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Prevalence of Plasmid-mediated Quinolone Resistance (PMQR) Genes and Beta-lactamase Genes among *Salmonella, Vibrio parahaemolyticus* and *Escherichia coli* from Shellfish

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Abstract

The objectives of the present study were to investigate the prevalence of plasmid-mediated quinolone-resistance (PMQR) genes and beta-lactamase genes in *Salmonella, Vibrio parahaemolyticus* and *Escherichia coli* from shellfish. A total of 35 *Salmonella*, 22 *Vibrio parahaemolyticus*, and 58 *Escherichia coli* of shellfish origin were used in this study. Antibiotic susceptibility testing of *Salmonella, V. parahaemolyticus*, and *E. coli* against different antibiotics was determined by disc diffusion method. The minimum inhibitory concentrations (MIC) of nalidixic acid and ciprofloxacin were analyzed by microdilution method for all nalidixic acid/ ciprofloxacin-resistant strains. All the isolates were screened for the presence of PMQR genes (*qnrA*, *qnrB*, *qnrS*, *qepA, aac(6')-Ib-cr*, and *oqxA*) and beta-lactamase genes (bla_{TEM}, bla_{SHV}, and bla_{CTXM-1}). The prevalence of nalidixic acid resistance was 5.7, 9, and 3.4% in *Salmonella, V. parahaemolyticus* and *E. coli*, respectively. Approximately, 4.5% of *V. parahaemolyticus* isolates were resistant to ciprofloxacin. The prevalence of PMQR harboring *Salmonella, V. parahaemolyticus*, and *E. coli* was 22, 9 and 3% respectively. The prevalence of beta-lactamase genes in *Salmonella, V. parahaemolyticus*, and *E. coli* were 17.14, 9 and 5.1%, respectively. The most prevalent PMQR and beta-lactamase genes were *qnrS* and bla_{TEM}, respectively. The co-occurrence of PMQR and beta-lactamase genes in *V. parahaemolyticus* and *Salmonella*, from shellfish, poses a public health concern.

Keywords: *Salmonella*; *Vibrio*; *Escherichia coli*; shellfish

Introduction

Salmonella, Vibrio parahaemolyticus and *Escherichia coli* are the most important causes of food-borne infections (WHO, 2017). *Salmonella* spp. are found in the intestinal tract of various warm and even cold-blooded animals and humans (Sterzenbach et al., 2013). *Vibrio parahaemolyticus* is a native of marine and coastal environments. Certain pathogenic *V. parahaemolyticus* strains are responsible for most seafood-related human infections (Yano et al., 2014). *E. coli* is a member of normal intestinal flora of warm-blooded animals and humans. However, diarrheagenic *E. coli* including Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC) are associated with foodborne infections.

Quinolones and fluoroquinolones are one of the broad-spectrum antibiotics, which are commonly used in the treatment of food-borne illness (Su et al., 2004; Hopkins et al., 2005). Food-borne infections with quinolones/fluoroquinolones-resistant food pathogens are a major threat to public health. World Health Organization (WHO, 2017) has categorized quinolones/ fluoroquinolones as critically important

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with the highest priority for human health. The acquisition of plasmid-mediated quinolone resistance (PMQR) genes is one of the mechanisms of quinolone resistance (Martínez-Martínez et al., 1998; Ruiz et al., 2012). The plasmid-mediated quinolone resistance (PMQR) mechanisms are controlled by *qnr* genes (target protection mechanisms), *qepA* genes (efflux pump), *oqx* genes (multidrug resistance pumps) and *aac(6')-Ib-cr* gene (enzymatic modifications) (Strahilevitz et al., 2009; Xiong et al., 2011; Aldred et al., 2014). Beta-lactam resistance can be associated with plasmid-mediated quinolone resistance (Basu & Mukherjee, 2018), increasing the risk of foodborne infections. Beta-lactamase genes such as bla_{CTXM-1}, bla_{TEM} and bla_{SHV} have been associated with resistance to penicillins, 1st, 2nd, and 3rd generation cephalosporins (Tansawai et al., 2018).

Several outbreaks caused by *Salmonella* spp., *V. parahaemolyticus* and *E. coli* associated with the consumption of shellfish have been reported worldwide (CDC, 1993; Yoon et al., 2008; Hongping et al., 2011; Guérin et al., 2017). Shellfish are filter feeders. Shellfish filter large quantities of water and thus concentrate the contaminants in water. They also concentrate pathogenic microbes present in the water including bacteria (Ripabelli et al., 1999). The emergence of antibiotic-resistant bacteria and transfer of these bacteria to humans via the food chain is a challenge to the field of food safety.

Several studies reported the prevalence of PMQR genes and beta-lactamase genes from aquatic environment (Benaicha et al., 2017; Yan et al., 2017), poultry (Campos et al., 2018; Ferreira et al., 2018; Niero et al., 2018) and clinical settings (Basu & Mukherjee, 2018; Mirzaii et al., 2018; Azargun et al., 2019). However, there are very few studies on the prevalence of PMQR and beta-lactamase genes in bacterial isolates from shellfish. The objectives of the present study were to investigate the prevalence of antibiotic resistance, PMQR genes and beta-lactamase genes in *Salmonella*, *Vibrio parahaemolyticus*, and *Escherichia coli* isolates from shellfish.

Materials and Methods

A total of 35 *Salmonella*, 22 *V. parahaemolyticus* and 58 *E. coli* strains were isolated from shellfish by our research group and maintained in the laboratory were used in this study. Shellfish samples were collected from Vembanad lake, South India.

Antibiotic susceptibility testing of *Salmonella*, *V. parahaemolyticus*, and *E. coli* against different antibiotics was determined by disc diffusion method (Bauer et al., 1966) on Mueller-Hinton agar (Hi-Media, India). The following antibiotics were tested: ampicillin (Amp, 10 mcg), cefotaxime (Ctx, 30 mcg), cefoxitin (Cx, 30 mcg), ceftazidime (Caz, 30 mcg), ceftriaxone (Ctr, 30mcg), ciprofloxacin, (Cip, 5 mcg), co-trimoxazole (Co, 25 mcg), gentamicin (Gen, 10 mcg), nalidixic acid (Na, 30 mcg), streptomycin (S, 10 mcg), tetracycline (Te, 30 mcg) and trimethoprim (Tr, 5 mcg). The results were interpreted according to the Clinical Laboratory Standards Institute guidelines (CLSI, 2010; 2012). The minimum inhibitory concentration (MIC) of nalidixic acid and ciprofloxacin were analyzed by microdilution method for all nalidixic acid/ciprofloxacin-resistant strains (Andrews, 2001). *E. coli* ATCC 25922 strain was used as the control strain. Multiple antibiotic resistance (MAR) index was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics used.

Plasmid DNA from the bacterial genome was extracted as per alkali lysis with SDS method: minipreparation (Sambrook & Russell, 2006). All plasmid yielded strains such as 17 *Salmonella*, 10 *Vibrio parahaemolyticus* and 5 *E. coli* were tested for PMQR genes. The PMQR genes such as *qnrA* (Cattoir et al., 2007), *qnrB*, *qnrS* (Benaicha et al., 2017), *qepA*, *aac(6')-Ib-cr* (Kim et al., 2011) and *oqxA* (Ni et al., 2016) were used in this study. PCR mixes of 25 μL final volume were prepared with 1 μL of total DNA, 0.2 μM of each primer and 12.5 μL of EmeraldAmp® GT PCR Master Mix (Takara, Japan). The cycling conditions were as follows: initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 30s, annealing (varied temperature, given in Suppl. Table 1) for 1 min, extension at 72°C for 1.5 min; and a final extension at 72°C for 7 min.

Beta-lactamase genes such as bla_{TEM}, bla_{SHV}, and bla_{CTXM-1} (Dallenne et al., 2010) were analyzed in this study. All plasmid yielded strains such as 17 *Salmonella*, 10 *Vibrio parahaemolyticus* and 5 *E. coli* were tested for beta-lactamase genes. PCR mixes of 25 μL final volume were prepared with 1 μL of total DNA, 0.2 μM of each primer and 12.5 μL of EmeraldAmp® GT PCR Master Mix (Takara, Japan). The cycling conditions were as follows: initial denaturation at 94°C for 7 min, 30 cycles of denaturation (94°C, 40 s), annealing (60°C, 40 s), extension (72°C, 1 min) and final extension (72°C,

75 min). The primers are shown in Supplementary Table 1.

Statistical analyses of the results were carried out using IBM SPSS version 22 (IBM Corporation, New York, USA). A Pearson's Chi-squared test was applied to test differences in the prevalence of antibiotic resistance among *Salmonella, V. parahaemolyticus,* and *E.coli*. It was also used to test the co-occurrence of PMQR and beta-lactamase genes among *Salmonella, V. parahaemolyticus* and *E. coli.* Statistical significance was set at $p<0.05$.

Results and Discussion

In this study, we have detected the presence of PMQR and beta-lactamase genes in *Salmonella*, *V. parahaemolyticus* and *E. coli* of shellfish origin. Many studies have raised a concern about the increasing quinolone-resistant bacteria (Ferreira et al., 2018; Luk-in et al., 2018; Azargun et al., 2019). Furthermore, the co-occurrence of PMQR and beta-lactamase genes complicates the treatment of infections.

Statistically significant differences ($p<0.05$) in the prevalence of antibiotic resistance among *Salmonella, V. parahaemolyticus* and *E. coli* isolates are indicated by superscript letters. [a] between *Salmonella* and *V. parahaemolyticus*, [b] between *Salmonella* and *E. coli*, [c] between *V. parahaemolyticus* and *E. coli*.

A total of 35 *Salmonella*, 22 *V. parahaemolyticus* and 58 *E. coli* were isolated from shellfish samples. Among *Salmonella* isolates, 5.7% were resistant to nalidixic acid. In contrast to results of the study, Hu et al. (2017) reported that nalidixic acid resistance is very common among *Salmonella*. Although fluoroquinolone resistance among *Salmonella* is increasing (Luk-in et al., 2018; Ma et al., 2018)

Table 1. Antibiotic resistance of *Salmonella, V. parahaemolyticus* and *E. coli* isolates. R: Resistant, I: Intermediate, S: Susceptible

Antibiotics	*Salmonella* (%) (n = 35)			*V. parahaemolyticus* (%) (n = 22)			*E. coli* (%) (n = 58)		
	R	I	S	R	I	S	R	I	S
Ampicillin [a, b, c]	25 (71.42)	0	12 (34.28)	22 (100)	0	0	16 (27.58)	3 (5.17)	39 (67.24)
Cefotaxime [b, c]	15 (42.86)	7 (22.86)	13 (37.14)	10 (45.45)	3 (13.63)	9 (40.9)	0	0	100
Cefoxitin [a, c]	3 (8.57)	0	32 (91.42)	14 (63.63)	0	8 (36.36)	2 (3.44)	0	56 (96.55)
Ceftazidime [a, c]	0	6 (17.14)	82.85	8 (36.36)	0	14 (63.63)	0	0	100
Ceftriaxone	1 (2.85)	2 (5.71)	32 (91.42)	0	2 (9.09)	20 (90.9)	0	0	100
Ciprofloxacin [a, c]	0 (0)	5 (14.28)	30 (85.71)	1 (4.54)	4 (18.18)	15 (68.18)	0	2 (3.44)	56 (96.55)
Co-trimoxazole [a, b, c]	0	2 (5.71)	33 (94.28)	6 (27.27)	2 (9.09)	14 (63.63)	3 (5.17)	0	55 (94.82)
Gentamicin [a, c]	0	0	100	7 (31.81)	0	15 (68.18)	1 (1.72)	0	57 (98.27)
Nalidixic acid	2 (5.71)	0	33 (94.28)	2 (9.09)	1 (4.54)	19 (86.36)	2 (3.44)	0	56 (96.55)
Streptomycin [a, b, c]	0	2 (5.71)	33 (94.28)	8 (36.36)	3 (13.63)	11 (50)	3 (5.17)	1 (1.72)	54 (93.1)
Tetracycline [c]	2 (5.71)	2 (5.71)	31 (88.57)	3 (13.63)	1 (4.54)	18 (81.81)	2 (3.44)	1 (1.72)	55 (94.82)
Trimethoprim [a, c]	2 (5.71)	0	33 (94.28)	4 (18.18)	1 (4.54)	17 (77.27)	4 (6.89)	0	54 (93.1)

Table 2. Characteristics of antibiotic-resistant *Salmonella, V. parahaemolyticus,* and *E. coli*. Amp, Amipicillin; Ctx, Cefotaxime; Cx, Cefoxitin; Caz, Ceftazidime; Ctr, Ceftriaxone; Cip, Ciprofloxacin; Cot, Co-Trimoxazole; Gen, Gentamicin; Na, Nalidixic acid; S, Streptomycin; Te, Tetracycline; Tr, Trimethoprim.

Strain No	Isolate	Resistance pattern	Mar index	MIC (Na)	MIC (Cip)	PMQR genes	Beta-lactamse genes
V40	*V. parahaemolyticus*	Amp-CazCipCtxCxGenNaT	0.75	> 128	2	*aac(6′)-Ib-cr, qnrB, qnrS*	$bla_{CTXM-1,}$ bla_{TEM}
S82	*Salmonella*	AmpCtrCtxCxNaTeTr	0.66	> 128	ND	*qnrB, qnrS*	bla_{CTXM-1}, bla_{TEM}, bla_{SHV}
V43	*V. parahaemolyticus*	AmpCazCtxCxGenNaTe	0.66	> 128	ND	*qnrA*	bla_{TEM}
S49	*Salmonella*	AmpCtxCxNaTeTr	0.5	> 128	ND	*qnrA, qnrS, oqxA,*	bla_{TEM}
EF2	*E. coli*	AmpCotGenNaSTr	0.5	> 128	ND	*aac(6′)-Ib-cr,oqxA*	bla_{TEM}
V1	*V. parahaemolyticus*	AmpCxNaSTr	0.41	> 128	ND	ND	ND
V31	*V. parahaemolyticus*	AmpCazCtxCxGenSTr	0.66	ND	ND	ND	ND
V17	*V. parahaemolyticus*	AmpCazCtxCxGenSTr	0.66	ND	ND	ND	ND
V29	*V. parahaemolyticus*	AmpCazCtxCxSTr	0.5	ND	ND	ND	ND
V23	*V. parahaemolyticus*	AmpCazCtxCxSTr	0.5	ND	ND	ND	ND
EF18	*E. coli*	CotNaSTeTr	0.41	ND	ND	*qnrA,qnrB*	ND
V5	*V. parahaemolyticus*	AmpCazCtxCxGen	0.41	ND	ND	ND	ND
V11	*V. parahaemolyticus*	AmpCtxCxSTe	0.41	ND	ND	ND	ND
V44	*V. parahaemolyticus*	AmpCazCxGen	0.33	ND	ND	ND	ND
V48	*V. parahaemolyticus*	AmpCtxCxGen	0.33	ND	ND	ND	ND
EF28	*E. coli*	AmpCotSTr	0.33	ND	ND	ND	bla_{TEM}
V28	*V. parahaemolyticus*	AmpCxSTr	0.33	ND	ND	ND	ND
V39	*V. parahaemolyticus*	AmpSTeTr	0.33	ND	ND	ND	ND
V6	*V. parahaemolyticus*	AmpCtxCx	0.25	ND	ND	ND	ND
S150	*Salmonella*	AmpCtxCx	0.25	ND	ND	*qepA, qnrA*	$bla_{TEM,}$, bla_{SHV}
EF100	*E. coli*	AmpCxTr	0.25	ND	ND	ND	bla_{TEM}
V9	*V. parahaemolyticus*	AmpCx	0.16	ND	ND	ND	ND
EFK3	*E. coli*	AmpCx	0.16	ND	ND	ND	ND
S53	*Salmonella*	AmpCtx	0.16	ND	ND	*aac(6′)-Ib-cr*	ND
S45	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
S32	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
S28	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
S27	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
S23	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
S2	*Salmonella*	AmpCtx	0.16	ND	ND	*aac(6′)-Ib-cr*	ND
S123	*Salmonella*	AmpCtx	0.16	ND	ND	*aac(6′)-Ib-cr, qepA, qnrA,qnrB, qnrS*	bla_{CTXM-1}, bla_{TEM}
S12	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
S118	*Salmonella*	AmpCtx	0.16	ND	ND	ND	ND
H77	*Salmonella*	AmpCtx	0.16	ND	ND	ND	bla_{TEM}
H124	*Salmonella*	AmpCtx	0.16	ND	ND	*qnrS*	bla_{TEM}
S42	*Salmonella*	Amp	0.08	ND	ND	*qepA*	ND

worldwide, in our study nalidixic acid-resistant *Salmonella* isolates were sensitive to ciprofloxacin. Several studies reported the prevalence of nalidixic acid-resistant *Salmonella* strains with reduced susceptibility to ciprofloxacin (Casas et al., 2016; Kuang et al., 2018). *Salmonella* isolates showed a high prevalence of resistance for ampicillin and cefotaxime, which was higher than those previously reported from aquaculture products including shellfish (Zhang et al., 2015). The highest prevalence of antibiotic resistance was observed for ampicillin (71.4%), followed by cefotaxime (42.8). The prevalence of cefoxitin, tetracycline, trimethoprim and ceftriaxone, resistance was low, with 8.5, 5.7, 5.7 and 2.8%, respectively (Table 1). About 8.5% of isolates were multi-drug resistant (resistant to 3 or more antibiotics). MAR index ranged from 0.16 to 0.66. The MICs of nalidixic acid for *Salmonella* were 128 mg l^{-1} for nalidixic acid resistant strains (n = 2) (Table 2).

More than 22% of *Salmonella* isolates harbored PMQR genes. Among the PMQR tested genes, *qnrS* (11.42%) was more frequent among *Salmonella* isolates, which was in agreement with previous studies (Murray et al., 2008; Luk-in et al., 2018). The prevalence of *qnrA*, *qepA*, and *aac(6')-Ib-cr* were 8.57% (3/35). The prevalence of *qnrB* and *oqxA* were 5.71% and 2.85%, respectively. In contrast to our study, Nsikan et al. (2019) reported a high prevalence of *qnrB* harboring *Salmonella* isolates. It was interesting to note that some of the ciprofloxacin intermediate strains also harbored PMQR genes. Several studies also reported the presence of PMQR genes in strains with reduced susceptibility to ciprofloxacin (Tran & Jacoby, 2002; Vinue et al., 2016; Benaicha et al., 2017). PMQR mechanisms favor the selection of lower-resistance mutations such as efflux pump overexpression (AcrAB, MdtE, YdhE), loss of porins (OmpF) or modifications in lipopolysaccharide biosynthesis (rfaD, rfaE) (Vinue et al., 2016). Hence it is difficult to detect PMQR-producing isolates in surveillance studies without using molecular methods (Rodriguez-Martinez et al., 2016). Regarding the prevalence of beta-lactamase genes, 17.14% of *Salmonella* isolates harbored bla_{TEM} and 5.71% of isolates had bla_{SHV} and $bla_{CTX\text{-}M\text{-}1}$. Approximately, 14.28% of isolates showed co-occurrence of PMQR and beta-lactamase genes. There was a statistically significant positive association between PMQR and beta-lactamase genes ($p<0.05$) (Table 3a). Ma et al. (2018) also reported the co-occurrence of PMQR and beta-lactamase genes in *Salmonella* isolates. *Aac (6')-Ib-cr* and *oqxA* harboring *Salmonella* isolates showed a negative association with the bla_{SHV} gene.

Among *V. parahaemolyticus* isolates, 4.5 and 9% were resistant to ciprofloxacin and nalidixic acid respectively. The prevalence of nalidixic acid-resistant isolates was higher than those reported from oysters in Korea (Kang et al., 2018). They followed the CLSI (2006) criteria for antibiotic susceptibility testing. In a study by Silva et al. (2018) all the *V. parahaemolyticus* isolates from molluscs were sensitive towards nalidixic acid. Among the various antibiotics,

Table 3a. Co-occurrence of PMQR and beta-lactamase genes among *Salmonella* isolates.

Beta-lactamase genes		PMQR genes																	
		aac(6')-Ib-cr			*qepA*			*qnrA*			*qnrB*			*qnrS*			*oqxA*		
		Present (n = 3)	Absent (n = 32)	*p*-value	Present (n = 3)	Absent (n = 32)	*p*-value	Present (n = 3)	Absent (n = 32)	p-value	Present (n = 2)	Absent (n = 33)	*p*-value	Present (n = 4)	Absent (n = 31)	*p*-value	Present (n = 1)	Absent (n = 34)	*p*-value
bla_{TEM}																			
	P (n = 6)	33.3% * (1)	15.6% (5)	0.005	66.6% * (2)	12.5% (4)	0.010*	100%* (3)	9.3% (3)	0.000	100% * (2)	12.1% (4)	0.000	100% * (4)	6.4% (2)	0.000	100% * (1)	14.7% (5)	0.000
	A (n = 29)	66.6% (2)	84.3% (27)		33.3% (1)	87.5% (28)		0	90.6% (29)		0	87.8% (29)		0	93.5% (29)		0	85.2% (29)	
bla_{SHV}																			
	P (n = 2)	0	6.2% * (2)	0.014	33.3%* (1)	3.2% (1)	0.000	33.3%* (1)	3.2% (1)	0.000	50% * (1)	3% (1)	0.000	25%* (1)	3.2% (1)	0.000	0	5.8%* (2)	0.014
	A (n = 33)	100% (3)	93.7% (30)		66.6% (2)	96.8% (31)		66.6% (2)	96.8% (31)		50% (1)	96.9% (32)		75% (3)	96.7% (30)		100% (1)	94.2% (32)	
$bla_{CTX\text{-}M\text{-}1}$																			
	P (n = 2)	33.3%* (1)	3.1% (1)	0.000	33.3%* (1)	3.2% (1)	0.000	33.3%* (1)	3.2% (1)	0.000	100%* (2)	0	0.000	50% * (2)	0	0.000	0	2.9% (1)	0.081
	A (n = 33)	66.6% (2)	96.8% (31)		66.6% (2)	96.8% (31)		66.6% (2)	96.8% (31)		0	100% (33)		50% (2)	100% (31)		100% (1)	97% (33)	

*Significant 'p' value; ($p<0.05$).

Table3b. Co-occurrence of PMQR and beta-lactamase gene among *V. parahaemolyticus* isolates.

Beta-lactamase gene		PMQR genes											
		aac(6')-Ib-cr			*qnrA*			*qnrB*			*qnrS*		
		Present (n = 1)	Absent (n = 21)	*p*-value	Present (n = 1)	Absent (n = 21)	p-value	Present (n = 1)	Absent (n = 21)	*p*-value	Present (n = 1)	Absent (n = 21)	*p*-value
bla_{TEM}	P (n = 2)	100% * (1)	4.7% (1)	0.000	100% * (1)	4.7% (1)	0.000	100%* (1)	4.7% (1)	0.000	100% * (1)	4.7% (1)	0.000
	A (n = 20)	0	95.2% (20)		0	95.2% (20)		0	95.2% (20)		0	95.2% (20)	
bla_{TEM}	P (n = 1)	100% * (1)	0	0.000	0	4.7% (1)	0.000	100% * (1)	0	0.000	100% * (1)	0	0.000
	A (n = 21)	0	100% * (21)		100% * (1)	95.2% (20)		0	100% * (21)		0	100% * (21)	

*Significant 'p' value; ($p<0.05$).

Table 3c. Co-occurrence of PMQR and beta-lactamase gene among *E. coli* isolates.

Beta-lactamase gene		PMQR genes											
		aac(6')-Ib-cr		*qnrA*	*qnrB*	*oqxA*							
		Present (n = 1)	Absent (n = 57)	*p*-value	Present (n = 1)	Absent (n = 57)	p-value	Present (n = 1)	Absent (n = 57)	*p*-value	Present (n = 1)	Absent (n = 57)	*p*-value
bla_{TEM}	P (n = 3)	0	5.2%* (3)	0.24	100% * (3)	5.2% (3)	0.000	100%* (1)	3.5% (2)	0.000	100% * (1)	3.5% (2)	0.000
	A (n = 55)	100% (1)	94.7% (54)		0	94.7% (54)		0	96.4% (55)		0	96.4% (55)	

*Significant 'p' value; ($p<0.05$).

resistance to ampicillin (100%) was most frequent followed by resistance to cefoxitin (63.6%), cefotaxime (45.4%) and gentamicin (45.4%). Several studies reported similar trends of antibiotic resistance against ampicillin and cefoxitin from seafood (Sudha et al., 2012; Saifedden et al., 2016). More than 63% (14/22) of isolates were multi-drug resistant. Mar index ranged from 0.16 to 0.75. High MAR index values were reported among *V. parahaemolyticus* from fish samples (Noorlis et al., 2011) and aquatic ponds (Daramola et al., 2009). The MICs of nalidixic acid for nalidixic acid-resistant *V. parahaemolyticus* (n = 2) were ranged from 0.5-128 mg l^{-1}. MICs of ciprofloxacin for ciprofloxacin-resistant *V. parahaemolyticus* (n = 1) was 2 mg l^{-1}. Nine percent of *V. parahaemolyticus* were PMQR positive. Approximately 4.5% of *V. parahaemolyticus* isolates harbored *qnrA*, *qnrB*, *qnrS* and *aac(6')-Ib-cr*. *qepA* and *oqxA* were absent among *V. parahaemolyticus* isolates. Although many studies have confirmed that the presence of *V. parahaemolyticus* in seafood could be a potential risk to consumers (Sudha et al., 2014; Yu et al., 2016), there is no report on PMQR harboring *V. parahaemolyticus* from seafood. The prevalence of bla_{TEM} and $bla_{CTX\text{-}M\text{-}1}$ was 9.09 and 5.54%, respectively. bla_{SHV} was absent in *V. parahaemolyticus.* Nine percent of isolates showed co-occurrence of PMQR and beta-lactamase genes. There was a statistically significant positive association between *aac (6')-Ib-cr* and *qnr* genes with bla_{TEM} and $bla_{CTX\text{-}M\text{-}1}$ ($p<0.05$) (Table 3b).

Among *E. coli* isolates, 3.4% were resistant to nalidixic acid. Only 27.5% of the *E. coli* isolates were found to be resistant to ampicillin. Resistance to trimethoprim, co-trimoxazole, streptomycin, cefoxitin, tetracycline and gentamicin, were lower, with percentages of 6.8, 5.1, 5.1, 3.4, 3.4 and 1.7%, respectively. Similar trends of the low prevalence of antibiotic resistance in *E. coli* from shellfish isolates have been reported (Van et al., 2008; Baliere et al., 2015). About 6.8% of isolates were multi-drug resistant. Mar index ranged from 0.16 to 0.5. The MICs of nalidixic acid for nalidixic acid-resistant

E. coli strains (n = 2) were ranged from 0.25-128 mg l^{-1}. More than 3% of *E. coli* isolates were PMQR positive. The prevalence of PMQR genes such as *aac (6')-Ib-cr*, *qnrA*, *qnrB*, *oqxA* was low among *E. coli* isolates with a percentage of 1.72%. *qepA* and *qnrS* genes were absent among *E. coli* isolates. Jiang et al. (2012) reported that *qnrS* and *qnrB* were dominant PMQR genes among *E. coli* isolates from farmed fish. Approximately 5.17% of *E. coli* isolates harbored bla_{TEM} gene. bla_{TEM-1} is a plasmid encoding ampicillin-resistant gene. Several studies reported the presence of bla_{TEM-1} gene in ampicillin-resistant isolates (Brinas et al., 2002; Henriques et al., 2006; Zou et al., 2011). None of the *E. coli* isolates harbored bla_{SHV} and $bla_{CTX-M-1}$. About 1.72% *E. coli* showed co-occurrence of PMQR and beta-lactamase genes. There was a statistically significant positive association between *aac(6')-Ib-cr*, *qnrA*, *qnrB*, *oqxA* genes with bla_{TEM} ($p<0.05$) (Table 3c).

This study, for the first time, identified the PMQR and beta-lactamase genes among major food-borne pathogens such as *Salmonella*, *V. parahaemolyticus*, and *E. coli* from shellfish, which may reduce the effectiveness of treatments. Food safety is a matter of concern in developing countries like India. There are no strict regulatory control measures for the proper management of shellfish growing and harvesting water environments in India. The results highlighted the fact that PMQR and beta-lactamase genes harboring bacteria may disseminate via the food chain and becomes a major threat to consumers. This study underlines the need for further studies on the risk associated with the consumption of contaminated shellfish.

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