxacin than *K.pneumoniae* strains, which were also resistant. Sanders et al reported *K.pneumoniae* with cross-resistance between betalactams and quinolone to be due to change in outer membrane protein(16). This could be the case in our study, but the fact that the levels of resistance to quinolone were high suggested another mechanism of resistance to quinolone, namely altered DNA gyrase. Pangon et al also reported a porin-deficient *K.pneumoniae* resistant to cephamycins(16). These findings substantially supported the evidence that mutational event on betalactamases may occur concurrently with the alteration in permeability. Studies concerning outer membrane protein of *E.coli* were more prevail, but few studies dealing with *E.coli* producing extended-spectrum betalactamase which also had alterations in outer membrane protein(18). We do not know this is the case in our strains, since, again, the levels of resistance to quinolone were too high for designation of decrease permeability.

The prevalence of *K.pneumoniae* producing extended-spectrum betalactamases in this location was quite high in comparing to other areas. Meyer et al(19)

and Coovadia et al (20), for example, separately reported the outbreaks in their institution of this organism that the prevalence were not as high. *K.pneumoniae* strains collected in this study were sporadic, not an outbreak. This may be called "endemic", but to the best of our knowledge, there is no cutoff percentage for dividing sporadicity and endemicity of these specific resistant problems. We believe that the prevalence of *E.coli* producing extended-spectrum betalactamases in this study was also high, although there is no contemporary reports on the prevalence of these strains.

From this study, it was found that the prevalences of *K.pneumoniae* and *E.coli* producing extended-spectrum betalactamases were high. These strains also should be called "true" multiply resistant. Further in depth studies on mechanisms of resistance and genetic control may be mandatory, but, the more urgent issue is the rational use of antimicrobial agents, especially cephalosporins group, since there is evidence that the inappropriate use is quite frequent, including this location(21).

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