Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2018.045

Antibiotic resistance and molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from fish pond

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Article info.

Received 28 Dec 2017
Revised 04 Jun 2018
Accepted 30 Nov 2018

Keywords

ESBL-producing Escherichia coli, extended-spectrum beta-lactamase genes, fish, the Mekong Delta, sul genes

ABSTRACT

Recently, extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli was isolated from cultured striped catfish, red tilapia and wild fish in the Mekong Delta, Vietnam. ESBL genes are located on plasmids, facilitating their spreads among Gram negative bacilli bacterial species. To better understand the dissemination of resistance genes in aquatic system, the antimicrobial susceptibility patterns and the molecular characteristics of ESBL-producing E. coli isolates were investigated using disk diffusion method and polymerase chain reaction. The results indicated that the proportion of antibiotic resistance of ESBL-producing E. coli was relatively high in most types of antibiotics except meropenem and cefoxitin. Considerably, multiple drugs resistance was recorded at high percentage, including 100% for ESBL-producing E. coli isolates of snakehead fish, 90% depended on the figure for striped catfish, 85% for ESBL-producing E. coli isolates of red tilapia, and 50% for that of wild fish. Besides, the number of ESBL-producing E. coli isolates carrying multiple ESBL genes were 90%, significantly higher than those of carrying single ESBL gene at just 10%. The B2 virulence group was mainly isolated from wild fish, which was higher compared to groups of culture fish. Moreover, the majority of isolates harbored multiple sulfonamides resistance genes (72.2%), which was significant higher compared to the percentages of isolates carrying single gene (27.8%). The study illustrated that there were the significant widespread of antibiotic resistant genes of ESBL-producing E. coli as well as a considerable ratio of multidrug resistance.

Cited as: Ly, T.H., Tuyet Hoa, T.T.T. and Huyen, H.M., 2018. Antibiotic resistance and molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from fish pond. Can Tho University Journal of Science. 54(8): 114-123.

1 INTRODUCTION

Enhancing production and increasing stocking density caused the fish more susceptible to diseases (Shoemaker *et al*., 2000), leading to the misuse and overuse of antibiotic in treatment of fish diseases. The regular use of antibiotics to treat diseases, especially the extended-spectrum beta-lactam antibiotic group, led to the increasing of antibiotic resistant bacteria capable of causing harm in human health (Carneiro *et al*., 2007), including *Escherichia coli.*

E. coli infection is innocuous to fish; however, it makes a significant influence on the products quality of fish due to its possibility of causing diseases in humans. The worst type of *E. coli*, known as *E.*

coli O157:H7, caused bloody diarrhea, sometimes kidney failure, and even death (Krystle and Alison, 2011). Besides, *E. coli* is capable of hydrolyzing the antibiotics of beta-lactam group based on the mechanism of releasing extended-spectrum beta-lactamase (ESBL). In which, the most common enzymes were TEM (temoniera), CTX-M (cefotaxime - Munich), SHV (sulfhydryl variable) which were encoded by the corresponding genes: bla_{TEM}, bla_{CTX-M}, bla_{SHV}. These genes are located on the plasmids; therefore, they are able to be transferred among different bacterial species (Huovinen, 2001; John, 2010).

Multiple resistance ability of *E. coli* carrying sulfonamide resistant genes to various antibiotic was more popular (Mitsuhashi, 1971). Considerably, resistance to sulfonamide antibiotics of *E. coli* had a common status due to the presence of *sul1, sul2* and *sul3* genes, which encodes dihydropteroate synthase (DHPS) to inactivate sulfonamide activity (Enne *et al*., 2001). *Sul1* gene was found on the conjugative plasmid and on the integron group 1 (Rådström *et al*., 1991; Antunes *et al*., 2005; Hammerum *et al*., 2006; Trobos *et al*., 2008). *Sul2* gene is considered previously to lie on the non-conjugative plasmid. Additionally, the *sul2* gene was also found on a variety of conjugative plasmids (Antunes *et al*., 2005; Bean *et al*., 2009) and related to the streptomycin resistance rate (Hammerum *et al*., 2006). *Sul3* gene was first described in pigs in Switzerland in 2003 and subsequently also found in humans and animals in many countries around the world (Antune *et al*., 2007). The *sul* genes had an ability to transfer from living organism via integrons, transposons or plasmids to more harmful bacteria in the human gut (Guerra *et al*., 2004). In addition, the presence of *sul* genes was unevenly distributed among bacterial populations (Kerrn *et al*., 2002; Antunes *et al*., 2005; Hammerum *et al*., 2006). Hammerum *et al*. (2006) showed that *E. coli* resistant to sulfonamide isolated from human, pork and pig manure had the presence of *sul1, sul2* and *sul3* genes. Of the 998 strains isolated from *E. coli*, 18% were isolated from humans, 20% from pork, and 26% from pig manure resistant to sulfonamide. This study was aimed to determine the antimicrobial susceptibility patterns and the genotype characteristics of ESBL-producing *E. coli* isolated from the cultured and wild fish in the Mekong Delta.

2 METHODS

2.1 Sources of bacteria

A total of 30 ESBL-producing *E. coli* isolates were recovered from the glycerol stock. These isolates were obtained from farmed fish (striped catfish and red tilapia) and wild fish (unidentified species) in the Mekong Delta from 2015 to 2016 from the project of Satreps. In addition, another 10 ESBL-producing *E. coli* isolates were also isolated from 2 snakehead fish farms in Dong Thap in 2017.

2.2 Isolation of *E. coli*

E. coli bacteria was isolated on chromagar ECC containing 1 µg cefotaxime (CTX) antibiotics and incubated in 37°C within 24 hours. Then 3 colonies of *E. coli* bacteria showed the blue color were transferred to a new chromagar ECC medium.

2.3 Antibiotic susceptibility test

Antibiotic susceptibility was tested with the Kirby Bauer disk diffusion method on Mueller-Hinton agar plates (Merck, Germany), according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2012). Combination antibiotic therapy, cephalosporin plus clavulanic acid was used to detect ESBL-producing *E. coli* and used cefoxitin antibiotic disk to detect AmpC. Antibiotic sensitivity method involves the following steps: (i) using sterile inoculating loop takes 4 to 5 colonies on overnight plates to dissolve into 2 mL sterile saline water and mix evenly with vortex mixer; (ii) evenly distributed on MHA surface; (iii) use thin wafers containing antibiotic placed on the MHA agar surface, including cefoxitin (FOX) 30 µg, cefotaxime (CTX) 30 µg, cefotaxime + clavulanic acid (CA) 30 µg + 10 µg, ceftazidime CAZ) 30 µg and ceftazidime + clavulanic acid 30 µg + 10 µg. Each antibiotic disc is 30 mm apart. After that, incubate at 37°C for 16-18h; (iv) Measure the zone of inhibition diameter of three antibiotic wafers FOX, CTX, and CAZ to determine the sensitivity or resistance of bacteria to antibiotics.

A total of thirteen antibiotics (Becton Dickinson, UK) were tested, including ampicillin (AMP, 10 µg), cefoxitin (FOX, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), meropenem (MEM, 10 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 50 µg), kanamycin (K, 30 µg), streptomycin (S, 10 µg), gentamicin (GM, 10 µg), tetracycline (TE, 30 µg), chloramphenicol (C, 30 µg), and trimethoprim-sulfamethoxazole (SXT, 1.25/23.7 µg).

2.4 PCR methods for detection of ESBL genes, phylogenetic group and sulfonamide resistance genes

Genomic DNA was extracted from the isolates as template for PCR assays by boiling method (Alexopoulou *et al*., 2006). In brief, a few of bacterial colonies was suspended in 500 µL TE buffer

(pH=8.0), boiled at 100ºC for 5 minutes, and centrifuged the tube at 10,000 rpm for 1 minute. The supernatant was stored at – 20ºC for further PCR analysis.

E. coli strain has been identified as presence of specific genes run as positive control. Multiplex PCR was carried out using specific primers for amplification of genes encoding enzymes: TEM, SHV and CTX-M (Monstein *et al*., 2009). The 25 µM reaction mixture contained distilled water, 2X PCR Master Mix, 1X Q-Solution, 1X Coral Load Dye, 0.2 µM Primer mix "ESBL multi v6" and extracted DNA (1 ng). The PCR conditions consisted of initial denaturation at 95ºC for 5 minutes followed by 25 cycles of 95ºC for 30 seconds, 60ºC for 90 seconds, 72ºC for 90 seconds and a final extension step at 68ºC for 10 minutes.

Table 1: List of PCR primers for ESBL gene analysis (Pitout and Laupland., 2004)

Primers	Sequence	Products
TEM	GGTCGCCGCATACACTATTCTC TTTATCCGCCTCCATCCAGTC	(372 bp)
SHV	CCAGCAGGATCTGGTGGACTAC CCGGGAAGCGCCTCAT	(231 bp)
CTX-M-1	GAATTAGAGCGGCAGTCGGG CACAACCCAGGAAGCAGGC	(588 bp)
CTX-M-2	GATGGCGACGCTACCCC CAAGCCGACCTCCCGAAC	(107 bp)
CTX-M-9	GTGCAACGGATGATGGTTCGC GAAACGTCTCATCGCCCATC	(475 bp)
CTX-M-8/25	GCGACCCGCGCGATAC TGCCGGTTTTATCCCCG	(186 bp)

E. coli strain has been identified as presence of specific genes run as positive control. Phylogenetic group was identified by PCR method (Clermont *et al.*, 2000). The 19 µM reaction mixture contains distilled water, 1X Ex Taq Buffer, 2 µM dNTP Mixture, 20 pmol of primer mix, 2.5 U Takara Ex Taq and extracted DNA (1 ng/µL). The PCR conditions consist of initial denaturation at 98ºC for 5 minutes followed by 35 cycles of 98ºC for 10 seconds, 57ºC for 30 seconds, 72ºC for 30 seconds and a final extension step at 72ºC for 7 minutes.

Table 2: List of PCR primers for phylogenetic analysis (Clermont *et al.*, 2000)

Primers	Sequence	Products
ChuA.1 ChuA.2	5'-GACGAACCAACGGTCAGGAT-3' 5'-TGCCGCCAGTACCAAAGACA-3'	279 bp
YjaA.1 YjaA.2	5'-TGAAGTGTCAGGAGACGCTG-3' 5'-ATGGAGAATGCGTTCCTCAA-3'	211 bp
TspE4C2.1 TspE4C2.2	5'-GAGTAATGTCGGGGCATTCA-3' 5'-CGCGCCAACAAAGTATTACG-3'	152 bp

E. coli strain has been identified as presence of specific genes run as positive control. Multiplex PCR was performed to detect the sulfonamide resistance genes, including *sul*1, *sul*2, *sul*3 (Kerrn *et al*., 2002). The PCR assays were carried out in a 25 µl reaction mixture, which included 3µl of template DNA (1 ng), 1X PCR buffer, 0.3µM dNTPs, 1.5 U Taq polymerase and 0.1µM each primer. The PCR conditions for detection of *sul* genes contained initial denaturing at 94ºC for 5 minutes, followed by 30 cycles of 94ºC for 30 seconds, 65ºC for 30 seconds, 72ºC for 60seconds, and a final extension step at 72ºC for 10 minutes.

Table 3: List of PCR primers for *sul1*, *sul2* và *sul3* genes analysis

Genes	Primers	Sequences	Products
Sul1	Sul1-F Sul1-R	5′-CGGCGTGGGCTACCTGAACG-3′ 5′-GCCGATCGCGTGAAGTTCCG-3'	433 bp (Kerrn *et al*., 2002)
Sul2	Sul2-F Sul2-R	5′-GCGCTCAAGGCAGATGGCATT-3′ 5′-GCGTTTGATACCGGCACCCGT-3′	293 bp (Kerrn *et al*., 2002)
Sul3	Sul3-F Sul3-R	5′-TCAAAGCAAAATGATATGAGC-3′ 5′-TTTCAAGGCATCTGATAAAGAC-3′	787 bp(Heuer and Smalla, 2007)

PCR products were analyzed by 2% agarose gel electrophoresis in 0.5X TAE buffer, and stained with ethidium bromide. DNA ladder 100bp were employed as a size marker.

2.5 Statistical analysis

The collected data were analyzed by chi-square test at $p<0.05$ for the significant level using SPSS16.0 software.

3 RESULTS

3.1 Susceptibility of ESBL-producing *E. coli* to antimicrobial agents

Antibiotic resistance pattern of the isolated ESBL-producing *E. coli* is presented in Table 4. The proportion of antibiotic resistance of ESBL-producing *E. coli* was relatively high in most type of antibiotics except MEM and FOX. In detail, MEM could not inhibit the bacteria, whereas there were two groups of fish resistant to FOX, with wild fish at 16.7% and snakehead fish at 20% of ESBL-producing *E. coli* isolates. Three types of antibiotics (ampicillin, cefotaxime and tetracycline) were found with the highest resistant frequencies up to 100%.

Moreover, the ESBL-producing *E. coli* isolated from wild fish and snakehead fish showed the most resistant ability to antibiotic groups in comparison with the isolates from striped catfish and red tilapia. In which, the majority of isolates from wild fish and snakehead fish were resistant to GM, CIP, NA and SXT at highest percentage of 100% with much higher than that of other species. In addition, all of isolates from wild fish (100%) resisted to S. Similarly, total isolates from snakehead fish (100%) completely resisted to ceftazidime, kanamycin and chloramphenicol, much higher than others ($p<0.001$).

Table 4: Percentage of ESBL-producing E. coli isolated from intestine of fish species exhibited resistance to antimicrobial agents

% resistance to		Bacteria isolated from fish species				
		Striped – catfish	Red – tilapia	Wild fish	Snakehead – fish	*p*
Beta – lactam	AMP	100	100	100	100	
	FOX	0	0	16.7	20	
	CTX	100	100	100	100	
	CAZ	70	64.3	50	100	<0.001
	MEM	0	0	0	0	
Aminoglycosides	S	40	92.9	100	80	<0.001
	K	50	78.6	50	100	<0.001
	GM	90	92.9	100	100	
Quinolones	CIP	90	92.9	100	100	
	NA	90	92.9	100	100	
Tetracycline	TE	100	100	100	100	
Phenicol	C	90	85.7	50	100	<0.005
Folic acid inhibitors	SXT	90	92.9	100	100	

AMP: ampicillin; FOX: cefoxitin; CTX: cefotaxime; CAZ: ceftazidime; MEM: meropenem; NA: nalidixic acid; CIP: ciprofloxacin; K: kanamycin; S: streptomycin; GM: gentamicin; TE: tetracycline; C: chloramphenicol; SXT: trimethoprim-sulfamethoxazole

The majority of the tested antibiotics in this research showed resistant higher frequency than in the other studies, such as CTX, GM, CIP, NA, TE, SXT, S, K and C (Su *et al*., 2012; Le *et al*., 2015; Nasreldin and Khaldoon, 2015). In particular, the average rate of ESBL-producing *E. coli* in this report was resistant to SXT at 95.73%, which had five times higher than that rate of *E. coli* isolated from fish (19%) (Le *et al*., 2015). The results indicated that there were increases the development of antibiotic resistant frequency in bacteria, including ESBL-producing *E. coli* isolated from fish.

Considerably, the frequency of multiple resistances (resistance to all six tested antibiotic groups) was high up to 90% (9/10), 85% (12/14) and 50% (3/6) for ESBL-producing *E. coli* isolates of striped catfish, red tilapia, and wild fish, respectively. Whereas, the frequency of multiple resistances was found at highest rate of 100% for ESBL-producing *E. coli* isolates from snakehead fish (Figure 1).

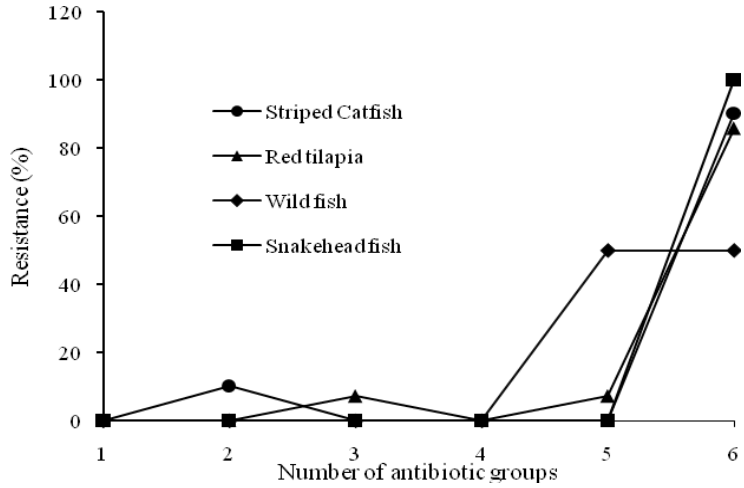

Fig. 1: Degree of MDR ESBL-*E. coli* isolated

This study gave information that all ESBL-producing *E. coli* isolated from different farming fish species were resistant to two or more type of antibiotics (100%). In particular, the extensively multidrug resistance phenotype of ESBL-producing *E. coli* (95.71%) was significantly higher than the obtained results in recent studies, e.g. 61 % for fishery products (Van *et al*., 2007a, 2007b; Le *et al*. 2015), 93.33% for mackerel (Nasreldin and Khaldoon, 2015). Moreover, the multidrug resistant strains were capable to transfer to human through the food chains (Heuer *et al*., 2009).

3.2 Molecular characterizations of ESBL-producing *E. coli*

The genotypes of ESBL-producing *E. coli* isolated from fish were shown in Table 5. The number of ESBL-producing *E. coli* isolates carrying multiple ESBL genes were 90% (36/40), with higher than those of carrying single ESBL gene at 10% (4/40). All of ESBL-producing *E. coli* isolated from wild fish, red tilapia and snakehead fish were harbored multiple ESBL genes. By contrast, there was 40% of ESBL-producing *E. coli* isolated from striped catfish were carried the single ESBL genes. *Bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ with band amplicon size from 107 bp to 588 bp were indicated in Figure 2.

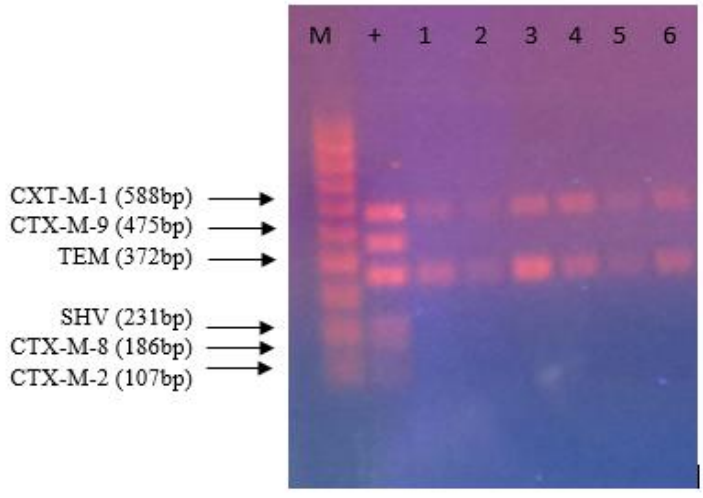

Fig. 2: Representative agarose gel electrophoregram of PCR products of *E. coli* carry ESBL gene

*Lane M: DNA marker; lane +: positive control (carry **bla$_{\text{CTX-M-1}}$, bla$_{\text{CTX-M-9}}$, bla$_{\text{CTX-M-8}}$, bla$_{\text{CTX-M-2}}$, bla$_{\text{SHV}}$,** and **bla$_{\text{TEM}}$** genes); lane 1, 2, 3, 4, 5, 6, 7: bla$_{\text{CTX-M-1}}$ and bla$_{\text{TEM}}$.*

Table 5: Prevalence of multiple ESBL-encoding genes ESBL-producing E. coli isolated from the fish samples

ESBL genotypes	Total	Bacteria isolated from fish species			
Multi-ESBL genes		**Striped catfish**	**Red tilapia**	**Wild fish**	**Snakehead fish**
CTX-M-1/CTX-M-9/TEM	0	0	0	0	0
CTX-M-9/TEM	7 (17.50%)	0	4 (28.57%)	3 (50%)	0
CTX-M-1/CTX-M-9	0	6 (60%)* 0	14 (100%) 0	6 (100%) 0	10 (100%) 0
CTX-M-1/TEM	29 (72.5%)	6 (60%)	10 (71.43%)	3 (50%)	10 (100%)
Single-ESBL genes					
CTX-M-1	3 (7.50%)	3 (30%)	0	0	0
CTX-M-9	0	0	0	0	0
TEM	1 (2.5%)	4 (40%) 1 (10%)	0 0	0 0	0 0
SHV	0	0	0	0	0
CRX-M-8/25	0	0	0	0	0
CTX-M-2	0	0	0	0	0
Total	40	10	14	6	10

**p<0.001 significantly different from other species*

Regarding to the differences among the group harbored single ESBL genes, the proportion of ESBL-producing *E. coli* strains carrying the CTX-M group was 7.5%, relatively higher than that of TEM group at 2.5%. In the group that harbored multiple ESBL genes, the CTX-M and TEM genotype were accounted for up to 90% of ESBL-producing *E. coli* isolates. Furthermore, the majority of ESBL-producing *E. coli* (72.5%) were encoded for the genotypes CTX-M-1 and TEM, significantly higher than the genotypes CTX-M-9 and TEM (17.5%). These findings demonstrated that bla_{TEM} and bla_{CTX-M} genes were the two dominant types in ESBL-producing *E. coli* isolated from fish.

These results were generally in accordance with the results obtained by Cao *et al.* (2002) who reported that the bla_{TEM} and bla_{CTX-M} genes were commonly found in different hosts and in different regions in the world. Besides, according to Asma (2006), CTX-M genotype of β-lactamases was the most frequent type of ESBL-producing strains worldwide. They were predominant in South America, the Far East and Eastern Europe, China, Japan, India, North America and Western Europe. Moreover, Nasreldin and Khaldoon (2015) reported that mackerel fish had 82% of ESBL-producing *E. coli* strains carrying CTX-M groups.

The recent investigation provides that the group of ESBL-producing *E. coli* isolated from fish harbored multiple ESBL genes with the frequency of 50%, which was equal to the group carried single ESBL genes (Le *et al*., 2015). In this research, there was a significant difference in the frequency: the group ESBL-producing *E. coli* harbored multiple ESBL genes (90%), much higher than the group carried single ESBL gene (10%). Host species and geographical areas were the factors influenced on the differences between multiple and single genes.

Phylogenetic analysis of ESBL- producing *E. coli* varied significantly among fish species ($p < 0.001$) (Table 6). ChuA, YjaA gene and clone TSPE4.C2 were identified upon the amplicon sizes of 279bp, 211bp and 152bp, respectively. The different profiles obtained by PCR for the phylogenetic groups are shown in Figure 3. The results demonstrated that phylogenetic group A and B1 were found in totally 4 fish groups whereas group D was observed only in wild fish at just 16.67% (1/6). In particular, the ESBL-producing *E. coli* identified as the group of B2, which was capable of infecting humans and causing disease for the gastrointestinal tracts (Jakobsen *et al*., 2010) was detected in wild fish (3/6) and red tilapia (2/14).

Table 6: Phylogenetic groups of ESBL-producing E. coli

Bacteria isolated fish species	Total	Phylogenetic group			
		A	B1	B2	D
Striped catfish	10	3 (30 %)	7 (70 %)	0	0
Red tilapia	14	4 (28.57 %)	8 (57.14 %)	2 (14.29 %)	0
Wild fish	6	1 (16.67 %)	1 (16.67 %)	3 (50 %)	1 (16.67 %)
Snakehead fish	10	8 (80 %)	2 (20 %)	0	0
P		<0.001	<0.001	<0.001	

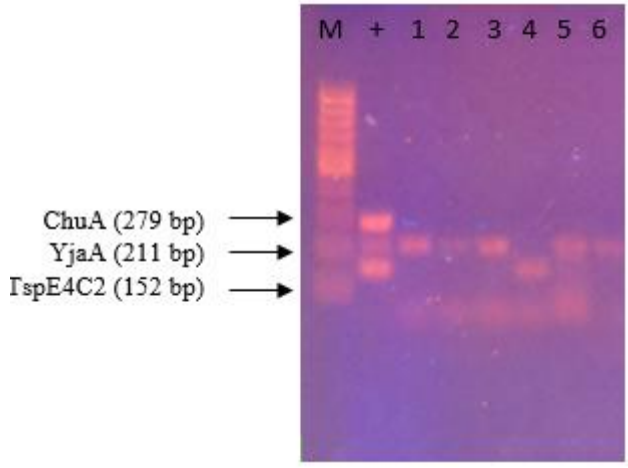

Fig. 3: Representative agarose gel electrophoregram of PCR products of phylogenetic groups LaneM: DNA marker; lane +: positive control; lane 1, 2, 3, 5 and 6: group A; lane 4,5: group B1

The most virulence genes were defined for phylogenetic groups B2 and D (Lillo *et al*., 2014), especially B2 was virulent for human (Jakobsen *et al*. 2010). Additionally, a total of ESBL-producing *E. coli* isolated from fish carrying B2 group were also harbored 2 sulfonamide resistance genes (*sul1* and *sul2*), and were resistant to at least eight types of antibiotics (Table 7). It is noticeable that the finding should be considered to reduce the risk of fish consumption for human.

Table 4: Data relate to phylogenetic groups B2 of ESBL-producing E. coli

Isolated species	Strain code	Phylogenetic groups	Sulfonamide genotype	Antibiotic resistances
Red tilapia	AGH.II.2.2.1	B2	*sul1* + *sul2*	CTX, CAZ, C, AMP, GM, S, TE, SXT, NA,CIP, K
	AGH.II.2.2.2	B2	*sul1* + *sul2*	CTX, CAZ, C, AMP, GM, S, TE, SXT, NA,CIP, K
Wild fish	ĐTS.I.1.1.1	B2	*sul1* + *sul2*	CTX, AMP, GM, S, TE, SXT, NA,CIP
	ĐTS.I.1.1.2	B2	*sul1* + *sul2*	CTX, AMP, GM, S, TE, SXT, NA,CIP, K
	ĐTS.I.1.1.3	B2	*sul1* + *sul2*	CTX, AMP, GM, S, TE, SXT, NA,CIP

The profile of ESBL-producing *E. coli* carrying sulfonamide resistance genes is shown in Table 8. The *sul1*, *sul2*, *sul3* genes were determined by PCR (Figure 4). The majority of ESBL-producing *E. coli* that was isolated from fish carried multiple sulfonamide resistance genes at 72.2% (26/37), significantly higher than the group carried single gene 27.8% (11/37). In particular, the considerable difference was recorded in the bacteria isolate from the group of red tilapia (78.57%) and snakehead fish (80%), followed by that of the group of striped catfish (57.14%) and wild fish (50%).

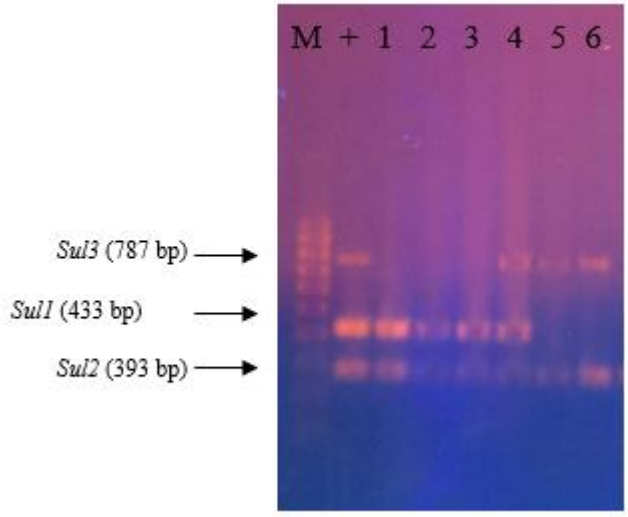

Fig. 4: Representative agarose gel electrophoregram of PCR products of sulfonamide genes Lane M: DNA marker; lane +: positive control; lane 1, 2, 3: *sul1* and *sul1*; lane 4: *sul1*, *sul2*, and *sul3* genes lane 5, 6: *sul2* and *sul3* genes

Table 8: Prevalence of multiple sulfonamide resistant genes in ESBL-producing E. coli isolated from the fish samples

Sulfonamide genotypes	Total	Bacteria isolated from fish species			
		Striped catfish	Red tilapia	Wild fish	Snakehead fish
Multi Sulfonamide genes					
sul1 + sul2	11	2	3	3	3
sul1 + sul3	1	4 (57.14%)[4] 0	11 (78.57%)[3] 1	3 (50%)[1] 0	8 (80%)[2] 0
sul2 + sul3	7	0	3	0	4
sul1 + sul2 + sul3	7	2	4	0	1
Single-Sulfonamide genes					
sul1	0	0	0	0	0
sul2	9	3 (42.86%)[d] 2	3 (22.34%)[c] 3	3 (50%)[a] 3	2 (20%)[b] 1
sul3	2	1	0	0	1
Total	37	7	14	6	10

1, 2, 3, 4p < 0.05, significantly different from the other species

a, b, c, d p < 0.001, significantly different from the other species

Regarding to the sulfonamide resistance genes, the majority of the ESBL-producing *E. coli* isolates harbored *sul1* and *sul2* genes 30.56% (11/37) which was higher than that figure for the group carrying *sul2* and *sul3* and the group carrying *sul1*, *sul2* and *sul3* 19.44% (7/37). Interestingly, the *sul2* gene was the most commonly found among these genes. The *sul2* was accounted for 9/11 cases in the single sulfonamide resistant genes and 19/26 cases for the multiple sulfonamide resistance genes. In addition, the *sul1* gene was not detected in single resistance gene as a whole.

This study showed that the prevalence of ESBL-producing *E. coli* carrying sulfonamide resistant genes was in order of *sul2*>*sul1*>*sul3* at 94.44%, 52,78% and 44.44%, respectively. The finding was similar to most previous reports (Trobos *et al*., 2008; Byrne-Bailey *et al*., 2009; Wu *et al*., 2010). Moreover, Wu *et al*. (2010) recorded that the proportion of *E. coli* that was isolated from human and animal in Denmark contained *sul1* (65%), *sul2* (45%) and *sul3* (12%). Moreover, the *sul2* gene was the most popular, which was consistent with a number of studies in Vietnam and in other countries (Enne *et al*., 2002; Blahna *et al*., 2006; Frank

et al., 2007). In Denmark, the *sul2* was found, higher than *sul1* among *E. coli* isolated from humans (Trobos *et al*., 2008). The *sul2* genes were also reported at high rates in the group of *E. coli* isolated from pigs, poultry, cattle, human feces, and urinary tract infections (Trobos *et al*., 2009). On the other hand, Arabi *el al*. (2015) determined the differences in the order, in which the most commonly found was*sul1*, followed by *sul2*, *sul3* genes from *E. coli* isolated from a sample source of hospital in Iran.

4 CONCLUSION

The proportion of antibiotic resistance of ESBL-producing *E. coli* isolated from snakehead fish, striped catfish, red tilapia and wild fish was relatively high in most types of antibiotics except MEM and FOX. According to the present research, MEM and FOX are potential to be applied in treating intestine relative bacteria due to its viability.

The study illustrated that there was the widespread of antibiotic resistant genes (beta-lactamase and sulfonamide resistance genes) of ESBL-producing *E. coli* as well as a considerable frequency of multidrug resistance genes. In further studies, a various of other fish species and considerable resistance genes should be examined in order to get to known efficiently about resistance genes characteristics.

ACKNOWLEDGMENT

The authors would like to thank College of Aquaculture and Fisheries – Can Tho University for the facilitation.

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