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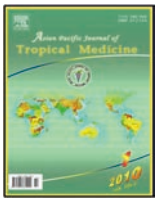

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Survey of epidemiology and bacteriology features of cholera in Iran

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ABSTRACT

Objective: To determine epidemiology and antimicrobial susceptibility patterns of *Vibrio (V.) cholerae* O1 biotype EL Tor in summer outbreak of 2008 in Iran. **Methods:** Stool samples were collected from patients suspected to have cholera admitted to hospitals and clinics. Specimens examined by conventional bacteriological methods. All isolates were sent to cholera reference laboratory for further confirmation, stereotyping and susceptibility testing. **Results:** A total of 220 patients were diagnosed as cholera. All cases confirmed by Iranian reference health laboratory. One hundred ninety nine of 220 *V. cholerae* serotypes were Inaba and 21 serotypes were Ogawa. All cases were reported from thirteen provinces. The majorities of cases were from Tehran, Qum and Zahedan provinces with 56, 26 and 25 cases respectively. 24(11%) of patients were under 15 years old and 196 (89%) of patients were older than 15 years.149 (68%) of patients were male and 71 (32 %) were female. 129(59%) of patients had Iranian nationality,79 (36.5%) were from Afghanistan and, 12 (5%) were from Pakistan. All isolates were resistant to co-trimoxazole, nalidixic acid, furazolidone, and intermediate to chloramphenicol and were susceptible to tetracycline, ciprofloxacin, and erythromycin. **Conclusion:** Our study reveals that in recent outbreak caused by *V. cholerae* EL Tor serotype Inaba is the predominant serotype. All isolates are resistant to co-trimoxazole, nalidix acid and furazolidon.

1. Introduction

Vibrio (V.) cholerae is a gram- negative comma shaped bacterium that causes cholera in human. Cholera is a wide-spread and severe disease and a global threat worldwide [1]. The main symptom of cholera is the production of life threatening watery diarrhea with varying degree of dehydration ranging from none to sever [2]. Since 1817, six pandemic have been reported and the seventh one is in progress [3]. Strains of *V. cholerae* belonging to serogroup O1 biotype El Tor and serogroup O139 have been described the causative agents of disease[3]. *V. cholerae* O139 responsible for non-O1 serotype isolated in 1992 in southern India and then spread rapidly up and down the coast of the Bay of Bengal reaching Bangladesh in December 1992. However, *V. cholerae* serogroup O1 still is the most common cause of cholera epidemics. Cholera is an endemic disease in Iran and is common in summer and early month of autumn. The causative serogroup in our country is O1 and we did not have any report for isolation of O139 serogroup in recent outbreaks [4-6].

The survival of *V. cholerae* in aquatic environment, and the potential role of the reservoir in subsequent disease outbreaks have been described in several studies [2]. Cholera disease have Owen epidemiologic features. Study and reconnection of different age groups at risk depends on epidemiologic pattern, causative serogroup, serotype, and source of infection. Drug resistance patterns are important parameters for designing preventative measures. The Islamic republic of Iran is at risk of cholera outbreaks especially spreading from neighboring country. The Center for disease control and prevention of Ministry health reported 12 epidemics of cholera since 1965. The recent epidemic occurred in 2005 with 1133 cases and 11 cases of death[2, 7, 8]. The aim of this study was to determine epidemiology and antimicrobial susceptibility patterns of *Vibrio cholerae* O1 biotype EL Tor in summer of 2008 in Iran.

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2. Materials and methods

Fecal specimens (stool or rectal swabs) were collected from patients that were admitted to hospital and clinics because of suspected cholera. Specimens were examined by conventional bacteriological methods. Briefly alkaline peptone water (APW) was used as an enrichment broth, and thiosulfate citrate bile salts sucrose agar (TCBS) as a selective agar medium. APW tubes were incubated at 37 ℃ for 6 to 8 hours. Subculture from APW was done on TCBA agar. All plates were incubated at 37 ℃ for 18-24h. Suspected sucrose fermenting colonies which were oxidase positive, indol positive and morphologically resembling to *V. cholerae* were identified biochemically and serologically[9, 10]. Serotype identification was based on agglutination test by using polyvalent and monovalent antisera as recommended by manufacture (Mast Diagnostic Group UK). A positive reaction with polyvalent antsera was considered as *V. cholerae* O1 isolate. Agglutination reaction with Inaba and Ogawa antisera were used for further serotyping.

All isolates were sent to cholera reference laboratory for confirmation, and susceptibility testing. Antimicrobial susceptibility testing was performed by disk diffusion methods as recommended by clinical laboratory standard institute (CLSI). Demographic data including clinical status, age, sex collected from questioner forms .The antimicrobial drugs testes included ampicillin (AM), co-trimoxazole (SXT), ciprofloxacin (CIP), tetracycline (TC), erythromycin (EM), choleramphenicol (C), cefexime (CFM) furazolidone (F) and nalidixic acid (NA). The E-test MIC (AB Biodisk , Solna, Sweden) method were used for detection of minimal inhibitory concentration (MIC) for erythromycin[11].

3. Results

From June to November 2008 all suspected patients of cholera were reported to the Center for Disease Control and Prevention of Ministry of Health and education. A total of 220 patients were clinically diagnosed as cholera with laboratory confirmation. Of the 220 isolates, 199 serotypes were Inaba and 21 serotypes were Ogawa.

The majority cases were from 3 of the 13 provinces where the data were collected from and included, Tehran (56), Qum (26) and Zahedan (25) cases. Of the 220 patients, 11% (24) were under 15 years old and 89% (196) were above 15years old. Of 220 patients, 149 (68%) were male and 71 (32%) were female. 129 (59%) of patients had Iranian nationality, 79 (36%) were from Afghanistan and 12 (5%) were from Pakistan. All isolates were resistant to co-trimoxazole, nalidixic acid, furazolidone and intermediate resistance to chloramphenicol. All isolates were susceptible to tetracycline, ciprofloxacin, and erythromycin. MIC for erythromycin ranged between 1-1.5 μg/mL.

The antimicrobial results and pulse field gel electrophoresis (PFGE) showed that all isolates had the same epidemiology and susceptibility patterns (Figure 1-4).

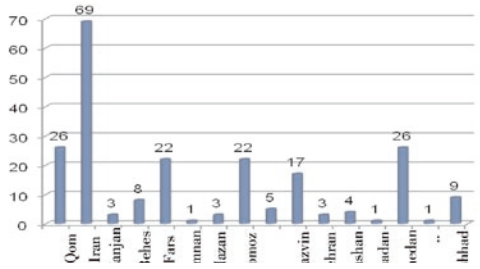

Figure 1. Reported cholera from unverisities.

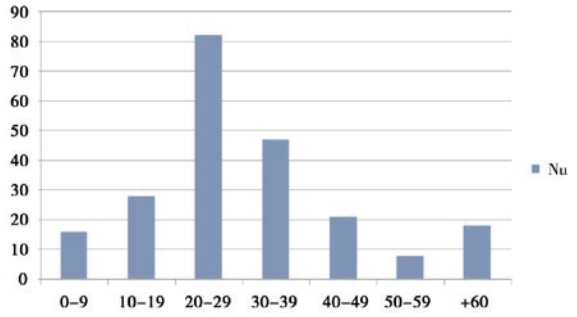

Figure 2. Age of cholear patients.

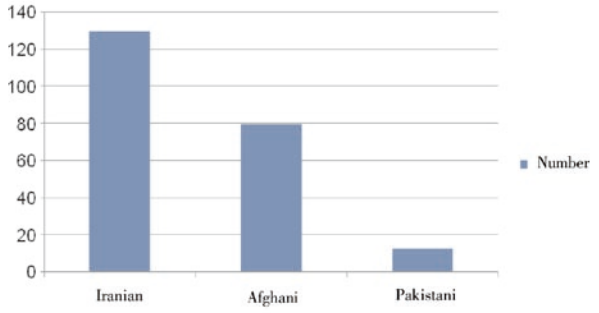

Figure 3. Nationality of patients.

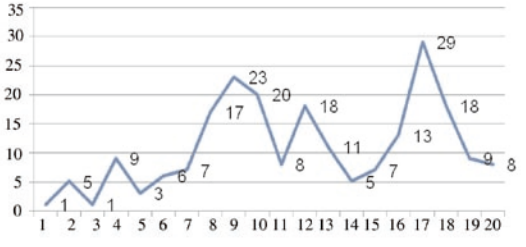

Figure 4. Weekly reported cholera cases in summer of 2008 in Iran.

4. Discussion

This is the second report of increased isolation of

of Inaba serotype from Iran. The first report was in 2005, when 1 104 cases of isolation reported [5,12]. Recent studies from Pakistan our eastern neighbor also showed an increased isolation of Inaba serotype [13]. The predominance of cholera due to *V. cholerae* O1 serotype Inba was also reported from India in recent years [14-16]. It needs molecular analysis such as PFGE to understand the widespread emergence of *V. cholerae* O1 serotype in different countries. Molecular studies on outbreak of 2005 in our country revealed similarity among *V. cholerae* O1 Inaba serotypes isolates. However there is not enough data in other countries for detection of origin of recent outbreaks.

It is assumed that the present Inaba serotype might have evolved from pre-existing *V. cholerae* O1 Ogawa isolates. *V. cholerae* O1 strains are known to interconvert and to switch between the two known serotypes, Ogawa and Inaba. Serotype switching may be related to immune pressure on the prevailing serotype as suggested by the observation of an epidemic in Latin America in 1991[16].

Data generated in the present study revealed that tetracycline, one of the main antimicrobials agent used for cholera treatment, was the most effective antibiotics. Our previous study in 2005 outbreak also showed that all isolated stains of *V. cholerae* were susceptible to tetracycline [7]. In our study all strains of *V. cholerae* that were susceptible to tetracycline were also susceptible to doxycycline. Doxycycline in our country is used as the first-line drug for treatment of cholera. Among third generation of cephalosporins, cefexim was effective against all isolates of *V. cholerae*, however this antibiotic is expensive and its supply is very limited. In contrast to other studies on diarrheal etiologies in our country that reported frequent resistance to ampicillin, all isolates of *V. cholerae* were susceptible to ampicillin. This antibiotic is an alternative drug for treatment of cholera in children, which all isolates are resistant to furazolidin. Among furoquinolnes, ciprofloxacin was the most effective antibiotic; however a significant limitation of fluoroquinolnes is their controversial use in children.

Resistance to co-trmoxazole was similar to results of prior study. The 100% resistance rate of *V. cholerae* to co-trmoxazole is due to widely use of this antibiotic for treatment of other diarrheal and urinary tract infections. Resistance of *V. cholerae* to nalidixic acid may due to widely use of this antibiotic in treatment of other infectious disease such as urinary tract infections and gastroenteritis. Intermediate resistance of *V. cholerae* to chloramphenicol is also notable, which make its usage limited. This can be partially explained by its frequent usage as a first line treatment for sever infectious diseases especially in children.

In general data obtained in this study and comparison of the results with that of our previous study in 2005, suggest that there is one main colonial strain circulating in recent outbreaks in our country .This hypothesis is confirmed also by molecular studies.

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