Biosafety of *Vibrio parahaemolyticus* from vegetables based on antimicrobial sensitivity and RAPD profiling

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Abstract: This study was undertaken to characterize the antibiotic resistance and randomly amplified polymorphic DNA (RAPD) profiles of *Vibrio parahaemolyticus* isolates from raw vegetable samples. A total of 46 isolates of *V. parahaemolyticus* recovered from raw vegetables samples and were confirmed by PCR were analyzed in this study. Most of the isolates were resistant to nalidixic acid (93.48%) and were the least resistant towards imipinem (4.35%). The MAR index results also demonstrated high individual and multiple resistances to antibiotics among the isolates. From the RAPD analysis, the size for RAPD fragments generated ranged from 250 bp to 1,500 bp, with most of the strains contained three major gene fragments of 350, 1,000 and 1,350 bp. The RAPD profiles revealed a high level of DNA sequence diversity within the isolates. Antibiotic resistance and RAPD proved to be effective tools in characterizing and differentiating the *V. parahaemolyticus* strains.

Keywords: Biosafety, *Vibrio parahaemolyticus*, RAPD, vegetables, antibiotic

Introduction

Pathogenic *V. parahaemolyticus* is one of the leading causes of bacterial gastroenteritis in many countries. The United States Centers for Disease Control and Prevention (CDC) reported that while infections due to *Shigella*, *Listeria*, *Eschericia coli* O157:H7 and *Salmonella* had decreased dramatically for 2005, infections due to *Vibrio* had increased (Chang *et al*., 2010). For a long time, *V. parahaemolyticus* has been known to be ubiquitously present in brackish and marine waters, and infection to human is frequently associated with the consumption of contaminated seafood or raw or undercooked shellfish (Guoxiang *et al*., 2009; Lynch *et al*., 2005). However, recent foodborne outbreaks throughout the world have been intensively linked to consumption of fresh fruits, vegetables and unpasteurized juices (Gorny, 2006). In Malaysia, an outbreak in Kedah in 2003 was reported to be caused by *V. parahaemolyticus* linked to 'kerabu tauge', a local dish mixed with vegetables (Mohamad *et al*., 2006). Cross-contamination of raw fruits and vegetables with seafood represents a potential mode of transmission of *V. parahaemolyticus* to humans (WHO/FSF/FSO, 1998). In our previous study (Tunung *et al*., 2010), there was a prevalence of *V. parahaemolyticus* in raw vegetables and other environmental samples.

It has been known that antibiotic therapy can reduce the duration and severity of symptoms of *Vibrio* infections in severe cases; however bacterial resistance to antibiotics has become an emerging medical issue threatening the public health due to the wide availability of antibiotics and sometimes misuse of drugs without proper prescription (Adeleye *et al*., 2008). Previous studies have shown that streptomycin, rifampicin, kanamycin, tetracycline and polymixin B were active against *Vibrio* spp. but a study by Ottaviani *et al*. (2001) showed that *V. parahaemolyticus* were resistant to penicillin, carbenicillin, ampicillin, cephalotin, kanamycin and rifampicin (Zulkifli *et al*., 2009). Zulkifli *et al*. (2009) also reported the resistance of *V. parahaemolyticus* towards tetracycline. The growing problems with antimicrobial drug resistance are beginning to erode our antibiotic armamentarium to combat antibiotic resistance and thus limiting

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therapeutic options to present-day clinicians (Zulkifli *et al*., 2009). Hence, surveillance of antimicrobial resistance is indispensable for empirical treatment of infections and important in preventing the spread of antimicrobial resistant microorganisms (Adeleye *et al*., 2008).

Nowadays epidemiological typing of pathogens has become increasingly important in public health control, in which molecular typing procedures are applied to show clonal and close relationship between isolates of one species. It is possible to identify pathogen reservoirs and to follow up the regional and global spread of pathogens, and some methods can give insights into the evolutionary dynamics of the bacterial genome. DNA-based procedures such as randomly amplified polymorphic DNA (RAPD) helps epidemiological investigations to be conducted more rapidly and thoroughly, and has been used for typing and differentiation of bacteria and for the study of genetic relationships between strains and species (Lesley *et al*., 2005). This present investigation will be using RAPD to generate polymorphism in DNA patterns amenable to the differentiation of the *V. parahaemolyticus* strains isolated. As a general, this study was embarked upon to determine the antimicrobial resistance patterns and RAPD profiles of the *V. parahaemolyticus* isolates recovered from vegetable samples.

Materials and Methods

V. parahaemolyticus *isolates*

A total of 46 isolates of *V. parahaemolyticus* (Table 1) were recovered from raw vegetables samples obtained from supermarkets and wet markets and kitchen simulation study (obtained from our previous study), and were further examined for antimicrobial sensitivity test and RAPD profiling. These strains have been confirmed by using specific-PCR targeting the species-specific *toxR* region in *V. parahaemolyticus* as mentioned by Kim *et al*. (1999) and Lesley *et al*. (2005). Isolates were revived from glycerol stocks using TSB (Bacto™, France) with 3% NaCl (Merck, Germany) and were incubated at 37°C for 18 to 24 hours in an orbital shaker.

Antibiotic Resistance

Susceptibility of the 46 isolates to antibiotics was determined by the disc diffusion tests based on the guidelines of The National Committee for Clinical Laboratory Standards (NCCCLS, 2001) and as previously described by Bauer *et al*. (1966), Tunung *et al*. (2007) and Zulkifli *et al*. (2009).

Table 1. *V. parahaemolyticus* isolates recovered from vegetable samples collected from pre-harvest, retail and domestic kitchen level

No.	Strain No.	Sample source	Sampling location
1	KS1	Four winged bean	Wet Market F
2	KS2	Four winged bean	Wet Market F
3	KS6	Four winged bean	Wet Market E
4	KS7	Four winged bean	Wet Market F
5	PH18	Cabbage	Packing house 1
6	SMB23	Cucumber	Supermarket B
7	SMB28	Cabbage	Supermarket B
8	SMB30	Tomato	Supermarket B
9	SMB31	Four winged bean	Supermarket B
10	SMB34	Cucumber	Supermarket B
11	SMB37	Japanese parsley	Supermarket B
12	WMC18	Japanese parsley	Wet Market C
13	WMC20	Cabbage	Wet Market C
14	WMC21	Tomato	Wet Market C
15	WMC22	Wild cosmos	Wet Market C
16	WMC26	Four winged bean	Wet Market C
17	WMC29	Indian pennywort	Wet Market C
18	WMC32	Sweet potato	Wet Market C
19	WMC33	Long bean	Wet Market C
20	WMC35	Wild cosmos	Wet Market C
21	WMC36	Japanese parsley	Wet Market C
22	WMC38	Cabbage	Wet Market C
23	WMC39	Indian pennywort	Wet Market C
24	WMC42	Four winged bean	Wet Market C
25	WMC44	Long bean	Wet Market C
26	WMC45	Cucumber	Wet Market C
27	WMC47	Japanese parsley	Wet Market C
28	WMD48	Japanese parsley	Wet Market D
29	WMD50	Cucumber	Wet Market D
30	WMD52	Sweet potato	Wet Market D
31	WMD53	Four winged bean	Wet Market D
32	WMD54	Tomato	Wet Market D
33	WMD55	Carrot	Wet Market D
34	WMD56	Indian pennywort	Wet Market D
35	WMD57	Cabbage	Wet Market D
36	WMD58	Cabbage	Wet Market D
37	WMD59	Indian pennywort	Wet Market D
38	WMD62	Four winged bean	Wet Market D
39	WMD63	Tomato	Wet Market D
40	WMD65	Japanese parsley	Wet Market D
41	WMD67	Cucumber	Wet Market D
42	WMD68	Cabbage	Wet Market D
43	WMD69	Indian pennywort	Wet Market D
44	WMD71	Tomato	Wet Market D
45	WMD147	Cabbage	Wet Market D
46	WMD148	Indian pennywort	Wet Market D

Disc diffusion method

All isolates were grown in TSB (Bacto™, France) with 3% NaCl (Merck, Germany) and were incubated at 37°C for 18 to 24 hours. The cultures were swabbed evenly on Mueller-Hinton (MH) agar plates (Merck, Germany) using sterile non-toxic cotton swab to form a uniform lawn of bacterial growth, which were then left to dry for 3 to 5 minutes before placing antimicrobial sensitivity discs. The culture *E. coli* ATCC 25922 was included as a control test in the susceptibility testing.

Antibiotic impregnated discs of 8 mm diameter containing 14 types of antibacterial agents (as

follows) were placed on the plates and incubated at 37°C overnight: Amoxycillin (AML, 25 µg), Ampicillin (AMP, 10 µg), Chloramphenicol (C, 30 µg), Erythromycin (E, 15 µg), Gentamicin (CN, 10 µg), Imipinem (IPM, 10 µg), Nalidixic acid (NA, 30 µg), Norfloxacin (NOR, 10 µg), Penicillin (P, 10 µg), Streptomycin (S, 25 µg), Sulphamethoxazole (RL, 25 µg), Tetracycline (TE, 30 µg), Tobramycin (TOB, 10 µg) and Vancomycin (VA, 30 µg). The antibiotic cartridges with commercially prepared antibiotic discs were purchased from Oxoid (Hamphire, United Kingdom).

After incubation, the diameter of inhibition zone was measured and compared with zone diameter interpretative chart from BBL "Sensi-Disc Antimicrobial Susceptibility Test Discs: Approved Standard, 1996" to determine the sensitivity of the isolates to the antibiotics. The bacteria will thus be classified into susceptible, intermediate or resistant.

Multiple Antibiotic Resistance (MAR)

Multiple antibiotic resistance (MAR) index of the isolates was determined as according to Krumperman (1983) and as previously described by Gwendellynne *et al*. (2005) and Tunung *et al*. (2007). The MAR index is defined as a/b, where 'a' represents the number of multiple antibiotics to which the particular isolates are resistant, and 'b' the number of multiple antibiotics to which the particular isolates are exposed.

Randomly Amplified Polymorphic DNA (RAPD)

Prior to amplification, the 46 *V. parahaemolyticus* strains were grown overnight and their genomic DNA were extracted by boil cell method as described by Tunung *et al.* (2010). A 1 ml portion of each MPN broth was subjected to centrifugation at 13, 400 x g for 1 min and the pellet was resuspended in 500 µl of sterile distilled water. The mixture was boiled for 10 min and then immediately cooled at − 20 °C for 10 min before it was centrifuged at 13, 400 x g for 3 min. The supernatant was kept for use in RAPD fingerprinting.

RAPD primer screening

For RAPD primer screening, optimization of the PCR was done using three randomly selected *V. parahaemolyticus* isolates to detect the polymorphism. A total of 10 types of 10 random primers with G+C content ranging from 50% to 70% were screened for the most suitable primer. The choice for the selected primer was based on the numbers of bands generated, with few low-intensity bands, and they provide reproducible and discriminatory patterns (Lesley *et al*., 2005). From the screening procedure, primer OPA8 (5'-TGGGGCTGTC-3'), OPA14 (5'-TTCCGAACCC-3') and OPA15 (5'-TCTGTGCTGG-3') were selected to be used on all isolates for the RAPD fingerprinting.

RAPD-PCR reaction

The PCR was performed in a 25 µl volume containing 1X PCR buffer, 6.0 mM of $MgCl_2$, 0.4 mM of dNTPs, 4 µM of primer, 2.5 units of *Taq* DNA polymerase, and 3 µl of DNA template. All reagents were purchased from Promega (Madison, WI, USA) while the primer was synthesized by 1st BASE. Amplifications were carried out in a Veriti™ 96 wells thermal cycler (Applied Biosystems, USA) with the following reaction conditions: pre-denaturation at 95°C for 1 min; 35 cycles of denaturation at 95°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min; and a final extension at 72°C for 10 min. The PCR products were stored at 4°C. Amplification products were fractionated by electrophoresis through 1.5% agarose gel with 0.5X TBE, using 80 Volts, and detected by staining with ethidium bromide. A 1 kb DNA ladder (Promega) was used as DNA size marker. The fragments were visualized under UV transilluminator (SynGene Gel Documentation System).

RAPD profile analysis

For the interpretation of the fingerprints, the GelCompare 4.2 software package (Applied Maths, Belgium) was used. Computer based normalization and interpolation of the DNA profiles, and numerical analysis using the Pearson product moment correlation coefficient, with 1% position tolerance, were performed. Dendrograms were constructed using the unweighted pair group linkage analysis method (UPGMA). For convenience, the correlation level was expressed as percentage similarity.

Results

The occurrence of antibiotic resistances in *V. parahaemolyticus* isolated from raw vegetables and environmental samples was assessed, in which a total of 46 *V. parahaemolyticus* isolates (Table 1) were tested against 14 antibiotics from different groups. The percentages of isolates resistant towards the antibiotic tested were analyzed and most of the isolates were resistant to Nalidixic acid (93.48%), Amoxycillin (80.43%), Sulphamethoxazole (73.91%), Tetracycline (71.74%), and Tobramycin (71.74%). The isolates showed intermediate resistance towards Streptomycin (65.22%), Gentamicin (54.35%), and Ampicillin (50.00%). About 34.78% of the isolates

were resistant to Vancomycin, 26.09% were resistant to both Erythromycin and Norfloxacin equally, while 15.22% were resistant to Penicillin and 6.52% to Chloramphenicol. The isolates were the least resistant towards Imipinem (4.35%).

From Table 2 which showed the profiles of the isolates based on their antibiotic resistance patterns, about 91.30% of the isolates showed multiple antibiotic resistances. Only four strains did not show multiple antibiotic resistances, in which one strain was not resistant to any of the antibiotics tested while the other three strains were resistant to only one or two antibiotics. From the typing of the isolates based on the similarity of resistance patterns, approximately 3 groups were classified in the present study. Notably, group II and III consist of *V. parahaemolyticus* isolated from the same sampling location, with a considerably high MAR index value of 0.57. Strains in the 3 groups were similarly resistant to Amoxycillin, Ampicillin, Gentamicin, Nalidixic acid, Streptomycin, Sulphamethoxazole and Tobramycin. The MAR index value shown was in the range of 0 to 1.0. One of the strains showed resistance to all of the antibiotics tested (MAR value 1.0), and one strain showed MAR index of value 0.0. Notably, 19.57% of the isolates had a MAR index value of 0.5 and 17.39% showed MAR index of 0.57. Strains with MAR index more than 0.2 were 91.34% (42/46).

To determine the clonal relatedness of the *V. parahaemolyticus* strains in this study, RAPD analysis was carried out using the three selected primers (OPA8, OPA14, and OPA15). Most of the strains were untypable (figures not shown) when subjected to RAPD, and only 19 strains were typable. From the typable strains, a majority were typable when using OPA8 compared to OPA14 and OPA15. The size for RAPD fragments generated ranged from 250 bp to 1,500 bp, with most of the strains contained three major gene fragments of 350, 1,000 and 1,350 bp.

All results from RAPD were analyzed with the GelCompare 4.2 software, and the overall achievable patterns were used to construct dendrograms using the UPGMA (unweighted pair group average method) clustering algorithms. Figure 1 showed the dendrogram constructed from RAPD profiles of the strains using OPA8 and OPA15 (strains using OPA14 were untypable). From the clustering in the dendrogram, the strains were divided into three major types (Type A, B and C) and a total of 16 profiles. Type A consisted of strains WMD63, WMD68, WMC32, WMC42, WMD50, WMD57, WMC 26, WMC22, and KS2. All of the strains in this group similarly originated from wet markets,

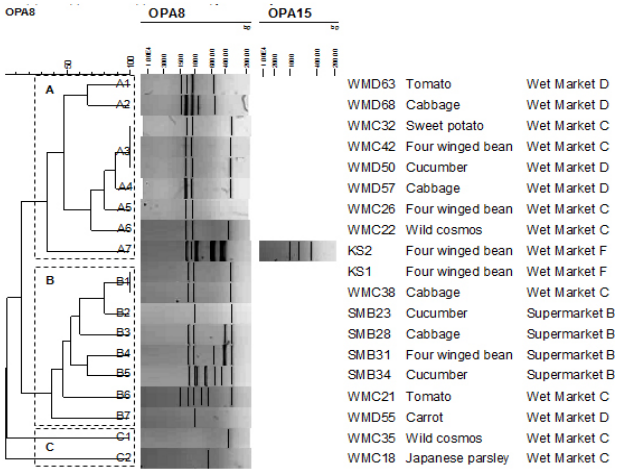

Figure 1. Dendrogram of RAPD profiles of *V. parahaemolyticus* strains using OPA8 and OPA15

particularly Wet Market D, Wet Market C, and one from Wet Market F. The strains were isolated from different types of vegetables, only two strains were from the same type of vegetable which was Cabbage. Type B consisted of strains KS1, WMC38, SMB23, SMB28, SMB31, SMB34, WMC21 and WMD55. The strains in this group originated from both wet markets and supermarket. Most of the strains were from Supermarket B, others were from Wet Market C, D, and F. In this group, two strains were isolated from Four winged bean, two strains from Cabbage, two strains from Cucumber, one from Tomato and another from Carrot. Type C consisted of two individual strains (WMC35 and WMC18), which were isolated from Wild cosmos and Japanese parsley respectively, and both originated from Wet Market C.

Table 3 showed the RAPD types and subtypes of the 19 typable strains based on the dendrogram. Type A was the major group, accounting for 47.37% (9/19) of all the typable strains, followed by Type B with 42.11% (8/19), and Type C with 10.53% (2/19). Subtype A3 and B1 were the major subtypes, accounting for 15.79% (3/19) and 10.53% (2/19) of all strains, respectively. Within the 15 typable strains originated from wet markets, Type A accounted for 60% (9/15), Type B accounted for 26.67% (4/15) and Type C accounted for 13.33% (2/15). All the 4 typable strains from supermarkets belonged to Type B, in which subtype B2, B3, B4 and B5 accounted for 25.0% (1/4) each, respectively.

Discussion

The role of the environment in the emergence and spread of antibiotic resistant bacteria and their possible pathways in contributing to the spread of resistance genes are not yet clear (Manjusha *et al*., 2005). In this study, we investigated the incidence of multiple

Table 2. Profiling of *V. parahaemolyticus* isolates based on antibiotic resistance patterns

Strain No.	MAR Index	Antibiotic Resistance Pattern[1]	Sample Source	Location	
WMC29	0	Not resistant to any antibiotics	Indian pennywort	Wet Market C	
WMD54	0.07	Na	Tomato	Wet Market D	
PH18	0.14	Na Te	Cabbage	Packing house 1	
WMD48	0.14	Na Rl	Japanese parsley	Wet Market D	
WMC39	0.21	Na Rl S	Indian pennywort	Wet Market C	
WMD59	0.21	Na Te Tob	Indian pennywort	Wet Market D	
WMC36	0.21	Ipm Na S	Japanese parsley	Wet Market C	
SMB30	0.21	Aml Na Te	Tomato	Supermarket B	
WMD53	0.29	Aml Amp E Te	Four winged bean	Wet Market D	
WMC20	0.29	Aml E Na Te	Cabbage	Wet Market C	
SMB34	0.29	Aml Na Nor Te	Cucumber	Supermarket B	
WMD62	0.29	Na Rl Te Tob	Four winged bean	Wet Market D	
WMC44	0.36	Na S Rl Te Tob	Long bean	Wet Market C	
KS7	0.36	Aml Amp Na Te Va	Four winged bean	Wet Market F	
WMD148	0.36	Aml Cn Na S Rl	Indian pennywort	Wet Market D	
WMC18	0.36	Aml Na Rl Te Va	Japanese parsley	Wet Market C	
WMD65	0.43	Aml Na S Rl Te Tob	Japanese parsley	Wet Market D	
WMD56	0.5	Aml Amp C Na Rl Te Tob	Indian pennywort	Wet Market D	
WMC26	0.5	Aml Amp Cn Na S Rl Tob	Four winged bean	Wet Market C	
KS2	0.5	Aml Amp E P Te Tob Va	Four winged bean	Wet Market F	
WMC35	0.5	Aml Amp Na Nor S Rl Te	Wild cosmos	Wet Market C	
WMD71	0.5	Aml Amp Na S Rl Te Tob	Tomato	Wet Market D	
SMB23	0.5	Aml Cn Na Rl Te Tob Va	Cucumber	Supermarket B	
KS6	0.5	Aml Cn Na S Rl Te Tob	Four winged bean	Wet Market E	
SMB31	0.5	Aml E Cn Na S Te Tob	Four winged bean	Supermarket B	
WMD69	0.5	Aml E Na S Rl Te Tob	Indian pennywort	Wet Market D	
WMC47	0.57	Aml Amp Cn Na Nor S Rl Tob	Japanese parsley	Wet Market C	I[2]
WMD55	0.57	Aml Amp Cn Na Nor S Rl Tob	Carrot	Wet Market D	
WMD58	0.57	Aml Amp Cn Na P Rl Te Tob	Cabbage	Wet Market D	
WMD50	0.57	Aml Amp Cn Na S Rl Te Tob	Cucumber	Wet Market D	II
WMD63	0.57	Aml Amp Cn Na S Rl Te Tob	Tomato	Wet Market D	
WMD67	0.57	Aml Cn Na Nor S Rl Te Tob	Cucumber	Wet Market D	
WMC42	0.57	Aml Cn Na S Rl Te Tob Va	Four winged bean	Wet Market C	
SMB37	0.57	Aml E Cn Na S Rl Te Tob	Japanese parsley	Supermarket B	
WMD57	0.64	Aml Amp Cn Na Nor S Rl Te Tob	Cabbage	Wet Market D	
WMC32	0.64	Aml Amp Cn Na Nor S Rl Tob Va	Sweet potato	Wet Market C	
KS1	0.64	Aml Amp Cn Na P S Rl Te Tob	Four winged bean	Wet Market F	
WMC38	0.64	Aml Amp Cn Na S Rl Te Tob Va	Cabbage	Wet Market C	
WMD147	0.64	Aml E Cn Na Nor S Rl Tob Va	Cabbage	Wet Market D	
WMC22	0.71	Aml Amp Cn Na Nor P S Rl Tob Va	Wild cosmos	Wet Market C	
WMD52	0.71	Aml Amp E Cn Na S Rl Te Tob Va	Sweet potato	Wet Market D	III
WMD68	0.71	Aml Amp E Cn Na S Rl Te Tob Va	Cabbage	Wet Market D	
WMC33	0.79	Aml Amp C Cn Na Nor P S Rl Tob Va	Long bean	Wet Market C	
WMC21	0.79	Aml Amp E Cn Na Nor S Rl Te Tob Va	Tomato	Wet Market C	
SMB28	0.79	Aml Amp E Cn Na P S Rl Te Tob Va	Cabbage	Supermarket B	
WMC45	1	Aml Amp C E Cn Ipm Na Nor P S Rl Te Tob Va	Cucumber	Wet Market C	

[1] = List of antibiotics used: Amoxycillin (AML), Ampicillin (AMP), Chloramphenicol (C), Erythromycin (E), Gentamicin (CN), Imipinem (IPM), Nalidixic acid (NA), Norfloxacin (NOR), Penicillin (P), Streptomycin (S), Sulphamethoxazole (RL), Tetracycline (TE), Tobramycin (TOB) and Vancomycin (VA).
[2] = Groups of strains based on the similarity of their resistance patterns (I, II, III)

antibiotic resistances in *V. parahaemolyticus* towards different antibiotics, and the results demonstrated high individual and multiple resistances to antibiotics among the isolates. This is not surprising as there are other reports on multi-drug resistance of *V. parahaemolyticus* isolated from raw foods even in

Table 3. RAPD types and subtypes of the 19 typable *V. parahaemolyticus* strains

Type	No. of strains (%)	Subtype	No. of strains (%)	Strain no.
A	9 (47.37)	A1	1 (5.26)	WMD63
		A2	1 (5.26)	WMD68
		A3	3 (15.79)	WMC32, WMC42, WMD50
		A4	1 (5.26)	WMD57
		A5	1 (5.26)	WMC26
		A6	1 (5.26)	WMC22
		A7	1 (5.26)	KS2
B	8 (42.11)	B1	2 (10.53)	KS1, WMC38
		B2	1 (5.26)	SMB23
		B3	1 (5.26)	SMB28
		B4	1 (5.26)	SMB31
		B5	1 (5.26)	SMB34
		B6	1 (5.26)	WMC21
		B7	1 (5.26)	WMD55
C	2 (10.53)	C1	1 (5.26)	WMC35
		C2	1 (5.26)	WMC18

neighbouring countries (Zulkifli *et al*., 2009). Foods contaminated with antibiotic resistant bacteria is a threat to public health as the antibiotic resistant determinants may be transferred to other bacteria of clinical significance, and *V. parahaemolyticus* is a candidate vehicle for such transfer because of its diversity and also because it can survive in the gastrointestinal tract of both humans and animals (Zulkifli *et al*., 2009).

In this study it was found that there was a high resistance (93.48%) towards Nalidixic Acid. This is in contrast with the findings by Zulkifli *et al*. (2009) and Manjusha *et al*. (2005), which reported a low resistance level of *Vibrio parahaemolyticus* towards Nalidixic Acid. Another case that could be highlighted here is their resistance towards Tetracycline (71.74%), which is alarmingly worrying since tetracycline is among the first-line drugs that were highly effective against *Vibrio* spp. (Han *et al*., 2007). Similarly, Adeleye *et al*. (2008) and Zulkifli *et al*. (2009) also reported a high resistance of *Vibrio parahaemolyticus* towards tetracycline.

Bacteria may be resistant to many drugs simultaneously or to the drugs from the same antibiotic group (Gwendelynne *et al*., 2005). Gwendelynne and co-workers (2005) have reported the resistance of *V. parahaemolyticus* towards β-lactams, however in this study there was an inconsistency in resistance of the isolates towards β-lactams. Some of the isolates were highly resistant towards Amoxycillin (80.43%) while some were highly susceptible towards Imipinem (4.35% resistance).

High MAR indices were detected in this study, ranging from 0 to 1.0, with more than 19.57% of the isolates had a MAR index value of 0.5 and 17.39% showed MAR index of 0.57. A high percentage of strains (91.34%) showed MAR index more than 0.2. MAR indices higher than 0.2 were rendered from high-risk sources (Gwendelynne *et al*., 2005). Antibiotics have been used since the 1950s to control certain bacterial diseases of high-value fruit, vegetable, and omamental plants, and until today the most commonly used antibiotics on plants is streptomycin with oxytetracycline to a minor extent (Kummerer, 2009). Several researchers have investigated the potential for a range of antibiotics to be taken up from soil by plants, and have assessed the potential significance of this exposure route in terms of human health. Some antibiotics are taken up by vegetables such as carrot roots (tuber), lettuce leaves and corn (Kummerer, 2009). Nonetheless, more studies on the elucidation of the antimicrobial susceptibilities of potential pathogenic *V. parahaemolyticus* are important for identification of more suitable and effective treatment of *V. parahaemolyticus* infections in humans and animals (Zulkifli *et al*., 2009).

Molecular typing is used for epidemiological studies as it provides the information on genetic relatedness of different bacterial strains, the source of infection, molecular markers of virulent and host specific strains (Chao *et al*., 2009). In this study, we found 16 RAPD profiles in which Type B was predominantly present in *V. parahaemolyticus* isolated from vegetables originated from Supermarket B. RAPD profiles type A, B and C were all present in *V. parahaemolyticus* isolated from vegetables originated from wet markets. The results indicated that most of the strains from the same type of sampling location were clustered into the same group, such as strains from supermarket (4 strains) in Type B and strains from wet markets (8 strains) in Type A. However, some strains from different sampling locations were found to be clustered in the same group, in which closely related strains were isolated from different locations (such as strains from wet markets and supermarkets in Type B), prompting a suggestion that cross-contamination occurred between wet market and supermarket. Chao *et al*. (2009) also reported that markets, hotels and restaurants caused the cross-contamination of *V. parahaemolyticus* between different seafood products.

From observation based on the vegetable types, only some vegetables of the same types were in the same group (for example Cabbage in Type A and Four winged bean in Type B), most of the vegetable types were widely spread. The genetic diversity of the strains on this study was not specifically associated with their origins. It is interesting to find that the strains in this study contained a major fragment of 1,000 bp, which is in concurrence with the study by Chang *et al*. (2010) whom reported the same major gene fragment in *V. parahaemolyticus* strains isolated from seawater, sediment and oysters collected

from Southern Taiwan. *V. parahaemolyticus* from seafood could be the source of cross-contamination to vegetables, which could be the most probable explanation in this case, because from our observation during sampling, some vegetable samples were displayed near seafood area.

Report on RAPD genotyping of *V. parahaemolyticus* from vegetable samples from other studies was difficult to be found; hence a comparison to the strains in our study could not be preceded. Nonetheless, the RAPD method has been successfully used for subdividing *V. parahaemolyticus* from different seafoods in India and for other organisms (Chang *et al*., 2010).

Conclusion

Continued monitoring of the antimicrobial susceptibility profiles of *V. parahaemolyticus* is important to better ensure vegetable safety, particularly, and retail and farm survey could be expanded to the national level. The subtyping data obtained from this study may also be useful as a comparison with the epidemiological data obtained from different countries and sources. This study provided new information with regards to the presence of *V. parahaemolyticus* in raw vegetable samples as well as its potential multiple antibiotic resistance and diverse heterogeneity.

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