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Antibiotic Susceptibility profile of *Vibrio parahaemolyticus* isolated from shrimp in Selangor, Malaysia

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Abstract

Vibrio parahaemolyticus is a halophilic Gram-negative bacterium that is considered among gastrointestinal pathogens. Thirty isolates were tested for their susceptibility using 14 different antibiotics. One *V. parahaemolyticus* isolate was resistant to 10 antibiotics (cefotaxime, ceftazidime, tetracycline, amikacin, ciprofloxacin, levofloxacin, ofloxacin, ampicillin, amoxicillin-calv-acid, and cefepime). The *V. parahaemolyticus* isolates were resistant to ampicillin (90%), amoxicillin–clavulanic acid (63.3%), cefotaxime (60%), ceftazidime (46.7%), cefepime (50%), tetracycline (36.6%), and amikacin (26.7%). However, the isolates were highly susceptible to imipenem (100%), and piperacillin and gentamicin (96.7%). Approximately 55% of the isolates showed a multiple antibiotic resistance (MAR) index of >0.2, thereby indicating the high risk of sources where these isolates originated. The occurrence of MAR asserted the importance of determining drug susceptibility and monitoring the antimicrobial resistance profile to improve and ensure food safety and public health.

Introduction

Since the discovery of penicillin by Alexander Fleming in 1928, bacteria have adapted defense mechanisms against antibiotic and continue to develop new resistance to survive. In 1937, sulfonamides were introduced as effective antimicrobial agents, but sulfonamide resistance was reported in the late 1930s (Davies and Davies, 2010). Since the 1960s, few new antibiotic drugs were discovered and modified chemically to treat infectious diseases, kill pathogens, and decrease side effects. Since the 1970s, the antibiotic therapy manufacture has combined drugs with different mechanisms of action to increase effectiveness. Antibiotic resistance becomes a global public health problem because of excessive misuse of antibiotics in humans, agriculture, and aquaculture, as well as usage to control bacterial infection, thereby contaminating sewage, wastewater, and river water. It directly influences the coastal sea area and aquaculture farms (Son *et al*., 2002; Thakur *et al*., 2003; Okoh and Igbinosa, 2010; Silvester *et al*., 2015).

Antimicrobial drugs are divided into two types according to their source. The first type is a natural antimicrobial drug produced from live organisms, such as cephalosporin and penicillin, which are produced from fungi. The second type is the chemical one, which is a synthetic antimicrobial drug (Riaz *et al*., 2011). Antimicrobial drugs are also divided according to the mechanism of action: inhibiting cell wall, cell membrane, protein synthesis, and nucleic acid synthesis (Harris, 1964). Recently, the most important issue of food safety concern is the increasing antibiotic resistance of foodborne pathogens, including aerobic and anaerobic bacteria (Yong *et al*., 2003; Zulkifli *et al*., 2009). Unfortunately, this multidrug resistance problem (bacteria can resist more than one antibiotic) is becoming further complicated because of the excessive usage of antibiotic (Livermore, 2003; Letchumanan *et al*., 2015). Additionally, the concern about antimicrobial resistant bacteria in aquaculture is not well documented (Cabello, 2006).

Foodborne disease is commonly associated with gastroenteritis symptoms, such as diarrhea, vomiting, headache, fever, and bloody diarrhea in severe cases. Although *V. parahaemolyticus* causing gastroenteritis is often self-limited, antibiotics, such as tetracycline, ciprofloxacin and cephalosporin (ceftazidime), are used as alternative treatment in

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severe cases (Zulkifli *et al*., 2009; Al-othrubi *et al*., 2014). Traditionally, *Vibrio* is considered highly susceptible to all antibiotics (Oliver, 2006), except that ampicillin resistance was determined in *V. parahaemolyticus* and *V. vulnificus* (Joseph *et al*., 1978; Zanetti *et al.*, 2001). During the past decades, food safety concern of antimicrobial resistance is increasing, and the emergence of antibiotic resistance of *V. parahaemolyticus* has become a serious threat in aquaculture industries (Tendencia and Peña, 2001; Han *et al.*, 2007). The prevalence of multidrug resistant *V. parahaemolyticus* is relatively high in Southeast Asia (Zulkifli *et al*., 2009). This chapter studies and contributes the antibiotic susceptibility of *V. parahaemolyticus* isolates by using the disc diffusion method (Bauer *et al*., 1966).

Material and Method

Bacterial isolates

A total of 30 *Vibrio parahaemolyticus* were isolated from shrimp samples purchased around Selangor, Malaysia. 10 g portion of each sample was placed in sterile stomacher bag added with 90 ml of tryptic soya broth (TSB) (BactoTM, France) with 3% NaCl (Merck, Germany) and blended in a stomacher for one minute. The pre-enrichment incubated in a shaker incubator at 200 rpm and 37°C for 18–24 h. Then pre-enrichment plated on CHROMagar™ *Vibrio* (CV) and incubated at 37°C for 18–24 h. The *V. parahaemolyticus* colonies appears with purple color on the CV agar, and the colonies were transferred to fresh CV agar for purification using a sterile toothpick. The bacterial culture was stored in 20% sterile glycerol under −20 °C for further experiments.

Antibiotic susceptibility

The susceptibilities of 30 isolates to antibiotics were determined via disk diffusion method (Bauer *et al.*, 1966) that is recommended by CLSI (CLSI, 2006). Briefly, colony was directly suspended into 4 mL of normal saline inoculum of 0.85% NaCl, which is equivalent to 0.5 McFarland standard. The inoculum was swabbed evenly on Mueller–Hinton (MH) agar plate (Merck, Germany) using a sterile cotton swab and left to dry for 3–5 min at room temperature. Before dispensing the antibiotic discs on the MH agar, the plates were incubated at 37°C overnight. Subsequently, the inhibition zone was measured, and the results were interpreted based on the CLSI recommendation (CLSI, 2010) M45-2A.

Fourteen antibiotic disks (Oxoid, UK) were used, 1: ampicillin, 10 µg (AMP 10); amoxicillin–clavulanic acid, 20/10 µg (AMC 30); piperacillin, 100 µg (PRL 100); imipenem, 10 µg (IPM 10); meropenem, 10 µg (MEM 10); amikacin, 30 µg (AK 30); gentamicin, 10 µg (CN 10); tetracycline, 30 µg (TE 30); ciprofloxacin, 5 µg (CIP 5); levofloxacin, 5 µg (LEV 5); ofloxacin, 5 µg (OFX 5); cefepime, 30 µg (FEP 30); cefotaxime, 30 µg (CTX 30); and ceftazidime, 30 µg (CAZ 30), as recommended by CLSI (CLSI, 2010) M45-2A.

Multiple antibiotic resistance index

MAR index is a useful tool that provides an excellent estimation about the origin of contamination (Krumperman, 1983). MAR index is calculated as the ratio of some resistance antibiotics to the total number of antibiotics to which isolates are exposed to (Osundiya *et al.*, 2013; Elexson *et al*., 2014).

Results

Antibiotic susceptibility test was performed on *V. parahaemolyticus* isolated from shrimp using 14 antibiotics selected from different groups. Referring to the Figure 1, *V. parahaemolyticus* isolates were the most resistant towards ampicillin (90%), followed by amoxicillin–clavulanic acid (63.3%), cefotaxime (50%), cefepime (50%), ceftazidime (46.7%), tetracycline (36.6%), and amikacin (26.7%). Some of the antibiotics, such as meropenem, ciprofloxacin, levofloxacin, and ofloxacin, had a slight resistance about 6%. However, the isolates were highly susceptible to imipenem (100%) and piperacillin and gentamicin (96.7%). Most of the isolates showed susceptibility to meropenem, ciprofloxacin, levofloxacin, and ofloxacin at 93.4%. In addition, the *Vibrio* isolates were moderately susceptible to amikacin, tetracycline, ceftazidime, and amoxicillin–clavulanic acid at 73.3%, 63.3%, 53.3%, and 36.7%, respectively. MAR index was obtained by calculating the ratio between the number of antibiotic and total number of antibiotic. About 96% of *V. parahaemolyticus* isolates demonstrated resistance to at least one antibiotic, and about 50% isolates demonstrated >0.2 MAR index (Figure 2). The indicated MAR was between 0.07 and 0.71. The highest MAR index was observed on isolate (Vp.027), which showed resistance to 10 antibiotics.

Discussion

V. parahaemolyticus is the causative agent of gastroenteritis, which is related to the consumption of contaminated seafood (Di Pinto *et al*., 2008). According to the Center for Disease Control and Prevention, most cases of *V. parahaemolyticus*

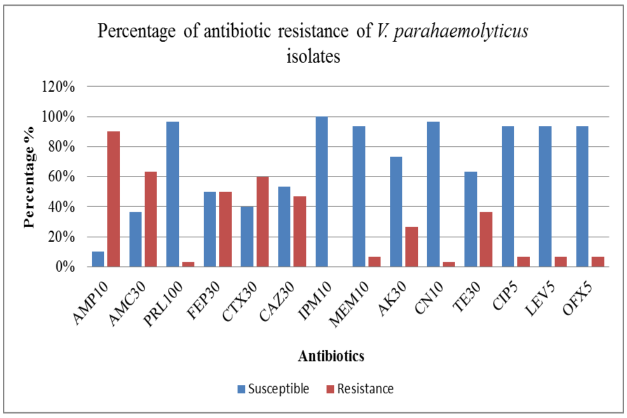

Figure1. Percentage of antibiotic susceptibility result

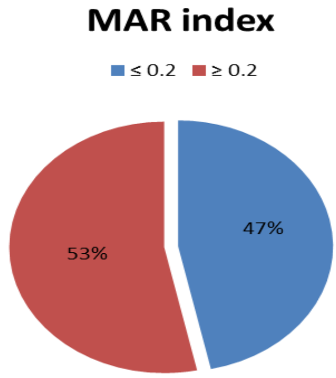

Figure 2. MAR index chart shows percentage of low and high risk

infection treatment are not necessary; the patient should drink plenty of fluid to avoid dehydration caused by diarrhea. In severe cases, antibiotics, such as tetracycline and ciprofloxacin, can be used based on the antimicrobial susceptibilities of organisms (Center for Disease Control and Prevention, 2006). *V. parahaemolyticus* isolates in this study showed resistance to ampicillin (90%) which was in agreement with other studies. *V. parahaemolyticus* isolates demonstrated general resistance to ampicillin at 80%–90% (Han *et al.*, 2007;; Al-othrubi *et al.*, 2014; Letchumanan *et al.*, 2014; Shaw *et al.*, 2014; Yu *et al.*, 2016). Since 1978, studies reported *Vibrio* resistance to ampicillin in the range of 40%–90% (Sudha *et al.*, 2014). This observation suggests that ampicillin has low-efficiency treatment of *V. parahaemolyticus* infection. Furthermore, *V. parahaemolyticus* isolates showed 50% resistant to AMC. However, other studies obtained 94%–99% susceptibility (Jiang *et al.*, 2014; Shaw *et al.*, 2014) and low resistance at about 6% (Yu *et al.*, 2016). The lack of data about AMC applied on *V. parahaemolyticus* causes the difficulty in making an accurate comparison with other studies.

Third- and fourth-generation cephalosporin (ceftazidime, cefotaxime, and cefepime), were applied, and isolates showed resistance at 46.7%, 60%, and 50%,, respectively. The result is relatively higher than that of other studies, who reported resistance average of 3%–46% (Noorlis *et al.*, 2011; Shaw *et al.*, 2014; Letchumanan *et al.*, 2015a; Zavala-Norzagaray *et al.*, 2015; Elmahdi *et al.*, 2016; You *et al.*, 2016). In Korea in 2012, *V. parahaemolyticus* isolates were detected resistant to cefotaxime and ceftazidime with a high percentage of 70%–80% (Jun *et al.*, 2012). By contrast, other reports showed that *V. parahaemolyticu*s has a high susceptibility to cephalosporin, which was recommended as a treatment for *Vibrio* infections (Zulkifli *et al.*, 2009; Liu *et al.*, 2013; Al-othrubi *et al.*, 2014; Yu *et al.*, 2016). The differences in the literature regarding the sensitivity of *V. parahaemolyticus* to cefotaxime may be related to the differences in geography. Furthermore, cefepime is considered one of the new fourth-generation of cephalosporin; even its low percentage of resistance could promote significant concerns. Nevertheless, the present study detected a moderate resistance to amikacin and tetracycline (26.7% and 36.6%, respectively).

V. parahaemolyticus isolates were sensitive to imipenem (100%); gentamicin and piperacillin (96.7%); and meropenem and quinolone (ciprofloxacin, levofloxacin, and ofloxacin) (93%). These results were in agreement with the literature stating that *V. parahaemolyticus* is susceptible to quinolone and tetracycline (Han *et al.*, 2007; Shaw *et al.*, 2014; Sudha *et al.*, 2014; Yu *et al.*, 2016). *V. parahaemolyticus* also showed high sensitivity to imipenem (Noorlis *et al.*, 2011; Letchumanan *et al.*, 2015a).

Antibacterial resistances are a complicated mechanism; many researchers have made assertion that we have to reduce and better use of antimicrobial to infection control. However, reduction of use antimicrobial is not a solution, might be because the bacteria already inherited the resistance by transferable genetic elements (Livermore, 2003). Liu *et al.* (2013), stated *V. parahaemolyticus* strain that carried a novel plasmid with multidrug resistance genes, and these genes most likely rendered transferable by genetic elements in *Vibrio* spp., that speed up the emergence of multidrug resistance in *Vibrio*. Thus, we have to take into consideration all factors which lead to increasing of bacterial resistance.

MAR index, which ranges from 0 to 1.0, is a helpful tool for analyzing health risk. The MAR index value (0.20) is differentiated between low and high risks; when the MAR value is >0.20, then the sample has high-risk for source contamination (Krumperman, 1983). Paul *et al.* (1997) stated that "the value of MAR index gives an indication that all isolates, somehow, originated from the environment where antibiotics are overused"; the MAR index is considered as a good tool for risk assessment. On the other hand, there are conflicting reports on antibiotic susceptibility and MAR from various geographical regions were reported. In our study, V. parahaemolyticus isolates from Selangor; Malaysia showed high frequency of MAR at about 96%. These results were in agreement with other studies around Malaysia (Tanil *et al.*, 2005; Zulkifli *et al.*, 2009; Baker-Austin *et al.*, 2009; Noorlis *et al*., 2011; You *et al*., 2016), thereby indicating that the aquatic environment in the sampling area may be affected and contaminated with antibiotics from human and animal sources. MAR assertion is important in determining drug susceptibility and monitoring the antimicrobial resistance profile to improve and ensure food safety and public health.

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