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นิพนธ์ต้นฉบับ
ORIGINAL ARTICLE

ลักษณะความไวของสารต้านจุลชีพและการทดสอบการสร้างเอนไซม์ Extended-spectrum β-lactamase Producing ของเชื้อ *Klebsiella pneumoniae* ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วยในโรงพยาบาลร้อยเอ็ด ประเทศไทย

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บทคัดย่อ

เชื้อ *K. pneumoniae* สายพันธุ์สร้างเอนไซม์เบตา-แลคทาเมส (ESBLKPs) และ เชื้อ *Klebsiella pneumoniae* ดื้อยาต้านจุลชีพหลายขนาน (MDRKP) พบได้บ่อยในประเทศไทย ผู้ป่วยที่ติดเชื้อชนิดนี้ส่งผลให้มีความยากในการรักษาและอาจส่งผลให้การรักษาล้มเหลว วัตถุประสงค์ของการศึกษาเพื่อ ศึกษาลักษณะของผลการทดสอบความไวของสารต้านจุลชีพของเชื้อ ESBLKPs ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วยที่เข้ารับการรักษาในโรงพยาบาลร้อยเอ็ด จังหวัดร้อยเอ็ด โดยดำเนินการเก็บข้อมูลระหว่างวันที่ 1 มกราคม พ.ศ. 2557 ถึงวันที่ 31 ตุลาคม พ.ศ. 2558 สิ่งส่งตรวจเพาะเชื้อประกอบด้วย ปัสสาวะ เสมหะ หนอง สารน้ำจากร่างกาย และเลือด การทดสอบความไวของยาต้านจุลชีพใช้วิธี Disk diffusion และทดสอบการสร้างเอนไซม์เบตา-แลคทาเมส ใช้วิธี combined disk diffusion โดยใช้เชื้อ *Klebsiella pneumoniae* ATCC 700603 เป็นสายพันธุ์อ้างอิง การตรวจวิเคราะห์ทางห้องปฏิบัติการตามมาตรฐานของ CLSI January, 2010 การวิเคราะห์ข้อมูล ใช้สถิติเชิงพรรณนา (ค่าเฉลี่ย, ส่วนเบี่ยงเบนมาตรฐาน, ร้อยละ) ผลการศึกษาพบว่า จากผู้ป่วย 125 คน ส่วนมากเป็นเพศหญิง ร้อยละ 50.4 อายุเฉลี่ย 62.2 ปี (SD =15.6) นอนรักษาตัวในโรงพยาบาลมากกว่า 7 วัน ร้อยละ 52.5 สิ่งส่งตรวจส่วนมากเป็นเสมหะคือ ร้อยละ 71.2 ผลการทดสอบความไวต่อสารต้านจุลชีพพบว่า 90 ตัวอย่าง เป็น ESBLKPs โดย ร้อยละ 99.0, 98.0, 97.0, 96.0, 98.0, 72.0, และ 81.0 มีความไวต่อยา imipenem, meropenem, ertapenem, doripenam, amikacin, gentamicin และ tigecycline ตามลำดับ และ 35 ตัวอย่างเป็น ESBL+ MDRKPs โดยร้อยละ 98.0, 97.0, 96.0, 96.0, 86.0 และร้อยละ 68.0 มีความไวต่อยา meropenem, doripenem, impipenem, ertapenem, amikacin และ tigecycline ตามลำดับ สรุปผลการศึกษา การศึกษาครั้งนี้แสดงให้เห็นว่าเชื้อ ESBLKPs และ ESBL+ MDRKPs มีความไวต่อยาต้านจุลชีพในกลุ่ม carbapenem, amikacin และ tigecycline ค่อนข้างสูง

คำสำคัญ : สารต้านจุลชีพ, เอนไซม์เบตา-แลคทาเมส, *Klebsiella pneumoniae*, การทดสอบความไว

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ORIGINAL ARTICLE

Antibiotic susceptibility patterns and detection of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* isolated from patients at Roi Et hospital, Thailand

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Abstract

Extended-spectrum β-lactamase producing *Klebsiella pneumoniae* strains (ESBLKPs) and multidrug resistant *Klebsiella pneumonia* (MDRKP) is frequently found in Thailand. The infected patients are difficult to treat and may lead to treatment failure. The aim of this study was to investigate the antibiotic susceptibility patterns of ESBLKPs from the clinical specimens of patients at Roi Et Hospital. The study was conducted between January1, 2014 to October 31, 2015. The clinical specimens were examined including urine, sputum, pus, body fluids and blood. The antimicrobial susceptibility testing was performed by disk diffusion, and β-lactamase enzymes produced were tested by combined disk diffusion methods and using *Klebsiella pneumoniae* ATCC 700603 to be a reference strain. All laboratory processes were performed according to guideline of CLSI January, 2010. Descriptive statistics (mean, standard derivation, percent) were used for data analysis. From 125 patients, most of them were female 50.4%, mean age 62.2 years (SD =15.6), more than 7 days admitted 52.5%. Clinical specimens were sputum 71.2%. Of 90 subjects were diagnosed with ESBLKPs for 98.0%, 98.0%, 98.0%, 97.0%, 97.0%, 72.0% and 81.0% susceptible to amikacin, imipenem, meropenem, ertapenem, doripenam, gentamicin andtigecycline respectively. Also, 35 subjects were identified as ESBL+ MDRKPs and 98.0%, 97.0%, 96.0%, 96.0%, 86.0%, 68.0% respectively which were susceptible to meropenem, doripenem, impipenem, ertapenem, amikacin and tigecycline respectively. In conclusion, this study revealed that ESBLKPs and ESBL+ MDRKPs were high susceptible to carbapenem, amikacin and tigecycline.

Keywords : Antimicrobial**,** Extended-spectrum β-lactamase**,** *Klebsiella pneumoniae***,** Susceptibility testing

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Introduction

The ESBLKPs infection is an opportunist pathogens frequently found in the hospital worldwide.[1,2] The incidence of hospital-acquired bacteremia caused from *K. pneumonia* in northeast Thailand was 13.9 %.[3] These pathogens can cause urinary tract infection, respiratory tract infection, blood stream infection, wound infection and soft tissue infection.[4-6] The ESBLKPs infection is a serious problem because it is difficult to treat and could cause treatment failure. These bacteria are resistant to many kinds of antibiotics.[7,8]

The risk factors of ESBLKPs infection were patients exposed to some types of invasive medical devices and improper use of antibiotics. The incidence of ESBLKPs infection in Thailand is very high.[9,10] Previous studies reported the risk factors of ESBLKPs infection and characterization of ESBLKPs genes. Moreover, there are a few reports on the antibiotic susceptibility patterns of ESBLKPs. The aim of this study was to investigate the antibiotic susceptibility patterns of ESBLKPs from the clinical specimens of patients at RoiEt Hospital.

Materials and Methods

Study subjects

This is a hospital-based study, and all data were collected at Roi Et Hospital, Roi Et Province, Thailand between January1, 2014 to October 31, 2015. From sample size calculation, the total numbers of subjects were 125 patients and all subjects were included in is study by random sampling technique. The inclusion criteria of subjects, all were admitted more than 48 hours, aged more than 15 years old and newly diagnosed with ESBLKPs infection. The clinical specimens used to study were sputum (n=89), urine (n=21), pus (n=5), blood (n=8) and body fluids (n=2).

The K. pneumoniae identification

The microbial isolation and identification were performed by using standard microbiological techniques. The clinical specimens were cultured by direct plating on sheep blood agar and MacConkey agar and then incubated for 48 hours. After 48-hour incubation, the suspected colonies of *K. pneumoniae* found as pink colonies on MacConkey agar were inoculated to tryptic soy broth, TSI (triple sugar iron agar), SIM (sulfide, indole, sotility) medium agar, LIA (lysine iron agar), citrate test, crease test, malonate test overnight. The results of biochemical test for *K. pneumoniae* showed TSI (A/A) with gas, catalase positive, oxidase negative, motile negative, indole negative, lysine positive, ornithine decarboxylase negative, citrate test positive, urease test positive and malonate test positive.

The Antibiotic susceptibility testing for K. pneumoniae

The antibiotic susceptibility test was performed by disk diffusion method, and all of the processes were followed guidelines of the Clinical and Laboratory Standards Institute 2010.[16] The antimicrobial drugs were used to test in the laboratory including amikacin (AK: 30 μg), ampicillin (AMP: 10 μg), cefotaxime (CTX:30 μg), ceftazidime (CAZ:30 μg), cefuroxime (CXM:30 μg), cephalotin (KF:30 μg), cotrimoxazole (SXT:25 μg), gentamicin (CN:10 μg), impipenem (IPM:10 μg), meropenem (MEM:10 μg), ertapenem (ETP:10 μg), doripenem (DOR:10 μg), tigecyclin (TGC:15 μg) and piperacilin/tazobactam (TZP:110 μg), cefoperazone/sulbactam (Sul=75 μg/15 μg) and ciprofloxacin (CIP=5 μg). The *K. pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 were used to reference strains.

The Extended-spectrum β-lactamase producing screening for K.pneumoniae

According to the CLSI 2010 guidelines, this study used the combination of disk diffusion method to detect ESBL that *K. pneumoniae* produces. In brief, the 0.5 McFarland standards were used to adjust the turbidity of *K. pneumoniae*. After adjusting and inoculation into the Muller Hinton agar plate, it was put in

pairs of disks contained cefotaxime (CTX: 30 μg), cefotaxime/clavulanuc acid (CTX/CLA 30/10 μg) and ceftazidime (CAZ: 30 μg), ceftazidime/clavulanuc acid (CAZ/CLA 30/10 μg). The positive result of ESBL compared to the disk without clavulanuc acid was defined as a ≥ 5 millimeters diameter of the inhibition zone. Also, the definition of multidrug resistant is *K. pneumoniae* resistant antibiotic more than three groups of antimicrobial agent have been testing in the laboratory.

Statistical analysis

The descriptive statistics used to analyze data are percentage, mean and standard deviation.

Ethics

This study was approved by The Ethical Committee of Roi Et Hospital. The reference number is 004/2559.

Results

The general characteristic of cases

The total number of subjects was 125 cases and most of them were female (50.4%), mean age was 62.2 years (±SD= 15.6). For marital status, they were married 76.0%, single 16.8% and divorce 7.2%. The occupations were agriculture 66.4%, unemployed 16.8%, employee 11.2%, government officer 3.2% and the business owner 2.4%. Ward of admitted were medicine 40.0%, intensive care unit 30.8%, surgical ward 19.2% and gynecological ward 10.0%. The clinical specimens were sputum 71.2%, urine 16.8%, blood 6.4%, pus 4.0% and body fluid 1.6 %. From all 125 cases, those who stayed in the hospital less than 7 days were 47.2 %, and more than 7 days were 52.8%. Most of them have co-morbidity 87.2% and complication 77.6%. Data are shown in **Table1.**

The antibiotic susceptibility pattern of Extended-spectrum β-lactamase K.pneumoniae producing strain

ESBLKPs were identified to 90 cases from 125 subjects. The antibiotics susceptibility patterns of ESBLKPs were high sensitivity to amikacin 98.0%, impipenem 98.0%, meropenem 98.0%, ertapenem 97.0%, doripenem 97.0%, tigecyclin 81.0% and gentamycin 72.0%. Otherwise ESBLKPs were resistance toampicillin 100%, cefotaxime 97.0%, ceftazidime 88.0%, cefuroxime 94.0%, cephalotin 96.0%, ciprofloxacin 58.0%, cotrimoxazole 77.0%, piperacilin/tazobactam 64.0%. Data have been shown in **Table 2.**

The antibiotic susceptibility pattern of Extended-spectrum β-lactamase with multidrug resistance *K.pneumonia*e producing strain.

Of 125 cases, 35 cases were identified for ESBLs+MDRKPs. The antibiotics susceptibility patterns of ESBL+MDRKPs were high sensitivity to meropenem 98.0%, doripenem 97.0%, impipenem 96.0%, ertapenem 96.0%, amikacin 86.0% and tigecyclin 68.0%. However, ESBL+MDRKPs is resistant to ampicillin 100%, cefotaxime 100%, ciprofloxacin 100%, ceftazidime 100%, cefuroxime 100%, cephalotin 100%, gentamicin 92.0%, cotrimoxazole 100% and piperacilin/tazobactam 73.0%. Data have been shown in **Table 3**.

Table 1 The general characteristics of cases

Variables	Cases	
	(n=125)	(%)
Gender		
Male	62	49.6
Female	63	50.4
Age		
<60	88	50.8
>60	85	49.2
Mean±SD	62.2±15.6	
Marital status		
Single	21	16.8
Couple	95	76.0
Divorce	9	7.2

Variables	Cases	
	(n=125)	(%)
Occupational		
Agriculture	83	66.4
Business	3	2.4
Government officer	4	3.2
Employee	14	11.2
Unemployed	21	16.8
Ward of admitted		
Gynecology	13	10.0
Surgical	25	19.2
Medicine	52	40.0
Intensive care unit	45	30.8
Specimens		
Sputum	89	71.2
Urine	21	16.8
Pus	5	4.0
Blood	8	6.4
Body fluid	2	1.6
Duration of admitted		
< 7 days	59	47.2
> 7 days	66	52.8
Co-morbidity		
No	16	12.8
Yes	109	87.2
Complication		
No	28	22.4
Yes	97	77.6

Table 2 The antibiotic susceptibility patterns of Extended-spectrum β-lactamase *K. pneumoniae* producing strain

Antibiotics	Resistance		Sensitivity	
	n	(%)	n	(%)
Amikacin	5	14.0	30	86.0
Ampicillin	35	100	0	0.0
Cefotaxime	35	100	0	0.0
Ceftazidime	35	100	0	0.0
Cefuroxime	35	100	0	0.0
Cephalotin	35	100	0	0.0
Ciprofloxacin	35	100	0	0.0
Cotrimoxazole	35	100	0	0.0
Gentamicin	32	92.0	3	8.0
Impipenem	2	4.0	33	96.0
Meropenem	1	2.0	34	98.0
Ertapenem	2	4.0	33	96.0
Doripenem	1	3.0	34	97.0
Tigecyclin	11	32.0	24	68.0
Piperacilin/ Tazobactam	26	73.0	9	27.0

Table 3 The antibiotic susceptibility patterns of Extended-spectrum β-lactamase with multidrug resistance *K. pneumoniae* producing strain

Antibiotics	Resistance		Sensitivity	
	n	(%)	n	(%)
Amikacin	2	2.0	88	98.0
Ampicillin	90	100	0	0
Cefotaxime	87	97.0	3	3.0
Ceftazidime	79	88.0	11	12.0
Cefuroxime	85	94.0	5	6.0
Cephalotin	86	96.0	4	4.0
Ciprofloxacin	52	58.0	38	42.0
Cotrimoxazole	69	77.0	21	23.0
Gentamicin	25	28.0	65	72.0
Impipenem	2	2.0	88	98.0
Meropenem	2	2.0	88	98.0
Ertapenem	3	3.0	87	97.0
Doripenem	3	3.0	87	97.0
Tigecyclin	17	19.0	73	81.0
Piperacilin/ Tazobactam	58	64.0	32	36.0

Discussion

This study has investigated the antimicrobial susceptibility patterns of *K.pneumoniae*. After analyzing, 90 subjects were ESBLKPs and 35 subjects were ESBL+MDRKPs and most of them were high susceptibility to carbapenam. It is consistent with previous studies that ESBLKPs have high sensitivity to carbapenam and carbapenam and had successful in treatment.[11, 12] Furthermore, this study found that amikacin was very sensitive to ESBLKPs and ESBL+MDRKPs. It is consistent with a previous study in Iran that amikacin was sensitive and effective for treatment of this pathogenic infection.[11] This study found that ESBLKPs is resistant to ciprofloxacin 50%, and ESBL+MDRKPs resistance 100% which is similar to a previous study in USA which they found ESBLKPs had high resistance to ciprofloxacin but no report for ESBL+MDRKPs.[13] Our study is also consistent with previous studies in Thailand, they found ESBL-producing *E. coli* and *K. pneumoniae* were highly susceptible to imipenem, meropenem and ertapenem but ESBL-producing *E. coli* was high resistance to cefotaxime and ceftriaxone.[14]

However, the combination of antimicrobial agents were a wide range of drug of choices for treatment of ESBLKPs infection. Previous studies found amikacin plus piperacillin/tazobactam, piperacillin-tazobactam plus gentamicin, ciprofloxacin plus amikacin and ciprofloxacin plus gentamicin were also a feasible empirical therapy for ESBLKPs infection.[15] Nowadays, carbapenem resistance of *K. pneumonia* (CREKPs) is a serious problem, and this study found 1-3% of it. To prevent and control the outbreak of CREKPs in the hospital, laboratory result of CREKPs, are reported to the department of Infection Control of Roi Et Hospital.

Conclusion

This study found that ESBLKPs and ESBL+ MDRKPs were high susceptible to carbapenem, amikacin and tigecycline.

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