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Antibiotic Resistance Profile and Diversity of Subtypes Genes in *Escherichia coli* Causing Bloodstream Infection in Northern Vietnam

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Keywords: Antibiotic resistance; Escherichia coli (E. coli); ESBL-producing; BSIs (bloodstream infections)

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Abstract

BACKGROUND: Evaluating the antibiotic susceptibility and resistance genes is essential in the clinical management of bloodstream infections (BSIs). But there are still limited studies in Northern Vietnam.

AIM: The aim of the study was to determine the antibiotic resistance profile and characteristics of subtypes genes in *Escherichia coli* causing BSIs in Northern Vietnam.

METHODS: The cross-sectional study was done in the period from December 2012 to June 2014 in two tertiary hospitals in Northern Vietnam. Tests were performed at the lab of the hospital.

RESULTS: In 56 *E. coli* strains isolating 39.29 % produced ESBL. 100% of the isolates harbored *blaTEM* gene, but none of them had the *blaPER* gene. The prevalence of ESBL producers and ESBL non-producers in *blaCTX-M* gene was 81.82%, and 73.53%, in *blaSHV* gene was 18.18% and 35.29%. Sequencing results showed three *blaTEM* subtypes (*blaTEM* 1, 79, 82), four *blaCTX-M* subtypes (*blaCTX-M*-15, 73, 98, 161), and eight *blaSHV* subtypes (*blaSHV* 5, 7, 12, 15, 24, 33, 57, 77). Antibiotic resistance was higher in ampicillin (85.71%), trimethoprim/sulfamethoxazole (64.29%) and cephazolin (50%). Antibiotics were still highly susceptible including doripenem (96.43%), ertapenem (94.64%), amikacin (96.43%), and cefepime (89.29%).

CONCLUSION: In *Escherichia coli* causing BSIs, antibiotic resistance was higher in ampicillin, trimethoprim/sulfamethoxazole and cephazolin. Antibiotics was highly susceptible including doripenem, ertapenem, amikacin, and cefepime.

Introduction

Escherichia coli (*E. coli*) took the highest position in causative gram-negative bacterium from bloodstream infection (BSIs) patients in Asia region [1]. It led to severe infections with a high rate of shock and mortality [2]. Currently, the worldwide incidence of *E. coli* BSI is still increasing over time [3] with the overall incidence increased year on year [4] that suggested an increasing burden of disease [5]. The estimation of infections worldwide showed that third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* caused 6.4 million (interval estimate 3.5-9.2) BSIs and 50.1 million (27.5-72.8) serious infections in 2014[6]. In addition, it was difficult to treat because of the emergence of multi-drug resistance (MDR) of *E. coli* [7]. Thus, evaluating antibiotic susceptibility is essential to decide what types of antibiotics and what appropriate doses that improving treatment efficiency and minimizing the antibiotic resistance rate. Over 20 years, the susceptibility of *E. Coli* BSIs was alarmed with the prevalence of antimicrobial-resistant isolates was increased [8]. In these cases, the patients had a worse prognosis with partial effect on correct empirical treatment [9]. Antimicrobial resistance-related encoding gene in each *E. coli* strain. Extended-spectrum β-lactamases

(ESBLs) was one of the most important genes [10]. It minimized the antibiotic efficiency in treatment [11]. Besides, the ability of inter-transmission within different *E. Coli* strains and transmission between *E. Coli* and other bacteria led to the state of becoming widespread resistance genes around the world. It becomes public-health concern [12] with increasing burden and cost of hospital-acquired infections [13], [14].

In Vietnam, there was one study in Northern Vietnam showed 25.1% of ESBLs among *Enterobacteriaceae* causing BSIs [15] but there are still limited studies in Northern Vietnam. Thus, this study aims to determine the antibiotic resistance profile and characteristics of subtypes genes in *Escherichia coli* causing bloodstream infections in Northern Vietnam.

Materials and Methods

The cross-sectional study was done in the period from 12/2012 to 6/2014 in two tertiary hospitals in Northern Vietnam (National Hospital of Tropical Diseases and 103 Military Hospital). Isolating from hospitalized BSIs patients in two hospitals 56 *E. coli* strains were inoculated in BHI Broth with 20% glycerol after being identified at the labs of these two hospitals.

Antimicrobial susceptibility assessed through MIC test by VITEK®2 Compact (BioMérieux, France and provided by DEKA *Limited Liability Company)* standardized by CLSI [16]. Antibiotics which has been used are *were (with number coding - abbreviation):* amikacin (1-AK), ampicillin (2-AM), ceftazidime (3-CAZ), ciprofloxacin (4-CIP), ceftriaxone (5-CRO), cefazolin (6-CZ), doripenem (7-DOR), ertapenem (8-ETP), cefepime (9-FEP), gentamycin (10-GM), levofloxacin (11-LVX), ampicillin/sulbactam (12-SAM), trimethoprim/sulfamethoxazole (13-SXT), tobramycin (14-TM) , piperacillin/tazobactam (15-TZP).

Using QIAamp DNA Mini Kit (USA) for DNA extraction (including isolation and quantification), we performed the experimental procedure according to manufacturer's instruction. PCR amplification performed in PCR master mix (Invitrogen – USA) that consisted of 200 µM of each dNTPs (dATP, dCTP, dGTP, dTTP), 100 pM primers, 1 U Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1,5 mM $MgCl_2$ and 10 µl DNA template. Specific primers for bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER} genes showed in Table 1. The experiments were performed using the protocol with 30 cycles that each of them consisted of 3 steps including denaturing (95°C for 30 seconds), annealing (58, 57, 60, 54°C for 30 seconds), elongating (72°C for 1 minute). PCR products were performed electrophoresis, imaged routinely and sequenced. The sequence of PCR products was compared with the original gene's sequence on GenBank to confirm bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{PER} gene.

Table 1: Specific primers for blaTEM, blaSHV, blaCTX-M, blaPER genes

Target gene	Primer	Nucleotide sequence (5' – 3')	Size (bp)	AT (°C)
bla_{TEM}	TEM-F	5' – TGC GGT ATT ATC CCG TGT TG – 3'	300	52.2
	TEM-R	5' – TCG TCG TTT GGT ATG GCT TC – 3'		
bla_{SHV}	SHV-F	5' – TCT CCC TGT TAG CCA CCC TG – 3'	600	51.2
	SHV-R	5' – CCA CTG CAG CAG CTG C – 3'		
bla_{CTX-M}	CTX-M-F	5' – CGA TGT GCA GTA CCA GTA A – 3'	650	60
	CTX-M-R	5' – TTA GTG ACC AGA ATC AGC GG – 3'		
bla_{PER}	PER-F	5' – ATG AAT GTC ATT ATA AAA GC – 3'	933	
	PER-R	5' – TTA ATT TGG GCT TAG GGC AGA A – 3'		

Statistical Analysis

The statistical analysis was conducted using the R language [17]. Graphics also were performed by R language (version 3.5.2). The analysis of such enormous volumes of information in the acquisition of data from 56 strains, each strain companion with subtype genes (three bla_{TEM} subtypes, four bla_{CTX-M} subtypes, eight bla_{SHV} subtypes) and 15 antibiotics with 3 level of resistance (susceptible, intermediate, resistance). For this reason, we used R language to analyze.

Results

Clinical characteristics of the patient in this study showed in Table 2.

Table 2: Clinical characteristics of patients

Age (subgroup)	
16-19	0 (0)
20-29	8 (14.29)
30-39	3 (5.36)
40-49	9 (16.07)
50-59	17 (30.35)
≥ 60	19 (33.93)
Gender	
Male	37 (66.07)
Female	19 (33.93)
History of medical condition	
Cirrhosis	13 (23.21)
Self-report alcoholism	10 (17.86)
Diabetes	8(14.29)
Hypertension	6 (10.71)
Long-term corticosteroid use	4 (7.14)
Renal failure	2 (3.57)
Pregnancy	2 (3.57)
Spinal cord injury	1 (1.79)
Urinary tract stone	1 (1.79)
Heart failure	1 (1.79)
Cancer	1 (1.79)
No	7 (12.5)
Time to hospitalization	
< 5	40 (71.43)
5-14	14 (25.00)
> 14	2 (3.57)

Among 56 *E. coli* strains isolated analyzed, 39.3% strains were identified as producing ESBL. Detail information of sequencing results showed in Table 3 highlighting three bla_{TEM} subtypes (bla_{TEM} 1, bla_{TEM} 79, bla_{TEM} 82), four bla_{CTX-M} subtypes (bla_{CTX-M}-15, bla_{CTX-M}-73, bla_{CTX-M}-98, bla_{CTX-M}-161), and eight bla_{SHV} subtypes (bla_{SHV}-5, bla_{SHV}-7, bla_{SHV}-12, bla_{SHV}-15, bla_{SH} -24, bla_{SHV}-33, bla_{SHV}-57, bla_{SHV}-77).

Table 3: ESBL-producing E. coli strains and ESBL encoding genes

Result	Number of strains (n = 56) Percentage (%)
ESBL-positive	22 (39.29 %)
ESBL-negative	34 (60.71 %)
bla$_{TEM}$	56
TEM-1	34
TEM-79	19
TEM-82	3
bla$_{CTX-M}$	43
CTX-M-15	12
CTX-M-73	11
CTX-M-98	17
CTX-M-161	3
bla$_{PER}$	0
bla$_{SHV}$	16
SHV-5	3
SHV-7	2
SHV-12	6
SHV-15	1
SHV-24	1
SHV-33	1
SHV-57	1
SHV-77	1
bla$_{TEM}$ + *bla*$_{CTX-M}$	32 (57.2 %)
bla$_{TEM}$ + *bla*$_{SHV}$	5 (8.9 %)
bla$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{CTX-M}$	11 (19.6 %)

The results of gene analysis revealed that 100% of isolates harbored *bla*$_{TEM}$ gene, but none of them had the *bla*$_{PER}$ gene (Table 4). The prevalence of *bla*$_{CTX-M}$ gene of overall strains, ESBL-producing, and non-ESBL-producing were 76.79%, 81.8%, and 73.5%, respectively. The prevalence of *bla*$_{SHV}$ gene among ESBL-producing and non-ESBL-producing strains were 18.2% and 35.3%. More information showed in Table 4.

Table 4: Encoding gene of ESBL subtypes

Gene	ESBL-positive (n = 22)				ESBL-negative (n = 34)			
	(+)		(-)		(+)		(-)	
	n	(%)	n	(%)	N	(%)	n	(%)
bla$_{PER}$			22	100			34	100
bla$_{TEM}$	22	100			34	100		
bla$_{TEM}$ + *bla*$_{CTX-M}$	18	81.82	4	18.18	25	73.53	9	26.47
bla$_{TEM}$ + *bla*$_{SHV}$	4	18.18	18	81.82	12	35.29	22	64.71
bla$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{CTX-M}$	3	13.64	19	86.36	8	23.53	26	76.47

Figure 1 showed a high prevalence of resistance to ampicillin (AM-85.7% of strains), trimethoprim/sulfamethoxazole (STX-64.3% of strains), cephazolin (CZ-50% of strains), ciprofloxacin (CP-35.7% of strains) and levofloxacin (LVX-35.7% of strains).

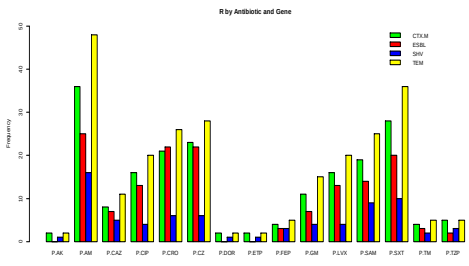

Figure 1: Antibiotic resistance profile; Antibiotics are 1 to 15 following 15 antibiotic have been coded Amikacin (1-AK); Ampicillin (2-AM); Ceftazidime (3-CAZ); Ciprofloxacin (4-CIP); Ceftriaxone (5-CRO); Cefazolin (6-CZ); Doripenem (7-DOR); Ertapenem (8-ETP); Cefepime (9-FEP); Gentamycin (10-GM); Levofloxacin (11-LVX); Ampicillin/Sulbactam (12-SAM); Trimethoprim/sulfamethoxazole (13-SXT); Tobramycin (14-TM); Piperacillin/Tazobactam (15-TZP)

Figure 2 showed highly active antibiotics such as doripenem (DOR-96.4% of strains), ertapenem (ETP-94.6% of strains), amikacin (AK-96.4% of strains), and cefepime (PEP-89.3% of strains). In each antibiotic, detail information of genes was shown.

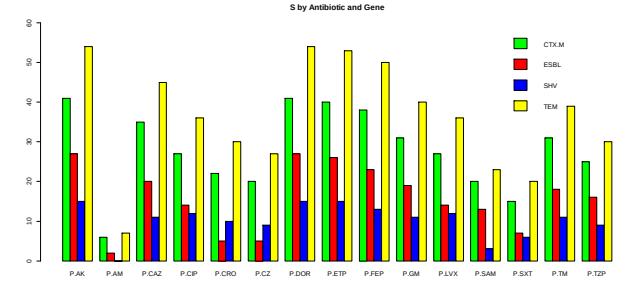

Figure 2: Antibiotic sensitivity profile; Antibiotics are 1 to 15 following 15 antibiotic have been coded Amikacin (1-AK); Ampicillin (2-AM); Ceftazidime (3-CAZ); Ciprofloxacin (4-CIP); Ceftriaxone (5-CRO); Cefazolin (6-CZ); Doripenem (7-DOR); Ertapenem (8-ETP); Cefepime (9-FEP); Gentamycin (10-GM); Levofloxacin (11-LVX); Ampicillin/Sulbactam (12-SAM); Trimethoprim/sulfamethoxazole (13-SXT); Tobramycin (14-TM); Piperacillin/Tazobactam (15-TZP)

Figure 3 showed that in patients who carried gene had high rate of antibiotic resistance with the main antibiotics were ceftazidime (3-CAZ), cefazolin (6-CZ), doripenem (7-DOR), gentamycin (10-GM), levofloxacin (11-LVX), ampicillin/sulbactam (12-SAM), trimethoprim/sulfamethoxazole (13-SXT) in line with *bla*$_{CTX-M}$ gene with the same allocation. Tobramycin (14-TM) with intermediate response had *bla*$_{SHV}$ and *bla*$_{TEM}$ as main genes. Figure 3 supported Figures 1 and 2 to visualize the association between gene and antibiotic resistance.

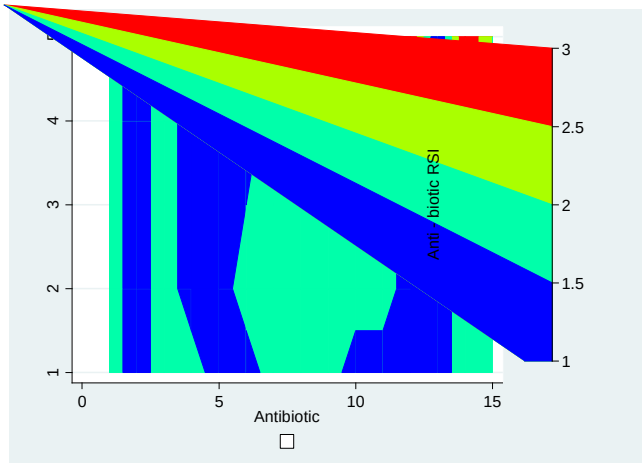

Figure 3: The antibiotic resistance level with genes. Gen antibiotic is 1 to 5 following CTX-M gene, ESBL gene, PER gene, SHV gene and TEM gene; Anti – biotic RSI is 1 to 3 following R (resistance), S (sensitive), I (intermediate); Antibiotics are 1 to 15 following 15 antibiotic have been coded Amikacin (1-AK), Ampicillin (2-AM), Ceftazidime (3-CAZ), Ciprofloxacin (4-CIP), Ceftriaxone (5-CRO), Cefazolin (6-CZ), Doripenem (7-DOR), Ertapenem (8-ETP), Cefepime (9-FEP), Gentamycin (10-GM), Levofloxacin (11-LVX), Ampicillin/Sulbactam (12-SAM), Trimethoprim/sulfamethoxazole (13-SXT), Tobramycin (14-TM) , Piperacillin/Tazobactam (15-TZP)

Table 5 clarified the detail of antibiotic resistance with the ESBL gene. While ESBL-positive strains were highly resistant to ampicillin (AM), ceftriaxone (CRO), cephazolin (CZ), and trimethoprim/sulfamethoxazole (SXT) at the rate of 100%, 100%, 100%, and 81.8%, respectively, ESBL-negative strains had a lower prevalence of resistance to these agents at the rate of 76.5%, 11.8%, 17.7%, and 53%, respectively. Both groups were susceptible to doripenem (DOR), ertapenem (ETP), and amikacin (AK) at the rate of more than 90%.

Table 5: The antibiotic resistance profile of ESBL subtype

Antimicrobial Agents	ESBL-positive (n = 22)			ESBL-negative (n = 34)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicilin			22 (100)	7 (20.59)	1 (2.94)	26 (76.47)
Ceftriaxone			22 (100)	30 (84.24)		4 (11.76)
Cephazolin			22 (100)	27 (79.41)	1 (2.94)	6 (17.65)
Trimethoprim/ sulfamethoxazole	4 (18.18)		18 (81.82)	16 (47.06)		18 (52.94)
Ampicilin/sulbactam	10 (45.45)		12 (54.55)	13 (38.24)	8 (23.53)	13 (38.24)
Ciprofloxacin	11 (50)		11 (50)	25 (73.53)		9 (26.47)
Levofloxacin	11 (50)		11 (50)	25 (73.53)		9 (26.47)
Piperacilline/ Tazobactam	11 (73.33)	2 (13.33)	2 (13.33)	19 (82.61)	1 (4.35)	3 (13.04)
Ceftazidime	15 (68.18)		7 (31.82)	30 (88.24)		4 (11.76)
Cefepime	18 (81.82)	1 (4.55)	3 (13.64)	22 (94.12)		2 (5.88)
Doripenem	22 (100)			32 (94.12)		2 (5.88)
Ertapenem	21 (95.45)	1 (4.55)	1 (4.55)	32 (94.12)		2 (5.88)
Amikacin	22 (100)			32 (94.12)		2 (5.88)
Gentamycin	15 (68.18)		7 (31.82)	25 (73.53)	1 (2.94)	8 (23.53)
Tobramycin	14 (63.64)	5 (22.73)	3 (13.64)	25 (73.53)	7 (20.59)	2 (5.88)

Discussion

ESBL-producing *E. coli* is common genotypes and its incidence varies from region to region. ESBLs are typically inhibitor-susceptible B-lactamases that are encoded by mobile genes with the bla_{CTX-M}, bla_{SHV}, and bla_{TEM} families were the most frequently. In our study, among 56 *E. coli* strains have been analyzed, 39.3% strains were identified as ESBL-producing. Our finding is higher than that of study in Northern, Vietnam (25.1% of strains produced ESBL among *Enterobacteriaceae*) [15]. Comparing with other countries, it is higher than Singapore (33%) [18], Chile (23.8%) and Brazil (12.8%) but lower than that of India (60%), Hong Kong (48%) [18] Mexico (48.4%) [19]. All cases with ESBL-producing *E. Coli* had bla_{TEM} gene and the 100% resistance to ampicillin was found that in line with the present study [20].

Our results about bla_{CTX-M} gene also corroborated another study that reported bla_{CTX-M} bla (beta-lactamase) gene was common in all the ESBL isolates [21]. This result is also in agreement with study Gurntke et al., of that among 19% ESBL-positive cases, bla_{CTX-M}-15 was the most common genotypes (60%), followed by bla_{SHV}-5 (27%) [22]. Other studies showed the same results with bla_{CTX-M}-14 (48% of the isolates) were the most frequent ESBL [11], [23]. It was observed that the predominant of subtypes bla_{CTX-M} gene was diverse from study to study. Analyzing 552 isolates from BSIs that resistance to third-generation cephalosporin showed more detail with bla_{CTX-M}-15 (50%), bla_{CTX-M}-14 (14%), bla_{CTX-M}-27 (11%) and bla_{CTX-M}-101 (5%) [24].

ESBL-producing *E. Coli* in BSIs have been shown a substantial increase in the 21st century [25]. Besides that, its burden was growing worldwide [26]. Finding the appropriate therapy became crucial and carbapenems emerged as 'best therapy' for ESBL-producing bacteria [25]. But in the time of antibiotics and resistance becoming popular, *E. Coli* also starts resistance to carbapenem that leading a high financial burden and increased mortality [27].

The knowledge of antibiotic resistance profile is key in clinical practice. The high rate of resistance to some routine antibiotic agents which were commonly used in most hospitals in our area was provided in this study. The results also showed that amikacin and carbapenems (doripenem and ertapenem) emerged as choices for empiric therapy instead. Sinha et al., showed similar findings with high prevalence of ESBL-positive, high rate of resistance to ampicillin (86%), ceftriaxone (80.6%), and fluoroquinolones (80%) and the clear choice for empirical treatment were carbapenems in these cases [21].

Knowing the risk factors of antibiotic resistance is crucial for management strategy. The time before hospitalization was an only independent risk factor among ESBL in BSIs [28] while previous use of oxyimino-beta-lactams was the only modifiable risk factor among nosocomial BSIs [11]. In our study, ESBL encoding genes showed high correlation with antibiotic resistance.

While ESBL-positive strains were highly resistant to ampicillin, ceftriaxone, cephazolin, and trimethoprim/sulfamethoxazole at the rate of 100%, 100%, 100%, and 81.8%, respectively, ESBL-negative strains showed a lower prevalence of resistance to these agents at the rate of 76.5%, 11.8%, 17.7%, and 53%, respectively. Both groups were susceptible to doripenem, ertapenem, and amikacin at the rate of more than 90%. This finding was similar to the study in Finland from 1999 to 2013 that showed most (88%) of the isolates reported as non-susceptible to third-generation cephalosporins had ESBL phenotype [29].

In conclusion, in *Escherichia coli* causing bloodstream infections, antibiotic resistance was higher in ampicillin, trimethoprim/sulfamethoxazole and cephazolin Antibiotics was highly susceptible including doripenem, ertapenem, amikacin, and cefepime.

Ethical approval

This study is approved by the ethics committee of National Hospital of Tropical Diseases and Military Hospital 103.

Ethical considerations

The protocol was approved by the Ethics Committee of both National Hospital of Tropical Diseases and 103 Military Hospital. The study was in line with the Declaration of Helsinki. Written informed consent has been provided to all participants with full explanation. After that, the blood samples were collected.

Informed consent

The consent and commitment were signed by the patients in the study.

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