Edible ice in Jakarta, Indonesia, is contaminated with multidrug-resistant *Vibrio cholerae* with virulence potential

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Consumption of street food is considered a major health risk in the absence of public-health inspection programmes in Indonesia. It is hypothesized that ice used in street food could be one of the major sources of *Vibrio cholerae* contamination. This study documented *V. cholerae* contamination in edible ice from different areas of Jakarta, the capital city of Indonesia, and attempted to characterize the virulence potential of the strains. A selective medium was used to isolate 98 *V. cholerae* strains and their identity was confirmed using biochemical assays. Serological tests classified the majority (78%) in the non-O1 serogroup. Multiplex PCR was used to detect the presence of *V. cholerae* virulence genes, namely *ctxA*, *ompU*, tcpA, ace, zot and *toxR*. The *toxR*, *ctxA*, *ompU* and zot genes were detected in 75, 26, 15 and 1% of isolates, respectively. The *ace* and tcpA genes were not detected in any of the isolates. The *ctxA* gene encoding the choler toxin subunit A, which has been associated only with clinical strains of O1, was present in both serogroups. The antibiotic-resistance profile showed that 65, 60, 52, 39, 37, 19 and 3% of the isolates were resistant to ampicillin, streptomycin, kanamycin, sulfamethoxazole–trimethoprim, erythromycin, tetracycline and ciprofloxacin, respectively. A large proportion of *V. cholerae* isolates came from west and south Jakarta, and these strains exhibited multidrug resistance to ampicillin, streptomycin, tetracycline, erythromycin, kanamycin and sulfamethoxazole–trimethoprim. Many of these isolates from west and south Jakarta also harboured *toxR*, encoding a regulator, and *ctxA*. The presence of multidrug-resistant *V. cholerae* with virulence genes in edible ice, which could cause a severe outbreak, reflects the poor water quality in Jakarta, and indicates an urgent need for better surveillance and management.

**INTRODUCTION**

Ice products are often used in street foods and are consumed by most people, especially in tropical countries such as Indonesia. Microbial infections acquired from contaminated ice are not uncommon and have been reported for *Escherichia coli* O157 : H7, *Legionella pneumophila*, *Salmonella enteritidis*, *Shigella flexneri*, and *Norwalk*-like virus (Kim & Harrison, 2008; Seo et al., 2006; Boccia et al., 2002; Graman et al., 1997). A significant number of cholera cases are due to consumption of contaminated water rather than person-to-person transmission (Schild et al., 2008).

The causative agent of cholera is *Vibrio cholerae*. Although it is a marine organism, it can also be found in fresh-water rivers. There are two *V. cholerae* serogroups, O1 and non-O1. Members of the O1 group are associated with epidemic cholera, whereas non-O1 members isolated from estuaries are associated with sporadic illness. The presence of virulence genes is usually associated with the O1 group (Sack et al., 2004). However, this scenario has been evolving slowly in recent years, and several studies have reported the presence of virulence genes in non-O1 environmental isolates (Kumar et al., 2008; Jagadeeshan et al., 2009; Kumar et al., 2010). The O1 serogroup is subdivided into the classical and El Tor biotypes. The classical biotype was responsible for the fifth and sixth cholera pandemics, whilst El Tor was the causative agent of the seventh pandemic (Sack et al., 2004). Outbreaks of acute diarrhoeal illness caused by the *V. cholerae* O1 or O139 serogroups have been reported (John & Jesudason, 1995; Sur et al., 2007).

Serogroup O1 is divided into two major serotypes, Ogawa and Inaba (Stroher et al. 1992). These serotypes differ by their terminal monosaccharides (Villeneuve et al., 1999). *V. cholerae* O1 Ogawa has been responsible for causing frequent diarrhoeal outbreaks over the last 10 years, although Inaba is becoming increasingly associated with cholera outbreaks (Chhotray et al., 2002). A third serotype, Hikojima, a variant of Ogawa (Ogg et al., 1978, 1979), has also been associated with outbreaks in Nigeria and India (Onyemelukwe & Lawande, 1991; Chandralakha et al., 2011).
Expression of virulence genes is a major contributing factor to the pathogenicity of V. cholerae. Some virulence-associated factors in V. cholerae are the ToxR regulator, cholera toxin (composed of CtxA and CtxB), toxin-coregulated pilus subunit (TcpA), outer-membrane protein (OmpU), accessory cholera toxin (Ace) and zonula occludens toxin (Zot). Expression of toxR is dependent on environmental conditions (Schild et al., 2008). ToxR regulates the expression of ctxA, tcpA and ompU (Walder & Mekalanos, 1994). Both cholera toxin and Ace have a classic enterotoxic effect (Schild et al., 2008; Huang et al., 2009; Morita et al., 2010), whereas Zot increases the permeability of the small intestine by affecting the structure of the tight junction, or zonula occludens (Singh et al., 2002). The majority of V. cholerae O1 strains contain genes for cholera toxin and/or the toxin-coregulated pilus (Kaper et al., 1995).

Antibiotic resistance is a major public-health concern in combating infectious diseases. There has been an increase in the incidence of antibiotic resistance in O1, O139 and non-O1 V. cholerae serogroups in both clinical and environmental isolates (Deashinta et al., 2007; Mandal et al., 2012; Rahmani et al., 2012; Panda et al., 2012). V. cholerae O1 strains that are resistant to cefotaxime, nalidixic acid, streptomycin, tetracycline and trimethoprim have been isolated from south India (Jagadeeshan et al., 2009), and Iwanaga et al. (2004) reported V. cholerae O1 strains from a patient in Laos resistant to chloramphenicol, tetracycline, streptomycin and trimethoprim.

Cholera has been categorized as one of the emerging and re-emerging diseases that cause severe diarrhoea. It threatens mortality in many developing countries and remains an important public-health problem (Fazil & Singh, 2011; Mandal et al., 2011). According to a recent survey on the incidence of cholera, Kolkata (India) leads, followed by Beira (Mozambique) and Jakarta (Indonesia) (Deen et al., 2008). Jakarta has the highest percentage of sporadic diarrhoeal illness compared with other areas in Indonesia (Simanjuntak et al., 2001). Consumption of ice contaminated with antibiotic-resistant bacteria may exacerbate the existing public-health problem. Thus, V. cholerae-contaminated ice products are likely to increase the risk of cholera transmission and may explain the sporadic outbreaks in Jakarta. In this study, we examined the contamination of V. cholerae in ice samples in Jakarta, characterized these isolates and determined their resistance profile, and analysed them for the presence of virulence genes.

METHODS

Sample collection. Ice cubes were collected from five regions of Jakarta: central, east, west, north and south (Fig. 1). We selected eight locations per region and sampling was repeated twice for each location. The samples were placed in a cooler box and transported immediately to the laboratory.

Isolation of V. cholerae. A previously established method for the isolation of V. cholerae from water samples was used (Hill et al., 2011; Ranbar et al., 2011). Briefly, the ice cubes were melted at room temperature and 25 ml was inoculated into 25 ml alkaline peptone water (Oxoid) and incubated at 37 °C with shaking at 120 r.p.m. for 18–24 h. The enrichment was performed in duplicate. A 2.0 ml sample of the inoculated alkaline peptone water was then centrifuged at 1500 g for 3 min and the supernatant was removed. The pellet was resuspended in 500 µl 0.85 % NaCl and subsequently diluted 1:1000. Each dilution was spread onto selective agar medium containing thiosulfate citrate bile salt (Oxoid) and incubated at 37 °C for 18–24 h.

Biochemical and serological assays. Suspected colonies of V. cholerae were grown in brain–heart infusion agar (Oxoid) at 37 °C for 18–24 h. The isolates were screened for oxidase, indole and lysine decarboxylase activity and for growth in Kligler iron agar (Oxoid) (Choopun et al., 2002). Polyvalent antisera (Biofarma) was used to determine the O1 and non-O1 serogroups. The O1 strains were analysed further using monovalent antisera to distinguish between Ogawa and Inaba (Ramamurthy et al., 1993). The identification and serogrouping of all strains were confirmed as described elsewhere (Chun et al., 1999).

Antimicrobial susceptibility testing. The antibiotic susceptibility of V. cholerae isolates was determined using a disc diffusion method (Kirby-Bauer) using commercial discs. A McFarland 0.5 standard was used to prepare V. cholerae inocula. Seven antibiotic discs (Oxoid) were used in this study: ampicillin (10 µg), streptomycin (10 µg), kanamycin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg) and sulfamethoxazole–trimethoprim (25 µg). The choice of antibiotics used to assay the resistance properties of V. cholerae was based on published articles (Smith et al., 2008; Ang et al., 2010; Nishibori et al., 2011). We used guidelines established by the Clinical and Laboratory Standard Institute (CLSI, 2005).

Multiplex PCR. Genomic DNA was extracted according to a method described by Murray & Thompson (1980). The 25 µl reaction mixture contained 1 U GoTaq DNA polymerase (Promega), 400 µM dNTPs (Promega), 1 µl each forward and reverse primer and 2.5 µl DNA template, adjusted to a final volume of 50 µl using nuclease-free water. The primer concentrations used in this study were 16 µM for ctxA and ompU, 30 µM for tcpA, ace and zot, and 50 µM for toxR (Eurogenetech). V. cholerae O1 strain 161532 was used as a positive control for toxR and ctxA (provided by Dr S. D. Putnam and Dr M. R. Kasper, US Naval Medical Research Unit 2, Jakarta, Indonesia). The primers used are listed in Table 1. PCR products were separated by 1.8 % agarose gel in 0.5 x TBE buffer, stained with ethidium bromide (0.5 µg ml⁻¹) and visualized using Gel Doc (Bio-Rad Laboratories).

RESULTS

Distribution of serogroups

A total of 40 samples was collected from five regions of Jakarta: central, east, west, north and south. A few colonies were selected from each sample for screening; in total, 98 V. cholerae isolates were characterized. The prevalence of V. cholerae was highest in west Jakarta with a total of 39 isolates, followed by south and central areas with 33 and 12 isolates, respectively (Table 2). North and east Jakarta had the lowest incidence with seven isolates from each region.

Of the 98 isolates, 22 % belonged to the O1 serogroup, but the majority (78 %) were non-O1 (Table 2). The occurrence of the non-O1 serogroup was higher than that of the O1 serogroup in all regions, except for north Jakarta.
where the prevalence of the O1 serogroup was 57\% (Table 2). Central Jakarta had the non-O1 \textit{V. cholerae} serotype exclusively. Within the O1 serogroup, Ogawa appeared to predominate at all localities except the south.

**Distribution of virulence genes**

A multiplex PCR assay was designed to detect six known virulence genes of \textit{V. cholerae}, namely \textit{ctxA}, \textit{ompU}, \textit{tcpA}, \textit{ace}, \textit{zot} and \textit{toxR} (Fig. 2, Table 2). Of the 98 isolates

**Table 1. Sequences of the primers used in this study**

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer sequence (5′→3′)</th>
<th>Expected amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ace-F</td>
<td>TAAGGATGTGCTTTATGATGGACACCC</td>
<td>316</td>
</tr>
<tr>
<td>ace-R</td>
<td>CGTGATGAATAAGATACCTCATAGG</td>
<td></td>
</tr>
<tr>
<td>ctxA-F</td>
<td>CGGGCAGATTCCTAGACCTCCTTG</td>
<td>564</td>
</tr>
<tr>
<td>ctxA-R</td>
<td>CGATGATCTGGAGCATTCGCCAC</td>
<td></td>
</tr>
<tr>
<td>tcpA-F</td>
<td>CAGGTAAGAAAAACCGGTCAGAG</td>
<td>620</td>
</tr>
<tr>
<td>tcpA-R</td>
<td>TTACCAAATGCAACGGGGAATG</td>
<td></td>
</tr>
<tr>
<td>toxR-F</td>
<td>CCCCTGGAGCTCCCCTAAGCAATAC</td>
<td>779</td>
</tr>
<tr>
<td>toxR-R</td>
<td>AGGTGTAACCCAGATGTGTTAAG</td>
<td></td>
</tr>
<tr>
<td>ompU-F</td>
<td>AGCGTGAAGCCAAATCAGATGTG</td>
<td>869</td>
</tr>
<tr>
<td>ompU-R</td>
<td>GGCGAAAGTTGGCTTGAGAAGT</td>
<td></td>
</tr>
<tr>
<td>zot-F</td>
<td>TCCTTAAAGCTCGGCGGTTT</td>
<td>947</td>
</tr>
<tr>
<td>zot-R</td>
<td>AACCCTTCTCTCTCTACTCCTCCTCCTACCA</td>
<td></td>
</tr>
</tbody>
</table>

F, Forward; R, reverse.
screened, toxR was found in 73 isolates (75%). Of the 73 isolates, 18 isolates (24.6%) belonged to the O1 serogroup and the rest were non-O1. The highest prevalence of toxR was seen in isolates from north, east and west Jakarta (Table 2). The ompU gene was found in 15 isolates (15%), and six of them belonged to the O1 serogroup. All of the isolates from east Jakarta harboured ompU, but none of the isolates from central Jakarta had this gene (Table 2).

None of isolates harboured the tcpA or ace gene, and only one isolate, from south Jakarta, had the zot gene (Table 2). The highest incidence (58%) of ctxA-positive isolates was from central Jakarta followed by the south and west. All isolates (100%) from east Jakarta harboured toxR and ompU, whereas only 58% of the isolates from central Jakarta had the toxR gene and none had the ompU gene. In west Jakarta, 85% of isolates harboured toxR but only 18% carried ctxA. Similarly, 61% of isolates from the southern region harboured toxR and only 33% carried ctxA. In north Jakarta, 86 and 14% of isolates harboured toxR and ompU, respectively, but none carried ctxA.

We found 25 isolates (26%) that were positive for ctxA, and of these only one was serogroup O1. The one isolate that was PCR positive for zot was negative for ctxA. We did not find any ctxA-positive isolates from north or east Jakarta (Table 2).

**Antibiotic-resistance profile**

Most of the isolates analysed (97%) were sensitive to ciprofloxacin (Table 3). The isolates from north, west and east Jakarta had the highest incidence of ampicillin resistance. The south and north Jakarta isolates exhibited high resistance to streptomycin, followed by kanamycin. All isolates from east Jakarta were resistant to both streptomycin and kanamycin. Many of them were also resistant to erythromycin (43%) and sulfamethoxazole–trimethoprim (29%).

All the isolates from north Jakarta were resistant to sulfamethoxazole–trimethoprim. Many were resistant to multiple antibiotics: kanamycin (86%), erythromycin

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**Table 2. Distribution of serogroup and virulence genes**

<table>
<thead>
<tr>
<th>Region in Jakarta</th>
<th>No. of isolates</th>
<th>Serology test (%)</th>
<th>Virulence gene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O1 Ogawa</td>
<td>O1 Inaba</td>
</tr>
<tr>
<td>North</td>
<td>7</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td>South</td>
<td>33</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>West</td>
<td>39</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>Centre</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>East</td>
<td>7</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>17</td>
<td>5</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Multiplex PCR for resistance genes. (a) Lanes: M, 1 kb DNA ladder (Biolabs); 1, negative control; 2, isolate IF (toxR+, zot+); 3, 4 and 7, isolates IB, LC and DD (toxR+, ompU+); 5 and 6, isolates JE5 and JA6 (toxR+). (b) Lanes: M, 1 kb DNA ladder (Biolabs); 1–4, isolates 9.2, O26, C42 and O25 (toxR+, ompU+); 5 and 6, isolates JH5 and JD6 (toxR+). (c) Lanes: M, 1 kb DNA ladder (Fermentas); 1, positive control (toxR+ and ctxA+, 779 and 564 bp, respectively); 2, negative control; 3–5, isolates D41, 7.23 and L41 (toxR+); 6 and 7, isolates I32 and 3.7 (toxR+, ctxA+).
(71%), and streptomycin (57%). In contrast to the north, all the isolates from the central region showed sensitivity to sulfamethoxazole–trimethoprim. Many of these (58%) also were resistant to ampicillin.

The isolates from the west and south of Jakarta were resistant to multiple antibiotics. The isolates from the south were resistant to streptomycin (85%), kanamycin (76%), sulfamethoxazole–trimethoprim (67%), ampicillin (55%) and erythromycin (33%). The isolates from the west were predominantly resistant to ampicillin (72%), but a large number of them were resistant to streptomycin (44%), tetracycline (38%), erythromycin (36%), kanamycin (28%) and sulfamethoxazole–trimethoprim (15%).

Of the O1 serogroup isolates, only two showed susceptibility to ampicillin and three to streptomycin. The rest of the O1 isolates were resistant to streptomycin. All of the ciprofloxacin-resistant isolates (3%) belonged to the non-O1 serogroups. Four isolates belonging to the Ogawa serotype were resistant to all of the antibiotics (ampicillin, streptomycin, kanamycin, sulfamethoxazole–trimethoprim, erythromycin and tetracycline) except for ciprofloxacin, and were toxR positive and ctxA negative. In contrast, four isolates belonging to non-O1 serogroup were susceptible to all of the antibiotics tested in this study. One harboured toxR and ctxA, whereas none of the genes tested for were detected in the remaining three.

**DISCUSSION**

Diarrhoea is a common cause of food-borne illness. There is a high incidence of sporadic diarrhoeal disease in Jakarta. To date, there has been no study to determine the source of these outbreaks. However, they may be due to consumption of food from street vendors. One of the major causes of food-borne illness originates from the use of contaminated water. In this study, we examined edible ice from five regions of Jakarta (east, west, north, south and central) for *V. cholerae* contamination. To date, there is no report connecting ice sold by street vendors and local outbreaks of cholera. We hypothesized that edible ice used in Jakarta could be the source of diarrhoea mediated by *V. cholerae*.

**Edible ice in Jakarta is contaminated with *V. cholerae***

In this study, we isolated 98 *V. cholerae* isolates from ice samples from all regions in Jakarta. The prevalence of *V. cholerae* was highest in west Jakarta, followed by south and central areas (Table 2). North and east Jakarta had the lowest incidence. These results are similar to our previous study on the rapid detection of *V. cholerae* from ice in Jakarta where the bacteria were isolated mostly in west Jakarta (Waturangi & Fransiska, 2009). The difference in *V. cholerae* prevalence between the various regions in Jakarta might be due to differences in the water sources used for ice making. A National Economic Social Survey (SUSENAS) revealed that the total proportion of households using tap water compared with groundwater varied with region, being 63% in the south, 54% in the east, 17% in the central region, 11% in the west and 1% in the north (Annual Statistics, 2011; Badan Pusat Statistik Republik Indonesia, http://www.bps.go.id/). The survey also found that most groundwater was contaminated with coliform bacteria. The *V. cholerae* contamination level was highest in west Jakarta compared with all other regions, although only 11% of the households use groundwater. It is also possible that these vendors may cross regional borders to set up their stalls. The north, which had only 1% groundwater users, had the lowest incidence of *V. cholerae*. The water source cannot explain why east Jakarta with 54% groundwater users also has the lowest incidence of *V. cholerae*.

**Prevalence of *V. cholerae* non-O1 serogroups in edible ice**

The majority of the isolates in this study belonged to the non-O1 serogroup. These results support previous findings that the non-O1 serogroup is distributed ubiquitously in the aquatic environment (Chakraborty et al., 2000; Rivera et al., 2001; Kumar et al., 2008). There are several reports of outbreaks or sporadic cases of gastroenteritis and extra-intestinal infections in humans caused by non-O1 serogroup *V. cholerae* (Bagchi et al., 1993; Ou et al., 2003). Although it was not clear whether the isolates in our study had caused any illness, the possibility cannot be ruled out.

**Virulence genes found in *V. cholerae* non-O1 serogroups**

Generally, non-O1 serogroup isolates do not harbour virulence genes (Sack et al., 2004), although this scenario continues to evolve, with these non-O1 environmental isolates acquiring virulence genes (Kumar et al., 2008; Jagadeeshan et al., 2009; Kumar et al., 2010). In our study, 75% of the isolates harboured the toxR gene, and it was distributed in both O1 and non-O1 serogroups of *V. cholerae* (Table 2). This is in

**Table 3. Percentage distribution of resistance isolates**

<table>
<thead>
<tr>
<th>Region in Jakarta</th>
<th>Antibiotic*</th>
<th>AM</th>
<th>TE</th>
<th>S</th>
<th>K</th>
<th>CIP</th>
<th>E</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>AM</td>
<td>71</td>
<td>43</td>
<td>57</td>
<td>86</td>
<td>0</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>South</td>
<td>AM</td>
<td>55</td>
<td>0</td>
<td>85</td>
<td>76</td>
<td>9</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>West</td>
<td>AM</td>
<td>72</td>
<td>38</td>
<td>44</td>
<td>28</td>
<td>0</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>Centre</td>
<td>AM</td>
<td>58</td>
<td>0</td>
<td>17</td>
<td>8</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>East</td>
<td>AM</td>
<td>86</td>
<td>14</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>43</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>AM</td>
<td>65</td>
<td>19</td>
<td>60</td>
<td>52</td>
<td>3</td>
<td>37</td>
<td>39</td>
</tr>
</tbody>
</table>

AM, Ampicillin; CIP, ciprofloxacin; E, erythromycin; K, kanamycin; S, streptomycin; SXT, trimethoprim–sulfamethoxazole; TE, tetracycline.

*Tests were carried out using a disc diffusion method.*
agreement with a study by Jagadeeshan et al. (2009), which showed the emergence of non-O1 V. cholerae carrying toxR. The toxR gene was not detected in three isolates of O1 serotype. This is curious, as most O1 strains are associated with pathogenicity and ToxR is an important regulator of virulence. Experiments are necessary to establish the virulence potential of this strain to cause diarrhoea. If it does not, then this strain is likely to be a precursor to the pathogenic variants, and it is possible that the presence or absence of toxR could be used as a diagnostic marker. As the V. cholerae toxR gene shares a high sequence identity with that of Vibrio parahaemolyticus and Vibrio mimicus, the primers used will need to be redesigned for specificity (Kim et al., 1999; Singh et al., 2002).

It has been reported that >95% of strains belonging to serogroups O1 and O139 produce cholera toxin, which is central to the diarrhoeal disease process (Kaper et al., 1995). In contrast, >95% of strains belonging to the non-O1 non-O139 serogroup do not produce cholera toxin. Faruque et al. (1998a) documented that most environmental strains tested did not produce cholera toxin. However, we found ctxA-positive isolates (26%) and they were distributed between the O1 and non-O1 serogroups. Our results are in agreement with several studies demonstrating the presence of virulence genes including ctxA in environmental isolates (Chakraborty et al., 2000; Jiang et al., 2003; Kumar et al., 2010). These ctxA-positive clones could easily give rise to illness.

All of the ctxA-positive isolates in this study were PCR negative for the CTXΦ toxin gene zot. The zot gene is almost always found in strains containing ctx but is rarely found in strains lacking ctx (Maiti et al., 2006). In this study, one zot-positive strain, which was negative for ctxA, was isolated. It is possible that a portion of the CTXΦ prophage genome may be missing, or may have been disrupted by deletion or insertion, suggesting that CTXΦ genes among environmental strains are likely to be defective (Jiang et al., 2003; Maiti et al., 2006). The Zot toxin may be associated with gastrointestinal symptoms induced by V. cholerae strains that do not express cholera toxin.

It has been found that ctxA transfer among V. cholerae does not always require the toxin-coregulated pilus, a homopolymer of TcpA pilin (Faruque et al., 1998b). Our results support these observations, as all of the ctxA-positive isolates were PCR negative for tcpA. However, the mechanism of TcpA-independent transfer among bacteria remains unclear. It is possible that these isolates may have lost tcpA after acquiring ctxA.

The presence of virulence genes in environmental isolates indicates the plasticity of the V. cholerae genome to acquire genes by horizontal gene transfer. This suggests that both oceans and rivers will contain pathogenic V. cholerae. This will only increase the potential of V. cholerae to cause severe diarrhoeal illness. Therefore, to prevent future outbreaks of diarrhoeal diseases, surveillance of environmental strains of V. cholerae will be extremely important.

Presence of resistance phenotypes in V. cholerae non-O1 serogroups

Multiple antibiotic resistance in bacterial pathogens is becoming a common phenomenon in developing countries, including South-East Asia. This is most likely to be related to the frequent use of over-the-counter drugs without proper or with no medical supervision. The changing landscape of antibiotic resistance in pathogenic bacteria associated with diarrhoeal patients in Indonesia was documented by Tjaniadi et al. (2003). The presence of ampicillin-resistant V. cholerae isolates in this study appeared to be similar to that found in Laos, where both non-O1 and non-O139 isolates were found to be resistant to ampicillin (Miyazato et al., 2004).

A 4 % increase in tetracycline resistance in V. cholerae was seen in Jakarta between 1996 and 2001 (Tjaniadi et al., 2003). In our study, 19 % of the isolates were resistant to tetracycline, a dramatic rise compared with 2001. This is perhaps not surprising as, in Indonesia, tetracycline has for many years been the drug of choice for cholera treatment. In addition, we found that 38.8 % of the isolates were resistant to sulfamethoxazole–trimethoprim, and this antibiotic is used as the second drug of choice in situations where V. cholerae is found to be resistant to tetracycline.

Among the fluoroquinolones, ciprofloxacin is frequently used in the treatment of cholera. Three non-O1 V. cholerae isolates were resistant to ciprofloxacin, whilst most of the isolates were sensitive to ciprofloxacin and some exhibited intermediate resistance. Similarly ciprofloxacin-resistant O139 V. cholerae strains have been isolated in India (Okuda et al., 2007).

A large number of the V. cholerae isolates were resistant to multiple antibiotics: ampicillin, streptomycin, tetracycline, erythromycin, kanamycin and sulfamethoxazole–trimethoprim. Further studies will be required to determine the mechanism of resistance.

Conclusions

O1 and non-O1 V. cholerae isolates are present in edible ice in Jakarta, indicating the poor quality of the water. Use of this contaminated water by street vendors is a major public-health problem. Most of the V. cholerae isolates in this study came from west and south Jakarta, and a large number of them were resistant to multiple antibiotics. In addition, many of these strains harboured toxR and ctxA encoding a regulator and cholera toxin subunit A, respectively. It is alarming to note the presence of multidrug-resistant non-O1 V. cholerae strains harbouring virulence genes. These genes could easily be transferred to O1 serogroups and may cause an epidemic. Alternatively, these non-O1 serogroup strains may have the potential for future cholera epidemics. This study clearly indicates that V. cholerae contamination is a serious public-health concern in Indonesia, and that there is a need for an increased surveillance and implementation of preventative measures.
**ACKNOWLEDGEMENTS**

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**REFERENCES**


contaminated well water.