Extended-Spectrum β-Lactamase-Producing *Klebsiella pneumoniae* from Sappasitthiprasong Hospital and Imipenem Activity

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Background: *Klebsiella pneumoniae is a common cause of nosocomial infection and is resistant to multiple antibiotics, including β-lactams. It excretes extended spectrum β-lactamase (ESBL), an enzyme capable of hydrolyzing almost all β-lactams. Carbapenems including imipenem are drugs of choice for the treatment of infections caused by ESBL-producing Enterobacteriaceae.*
Objective: *This study aimed to determine minimum inhibitory concentration (MIC) of imipenem in ESBL-producing K. pneumoniae isolates from patients admitted to Sappasitthiprasong Hospital.*
Material and Method: *A total of 250 non-repetitive K. pneumoniae isolates were collected from patients admitted to Sappasitthiprasong Hospital between September 2014 and October 2015 and then screened for ESBL production by double disk synergy test and determined for antimicrobial susceptibility by disk diffusion method. A total of 100 ESBL-producing K. pneumoniae isolates were determined for the MICs of imipenem and ceftazidime. The resistant strain was further to determined for MIC of meropenem.*
Results: *All ESBL-producing K. pneumoniae were resistant to ceftazidime in vitro with MICs of >64 μg/mL. Ninety-nine isolates expressing ESBL were susceptible to imipenem with MIC range of 0.25-2 μg/mL. One out of the 100 ESBL-positive K. pneumoniae strains was resistant to imipenem with MIC of >64 μg/mL. It was resistant to all antibiotics, including meropenem.*
Conclusion: *ESBL-producing K. pneumoniae can hydrolyze ceftazidime. However, imipenem was effective against ESBL-producing K. pneumoniae strains. Further characterization of ESBL types is required.*

Keywords: *Klebsiella pneumoniae, ESBL, Imipenem*

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K. pneumoniae is the most common cause of bacterial pneumonia. This pathogen is associated with nosocomial infection and was reported to express the gene encoding β-lactamase. Extended spectrum β-lactamase (ESBL) was also reported which renders them resistant to extended-spectrum cephalosporins, included ceftazidime. ESBL is plasmid-mediated and confers multi-drug resistance, including multiple β-lactam antibiotics. These situations limit therapeutic options for treatment.

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Carbapenems are a group of broad spectrum antibacterial activity used for treatment of infections caused by multi-drug resistance Gram-negative bacteria, including ESBL-producing strains. The therapeutic choice of ESBL-producing *K. pneumoniae* is carbapenems, such as imipenem or meropenem. There are many reports of Gram negative bacteria resistant to carbapenems due to carbapenemases production, which have been increasingly reported in worldwide. Imipenem resistant-*K. pneumoniae* has been isolated. These strains possessed a transmissible plasmid mediated AmpC-type β-lactamase. The emergence of imipenem-resistant ESBL-producing *K. pneumoniae* has a serious impact on therapeutic options. However, there was no information of imipenem-resistant ESBL-producing *K. pneumoniae* in Sappasitthiprasong

Hospital, a medical school with a high rate of multidrug resistant strains.

The aim of this study was to determine the prevalence of multidrug-resistant *K. pneumoniae* and the imipenem minimum inhibitory concentration (MIC) in ESBL-positive *K. pneumoniae* isolates from patients admitted to Sappasitthiprasong Hospital. It is anticipated that this information will assist medical staff in therapeutic choices.

Material and Method

Bacterial strains

A total of 250 *K. pneumoniae* isolates were obtained from patients admitted to Sappasitthiprasong Hospital from September 2014 to October 2015. All isolates were tested biochemically to confirm *K. pneumoniae*.

Standard disk diffusion test

The disk diffusion tests were performed on Mueller Hinton agar (Hardy Diagnostics, Santa Maria, USA) using the Kirby-Bauer method. *Escherichia coli* ATCC 25922 was used as a control with an expected inhibition zone of each antimicrobial. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI)[1]. Antimicrobial disks (Hardy Diagnostics, Santa Maria, CA, USA) included cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), amikacin (30 µg), and ciprofloxacin (10 µg).

ESBL screening

ESBL expression was detected phenotypically by double disk synergy test. *K. pneumoniae* were grown on blood agar and incubated at 37°C for 24 hours. The single colonies were further sub-cultured on Tryticase soy broth and incubated at 37°C for 3 hours and adjusted to McFarland No. 0.5. The bacterial suspension was further applied onto Mueller Hinton

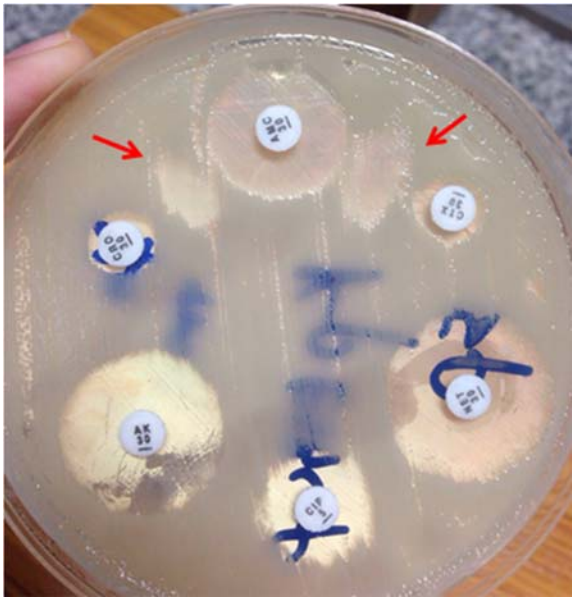

Fig. 1 ESBL-producing *K. pneumoniae* by double disk synergy.

Table 1. Antimicrobial susceptibility of *K. pneumoniae* by standard disk diffusion

Antimicrobial agents	Susceptibility		
	Total *K. pneumoniae* (250 isolates)	Total *K. pneumoniae* (250 isolates)	
		ESBL producer (100 isolates)	Non-ESBL producer (150 isolates)
Cefotaxime	99 (39.6%)	0 (0%)	99 (66%)
Ceftazidime	107 (42.8%)	0 (0%)	107 (71.33%)
Ceftriaxone	79 (31.6%)	0 (0%)	79 (52.67%)
Gentamycin	82 (32.8%)	37 (37%)	45 (30%)
Imipenem	241 (96.4%)	94 (94%)	147 (98%)
Amikacin	217 (86.8%)	67 (67%)	150 (100%)
Ciprofloxacin	60 (24.0%)	7 (7%)	53 (35.33%)
Trimethoprim/sulfamethoxazole	45 (18.0%)	5 (5%)	40 (26.67%)

(MH) agar. Amoxicillin/clavulanic acid (20/10 μg) disk was centrally placed on MH agar and disks of cefotaxime and ceftriaxone were each placed 15 mm apart from the central disk. The plates were incubated at 37°C overnight.

Minimal inhibitory concentration determination

Minimal inhibitory concentrations (MIC) of imipenem and ceftazidime for ESBL-producing *K. pneumoniae* isolates were tested by agar dilution test. The concentrations of antimicrobials were 0.25-64 μg/mL. The MIC results of clinical isolates and controls were interpreted as recommended by CLSI[1]. The resistant strain was further tested MIC of meropenem.

Results

Antimicrobials to K. pneumoniae

Standard disk diffusion test

All 250 isolates were screened for susceptibility to a variety of antibiotics including cefotaxime, ceftazidime, ceftriaxone, imipenem, trimethoprim/sulfamethoxazole, amikacin, and ciprofloxacin. Trimethoprim/sulfamethoxazole had the lowest percentage of susceptibility rate 18.0%. However, cefotaxime, ceftazidime, ceftriaxone, and ciprofloxacin also had low percentage of susceptibility against *K. pneumoniae* (39.6%, 42.8%, 31.6%, and 24.0%, respectively). Imipenem was the most effective antibiotic against *K. pneumoniae* (96.4%).

ESBL-producing K. pneumoniae

By double disk synergy test, ESBL was expressed in 40% of the isolates (100/250 isolates).

Imipenem MIC of ESBL-producing K. pneumoniae

Most of the ESBL-producing *K. pneumoniae* isolates were susceptible to imipenem with MIC range of 0.25-2 μg/ml. Only 1 out of 100 ESBL-producing *K. pneumoniae* strains exhibited high level resistance to imipenem with MIC of >64 μg/ml.

Discussion

K. pneumoniae has emerged as an important cause of hospital-acquired infections, especially with patients admitted to intensive care units, and the mortality rate can be as high as 70%[2,3]. ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillin, first-, second-, and third-generation cephalosporins, and aztreonam by hydrolysis of these antibiotics. ESBLs have emerged by the selective pressure of extensive use of antimicrobials, especially in intensive care units, medical teaching schools, and tertiary care hospitals. *K. pneumoniae* have become highly resistant to antibiotics and remain the major ESBL-producing organisms isolated worldwide. ESBL-producing *K. pneumoniae* was first reported in 1983 in Germany[4]. Prevalence of ESBLs varies from place to place, being as low as 1.5% in Germany to as high as 39-47% in Russia, Poland, and Turkey[5]. ESBL-producing *K. pneumoniae* was isolated in 16% of cases (28/176) in a study at a tertiary care hospital in India[6]. In Thailand, there are many reports of ESBL production from medical teaching schools, especially *Escherichia coli* and *K. pneumoniae*. ESBL-producing *K. pneumoniae* were isolated from 26% from patients attending Siriraj Hospital during 2000-2001[7]. These isolates were non-susceptible to cephalosporins, cephamycin, and aztreonam. Imipenem was highly active against those ESBL-producing *K. pneumoniae* [7]. Prevalence of ESBL-producing *K. pneumoniae* from the Emergency Room at Ramathibodi Hospital was found to be 1% in 2011, and the report found bla_{TEM}, bla_{CTX-M}, and bla_{SHV} located both on chromosome and plasmid[8].

This paper's research found 40% of the strains of ESBL-producing *K. pneumoniae* by phenotypic study. Those ESBL-producing *K. pneumoniae* isolates were non-susceptible to cefotaxime, ceftazidime, and ceftriaxone. Only imipenem was highly active against these ESBL-producing *K. pneumoniae*. However, we found 1 isolate (1/100 isolates) that was resistant to imipenem with MIC of >64 μg/ml. This isolate was resistant to all antimicrobials tested including trimethoprim/sulfamethoxazole, amikacin, and ciprofloxacin. Treatment with ESBL-producing *K. pneumoniae* was difficult because the organisms were frequently resistant to multiple antibiotics. However, ESBL-producing *K. pneumoniae* may appear susceptible to a combination therapy with β-lactams/β-lactamases inhibitors, third, and fourth generation cephalosporins, aminoglycosides, and quinolone in vitro. We found susceptibility rates for these antibiotics to be between 0% and 67%, especially third generation cephalosporins that show various susceptibility patterns. Those ESBL positive *K. pneumoniae* strains were non-susceptible to ceftriaxone and ceftazidime. Aminoglycoside was more effective against ESBL-producing *K. pneumoniae* than third generation cephalosporins, which were not effective at all. Gentamicin and amikacin (both aminoglycosides) were 37% and 67% effective respectively against ESBL-

producing *K. pneumoniae.*

However, double disk synergy method was not a standard method for ESBL detection. The sensitivity of this test may be reduced when ESBL activity is very low. In addition, the synergy between the amoxicillin/clavulanate disk and the indicator cephalosporin may be overlooked if the inoculum is too heavy or if the disks are too far from each other[10]. This study screen detected for ESBL producing *K. pneumoniae*. A further study is required for genotype.

Carbapenem was a good, effective regimen to emerging serious Gram negative infection especially, serious infection with ESBL-producing *K. pneumoniae*. Carbapenemase have been increasingly reported in *Enterobacteriaceae* including *K. pneumoniae.* There are a variety of carbapenemase, such as class A carbapenemases, class B metallo-β-lactamases, and class D enzymes of the OXA-48 type. Each type of carbapenemases can hydrolyze in the different antimicrobial agents[9]. *Klebsiella pneumoniae* carbapenemase (KPC) can hydrolyze cephalosporins, cephamycins, monobactams, and carbapenems. Imipenem was effective against ESBL-producing *K. pneumoniae*. However, in this study we found one isolate that had imipenem MIC >64 μg/ml and this was resistant to all antimicrobials. It is possible that carbapenemase-producing *K. pneumoniae* or largely carbapenem-resistant Enterobacteriaceae (CRE) is now emerging and further study is required. The high MIC of imipenem will further detect for KPC or New Delhi metallo-β-lactamase (NDM) as epidemiology data, they are the most prevalent carbapenemase in Thailand. In addition, carbapenem and *K. pneumoniae* are causes of alarm for the Bureau of Drug Control Food and Drug Administration, Ministry of Public Health of Thailand for antimicrobial resistant bacteria. Continued monitoring of the susceptibility pattern of *K. pneumoniae* will provide invaluable information in proper clinical management.

Conclusion

The prevalence of ESBL-producing *K. pneumoniae* was 40%. Most isolates were resistant to ceftazidime and ceftriaxone. Imipenem was effective against ESBL-producing *K. pneumoniae*.

What is already known on this topic?

The high prevalence of ESBL producing *K. pneumoniae* was isolated by pheotypic methods and these isolates were susceptible to imipenem. Imipenem was effective against ESBL producing *K. pneumoniae*. However, we did not do genotype study.

What this study adds?

The susceptible pattern of ESBL producing *K. pneumoniae* was presented and imipenem was an effective antibiotic.

Acknowledgements

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Potential conflicts of interest

None.

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เชื้อ Klebsiella pneumoniae สร้างเอนไซม์ Extended spectrum β-lactamase ที่แยกได้จากโรงพยาบาลสรรพสิทธิ์ประสงค์ ต่อฤทธิ์ของยา imipenem

ภาวนา พนมเขต, ศุทธินี ธิราช, สุรศักดิ์ แว่นรัมย์, มารุตพงศ์ ปัญญา, จิราพร นิสกุล

ภูมิหลัง: *Klebsiella pneumoniae เป็นสาเหตุทั่วไปของโรคติดเชื้อในโรงพยาบาลและดื้อต่อยาต้านจุลชีพหลายชนิดรวมถึงยากลุ่มเบต้าเลคเทม เชื้อ K. pneumoniae หลั่งสาร extended spectrum β-lactamase (ESBL) เป็นเอนไซม์ที่สามารถย่อยยากลุ่มเบต้าเลคเทมเกือบทั้งหมด ยากลุ่ม carbapenems เช่น imipenem เป็นยาทางเลือกสำหรับรักษาโรคติดเชื้อจากแบคทีเรียวงศ์ Enterobacteriaceae ที่ผลิตเอนไซม์ ESBL*

วัตถุประสงค์: *เพื่อตรวจหาความเข้มข้นต่ำสุดของยา imipenem ในเชื้อ ESBL-producing K. pneumoniae ที่แยกได้ผู้ป่วยที่เข้ารับการรักษาในโรงพยาบาลสรรพสิทธิประสงค์*

วัสดุและวิธีการ: *เชื้อ K. pneumoniae จำนวน 250 สายพันธุ์ ที่แยกได้จากผู้ป่วยที่เข้ารับการรักษาในโรงพยาบาลสรรพสิทธิประสงค์ ช่วงเดือนกันยายน พ.ศ. 2557 ถึง ตุลาคม พ.ศ. 2558 ตรวจคัดกรองหาการสร้าง ESBL ด้วยวิธี disk diffusion และตรวจหาความไวของยาปฏิชีวนะต่อเชื้อ K. pneumoniae ที่สร้าง ESBL จำนวน 100 สายพันธุ์ถูกนำมาตรวจหาความเข้มข้นต่ำสุดของยา imipenem และ ceftazidime สายพันธุ์ที่ดื้อต่อ Imipenem และ Ceftazidime จะนำมาทดสอบหาขวามเข้นข้นต่ำสุดของยา Meropenem ต่อไป*

ผลการศึกษา: *เชื้อ K. pneumoniae ที่สร้าง ESBL ทุกสายพันธุ์ดื้อต่อยา ceftazidime ในหลอดทดลอง และมีค่า MIC มากกว่า 64 ไมโครกรัมต่อมิลลิลิตร เชื้อ K. pneumoniae ที่สร้าง ESBL 99 สายพันธุ์ ยังมีความไวต่อยา imipenem มีค่า MIC อยู่ระหว่าง 0.25-1 ไมโครกรัมต่อมิลลิลิตร 1 ใน 100 ของเชื้อ K. pneumoniae ที่สร้าง ESBL ดื้อต่อยา imipenem ค่า MIC ของเชื้อคือมากกว่า 64 ไมโครกรัมต่อมิลลิลิตร และเชื้อดังกล่าวดื้อต่อยาปฏิชีวนะทุกชนิดรวมทั้งยา meropenem*

สรุป: *เชื้อ K. pneumoniae ที่สร้าง ESBL สามารถย่อยยา ceftazidime แต่อย่างไรก็ตามยา imipenem เป็นยาที่มีประสิทธิภาพในการต้านเชื้อ K. pneumoniae ที่สร้าง ESBL ชนิดของ ESBL ต้องการทำการศึกษาเพิ่มเติม*