VIBRIO CHOLERAE AND ITS TYPING PROFILE

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ABSTRACT

A research to type clinical isolate of Vibrio cholerae kept as stock culture at Institute of Tropical Disease Airlangga University had been conducted in Surabaya, Indonesia. A total of 77 stocked culture had been serotyped by a slide agglutination test with polyvalent O1 and continued with monovalent antisera, and biotyped biochemically based on the VP reaction. All examined isolates belong to serogroup O1, 76/77 (99%) of the isolates are Ogawa serotype, and 53/77 (68%) are eltor biotype. Another 1 isolate does not belong to serogroup O1. It means that people are faced to two kinds of potential epidemic strains of Vibrio cholerae O1.

Keywords: Vibrio cholerae O1, serotypes and biotypes, Surabaya, Indonesia

INTRODUCTION

Cholera is a major cause of illness in developing world (WHO 2006). In 2006, World Health Organization reported that 236,896 cases of cholera occurred in 52 countries, a 79% increase over 2005 (WHO 2006). Even though there is increasing in the understanding of the molecular basis of Vibrio cholerae pathogenicity we do not fully understand the cause of seasonal epidemics in cholera-endemic areas nor the factors that drive epidemics (Franco et al. 1997). The association between Vibrio cholerae and aquatic environments has long been studied, but emphasis has been almost exclusively placed on coastal areas such as the Bay of Bengal, the point of origin of cholera (Colwell 2004). Recent studies have investigated environmental and climatic factors that may encourage the spread of cholera in African countries (Magny et al. 2007; Mendelson and Dawson 2007). Those studies also focused on coastal areas. Little is known about the epidemiology of cholera in inland areas of Africa (Bompangue et al. 2008). In the Democratic Republic of Congo (DRC), dozens of emergency programs have been implemented by humanitarian organizations, national health services, and international agencies; however they failed to achieve long-term control of cholera epidemics (Bompangue et al. 2008).

Faced with first emergence of cholera in Madagascar and its rapid spread, medical authorities reacted immediately by using doxycycline as chemoprophylaxis (Dromigny et al, 2002). This policy is essentially contrary to World Health Organization recommendations (WHO, 1993). Two years after the epidemic began, 15.7% of the 351 strains isolated were found to be tetracycline resistant (cross-resistance with doxycycline) and can not be recommended any longer for treating severe cases of cholera (Dromigny et al. 2002). This may represent a critical public health problem. In order to increase our knowledge in controlling cholera outbreak, we analyzed the serotypes and biotypes of Vibrio cholerae isolates kept as stocked culture at Institute of Tropical Disease Surabaya, Indonesia.

MATERIALS AND METHODS

The bacteria used in this study are stock cultures of clinical isolates of Vibrio cholerae that were collected 4.5 years since 1993. As many as 77 strains of Vibrio cholerae O1 were recultured on to new Heart Infusion broth and were incubated overnight at 37°C. Any growth in the broth culture was then streaked on to Thiosulfate Bile salt Sucrose agar and incubated overnight at 37°C. The characteristic flat yellow colony on the TCBS agar was then biochemically identified by routine laboratory procedures. Identified Vibrio cholerae were streaked on to Heart Infusion agar and incubated overnight at 37°C for further serotyping and biotyping. In serotyping all identified Vibrio cholerae were serotyped by a slide agglutination test using commercial polyvalent O1 and monovalent antisera (Denkaseiken). In biotyping all identified Vibrio
**cholerae** were biotyped biochemically based on the Voges Proskauer reaction.

**RESULT**

Seventy seven of stocked culture had been recultured on to new media and reidentified biochemically as *Vibrio cholerae*. Then serogrouped by Polyvalent O1 antisera and then serotyped by monovalent antisera. The results are seen on the table below. We also found 1 isolate of non O1 *Vibrio cholerae* isolated In 1995.

**DISCUSSION**

*Vibrio* species are included in the family of *Vibrionaceae* together with *Aeromonas* and *Plesiomonas*. The majority of clinical infections caused by *Vibrio* species are enteric in nature, ranging from the dread Asiatic cholera to isolated sporadic cases of diarrhea. In contrast, the *Aeromonas* and *Plesiomonas* have been isolated from a variety of other sources, such as blood, spinal fluid, and urine, as well as feces (Joklik et al. 1988). All members of this family are Gram-negative, facultative microorganisms and do not have exacting nutritional requirements (Joklik et al. 1988). The genus *Vibrio* contains some of the most important intestinal pathogens of man including the cause of epidemic asiatic cholera, *Vibrio cholerae*. Another intestinal pathogen, *Vibrio parahaemolyticus*, is the leading cause of diarrhea in Japan (Joklik et al. 1988) and could also be found in Surabaya, Indonesia (Wasito 1981).

A major concern of early cholera investigation was the concept that *Vibrio cholerae* strains could be divided into two groups: those in serogroup O1, and those in “non-agglutinating” or “noncholera” vibrios (Morris 1994). There are 3 serotypes in serogroup O1, namely Ogawa, Inaba, and Hikojima (Joklik et al. 1988). It is believed that there are seroconversions among these serotypes. Each serotype is divided into 2 biotypes, namely classic biotype and eltor biotype (Chart and Shears, 2003). Until the appearance of *Vibrio cholerae* O139 in 1992, *Vibrio cholerae* O1 was the only serotype responsible for cholera. The emerging of noncholera vibrio as a cause of cholera in human population in the end of the last century aware us to always monitor this strain by well conductive surveillance studies in the world. This noncholera vibrio is assigned now as *Vibrio cholerae* O139 and is related to the eltor biotype (Chart & Shears 2003; Morris 1994).

<table>
<thead>
<tr>
<th>Year</th>
<th>Ogawa serotype</th>
<th>Inaba serotype</th>
<th>Hikojima serotype</th>
<th>Total</th>
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<tr>
<td></td>
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<td>1993</td>
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<td>17</td>
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<td>1995</td>
<td>4</td>
<td>18</td>
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<td>22</td>
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<td>1996</td>
<td>0</td>
<td>4</td>
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<td>4</td>
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<td>- June 1997</td>
<td>0</td>
<td>3</td>
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<td>3</td>
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<td></td>
<td>24</td>
<td>52</td>
<td>1</td>
<td>77</td>
</tr>
</tbody>
</table>

Table 1. Frequencies of *Vibrio cholerae* O1 serotypes and biotypes kept in the laboratory since 1993 to June 1997

This study revealed that 76/77 (99%) of the clinical isolates are *Vibrio cholerae* serotype Ogawa and 52/76 (68%) of this serotypes are belong to eltor biotypes. There was only 1 *Vibrio cholerae* serotype Inaba and it was eltor biotype. It was well known that until 1961, the eltor biotype was isolated only in Sulawesi, Indonesia, and had caused four localized epidemics between 1937 and 1958 (Chart and Shears 2003). Based on the above opinion and fact, it is not impossible that we are faced with potential epidemic strains of *Vibrio cholerae* O1.

Humans are the only known natural hosts of *Vibrio cholerae*. Transmission is by ingestion, through contaminated water or food. The infective dose is high, up to $10^{11}$ bacteria being required. The incubation period ranges from a few hours to 5 days. Most are free from infection within 2 – 3 weeks, and there have been few examples of persistent carriage. For eltor biotypes, the ratio of carriers to cases may range from 30 : 1 to 50 : 1, compared with 5 : 1 for classic biotypes. Ector biotypes can also survive for longer periods in the environment (Chart and Shears, 2003). These factors give eltor biotypes an epidemiological advantage in the spread of the disease, which has occurred in the seventh pandemic and has contributed to the displacement of classic biotypes by eltor biotypes. Only in parts of southern Bangladesh has the classic biotypes persisted (Chart and Shears 2003; Stine 2008).
CONCLUSION

Current data indicate that these two kinds of biotypes are exist in Surabaya, with eltor biotypes slightly greater than classic biotypes. While Ogawa serotypes are most dominant serotype found in this area. It means that people are faced to two kinds of potential epidemic strain of Vibrio cholerae O1

REFERENCES


