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Short communication

Mismatch between antimicrobial resistance phenotype and genotype of pathogenic *Vibrio parahaemolyticus* isolated from seafood

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ABSTRACT

Antimicrobial resistance phenotypes (18 antimicrobials; disk diffusion method) and genotypes (38 antimicrobial resistance genes; PCR) of 20 pathogenic *Vibrio parahaemolyticus* strains isolated from seafood in Shanghai wholesale markets between 2009 and 2013 were evaluated. Seventeen isolates (85%) were resistant to one or more antimicrobials, and highest resistance was observed to ampicillin (85%) and cephazolin (30%). And the isolates with *tdh* displayed higher resistant rates than isolates with *trh*. Eight antimicrobial resistance genes (*str*B, *aad*A2, *str*A, *tet*A, *flo*R, *sul*I, *sul*II, and *sul*III) were detected in these isolates. Surprisingly, the antimicrobial resistance phenotypes and genotypes of these isolates were not consistent: some isolates were resistant to β-lactam or aminoglycoside, whereas the corresponding genes were negative. Comparatively, aminoglycoside, tetracycline and chloramphenicol resistance genes occurred in susceptibility isolates. This research reveals the mismatch phenomenon between the antimicrobial resistance phenotype and genotype of seafood-derived pathogenic *V. parahaemolyticus*, and that susceptibility isolates might be a potential risk source for storage and transmission of resistance genes.

1. Introduction

Antimicrobial resistance of pathogenic bacteria constitutes one of the most serious threats to human health in recent decades (Koser, Ellington, & Peacock, 2014). Improper or increased application of antimicrobials in aquaculture leads to the evolution and spread of bacterial antimicrobial resistance, which is a great hazard to public health due to transmission of resistant pathogens to humans via consuming with seafood (Yutaka et al., 2014). Antimicrobial resistance genes (ARGs) are emerging contaminants posing a potential worldwide human health risk (Allen et al., 2010). Recent studies have shown that seafood-derived pathogenic bacteria are capable of serving as reservoirs of resistance genes and might facilitate the dissemination of ARGs (Allen et al., 2010; Yutaka et al., 2014). Therefore, monitoring of antimicrobial resistance and ARGs in pathogens isolated from seafood is very important for improvement of seafood quality and protection of human health.

Vibrio parahaemolyticus is a notorious seafood-borne pathogenic bacterium throughout the world and the leading cause of seafood-associated illness and death after consumption of raw or undercooked seafood in China (Wu, Wen, Ma, Ma, & Chen, 2014; Xu et al., 2014). A total of 322 gastroenteritis outbreaks due to *V. parahaemolyticus* were reported in China during 2003–2008, resulting in 9041 illnesses and 3948 hospitalizations (Wu et al., 2014). This organism bearing the *tdh* or *trh* genes is generally pathogenic to humans and responsible for the vast majority of clinical cases (Chonchanok et al., 2013; Su & Liu, 2007). These two genes encode the thermostable direct haemolysin (TDH) and the TDH-related haemolysin (TRH), respectively (Su & Liu, 2007), which are major virulence factors of *V. parahaemolyticus*. Antimicrobial resistant pathogenic *V. parahaemolyticus* will increase food insecurity and lead to a serious clinical problem in treatment (Yutaka et al., 2014). Nevertheless, since most strains of *V. parahaemolyticus* isolated from the environment or seafood are

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not pathogenic, very few studies have focused on the antimicrobial resistance and resistance genes for seafood-derived pathogenic *V. parahaemolyticus*. Additionally, to our knowledge, no similar studies have been completed in Shanghai, the most prosperous city in China, which has enormous seafood production and consumption annually.

In this study, we evaluated the antimicrobial resistance phenotype and genotype of pathogenic *V. parahaemolyticus* recovered from seafood in Shanghai wholesale markets during 2009–2013 and gained a better understanding of relationship between antimicrobial resistant phenotypes and resistance genotypes of these isolates. The results will provide important information for understanding antimicrobial resistance of pathogenic *V. parahaemolyticus* derived from seafood for this region, and be helpful in further studies associated with resistant bacteria.

2. Materials and methods

2.1. Bacterial strains

As shown in Table S1, a total of 20 pathogenic *V. parahaemolyticus* isolates were obtained from four types of seafood (*Macrobrachium nipponense*, *Litopenaeus vannamei*, *Crassostrea gigas*, *Penaeus monodon*), which were collected from wholesale markets during 2009–2013 in Shanghai. The presumptive *V. parahaemolyticus* isolates were confirmed by the presence of the species-specific gene *tlh* by using the polymerase chain reaction (PCR) according to Kaysner and DePaola (2001) and the API 20E system (bioMérieux, Inc., Durham, NC, USA). The pathogenic isolates were determined by the presence of virulence genes *tdh* and *trh* by using PCR reaction (Kaysner & DePaola, 2001).

2.2. Screening for antimicrobial resistance

Antimicrobial susceptibilities of *V. parahaemolyticus* isolates were tested by the Kirby–Bauer disk diffusion method on Mueller-Hinton agar (MHA) (OXOID Limited, China) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013). The 18 common antimicrobials used in this study belonging to 6 classes were: β-lactam (ampicillin: AMP, Amoxicillin-clavulanic: AMC, piperacillin: PRL, cefotaxime: CTX, ceftazidime: CAZ, cefoxitin: FOX, cephazolin: KZ, imipenem: IPM, meropenem: MEM), aminoglycoside (amikacin: AK, centamincin: CN, Kanamycin: K, streptomyein: S), tetracycline (tetracycline: TET), quinolone (ciprofloxacin: CIP, levofloxacin: LEV), sulfonamides (trimethoprim -sulfamethoxazole: SXT), chloramphenicol (chloramphenicol: CMP). The results of susceptibility testing were interpreted according to methods recommended by the CLSI (CLSI, 2013). *Escherichia coli* ATCC 25922 was used as the reference strain. The antimicrobial resistance of each isolate was performed with three repetitions.

2.3. DNA extraction and determination of ARGs

Genomic and plasmid DNA were extracted from 20 pathogenic strains by TIANamp Bacteria DNA Kit (Tiangen Biotech Beijing Co., Ltd., China) and TIANprep Mini Plasmid Kit (Tiangen Biotech Beijing Co., Ltd., China) respectively according to the manufacturer's instructions and stored at −20 °C prior to PCR analysis. PCR detection assays were used to determine the presence or absence of 38 ARGs belonging to 6 classes. Primers of all target ARGs were synthesized based on the published literature, with details listed and described in Table S2. PCR fragments were sequenced for both strands by Sangon Biotech (Sangon Biotech, Shanghai) and analyzed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

3. Results and discussion

3.1. Antimicrobial resistance phenotypes of pathogenic Vibrio parahaemolyticus

A total of 20 pathogenic *V. parahaemolyticus* isolates were obtained from seafood samples. Ten were test-positive for virulence gene *tdh*, and the other 10 were test-positive for virulence gene *trh*. The percentages of 20 pathogenic *V. parahaemolyticus* isolates resistant to 18 commonly used antimicrobials agents belonging to 6 classes were shown in Table 1. Among 20 isolates, seventeen isolates were tested for resistance to at least one of the antimicrobial agents resistant. Resistance was observed to ampicillin (85%), cephazolin (30%), amoxicillin-clavulanic (10%), piperacillin (10%), ciprofloxacin (5%), levofloxacin (5%), amikacin (5%), gentamincin (5%) and trimethoprim-salfamethoxazole (5%), respectively. And multi-drug resistance (MDR, defined as resistance to 3 or more different antimicrobials) was found in 2 *V. parahaemolyticus* strains (10%). Isolate VPD18 presented resistant to 6 antimicrobial agents (AMP, AMC, PRL, KZ, CIP and LEV) and isolate VPD14 to 3 antimicrobial agents (AMP, PRL and AK). Our results also showed that there were differences in the levels of antimicrobial resistance for each of the *V. parahaemolyticus* virulence types. Compared to isolates with *trh*, the isolates with *tdh* displayed higher resistant rates.

Our findings showed lower prevalence of resistance among pathogenic *V. parahaemolyticus* than those in most similar studies (Baker et al., 2008; Chao et al., 2009; Donatella et al., 2013). Firstly the antimicrobial resistance in this study was compared to the previous studies conducted on seafood-derived pathogenic *V. parahaemolyticus* isolates. Baker-Austin et al. (2008) tested the susceptibility of pathogenic *V. parahaemolyticus* strains from coastal water and sediment in USA, and reported that isolates were resistant to more antimicrobials (5.8 antimicrobials per isolates) than these in our study (1.6 antimicrobials per isolates). Donatella et al. (2013) found pathogenic *V. parahaemolyticus* from shellfish in Italy to be approximately 63.3% and 70% resistant to streptomycin and kanamycin respectively, while no resistance to these two antimicrobials in present study. Resistance were also compared with clinical pathogenic *V. parahaemolyticus* isolates. Chao et al. (2009) reported higher percent resistance of clinical pathogenic *V. parahaemolyticus* strains to chloramphenicol and streptomycin. Although seafood-derived pathogenic *V. parahaemolyticus* isolates in this study showed lower prevalence of resistance to antimicrobials than that was noted in previous researches (Baker et al., 2008; Chao et al., 2009; Donatella et al., 2013), these antimicrobials, which the isolates are resistant to in present study, are considered as some of the best defenses against the severe infections that these organisms can elicit. Additionally pathogenic *V. parahaemolyticus* may cause deleterious health effects, particularly if the strains involved are resistant to clinically important antimicrobials (Shaw et al., 2014). Based on these results, it can be surmised the antimicrobial resistance of pathogenic *V. parahaemolyticus* in Shanghai should be cause for concern.

3.2. Antimicrobial resistance genotypes of pathogenic V. parahaemolyticus

Thirty-eight antimicrobial resistance genes belonging to 6 classes detected in 20 pathogenic *V. parahaemolyticus* isolates are shown in Table 2. The PCR method employed to determine the presence of ARGs was based on genomic DNA of 20 isolates. This study also considered that plasmids may account for resistance genes, but no plasmid (≤10 kb) was detected in these isolates by using the TIANprep Mini Plasmid Kit (data not shown). And for the