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Molecular Typing of β-Lactamase and Tetracycline Resistant *Escherichia coli* Strains Isolated from Imported Shrimp

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Abstract

The misuse of antibiotics in commercial aquaculture may result in the selection of antibiotic-resistant bacteria. A study was undertaken to isolate and characterize the prevalence of β-lactam and tetracycline-resistant *Escherichia coli* from imported shrimp. All 55 strains of *E. coli* isolated from 207 shrimp samples were resistant to ampicillin, penicillin and tetracycline. These isolates were screened for 11 different β-lactam resistance genes by PCR. Oligonucleotide primers specific for the amplification of a 550-bp portion of the bla_{CTX-M} gene amplified this gene from 31 of the 55 (56%) isolates. Oligonucleotide primers specific for the amplification of a 851-bp portion of the bla_{TEM} gene amplified the genes from 9 (16%) of the isolates. Six (11%) were found to harbor both the 550-bp bla_{CTX-M} and the 851-bp bla_{TEM} genes. Template DNA were also screened for the presence of 6 different tetracycline resistance (*tet*) genes, PCR detected the presence *tet*B in a majority (39/55, 71.0%) of the isolates, followed by *tet*A (13.0%). Eleven percent contained both *tet*A and *tet*B genes. The PCR based Replicon Typing Method (PBRT) was used for typing plasmids from the 55 isolates by targeting the replicons of 15 major known plasmid families. Majority (44/55, 80.0%) of the isolates contained B/O plasmid replicon. Seven (12%) isolates also contained plasmids of the FIA family and 5 (9%) isolates contained both B/O and FIA plasmid families. The β-lactam and tetracycline resistant determinants were successfully transferred to *E. coli* J53 by conjugation. Our results indicate that imported shrimp may be a reservoir of the known β-lactam and tetracycline-resistance determinants.

Introduction

Asian nations, such as Thailand, Vietnam, Indonesia, China, India and Bangladesh, currently produce nearly 80% of the world's farmed shrimp [1-3]. These Asian nations earn more than $5 billion by exporting 1.3 billion of farm-raised shrimp. Intense aquaculture demands the implementation of modern technologies to enhance production and profits [4,5]. However, aquaculture production is affected by several bacterial diseases. Thus, large amounts of antibiotics are broadly used in aquaculture farming to promote growth and to retard the incidence and effects of diseases caused by overcrowded aquaculture ponds [1,2]. The excess use of antibiotics in aquaculture ponds may select bacteria resistant to multiple antibiotics [1,5]. Several public health agencies in the United States limit the presence of antibiotic residues in food-producing animals to decrease the prevalence of antibiotic-resistant microflora in food-producing ecosystems [6].

β-Lactam antibiotics (including penicillin derivatives, cephalosporins, monobactams and carbapenems) and tetracyclines are widely used for the treatment of urinary tract, respiratory tract, the intestines, certain inflammatory disorders and numerous other life threatening infections [7]. However, the widespread use of the same types of drugs in aquaculture ponds to prevent the outbreak of diseases may select bacteria resistant to these life-saving drugs and reduce the efficacy of these drugs in clinical treatment of infectious diseases [1,5,6].

Several mechanisms of resistance to β-lactam and tetracycline antibiotics have been reported [8-10]. Additional mechanisms, such as altered cell membrane permeability and overexpression of multidrug efflux pumps, also contribute to high-level bacterial resistance to these antibiotics. However, limited information is available on the occurrence and prevalence of β-lactam and tetracycline-resistant determinants in *E. coli* in imported shrimp. There is also a paucity of information on the types of plasmid replicon prevalent in aquaculture ecosystem. Such information is indispensable for understanding the epidemiological dynamics and to devise interventional strategies to curtail the dissemination of specific plasmid replicons to other ecosystems. In this report, we describe the occurrence and prevalence of different β-lactam and tetracycline resistance determinants and plasmid replicons in *E. coli* isolated from imported shrimp samples.

Materials and Methods

Bacterial strains: All the strains of *E. coli* used in this study were isolated from shrimp (*Penaeus monodon*) imported to the US. The isolation, characterization and identification of these isolates were described earlier [11]. All isolates were stored in Luria Broth (LB) containing 20% glycerol at -70˚C and were grown overnight at 37˚C in LB or on Trypticase Soy Agar (TSA) plates supplemented with 5% sheep blood.

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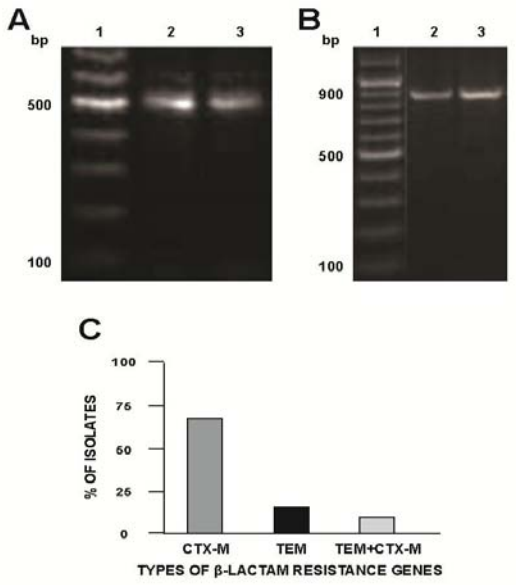

Figure 1: Detection and quantification of β-lactamase genes in the template DNA of the *E.coli* isolated from imported shrimp. (A) lane 1, 100-bp molecular weight marker; lanes 2-3, 550-bp bla_{CTX-M} amplified from the template DNA. (B) lane 1, 100-bp molecular weight marker; lanes 2-3, 851-bp bla_{TEM} amplified from the template DNA of the isolates. (C) Quantification of the occurrence of bla_{CTX-M}, bla_{TEM} and a combination of the genes in the template DNA of the isolates.

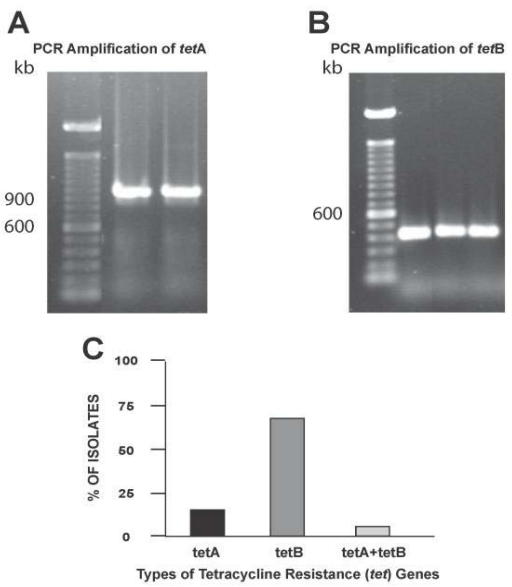

Figure 2: Detection and quantification of tetracycline resistant (*tet*) genes in the template DNA of the *E. coli* isolated from imported shrimp. (A) lane 1, 100-bp molecular weight marker; lanes 2-3, 851-bp *tet*A amplified from the template DNA (B) lane 1, 100-bp molecular weight marker; lanes 2-4, 436-bp region of the *tet*B (C) Quantification of the occurrence of *tet*A, *tet*B and a combination of these genes in the template DNA of the isolates.

Determination of antibiotic susceptibility and the minimum inhibitory concentration (MIC) of the isolates: The antibiotic susceptibility of each isolate was determined by disk diffusion assay [12] and was interpreted as per the criteria specified by the Clinical and Laboratory Standards Institute (CLSI). The MICs for the antibiotics (tetracycline, ampicillin and penicillin) were determined by broth dilution using Mueller-Hinton broth [13].

Genomic DNA extraction

Genomic DNA was extracted from cells grown overnight at 37˚C with the QIAamp DNA Mini Prep Kit (Qiagen, Valencia, CA).

Detection of β-lactamase genes from template DNA

The presence of various β-lactam resistance genes in the template DNA was determined by PCR [14]. The primers used for the amplification of these genes are listed in Table 1. PCR amplification of the β-lactam resistance genes was carried out in a reaction volume of 25 µl using a PCR Kit (Applied Biosystems, Foster City, CA). The thermal cycling conditions consisted of an initial denaturation of 94˚C for 2 min followed by 35 cycles of amplification. Each cycle consisted of 94˚C denaturation for 30 s, annealing for 1˚C below the lowest T_m of a given primer pair, and 72˚C extension for 1 min. The amplified PCR products were maintained at 4˚C. A reagent blank contained all the components of the reaction mixture except template DNA, for which sterile distilled water was substituted. The PCR products were subjected to electrophoresis on 1.2% agarose gels in 1 x Tris-Borate-EDTA (TBE) buffer, visualized with UV, and photographed using an Eagle Eye II gel documentation system (Stratagene, La Jolla, CA). A 100-bp DNA ladder (Thermo Fisher Scientific, Grand Island, NY) was used as the size standard.

Detection of tetracycline resistance (*tet*) genes by PCR

The various *tet* genes were individually amplified by PCR [10] with oligonucleotide primers (Table 2). The universal PCR amplification included 30 thermal cycles of 30 s at 94˚C, 60 s at 55˚C, and 60 s at 72˚C, with an additional extension in the last cycle for 300 s at 72˚C. The amplified PCR products were maintained at 4˚C. PCR products were subjected to electrophoresis on 1.2% agarose gels in 1 x TBE buffer, visualized with UV, and photographed using the Eagle Eye II gel documentation system.

Isolation of plasmids

Plasmid DNA was isolated using a modified alkaline lysis method [14]. Samples were analyzed by electrophoresis in 1 X Tris acetate-EDTA buffer at 64 V for 2 h on 1.0% agarose gels. A supercoiled DNA ladder (Invitrogen, Carlsbad, CA) was used as a molecular weight marker.

Plasmid typing

Plasmids were typed by the PCR-Based Replicon Typing (PBRT) method [14,15] with primers listed in Table 3.

Conjugation

Four tetracycline, ampicillin resistant *E.coli* strains isolated from shrimp aquaculture samples were selected as donors. A tetracycline-sensitive *E. coli* J53 strain was used as a recipient for conjugation experiments to determine transferability of β-lactamase and tetracycline resistant genes. The broth mating conjugation method was performed as previously described [16]. LB agar plates containing tetracycline (256 µg/mL) and sodium azide (800 µg/mL) were used

Table 1: Oligonucleotide primers used in the amplification of β-lactam resistance genes from *E. coli* strains isolated from imported shrimp.

Primers	Nucleotide Sequence	Target gene	Size (bp)
bla_{OXAF}	GCAGCGCCAGTGCATCAAC	OXA-1	198
bla_{OXAR}	CCGCATCAAATGCCATAAGTG		
bla_{PSEF}	AGTAGGGCAGGCAATCACAC	PSE-1	421
bla_{PSER}	GCGATCCGCAATGTTCCATC		
bla_{SHVF}	GCAAAACGCCGGGTTATTC	SHV-1	940
bla_{SHVR}	GGTTAGCGTTGCCAGTGCT		
bla_{TEMF}	ATGAGTATTCAACATTTCCG	TEM	851
bla_{TEMR}	TTAATCAGTGAGGCACCTAT		
bla_{CTXMF}	CGCTTTGCGATGTGCAG	CTX-M	550
bla_{CTXMR}	ACCGCGATATCGTTGGT		
bla_{CTXM9F}	GTGACAAAGAGAGTGCAACGG	CTXM9	856
bla_{CTXM9R}	ATGATTCTCGCCGCTGAAGCC		
bla_{CMYF}	TGGCCAGAACTGACAGGCAAA	CMY2	462
bla_{CMYR}	TTTCTCCTGAACGTGGCTGGC		
bla_{FOXF}	AACATGGGGTATCAGGGAGATG	FOX	190
bla_{FOXR}	CAAAGCGCGTAACCGGATTGG		
bla_{IMPF}	CATGGTTTGGTGGTTCTTGT	IMP	447
bla_{IMPR}	ATAATTTGGCGGACTTTGGC		
bla_{KPCF}	CAGCTCATTCAAGGGCTTTC	KPC	533
bla_{KPCR}	AGTCATTTGCCGTGCCATAC		
bla_{VIMF}	AGTGGTGAGTATCCGACAG	VIM	261
bla_{VIMR}	ATGAAAGTGCGTGGAGAC		

to select transconjugants harboring genes conferring β-lactam and tetracycline resistance.

Results

Determination of the Minimum Inhibitory Concentrations (MICs) of the isolates for ampicillin, penicillin and tetracycline

Wide ranges of MICs were observed for all three antibiotics tested. Thirty-two of the 55 strains (58%) were resistant to the highest concentration of ampicillin (256 μg/mL) tested; and 42 (76%) of the strains were resistant to the highest concentration of penicillin (256 μg/mL) tested; and 14 (25%) were resistant to the highest concentration of tetracycline (256 μg/mL) tested. Thirteen of the 55 isolates (24%) were resistant to the highest concentrations (256 μg/mL) for all three antibiotics tested and 15 of the 55 isolates that had MICs of 256 μg/mL for the two β-lactam antibiotics and variable MICs of 32-192 μg/mL for tetracycline (data not shown).

PCR amplification of β-lactam and tetracycline (*tet*) resistance genes from the template DNA by PCR

Template DNAs from the 55 β-lactam and tetracycline-resistant *E. coli* strains from the imported shrimp were screened for the presence of 11 different β-lactam resistance genes. Oligonucleotide primers specific for 550-bp portion of the bla_{CTX-M} gene amplified the gene from the template DNA of 31 of the 55 (56%) of the isolates (Figure 1A). Oligonucleotide primers specific for a 851-bp portion

Table 2: Oligonucleotide primers for the amplification of tetracycline resistance (tet) genes from *E. coli* strains isolated from imported shrimp.

Primers	Nucleotide Sequence	Target gene	Size (bp)
*tet*AF	GCTACATCCTGCTTGCCTTC	*tet*(A)	211
*tet*AR	GCATAGATCGCCGTGAAGAG		
*tet*BF	TCATTGCCGATACCACCTCAG	*tet*(B)	391
*tet*BR	CCAACCATCATGCTATTCCATCC		
*tet*CF	CTGCTCGCTTCGCTACTTG	*tet*(C)	897
*tet*CR	GCCTACAATCCATGCCAACC		
*tet*DF	TGTGCTGTGGATGTTGTATCTC	*tet*(D)	844
*tet*DR	CAGTGCCGTGCCAATCAG		
*tet*G_F	GCGCTNTATGCGTTGATGCA	*tet*(G)	803
*tet*G_R	ATGCCAACACCCCCGGCG		

Table 3: Primers used in PCR for detection of plasmid replicon typing in *E. coli* strains isolated from imported shrimp.

Replicon	Nucleotide Sequence	Target gene	Size (bp)
HI1	F-GGAGCGATGGATTACTTCAGTAC	*parA-parB*	471
	R-TGCCGTTTCACCTCGTGAGTA		
HI2	F-TTTCTCCTGAGTCACCTGTTAACAC	*iterons*	644
	R-GGCTCACTACCGTTGTCATCCT		
I1	F-CGAAAGCCGGACGGCAGAA	*RNAI*	139
	R-TCGTCGTTCCGCCAAGTTCGT		
X	F-AACCTTAGAGGCTATTTAAGTTGCTGAT	*ori* ν	376
	R-TGAGAGTCAATTTTTATCTCATGTTTTAGC		
L/M	F-GGATGAAAACTATCAGCATCTGAAG	*rep A, B, C*	785
	R-CTGCAGGGGCGATTCTTTAGG		
N	F-GTCTAACGAGCTTACCGAAG	*rep A*	559
	R-GTTTCAACTCTGCCAAGTTC		
FIA	F-CCATGCTGGTTCTAGAGAAGGTG	*iterons*	462
	R-GTATATCCTTACTGGCTTCCGCAG		
FIB	F-GGAGTTCTGACACACGATTTTCTG	*rep A*	702
	R-CTCCCGTCGCTTCAGGGCATT		
W	F-CCTAAGAACAACAAAGCCCCCG	*rep A*	242
	R-GGTGCGCGGCATAGAACCGT		
FIC	F-GTGAACTGGCAGATGAGGAAGG	*rep A2*	262
	R-TTCTCCTCGTCGCCAAACTAGAT		
A/C	F-GAGAACCAAAGACAAAGACCTGGA	*rep A*	465
	R-ACGACAAACCTGAATTGCCTCCTT		
T	F-TTGGCCTGTTTGTGCCTAAACCAT	*rep A*	750
	R-CGTTGATTACACTTAGCTTTGGAC		
FIIA (FIIS)	F-CTGTCGTAAGCTGATGGC	*rep A*	270
	R-CTCTGCCACAAACTTCAGC		
K/B	F-GCGGTCCGGAAAGCCAGAAAAC	*RNAI*	160
	R-TCTTTCACGAGCCCGCCAAA		
B/O	F-GCGGTCCGGAAAGCCAGAAAAC	*RNAI*	159
	R-TCTGCGTTCCGCCAAGTTCGA		

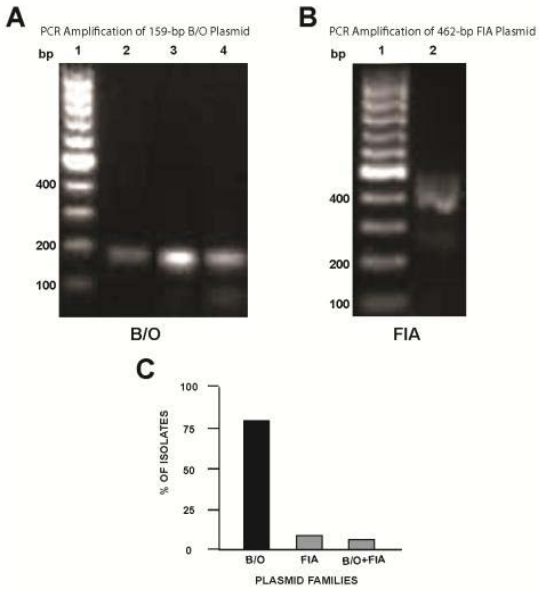

Figure 3: Profiles of plasmids of the 55 *E. coli* strains isolated from imported shrimp. Lane 1, strain EC120, lane 2, strain EC126, lane 3, strain EC221, lane 4, strain EC123, lane 5, strain EC338, lane 6, strain EC827, lane 7, strain EC803, lane 8, strain EC01, lane 9, strain ECIS801, lane 10, strain EC503, lane 11, strain EC510.

of the bla_{TEM} gene amplified the gene from the template DNA of 9 (16%) of the isolates (Figure 1B). Six (11%) of the isolates harbored both the 550-bp bla_{CTX-M} and the 851-bp bla_{TEM} genes in the template DNA (Figure 1C). PCR failed to amplify the other nine β-lactam resistance genes from the template DNA of any of the 55 isolates. The template DNA was also screened for the 5 *tet* genes (*tet*A, *tet*B, *tet*C, *tet*D and *tet*G). Oligonucleotide primers specific for a 957-bp region of *tet*A and for a 436-bp region of *tet*B successfully amplified the PCR amplicons from the template DNA of 7 (13%, Figure 2A & 2C) and 39 (71%, Figure 2B & 2C) of the isolates respectively. Additionally, 5 (9%) of the strains harbored both *tet*A and *tet*B genes (Figure 2C). PCR primers specific for the amplification of *tet*C, *tet*D, and *tet*G failed to amplify these genes from the template DNA of any of the isolates.

Plasmid identification and typing by PCR

Primers specific for a 159-bp portion of the B/O plasmid [15] were amplified from the template DNA of 44 (80%) isolates (Figure 3A-C). Additionally, oligonucleotides specific for the FIA plasmid successfully amplified a 462-bp region of the replicon from the template DNA of 5 (7%) isolates (Figure 3B-C). PCR also indicated that 4 (7%) strains simultaneously harbored B/O and FIA replicons (Figure 3C). The oligonucleotide primers failed to amplify any of other 13 plasmid families from the template DNA of the isolates.

Characterization of plasmids isolated from β-lactam and tetracycline-resistant *E. coli* isolated from shrimp

Seventeen of the fifty-five isolates did not contain any plasmids. Eleven distinct plasmid profiles were observed in the 38 isolates; the plasmids varied in size from 2.0 to greater than 16.0 kb (Figure 4). Strain EC 120 (lane 1) differed from other strains by harboring two distinct plasmid; one plasmid measured ca. 14.0 kb while the other

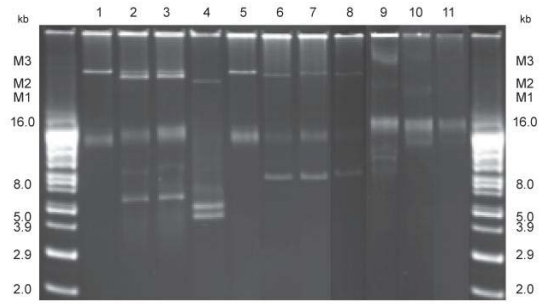

Figure 4: Detection and quantification of the various plasmids families by the PCR-based replicon typing. (A) lane 1, 100-bp molecular weight marker; lanes 2-4, 159-bp B/O plasmid. (B) lanes 1; 100-bp molecular weight marker; lane 2, 462-bp FIA plasmid (C) quantification of the different plasmid families occurring in the template DNA of the isolates.

was a Mega Plasmid (M3) measuring above 16.0 kb. Strains EC126 and EC 221 had distinct multiple plasmids measuring 6.0 kb to above 16.0 kb (Figure 4, Lanes 2-3). Strain EC126 had plasmids measuring 6, 9, 14 kb and 2 mega plasmids above 16.0 kb. Strain EC221 (lane 3) had plasmids measuring 6, 10 and 14 kb and two mega plasmids measuring above 16.0 kb. Strain EC123 had two plasmids measuring 5.0 and 6.0 kb and a mega plasmid measuring above 16.0 kb (Figure 4, lane 4). Strain EC338 (lane 5) had two distinct plasmids. One plasmid measuring 14.0 kb and the other a mega plasmid measuring above 16.0 kb. Strains EC827, EC803 and EC01 contained plasmids of various sizes (lanes 6-8). Strains EC827 and EC803 had two identical plasmids measuring ca. 8.0 kb, 12.0 kb and mega plasmids of different sizes measuring above 16.0 kb. Strain EC803 had plasmids measuring 9.0 kb, 12.0 kb and a mega plasmid. Strains ECIS801, EC503 and EC510 had plasmids of variable sizes (Figure 4; lanes 9-11). Strain ECIS801 (lane 9) had multiple plasmids measuring 8.0, 10.0, and 16.0 kb and 2 mega plasmids measuring above 16.0 kb. Strain EC503 had 2 plasmids measuring 13.0 and 16.0 kb and one mega plasmid. Strain EC510 had 2 plasmids measuring 13.0 and 16.0 kb.

Horizontal transfer of β-lactam and tetracycline resistance phenotypes and genotypes

Four strains (*E. coli* strain 120, 123, 126 and 338) that were resistant to 256 µg/mL of tetracycline but sensitive to 800 µg/mL of sodium azide were selected as donors. Our preliminary analysis indicated that these strains harbored bla_{tem}, bla_{ctx-m} and *tet*B genes. In addition, these strains also carried B/O plasmid. Transconjugants were only obtained when mating donor strains *E. coli* 126 and 338 with the recipient strain J53 for 24 h. No transconjugants were obtained when using strains 120 and 123 as donors. Our experiments also indicated higher rates of transconjugants were obtained when using *E. coli* strain 338 than *E.coli* strain 126. Serial dilution techniques indicated the rates of transconjugation to be 4.9×10^4 when using *E. coli* 126 when compared to the conjugation rates of 2.46×10^5 when using *E. coli* 338. All transconjugants were resistant to 256 µg/mL of tetracycline, ampicillin and 800 µg/mL of sodium azide, respectively. Template DNA of transconjugants from both strain 126 and 338 were positive for the presence of the 550-bp region of the bla_{CTX-M}, 436-bp region of the *tet*B and the 159-bp region of the B/O plasmid.

Discussion

The isolation of multiple antibiotic-resistant *E. coli* strains,

resistant to high concentrations of β-lactam and tetracycline antibiotics, from imported shrimp suggest the usage of these antibiotics in commercial aquaculture ponds. The usage of these drugs may have played a key role in the selection of these resistant strains. These strains, as a reservoir of multiple antibiotic-resistance genes, may transfer their resistance markers to other ecosystems and thus become a potential public health hazard [17,6].

The detection, characterization and classification of β-lactamases have been well documented [18-20]. These enzymes include the Extended Spectrum β-Lactamases (ESBLs), observed as TEM-1, TEM-2 or SHV-1; more than 150 different TEM and SHV-type ESBLs have been reported [21,19,9]. TEM-1 is the most commonly encountered β-lactamase in Gram-negative bacteria; up to 90% of ampicillin resistance in *E. coli* is due to the synthesis of TEM-1 [19,9]. This enzyme is also responsible for ampicillin and penicillin resistance in *Haemophilus influenzae* and *Neisseria gonorrhoeae* [19]. Unlike TEM-ESBLs, SHV-ESBLs are predominantly found in *Klebsiella pneumoniae* and are known to regulate resistance to ampicillin and penicillin [19]. Very few variants of SHV-lactamases have been reported. Both TEM and SHV-ESBLs are borne on transmissible plasmids that frequently co-transfer resistance to other classes of antibiotics [19,9,22]. Recently, both TEM and SHV-derived ESBLs have been joined by the CTX-M family of β-lactamases in *E. coli* [19,20]. The CTX-M lactamase has less than 40% similarity to TEM and SHV-lactamases. Results from our investigations indicate that the bla_{CTX-M} gene is the predominant ESBL gene in the template DNA of the 55 aquaculture isolates. It occurred in 56.0% of the isolates, followed by bla_{TEM}, which was found in a minority (16.0%) of the isolates. However, these results contrast with an earlier report from India [17] that indicated the predominant occurrence of bla_{TEM} (100%) and low occurrence (16%) of bla_{CTX-M} ESBLs in the template DNA of *E. coli* isolated in aquatic environments. A report from aquaculture from China [23] indicated the predominant occurrence of bla_{TEM} followed by bla_{SHV}. Few isolates in these previous studies harbored bla_{CTX-M}. Additionally, results from our investigation indicate that 9 of the isolates had an MIC of 256 μg/ml for both ampicillin and penicillin. These isolates were found to harbor both bla_{CTX-M} and bla_{TEM}, indicating that the concomitant occurrence of these two genetic determinants may be required for the higher MICs of these isolates. Results from all of these investigations clearly indicate that aquaculture ecosystems are reservoirs of ESBLs.

The occurrence and prevalence of tetracycline resistance in aquaculture ecosystems has been well documented. Furushita et al., [24] studied the prevalence of *tet* determinants in Gram-negative bacteria isolated from seafood. They reported that 43% of the isolates harbored *tet*B determinants, followed by *tet*C and *tet*D determinants. Another study [10] indicated that 77% of the *E. coli* isolates from catfish (Ictalurus punctatus) harbored *tet*B, followed by *tet*A (25%) and *tet*C (5%). An Australian investigation of Gram negative bacteria isolated from aquaculture sediments [25] indicated that *tet*M (50%) was the predominant tetracycline resistance determinant followed by *tet*E (45%). Results from our current investigation indicated that *tet*B (71%) was the dominant tet determinant, followed by *tet*A. Findings from all these investigations indicate that aquaculture may be a reservoir of a variety of *tet* genes with wide regional and continental differences in prevalence and distribution patterns of *tet* determinants.

Enterobacteriaceae producing ESBLS (extended spectrum β-lactamases) are a major public health problem and are responsible for triggering many outbreaks as well as sporadic infections worldwide [18,10,20,9]. The extensive prevalence of ESBLs is often due to horizontal gene transfer *via* plasmids [20,26,27,28,29,23]. Several studies have reported that the replicon types most frequently detected in ESBLs among the *Enterobacteriaceae* belong to the incompatibility (Inc) group, which includes F, A/C, L/M, I1, H12 and N [26]. IncF and IncI1 are the most frequently reported replicon types associated with the dissemination of ESBLs [29-31]. ESBL bla_{CTX-M} has been found on an IncF plasmid belonging to type FII in combination with FIA [32,33]. These plasmid types with different replicon types have been identified in strains isolated from different origins (environmental, livestock and humans). Results from our investigation indicate that plasmids were present in a majority of isolates and measured from 5.0 to 16.0 Kb with 1-3 megaplasmids. In contrast to several earlier investigations, none of the isolates in our study were found to harbor any of the replicon types F, A/C, L/M, I1 or H12. We for the first time report the occurrence of the B/O replicon type in ESBL strains of *E. coli* from aquaculture ecosystem. Our results indicate that 80% of the *E. coli* strains harbored replicon type B/O; 7% of the isolates contained the incompatibility (Inc) group FIA; and some isolates (4%) carried both types. It is possible that B/O type plasmids may play a role in the dissemination of ESBLs but also *tet* genes in shrimp aquaculture. Since we were unable to amplify the FIA replicon type plasmid from the template DNA of any of the transconjugants, it is possible that these plasmids may not harbor the *tet* genes or ESBL resistant determinants.

The prevalence of ESBL-bla_{CTX-M} and to a minor extent, bla_{TEM}, along with the presence of *tet*B and *tet*A in the template DNA of most isolates, may be a public health concern and highlights the significance of aquaculture ecosystems as reservoir of these resistance determinants. Thus, concerted efforts should be made by all global public health agencies to more closely monitor and introduce control measures to reduce the prevalence of antibiotic resistance in aquaculture ecosystems, as this represents a major pool of resistance determinants likely to pose a threat to the clinical use of these life-saving drugs to treat human illnesses.

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