Association of ESBL and CTX-M gene with the clinical outcome of patients suffer from bloodstream infections caused by *Escherichia coli* and *Klebsiella pneumoniae*

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Summary

Objective: To evaluate the value of CTX-M on the clinical outcome of bloodstream infection (BSI) patients caused by *E. coli* and *K. pneumoniae*. *Subject and method:* A total of 165 BSI patients were treated at 108 Military Central Hospital from Oct. 2014 to May 2016, including 115 cases caused by *E. coli* and 50 cases caused by *K. pneumoniae*. All strains were identified and performed antibiotic susceptible test by Vitek II system (Bio-Merieux, USA). CTX-M gene encoding ESBL was screened using polymerase chain reaction method. *Result:* In total, the percentage of ESBLs producing strains was 47.3% and CTX-M was detected in 62.4% (103/165) all of strains. We observed that there was not significant difference with the clinical outcome between BSI patients caused by CTX-M positive strains (septic shock 22.3% and fatality 21.4%) and BSI patients caused by cephalosporin resistant strains (17.9% - 22.5% with shock and 17.9% - 22.5% with fatality). However, when we analyzed the subgroup of BSI patients caused by cephalosporin susceptible strains. The proportion of shock and fatality in groups with CTX-M positive were higher than that in group with CTX-M negative (21.6% - 33.3% vs 14.6% - 19.2% with shock and 18.9% - 26.7% vs 10.4 - 15.4% with fatality). Additionally, the length of hospital stay in groups with CTX-M positive were significant longer than that in group with CTX-M negative. *Conclusion:* Our data showed a high prevalence of ESBL producing strains causing BSI patients, and also high prevalence of CTX-M positive strains. Moreover, in BSIs caused by cephalosporin susceptible strains, the clinical outcome of group with CTX-M positive were worse than that of group with CTX-M negative.

Keywords: Cephalosporin resistance, CTX-M beta-lactamase, bloodstream infections (BSIs).

1. Background

Bloodstream infections (BSIs) that are mostly caused by multidrug resistant pathogens is of growing importance worldwide, including those with critical and high priority levels. The most common cause of both community and hospital-acquired BSI in South-East Asia are *Escherichia coli* and *Klebsiella pneumoniae* (families of *Enterobacteriaceae*) and their antibiotic resistance are increasing, such as extended spectrum beta lactamase (ESBL) [1, 2].

Wide dissemination of beta-lactamase producing strains, and increased incidences of hospital-acquired ESBL producing *E. coli* and *K. pneumoniae* infections have been documented. Reports for the years 2002 until 2011 suggest an exponential increase in ESBL resistances from 20 to 45% in the Asia-Pacific region, and from 39 to 55% in South-East Asia [2]. Especially the Philippines and Vietnam have been reported high burden of ESBL-producing *E. coli* and *K.*

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pneumoniae infections with prevalence of 59% and 81%, respectively. The most common resistance factors in clinical settings worldwide are the CTX-M beta-lactamases [3].

In clinical practice, antibiotic therapy for BSI patients bases on phenotypic resistance and the reports demonstrated that multidrug resistance bacterial infections associated with poor outcome of BSI patients [4]. With genetic resistance such as CTX-M, there are not all of them to express phenotype and the less reports to evaluate of genotypic resistance on the outcome of BSIs. Therefore, the purpose of this study was to evaluate the role of CTX-M on the outcome of bloodstream infections caused by *E. coli* and *K. pneumoniae*.

2. Subject and method

2.1. Subject

A total of 165 patients with bloodstream infections were treated at 108 Military Central Hospital in Hanoi, from Oct. 2014 to May 2016, including 115 cases caused by *E. coli* and 50 cases caused by *K. pneumoniae*. BSIs were diagnosed by following the criteria of the Surviving Sepsis Campaign guidelines [5].

2.2. Method

This is observational study with combination between clinical reports and laboratory. To evaluate the influence of CTX-M on the outcome, we compared the ratio of septic shock and fatality between BSI patients caused by phenotypic resistant strains and CTX-M positive strains. We also analysed subgroup of patients caused by cephalosporin susceptible strains to compare the clinical outcome (shock, fatality and length of hospital stay) between group with CTX-M positive and CTX-M negative.

All strains were identified and performed antibiotic susceptible test by Vitek II system (BioMerieux, Lyon, France). The following antimicrobial substances were included: Cefotaxime (CTX), ceftazidime (CAZ), and ESBL phenotyping. We also detected CTX-M gene of each strains by polymerase chain reaction (PCR) method.

2.3. Statistical analysis

Statistical analyses were performed using the SPSS software v.23.0 (IBM Corporation, Chicago, IL, USA). Categorical variables are given as frequencies with percentages and comparisons of categorical variables between groups were performed using Chi-square and Fisher's exact tests. The level of significance was set at p-values<0.05.

2.4. Ethical statement

Ethical approval was obtained from the Ethics Committee and the Review Board of the 108 Military Central Hospital. All patient data were anonymized prior to the analysis.

3. Result

3.1. General characteristics of patient cohort

Table 1. General characteristics

General characteristics	*E. coli* BSI (n = 115)	*K. pneumoniae* BSI (n = 50)	Total (n = 165)
Sex (male) n (%)	70 (60.9)	37 (74.0)	107 (64.8)
Ages (mean ± SD) (years)	62.3 ± 16.2	62.0 ± 17.2	62.3 ± 16.5
Over 60 years n (%)	62 (53.9)	26 (52.0)	88 (53.3)
Pre-existing conditions n (%)	73 (63.5)	27 (54.0)	100 (60.6)
Primary source infection n (%)	95 (82.6)	44 (88.0)	139 (84.2)

Length of hospital stay (days)	19.9 ± 14.7	27.7 ± 27.6	22.3 ± 19.8
Septic shock n (%)	19 (16.5)	14 (28.0)	33 (20.0)
Fatality n (%)	18 (15.7)	12 (24.0)	30 (18.2)

Most of the patients were elderly with pre-existing conditions. The ratio of septic shock and fatality were 20.0% and 18.2%, respectively.

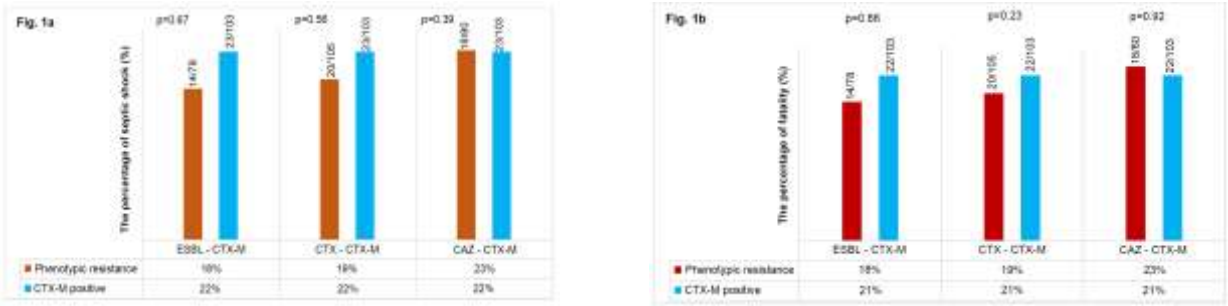
3.2. Distribution of phenotypic and genotypic resistance

Table 2. Distribution of phenotypic and genotypic resistance

Characteristics	*E. coli* BSI (n = 115)	*K. pneumoniae* BSI (n = 50)	Total (n = 165)
Phenotypic resistance			
ESBL n (%)	67 (58.3)	11(22.0)	78 (47.3)
CTX n (%)	81 (70.4)	24 (48.0)	105 (63.6)
CAZ n (%)	53 (46.1)	27 (54.0)	80 (48.5)
Genotypic resistance			
CTX-M n (%)	80 (69.6)	23 (46.0)	103 (62.4)

We observed 47.3% strains producing ESBL (53.8% with *E. coli* and 22.0% with *K. pneumoniae*), 63.6% resistance to CTX and 48.5% resistance to CAZ. We also detected CTX-M genes of 62.4% (103/165) all of strains, including 69.6% (80/115) *E. coli* and 46.0% (23/50) *K. pneumoniae*.

3.3. The association of cephalosporin resistance and CTX-M on the clinical outcome

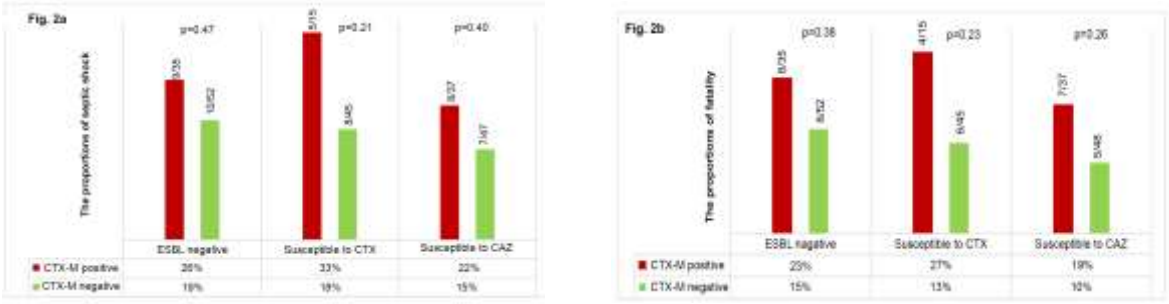


Note: ESBL: Extended spectrum beta-lactamase; CTX: Cefotaxime; CAZ Ceftazidime.

Figure 1. Comparison of the percentage of septic shock (Figure 1a) and fatality (Figure 1b) of BSI patients based on cephalosporin resistance strains and CTX-M positive strains

The percentage of shock and fatality of BSI patients caused by strains with cephalosporin resistance (ESBL, CTX and CAZ) were 17.9% - 22.5% and 17.9% - 22.5%, respectively. There was not significant difference with the outcome (septic shock 22.3% and fatality 21.4%) of BSI patients caused by CTX-M positive strains.

3.4. The influence of CTX-M on the outcome of susceptible phenotype group



Note: ESBL: Extended spectrum beta-lactamase; CTX: Cefotaxime; CAZ Ceftazidime.

Figure 2. The contribution of CTX-M to the outcome of BSI patients caused by cephalosporin susceptible strains, Figure 2a for ratio of shock and Figure 2b for fatality

In the subgroup of BSI patients caused by cephalosporin susceptible strains, the proportion of shock and fatality in groups with CTX-M positive were higher than that in group with CTX-M negative (21.6% - 33.3% vs 14.6% - 19.2% with shock and 18.9% - 26.7% vs 10.4 - 15.4% with fatality). However, the difference was not significant with p>0.05.

Table 3. The length of hospital stay in patients caused by cephalosporin susceptible strains

Phenotypic resistance	Length of hospital stay (days) (Mean ± SD (n))		
	CTX-M positive	CTX-M negative	p
ESBL negative	31.9 ± 29.1 (n = 35)	15.3 ± 9.9 (n = 52)	0.001
Susceptible to CTX	26.4 ± 16.8 (n = 15)	13.9 ± 8.0 (n = 45)	0.001
Susceptible to CAZ	20.5 ± 14.8 (n = 37)	14.1 ± 7.7 (n = 48)	0.011

In BSI group caused by cephalosporin susceptible strains, the length of hospital stay was significant longer in group with CTX-M positive than group with CTX-M negative with p<0.05.

4. Discussion

The *E. coli* and *K. pneumoniae* are common pathogen found in BSI patients and these are also common species producing CTX-M ESBL. The genetic basis of antibiotic resistance is rarely assessed in the clinical practice. We evaluated the antibiotic resistance patterns of *E. coli* and *K. pneumoniae* strains isolated from BSI patients in our hospital and screened for CTX-M beta-lactamase by applying an in-house PCR assay. We observed a high prevalence of ESBL-producing trains (47.3%) and resistance to CTX (63.6%), CAZ (48.5%) and CTX-M positive strains (62.4%). In the subgroup of BSI caused by cephalosporin susceptible strains, the outcome of group with positive CTX-M was worse than that of negative CTX-M.

The overall proportion of ESBLs producing strains was similar to that observed in the Asian-Pacific region from 2010 to 2013, it has been showed the highest rate of ESBL-producing *Enterobacteriaceae* in China (67%), followed by Thailand (50%) and Vietnam (48%) [6]. Studies in developed countries has reported a far lower rate of 2 - 4% [7]. Compliance with antibiotic stewardship might account for these differences, while high antibiotic use in Vietnam, especially of broad-spectrum cephalosporins, and inappropriate indications for antibiotic prescriptions might cause the high rates of resistance [8]. The CTX-M-type beta-lactamases play a major role as emerging and spreading resistance factors in *Enterobacteriaceae* [3]. In

the present study the rates of CTX-M (62.4%) were similar to other reports in Vietnam [9].

Currently, time-consuming phenotypic culture-based methods are the diagnostic standard. In contrast, genetic testing of antibiotic resistance can be completed in just hours and, thus, is an attractive alternative [10], especially in emergency situations such as sepsis and septic shock. All participants of this study were treated with antibiotics based on their resistance phenotype. We observed a higher percentage of shock and fatalities in the patients with CTX-M positive strains, although the strains were susceptible to cephalosporins. Although, the difference of shock and fatality was not significant with $p>0.05$, the length of hospital stay was significant longer in group with CTX-M positive than group with CTX-M negative with $p<0.05$. That can be explained in case of phenotypic susceptible strains, that carry genes encoding antibiotic resistance (such as CTX-M) to increase expression of resistant genes under pressure of antibiotic therapy. In that case the antibiotic therapy guided by phenotypic resistance may not be suitable and consequently the outcome was worst. Palmer et al [11] observed that fitness-expression functions of single-drug and multidrug resistant genes changed dramatically when expressed under antibiotic treatment and mutations of resistant genes can contribute to resistance by altering expression. While our results did not reveal significant differences of the rate of shock and fatal outcome due to a rather small number of participants, they allow to attribute an important role of genotype determination in the clinical settings to guide antibiotic therapy and comply with antibiotic stewardship.

This study is one of a few studies that focus on the role of genetic resistance, such as CTX-M on the outcome of BSI patients. Thus, it may suggest to use genetic resistance to guide empirical antibiotic therapy for patients with BSI. A limitation of the study is the lack of data on other genes encoding beta-lactamases.

5. Conclusion

Our data showed a high prevalence of ESBL producing strains causing BSI patients, and also high prevalence of CTX-M positive strains. Moreover, in BSIs caused by cephalosporin susceptible strains, the clinical outcome of group with CTX-M positive were worse than that of group with CTX-M negative.

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