Antimicrobial Resistance Patterns of Isolated *Vibrio cholerae* Strains

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Abstract

**Background:** Cholera is a potentially life-threatening acute diarrheal disease caused by the toxigenic bacteria, *Vibrio cholerae*. Antibiotics should be selected using local antibiotic susceptibility testing patterns.

**Objectives:** This study was performed to identify the patterns of antimicrobial resistance in isolates collected from laboratory-confirmed cases of cholera during three years, from 2011 to 2013.

**Materials and Methods:** All isolates at the Health Reference Laboratory were tested by the Minimum Inhibitory Concentration (MIC) Test using Liofilchem against ciprofloxacin, nalidixic acid, cefixime, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, and erythromycin. The following organisms were used as quality control strains for MIC E-testing; *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), and *Pseudomonas aeruginosa* (ATCC 27853).

**Results:** Results of susceptibility testing showed complete sensitivity to ciprofloxacin, cefixime and ampicillin for both isolated Inaba and Ogawa serotypes except all isolated Inaba serotypes from year 2011, which were resistant to cefixime. These resistant Inaba serotypes were not isolated in the next year. Inaba serotypes showed an increased resistance rate of up to 100% to nalidixic acid, tetracycline and trimethoprim-sulfamethoxazole, while Ogawa serotypes were 100% sensitive at the end of year 2013. The susceptibility pattern of erythromycin was similar in these two types. Sensitivity to erythromycin was decreased in both Inaba and Ogawa serotypes.

**Conclusions:** The analyzed results indicate that tetracycline should not be considered as a first line antibiotic therapy for patients infected with Ogawa serotypes. Also, national guidelines for confirmation of cholera should be improved by responsible authorities to cover new resistance during outbreaks.

**Keywords:** Cholera, Epidemiology, Antimicrobial Pattern Susceptibility Test

1. Background

More than 200 serogroups of *Vibrio cholerae* have been identified, but serogroups O1 and O139 are the most common causative agents of cholera epidemics (1). The O1 serogroup has two biotypes (classical and El Tor), each of which has three serotypes, Ogawa, Inaba and Hikojima, although the last serotype is extremely rare. *Vibrio cholerae* O1 strains have the ability to interchange between the Ogawa and Inaba serotypes (1).

Administration of an oral antibiotic for a patient with cholera with moderate or severe dehydration is necessary. An effective antibiotic can reduce the volume of diarrhea in patients with severe cholera, hence shortening the period of illness (2, 3). In addition, it usually stops the diarrhea within 48 hours, thus shortening the period of hospitalization.

Occurrence of frequent outbreaks and multiple antibiotic resistances of cholera have been reported with increasing frequency in Iran (4-7). The last cholera resistance pattern was reported by the study of Rahbar et al., during the year 2010. They reported, that all isolates were resistant to co-trimoxazole, nalidixic acid and furazolidone (8). The evolution of multidrug-resistant *V. cholerae* strains over the last decade, particularly in Asian countries, poses a great threat to the clinical diagnosis and treatment of this disease (9-11).

Rapid diagnosis and the emergence and spread of multidrug resistant *V. cholerae* with resulting outbreaks across the globe can undermine the success of antimicrobial therapy (12, 13). However, there is great variation in patterns of antibiotic resistance at different times and places, with multiple antibiotic-resistant *V. cholerae* commonly found during each outbreak.

2. Objectives

Antibiotics should be selected using local antibiotic susceptibility patterns. Therefore, it is necessary to monitor antimicrobial resistance patterns of this potentially life-threatening pathogen. This study was performed to identify the patterns of antimicrobial resistance in collected isolates from laboratory-confirmed cases of cholera, during three years, from 2011 to 2013.
3. Materials and Methods

All patients suspected of cholera were entered in this study. All *V. cholerae* isolates were diagnosed at local laboratories of each province, based on standard procedures (1, 14). The first five diagnosed cholera specimens were transferred to the Health Reference Laboratory of each province for re-identification; these laboratories served as referral laboratories for final confirmation, and were part of an established surveillance system by the Ministry of Health and Higher Education. These specimens were examined for specific serogroups by O1 polyvalent and Ogawa/Inaba monospecific antisera (BD, Becton Dickinson Co. USA) and after identification they underwent standard biochemical and bacteriological tests (1, 14, 15).

3.1. Assessing Antimicrobial Susceptibility of the Isolates

All identified isolates were tested by the Minimum Inhibitory Concentration (MIC) Test Strip Method using Liofilchem (CE IVD approved, Italy) against ciprofloxacin (CIP), nalidixic acid (NA), cefixime (CFM), ampicillin (AMP), tetracycline (TE), trimethoprim-sulfamethaxazole (SXT), and erythromycin (E), according to the manual of the kit. The following organisms were used as quality control strains for MIC E-testing: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), and *Pseudomonas aeruginosa* (ATCC 27853).

4. Results

A total of 2498 cholera patients were registered during the study period by the Center for Disease Control of the Ministry of Health and Medical Education. However, only 215 specimens were sent to the Health Reference Laboratory for re-identification based on the Guidelines. Finally, 192 specimens were confirmed and 23 specimens were not confirmed as *V. cholerae* serotype O1. These samples belonged to 26 out of 31 provinces. Number of Ogawa and Inaba serotypes was 121 (63%) and 71 (37%), respectively (Table 1).

The mean age of the confirmed patients was variable in each year. The mean age was 37.65 ± 17.57, 29.5 ± 16.51 and 25.52 ± 11.7 for years 2011, 2012 and 2013, respectively.

Results of the susceptibility testing showed complete sensitivity to ciprofloxacin, cefixime and ampicillin for both isolated Inaba and Ogawa serotypes except all Inaba serotypes isolated during 2011 that were resistant to cefixime. Resistant Inaba serotypes were not isolated in the next two years.

### Table 1. Frequency of Inaba and Ogawa Serotypes During the Study Period

<table>
<thead>
<tr>
<th>Serotype</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inaba</td>
<td>4</td>
<td>7</td>
<td>60</td>
<td>71</td>
</tr>
<tr>
<td>Ogawa</td>
<td>96</td>
<td>22</td>
<td>3</td>
<td>121</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>29</td>
<td>63</td>
<td>192</td>
</tr>
</tbody>
</table>

### Table 2. Results of the Susceptibility Test for Isolated Ogawa Strain During Years 2011 to 2013<sup>a</sup>

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>99</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>3</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>Cefixime</td>
<td>99</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>99</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>48</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SXT</td>
<td>4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>58</td>
<td>91</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviation: SXT, trimethoprim-sulfamethaxazone.

<sup>a</sup>Data are presented as %.

### Table 3. Results of the Susceptibility Test for Isolated Inaba Strain During Years 2011 to 2013<sup>a</sup>

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefixime</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>75</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>SXT</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>100</td>
<td>86</td>
<td>23</td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviation: SXT, trimethoprim-sulfamethaxazone.

<sup>a</sup>Data are presented as %.
Although Inaba serotypes showed an increased resistance of up to 100% to nalidixic acid, tetracycline and SXT, Ogawa serotypes were 100% sensitive at the end of 2013 (Tables 2 and 3). Sensitivity to erythromycin was also decreased in both Inaba and Ogawa serotypes at the end of 2013 and was different in provinces (Table 4).

### 5. Discussion

Patterns of antibiotic resistance of *V. cholerae* have changed during the recent years. There are many reports, which have documented resistance of *V. cholerae* to antibiotics such as tetracycline and fluoroquinolones (16, 17). The first susceptibility testing of isolated *V. cholerae* strains was performed in year 2005, during the cholera epidemic (18). One-hundred serotypes of Inaba were examined in this study; all strains were resistant to co-trimoxazole, nalidixic acid and furazolidon. However, all strains were sensitive to tetracycline, ciprofloxacin erythromycin and ampicillin. The results of this study were confirmed by other investigators later on (19).

According to the report of Rahbar et al. (8), all *V. cholerae* isolates were sensitive to tetracycline. However, our results showed that resistance to tetracycline increased from 25% for Inaba strains in 2011 to 100% in 2013. However, we applied the MIC test strip method while Rahbar et al. did not. The sensitivity pattern of Ogawa should be re-evaluated and may not reflect its true pattern because of the very low number of Ogawa isolates in this study.

Both Ogawa and Inaba strains did not show any resistance to erythromycin during the study period, yet we observed a considerable intermediate rate that increased to 71% and 100% for Inaba and Ogawa isolates, at the end of the study. Therefore, another item that needs to be considered is the procedure performed for susceptibility testing between the study performed in 2005 and the current study.

Overall, it seems that there was a paradox between results of susceptibility testing of *V. cholerae* serotypes against tetracycline and erythromycin in our study. One reason for the differences between antibiotic resistance of Ogawa and Inaba isolates may be related to the source of organisms. Our country is the neighboring country of Pakistan and Afghanistan, in which cholera is an epidemic disease. Mafi et al. suggested that outbreaks in Baluchistan might have been caused by immigration from Pakistan and Afghanistan (4).

Our study revealed that the majority of Inaba and Ogawa serotypes were susceptible to ciprofloxacin, cefixime and ampicillin, and were resistant to nalidixic and co-trimoxazole, which is in agreement with other previous studies. In a study from Pakistan, *V. cholerae* was identified in 245 cases out of 3292 stool specimens; all samples being Inaba strains (7.4%) (20). They reported that Inaba isolates had 100% and 3% resistance rates to cotrimoxazole and chloramphenicol, respectively and no resistance to ampicillin, tetracycline and ofloxacin. Similar results were reported by Rahbar et al. during the year 2005, with the dominant strains being Inaba, suggesting the above-mentioned theory about the route of outbreak (18). Resistance rate to the following antibiotics, tetracycline, ampicillin, co-trimoxazole, and nalidixic acid, also had the same pattern in India (21).

Antibiotics resistance of *V. cholerae* in other countries is a major problem as well. In a reported survey by the World Health Organization (WHO), all strains were susceptible to tetracycline and resistant to nalidixic acid from 2004 to 2005 outbreaks in Cameroon (22), which is in agreement with the study of Rahbar et al. (18). In another study on the outbreak in South Africa during 2008-2009, the majority of serotypes were Ogawa. In this study all strains were reported susceptible to ciprofloxacin, ampicillin, and erythromycin, while all strains were resistant to co-trimoxazole and nalidixic acid (23). In Haiti, cholera outbreak in 2010 with the typical outbreak strain of 2010EL-1786, displayed resistance to streptomycin, sulfisoxazole, trimethoprim/sulfamethoxazole, and nalidixic acid, and decreased susceptibility to ciprofloxacin and chloramphenicol and tetracycline (24).

Another parameter that needs to be considered is our national guidelines regarding re-identification of cholera. As mentioned before, the Center for Disease Control only requires the first five isolates of *V. cholerae* specimens for re-identification. This guideline is obviously based on ensuring true identification, and does not monitor resistance patterns. Susceptibility patterns may change...
during any outbreak and may not to be the same during early and late of outbreaks.

5.1. Conclusions

In conclusion, Tetracycline should not be considered as the first choice for antibiotic therapy of patient’s infected with Ogawa serotypes. Also, national guidelines for confirmation of cholera should be updated by responsible authorities, to be able to cover new resistance patterns during outbreaks.

Acknowledgments

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Footnotes

Authors’ Contribution: Contribution of each author in this report was Roghie Saboorian for laboratory examination, Mohamad Rahbar for direct observation and confirmation of the tests, and Masood Hajia for the analysis of results and preparing the reports.

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References