Prevalence and Susceptibility Pattern of Extended Spectrum β-lactamases Producing In *Escherichia coli* and *Klebsiella pneumoniae* at Maharat Nakhon Ratchasima Hospital, Thailand

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Abstract:

The ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are important pathogens which cause infectious diseases. The prevalence of these organisms have increased in trend worldwide. In addition, the ESBL-producing bacteria are resistant to multiple antibiotics. Thus, this study was aimed to determine the prevalence and susceptibility pattern of ESBLs-producing *E. coli* and *K. pneumoniae* in patients at Maharat Nakhon Ratchasima Hospital, Thailand. A total of 22,865 isolates of *E. coli* and 26,919 isolates of *K. pneumoniae* were isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014. The ESBL-producing strains were found in 9,395 (41.1 %) isolates of *E. coli* and 12,152 (45.1 %) isolates of *K. pneumoniae*. Furthermore, the ESBL-producing *E. coli* were most commonly found in urine (49.7 %), followed by pus (19.1 %), blood (15.2 %) and sputum (9.6 %). The ESBL-producing *K. pneumoniae* isolates were most commonly found in sputum and then urine, pus and blood. The percentages are 69.8, 13.8, 6.7 and 5.8 percent, respectively. The susceptibility pattern of these organisms were found susceptible to amikacin, netilmicin, cefoxitin, augmentin, gentamicin and trimethoprim-sulfamethoxazole but these organisms were resistant to ampicillin, cefazolin, cefuroxime and ceftriaxone. However, this study found that the ESBL-producing *E. coli* and *K. pneumoniae* were susceptible to ertapenem, imipinem and meropenem.

Key Words: Extended spectrum β-lactamases, ESBL, *Escherichia coli, Klebsiella pneumoniae*, Prevalence, Susceptibility

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บทคัดย่อ: ความชุกและแบบแผนความไวต่อยาต้านจุลชีพของเชื้อ *Escherichia coli* และ *Klebseilla pneumoniae* ที่สร้างเอนไซม์ Extended spectrum β-lactamases (ESBL) ในโรงพยาบาลมหาราชนครราชสีมา, ประเทศไทย

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เชื้อ *Escherichia coli* และ *Klebsiella pneumonia* ที่สร้างเอนไซม์ Extended spectrum β-lactamases (ESBL) เป็นเชื้อก่อโรคที่สำคัญที่เป็นสาเหตุก่อให้เกิดโรคติดเชื้อโดยความชุกของเชื้อเหล่านี้มีแนวโน้มเพิ่มขึ้นในทั่วโลก นอกจากนี้เชื้อแบคทีเรียที่สร้างเอนไซม์ ESBL ยังดื้อต่อยาต้านจุลชีพหลายชนิด ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาความชุกและแบบแผนความไวต่อยาต้านจุลชีพของเชื้อ *E. coli* และ *K. pneumoniae* ที่สร้างเอนไซม์ในผู้ป่วยโรงพยาบาลมหาราชนครราชสีมา, ประเทศไทย โดยทำการศึกษาเชื้อ *E. coli* จำนวน 22,865 สายพันธุ์ และเชื้อ *K. pneumoniae* จำนวน 26,919 สายพันธุ์ ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วยในโรงพยาบาลมหาราชนครราชสีมา ตั้งแต่ปี พ.ศ. 2553 ถึงปี พ.ศ. 2557 พบเชื้อ *E. coli* ที่สร้างเอนไซม์ ESBL จำนวน 9,395 (41.1 %) สายพันธุ์ และเชื้อ *K. pneumoniae* ที่สร้างเอนไซม์ ESBL จำนวน 12,152 (45.1%) สายพันธุ์ นอกจากนี้จากการศึกษายังพบว่า เชื้อ *E. coli* ที่สร้างเอนไซม์ ESBL พบมากที่สุดในสิ่งส่งตรวจที่เป็นปัสสาวะ (49.7%) รองลงมาคือ แผลหนอง (19.1%), เลือด (15.2%) และเสมหะ (9.6%) ส่วนเชื้อ *K. pneumoniae* ที่สร้างเอนไซม์ ESBL พบมากที่สุด ในสิ่งส่งตรวจที่เป็นเสมหะรองลงมาคือ ปัสสาวะ แผลหนอง และเลือด คิดเป็นร้อยละ 69.8, 13.8, 6.7 และ 5.8 ตามลำดับ นอกจากนี้การศึกษาแบบแผนความไวต่อยาต้านจุลชีพพบว่าเชื้อ *E. coli* และ *K. pneumoniae* ที่สร้างเอนไซม์ ESBL มีความไวต่อยา amikacin, netilmicin, cefoxitin, augmentin, gentamicin และ trimethoprim-sulfamethoxazole ในขณะที่เชื้อเหล่านี้ดื้อต่อยา ampicillin, cefazolin, cefuroxime และ ceftriaxone อย่างไรก็ตามในการศึกษาครั้งนี้ยังพบว่า เชื้อ *E. coli* และ *K. pneumoniae* ที่สร้างเอนไซม์ ESBL ยังมีความไวต่อยา ertapenem, imipinem และ meropenem

Introduction

Extended spectrum β-lactamases (ESBLs) are enzymes that are able to hydrolyze antibiotics belonging to β-lactam group such as penicilins, cephalosporins (except cephamycin and carbapenems) and mono-bactams[1-4]. However, these enzymes are inhibited by lactamase inhibitor (clavulanic acid)[5, 6] In addition, ESBLs are most commonly found in the family Entero-bacteriaceaes especially *Escherichia coli (E. coli)* and Klebsiella pneumoniae *(K. pneumoniae)* and other members in the family Enterobacteriaceaes such as Salmonella species, Proteus species, Enterobacter species etc[7]. The ESBL-producing E. coli was firstly isolated in 1987[8] and K. pneumoniae was firsty isolated in 1983[9]. After that, the prevalence of these organisms was reported worldwide.

ESBLs are the derivative of TEM or SHV enzymes[10]. Moreover, the structural characteristics, function, specificity of hydrolysis and level of expression of enzymatic activity have effect to the ability resistance

to antibiotics of bacteria[11]. So for there worldwide have been many reports of infections caused by ESBLs-producing bacteria especially ESBLs-producing *E. coli* and *K. pneumoniae*. In addition, Thailand has reports outbreaks of ESBLs-producing *E. coli* and *K. pneumoniae*. Thus, this study aimed to determine the prevalence and susceptibility pattern ofESBL s-producing *E. coli* and *K. pneumoniae* in patients at Maharat Nakorn Ratchasima Hospital in Thailand.

Materials and Methods

Bacterial isolates

A total of 22,865 isolates of *Escherichia coli* (*E. coli*) and 26,919 isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) were isolated from patients at Maharat Nakhon Ratchasima Hospital in Nakhon Ratchasima province, Thailand from 2010 to 2014. These bacteria were isolated from several specimens; urine, sputum, blood, pus, body fluid and etc. All isolates were identified by standard biochemical tests. Moreover, all isolates were tested ESBLs and antimicrobial susceptibility.

Antimicrobial susceptibility testing

All bacterial isolates were tested antimicrobial susceptibility by the Kirby-Bauer disk diffusion method[12]. All bacteria were adjusted to the turbidity of 0.5 McFarland standards and swabbed onto the surface of a Muller-Hinton agar plate. After that, the disks were pressed onto plate that previousy were prepared and incubated at 35±2°C for 18 hours. The results of susceptibility were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antimicrobial agents were used for susceptibility testing; ampicillin (10μg), cefazolin (30μg), gentamicin (10μg), trimethoprim-sulfame-thoxazole (25μg), amikacin (30μg), nettil-micin (30μg), augmentin (20μg of amoxicillin and 10μg of clavu-lanate), cefoxitin (30μg), cefuroxime (30μg) and ceftriaxone (30μg). The reference strain *E. coli* ATCC 25922 was used as the quality control strain for the susceptibility testing.

Test for ESBL production

ESBL production was screened by double disk diffusion method. This method was recommended by CLSI. A disk of augmentin (20 μg of amoxicillin and 10 μg of clavulanic acid) was placed the side of ceftriaxone (30 μg). The distance from center to center of the both disks was about 30 mm. The presence of ESBL is indicated by clearing the area between the both disks (synergist zone).

The combination disk method is tested for ESBL production that CLSI recommended. Disk containing cefotaxime (30 μg) and ceftazidime (30 μg) of which inhibition zone was compared with cefotaxime-claulanic acid (30 μg/10 μg) and ceftazidime-clavulanic acid (30 μg/10 μg), respectively. A difference of ≥5 mm. in diameter of the inhibition zone was considered positive for ESBL production.

Results

This study, a total of 22,865 isolates of *E. coli* and 26,919 isolates of *K. pneumoniae* were isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014. The study found 9,395 (41.1%) isolates of *E. coli* that were ESBL-producing bacteria. The prevalence of ESBL-producing *E. coli* isolates ranged from 31.4-46.0 percent. Moreover, the prevalence had trend to increase and the results were

Table 1 The prevalence of extended spectrum β-lactamasesproducing in Escherichia coliisolated from patients in Maharat Nakhon Ratchasima Hospital from 2010 to 2014

	E. coli		
Years	**No. of isolates**	**ESBL**	**Percentage of ESBL-producing bacteria (%)**
2010	4,707	1,479	31.4
2011	4,170	1,771	42.5
2012	4,709	2,022	42.9
2013	4,687	2,010	42.9
2014	4,592	2,113	46.0
Total	22,865	9,395	41.1

shown in the table 1 and figure 1. The ESBL-producing *K. pneumoniae* were found in 12,152 (45.1%) isolates. The prevalence of ESBL-producing *K. pneumoniae* ranged from 41.6- 50.6 percent that they have highest prevalence in 2011 and decrease after 2011. However, the prevalence range of ESBL-producing *K. pneumoniae* was relatively stable. The results were shown in the table 2 and figure 2.

The number of ESBL-producing *E. coli* and *K. pneumoniae* were isolated from several specimens. The ESBL-producing *E. coli* were most commonly found in urine (49.7 %), followed by pus (19.1 %),

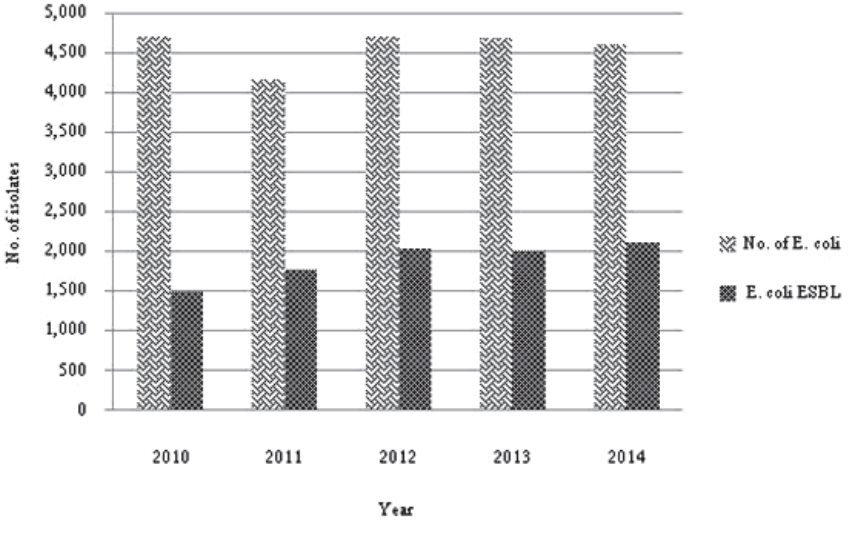

Figure 1 The prevalence range of extended spectrum β-lactamases producing *Escherichia coli* isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014.

Table 2 The prevalence of extended spectrum β-lactamases producing Klebsiellapneumoniaeisolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014.

	K. pneumoniae		
Years	**No. of isolates**	**ESBL**	**Percentage of ESBL-producing bacteria (%)**
2010	5,058	2,298	45.4
2011	5,232	2,647	50.6
2012	5,648	2,585	45.8
2013	5,513	2,345	42.5
2014	5,468	2,277	41.6
Total	26,919	12,152	45.1

blood (15.2 %) and sputum (9.6 %). The ESBL-producing *K. pneumoniae* isolates were most commonly found in sputum and then urine, pus and blood. The percentages were 69.8, 13.8, 6.7 and 5.8 percent, respectively. The results were shown in the table 3-4 and figure 3-4.

The antibiotic susceptibility testing of non- ESBL-producing *E. coli* and *K. pneumoniae* isolates were found more than 40 percent susceptible to cefazolin, genta-micin, trimethoprim-sulfamethoxazole, amikacin, netilmicin, cefuroxime, cefoxitin, augmentin and ceftriaxone. These organisms were susceptible

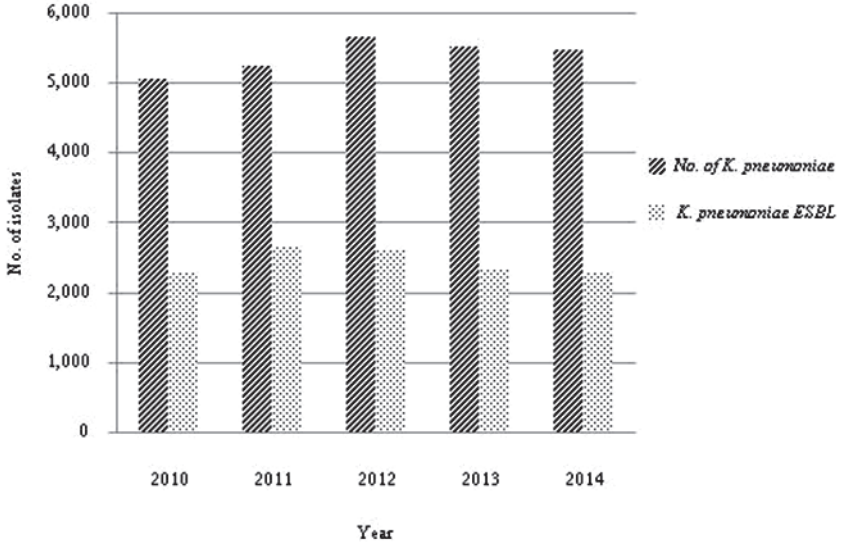

Figure 2 The prevalence range of extended spectrum β-lactamases producing *Klebsiella pneumoniae* isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014

Table 3 The specimens of extended spectrum β-lactamasesproducing in *Escherichia coli* isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014 (n = 9,395)

	E. coli	
Specimens	**Number of ESBL-producing bacteria**	**Percentage of ESBL-producing bacteria (%)**
Urine	4,674	49.7
Pus	1,798	19.1
Blood	1,430	15.2
Sputum	900	9.6
Others	593	6.3
Total	9,395	100

to ampicillin were 19.1 % in *E. coli* and 0.7 % in *K. pneu-moniae.* The results were shown in the table 5.

The antibiotic susceptibility testing of ESBL-producing *E. coli* and *K. pneumoniae* isolates were resistant to ampicillin, cefazolin, cefuroxime and ceftriaxone. The ESBL-producing *E. coli* were suscep-tible to amikacin, netilmicin, cefoxitin, augmentin, gentamicin and trimethoprim-sulfamethoxazole. The percentages of susceptibility were 96.3, 82.3, 74.0, 43.0, 34.8 and 27.7 percent, respectively. Furthermore, ESBL-producing *E. coli* were susceptible to ertapenem (98.5%), imipenem (99.8%) and meropenem (99.8 %).

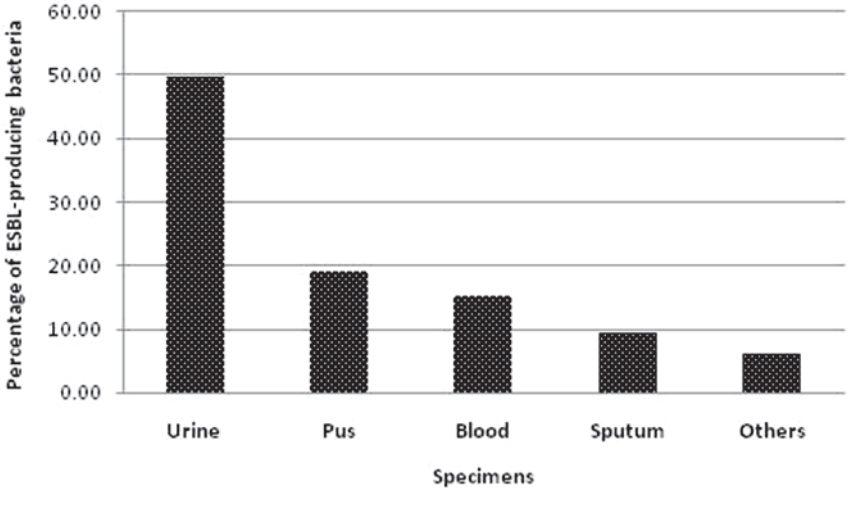

Figure 3 The numbers of extended spectrum β-lactamases producing *Escherichia coli* found in specimens from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014.

Table 4 The specimens of extended spectrum β-lactamases producing *Klebsiella pneumoniae* isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014 (n = 12,152)

	K. pneumoniae	
Specimens	**Number of ESBL-producing bacteria**	**Percentage of ESBL-producing bacteria (%)**
Urine	1,679	13.8
Pus	813	6.7
Blood	710	5.8
Sputum	8,479	69.8
Others	471	3.9
Total	12,152	100

The ESBL-producing *K. pneumoniae* were susceptible to amikacin, netilmicin, cefoxitin, gentamicin, trime-thoprim-sulfamethoxazole and augmentin. The percentages of susceptibility ware 92.7, 72.2, 70.3, 40.3, 17.9 and 14.9 percent, respectively. In addition, ESBL-producing *K. pneumoniae* were susceptible to ertapenem (95.5%), imipenem (99.1%) and meropenem (98.1%). The results of antibiotic susceptibility were shown in the table 6.

Discussions

Extended spectrum β-lactamases (ESBLs)

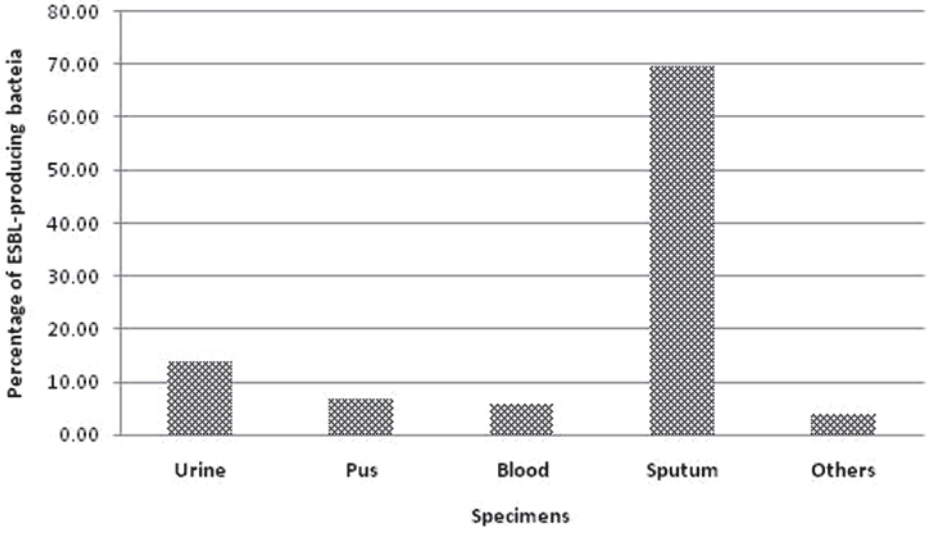

Table 4 The specimens of extended spectrum β-lactamases producing *Klebsiella pneumoniae* isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014 (n = 12,152)

Table 5 The antibiotic susceptibility pattern of non-ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014.

Antimicrobial Agent	Susceptibility of non-ESBL producing bacteria					
	E. coli			*K. pneumoniae*		
	Number of test	non-ESBL	Percentage of susceptibility (%)	Number of test	non-ESBL	Percentage of susceptibility (%)
Ampicillin	9,605	1,834	19.1	13,296	88	0.7
Cefazolin	4,532	2,857	63.0	6,454	5,558	86.1
Gentamycin	9,639	7,899	81.9	13,338	12,691	95.1
Trimethoprim-sulfamethoxazole	9,466	4,381	46.3	13,065	11,364	87.0
Amikacin	9,642	9,533	98.9	13,332	13,050	97.9
Netilmicin	9,339	9,031	96.7	13,177	12,804	97.2
Cefuroxime	9,373	7,231	77.1	13,197	9,794	74.2
Cefoxitin	9,601	8,694	90.6	13,312	12,011	90.2
Augmentin	9,650	7,056	73.1	13,344	11,685	87.6
Ceftriaxone	9,642	9,020	93.5	13,332	12,606	94.6
Ertapenem	1,903	1,792	94.2	1,504	993	66.0
Imipenem	1,942	1,863	95.9	1,528	1,094	71.6
Meropenem	1,907	1,826	95.8	1,497	1,063	71.0

are enzymes that are able to hydrolyze antibiotics belonging to β-lactam group such as penicilins, cephalosporins (except cephamycin and carbapenems) and mono-bactams. However, these enzymes are inhibited by lactamase inhibitor (clavulanic acid). The ESBL-producing bacteria which are most commonly found in family Enterobacteriaceaes are cause of infectious disease and become a problem worldwide. In addition, they worldwide were reported the prevalence of ESBL-producing bacteria that sincrease trend. The prevalence of ESBL-producing bacteria in Europe was increased and higher than that in the USA but lower than that in South America and Asia[7]. In Europe, ESBL-producing *E. coli* and *K. pneumoniae* isolates were increased from 2.1% to 10.8% and 9.0% to 13.6% in 1997 to 2004, respectively[13]. The Latin America has higher rate of these organisms in 2008 when compared with previous years. The ESBL-producing *E. coli* and *K. pneumoniae* were isolated from intra-abdominal infections (IAI) in Latin America where SMART (The Study for Monitoring Antimicrobial Resistance Trends) showed 26% of *E. coli* and 35% of *K. pneumoniae.* These increased when they were compared with 10% of *E. coli* and 14% of *K. pneumoniae* in 2003, and 10% of *E. coli* and 18% of *K. pneumoniae* in 2004[14-16]. Moreover, the

Table 6 The antibiotic susceptibility pattern of extended spectrum β-lactamasesproducing Escherichia coli and Klebsiellapneumoniae isolated from patients at Maharat Nakhon Ratchasima Hospital from 2010 to 2014.

Antimicrobial Agent	Susceptibility of non-ESBL producing bacteria					
	E. coli			*K. pneumoniae*		
	Number of test	ESBL	Percentage of susceptibility (%)	Number of test	ESBL	Percentage of susceptibility (%)
Ampicillin	8,990	12	0.1	12,042	6	0.0
Cefazolin	4,369	8	0.2	5,257	11	0.2
Gentamycin	8,977	3,128	34.8	12,027	4,852	40.3
Trimethoprim-sulfamethoxazole	8,809	2,437	27.7	11,758	2,107	17.9
Amikacin	8,996	8,662	96.3	12,044	11,163	92.7
Netilmicin	8,736	7,194	82.3	11,922	8,611	72.2
Cefuroxime	9,499	0	0.0	12,220	0	0.0
Cefoxitin	9,017	6,676	74.0	12,045	8,470	70.3
Augmentin	8,994	3,864	43.0	12,042	1,790	14.9
Ceftriaxone	9,499	0	0.0	12,220	0	0.0
Ertapenem	8,955	8,822	98.5	11,977	11,440	95.5
Imipenem	8,969	8,950	99.8	11,985	11,872	99.1
Meropenem	8,745	8,724	99.8	11,703	11,482	98.1

percentage of ESBL-producing *K. pneumoniae* were found highest in Latin America (45%), followed by the Western Pacific region (25%), Europe (23%), the USA (8%) and Canada (5%)[7]. In Asia, since 1990s the ESBL-producing bacteria had increased rate in India and Pakistan[17]. The prevalence of ESBL-producing *K. pneumoniae* in northern Taiwan increased from 3.4% in 1993 to 10.3% in 1997[18]. In Hong Kong, the incidences of ESBL-producing *E. coli* and *K. pneumoniae* were higher from 1.6% to 11% and 2.6% to 13% in 1990 to 1999, respec-tively[17]. Moreover, the prevalence of these organisms was increased from 0.4% to 1.7% in *E. coli* and 0.6% to 7.2% in *K. pneumoniae* in 1990 to 1995 and this report was studied by Kawakami et al. in Tokyo, Japan[19]. The prevalence of ESBL- producing *E. coli* and *K. pneumoniae* were different in each country. However, all countries have raised trend of ESBL. In this study, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* isolates were increased ranging from 31.4-46.0% in *E. coli* and 41.6-50.6 % in *K. pneumoniae*, the mean were 41.1% and 45.1%, respectively. The result showed *K. pneumoniae* isolates had higher prevalence than *E. coli.* These results have similarity to other country in Asia. The rate of ESBL-producing *E. coli* and *K. pneumoniae* were 7.5% and 22.8% in south Korea,

respectively[20]. In Thailand, the prevalence of ESBL -producing bacteria in Siriraj Hospital were found 33.3% in *E. coli* and 56.9% in *K. pneumoniae* in 2003[21]. In addition, a teaching hospital in southern Thailand found the rates of these organisms 5.1% in *E. coli* and 44.4% in *K. pneumoniae*[22]. In Songklanagarind Hospital the rate of prevalence of ESBL-producing *E. coli* and *K. pneumoniae* were 19% and 32%, respectively[23]. Furthermore, at Queen Sirikit National Institute of Child Health found the rate of 27% in E. coli and 64% in K. pneumoniae[24]. The overall rate of prevalence of ESBL-producing bacteria in Thailand *K. pneumoniae* was found more than *E. coli*[25, 26]. However, the s rate of prevalence of ESBL-producing *E. coli* was more than *K. pneu-moniae*[1].

In this study, the ESBL-producing *E. coli* isolates were most commonly found in urine, followed by pus, blood, and sputum so *E. coli* was important pathogen in urinary tract. While ESBL-producing *K. pneumoniae* were most commonly found in sputum, followed by urine, pus and blood so *K. pneumoniae* is important pathogen in respiratory tract. These study results have similarity to other study in Thailand[24-26]. Moreover, the ESBL-producing *E. coli* and *K. pneumoniae* were found in the respiratory tract (42.5%), urinary tract (33.7%), surgical wound (12.9%), skin and soft tissue (5.7%) and blood stream (5.2%) in regional and provincial government hospital in Thailand[1].

The ESBL-producing *E. coli* was firstly isolated in 1987[8] while *K. pneumoniae* was firstly isolated in 1983[9]. After that, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* were increased worldwide. At present, the high rates of ESBL-producing bacteria are important problem for choosing antibiotics for therapy because these organisms resist to multiple antibiotics. Thus, the studies about susceptibility pattern of ESBL-producing bacteria are important and useful for antibiotics therapy. This study showed non-ESBL-producing *E. coli* and *K. pneumoniae* were susceptible to all antibiotics that were used in this study. While, the ESBL-producing *E. coli* and *K. pneumoniae* were found susceptible to amikacin, netilmicin, cefoxitin, augmentin, gentamicin and trimethoprim-sulfamethoxazole but these organisms resisted to ampicillin, cefazolin, cefuroxime and ceftriaxone. Moreover, the percentages of susceptibility to these antibiotics are different from other study[24, 26, 27]. Furthermore, non-ESBL-producing *E. coli* and *K. pneumoniae* were susceptible to ertapenem, imipenem and meropenem but the percentage of susceptibility of these organismsis lower than the ESBL-producing *E. coli* and *K. pneumoniae*. The ESBL-producing *E. coli* were found susceptible to imipenem (99.8%) and meropenem (99.8%). This result has similarity to other study in Thailand[24, 26].The percentages of susceptibility of ESBL-producing *K. pneumoniae* is less than ESBL-producing *E. coli*. However, both imipenem and meropenem are effective against ESBL-producing *E. coli* and *K. pneumoniae*.

In conclusion, the ESBL-producing *E. coli* and *K. pneumoniae* are important pathogens and cause of infectious diseases. Moreover, these organisms resist to multiple antibiotics that is a problem of antibiotic therapy. Thus, the study about prevalence and suscep-tibility pattern of ESBL-producing *E. coli* and *K. pneumoniae* is important and useful for choosing antibiotics for therapy of clinician. In addition, the microbiology laboratory has important role in identification and susceptibility testing in order to

derive good results. Therefore, the participation of several divisions is important in order to control, reduce and prevent ESBL-producing *E. coli* and *K. pneumoniae* spread in our hospital.

Acknowledgements

The author would like to thank Miss Waraporn Sititerm for data collection in this study.

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