

Antibiotic Susceptibility of *Vibrio cholerae* Strains isolated in Makkah, Saudi Arabia

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ABSTRACT. The antimicrobial activity of 15 antibiotics against 73 *Vibrio cholerae* O1 strains Ogawa type, El Tor biotype was determined using the disc diffusion method. Most of the isolates (91.8%) were resistant to trimethoprim/sulphamethoxazole, 82.2% to streptomycin, 61.6% to furazolidone, 57.5% to tetracycline, 54.8% to rifampicin, 39.7% to erythromycin, and 27.4% to ampicillin. Less than or equal to 3% were resistant to gentamicin, chloramphenicol, and neomycin. All were sensitive to ciprofloxacin, ofloxacin, nalidixic acid, norfloxacin, and tobramycin.

Keywords: *Vibrio cholerae*, Antibiotic susceptibilities.

Introduction

Cholera continues to be an important cause of morbidity and mortality in many areas of Asia and Africa. The disease has long been recognized as endemic in the Indian sub-continent and there has been concern recently that endemic foci may also have become established in parts of Africa^[1].

Antibiotic treatment of cholera is known to shorten the duration of the illness and also decrease the period during which vibrios are excreted. Tetracycline remains the antibiotic of choice and prior to 1977 there were no reports of widespread clinically significant resistance of cholera to this drug^[2]. Strains of El-Tor *Vibrio cholerae* resistant to tetracycline were reported^[1,3]. An epidemic of *Vibrio cholerae* resistant to several antibiotics on a pediatric ward at a hospital in Thailand was also described^[4].

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However, there have been numerous sporadic reports from various parts of the world of strain of *Vibrio cholerae* carrying plasmid mediated resistance to several antibiotics including tetracycline^[1].

The purpose of this study was to determine the *in-vitro* activity of the most commonly prescribed antibiotics against *Vibrio cholerae* 01 in order to select the proper antibiotics for treatment.

Materials and Methods

Patients, Specimens, and Bacterial Identification: Seventy-three *Vibrio cholerae* isolates were obtained from patients admitted to the King Abdulaziz Hospital, Makkah (30 isolates) and from cases referred to the Ministry of Health central laboratory in Makkah (43 isolates). Cases were collected during the 1994 pilgrimage (Hajj) season. *Vibrio cholerae* was identified by growth on thiosulfate-citrate-bile-sucrose agar plate, morphology, Gram's stain, and oxidase reaction. The identity of *Vibrio cholerae* 01 was thereafter confirmed serologically using polyvalent 01 and monospecific ogawa antisera (Denka Seiken, Japan).

Antibiotics: Antibiotic discs used were: ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), streptomycin (10 µg), tobramycin (10 µg), neomycin (30 µg), trimethoprim/sulphamethoxazole (25 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), ofloxacin (5 µg), nalidixic acid (30 µg), rifampicin (30 µg), erythromycin (15 µg), and furazolidone (50 µg). All were obtained from Oxoid, U.K.

Susceptibility Testing: Antibiotic disc susceptibility testing was performed according to the procedure described by the National Committee for Clinical Laboratory Standards^[5]. Prior to susceptibility testing, the isolates were cultured on nutrient agar plates and incubated at 35°C for 24 hrs. The organisms were subsequently inoculated into Mueller-Hinton broth and incubated at 35°C for 3-5 hrs until standard bacterial suspensions equivalent in turbidity to a 0.5 McFarland standard (10⁸ cfu/ml) were obtained. Then they were inoculated into Mueller-Hinton agar plates (Difco, Detroit, Michigan, U.S.A.) pH 7.2-7.4. The susceptibility plates were incubated for 18-24 hrs at 35°C.

Inhibition zone diameters were measured using sliding calipers to determine the susceptibility of the micro-organism to each antimicrobial agent.

Escherichia coli (ATCC 25922) and *Vibrio cholerae* 01 (H 218) were used as controls.

Results

Thirty *Vibrio cholerae* 01 strains isolated from diarrhoeal patients admitted to the

King Abdulaziz Hospital in Makkah during the pilgrimage (Hajj) in 1994 and 43 others kindly provided by the Central Health Laboratory in Makkah, were studied. All isolates were ogawa type, biotype El Tor and susceptible by the disc diffusion method to ciprofloxacin, norfloxacin, ofloxacin, nalidixic acid, and tobramycin. Sixty-seven (91.8%) of the isolates were resistant to trimethoprim/sulphamethoxazole, 60 (82.2%) were resistant to streptomycin, 45 (61.6%) to furazolidone, 42 (57.5%) to tetracycline, 40 (54.8%) to rifampicin, 29 (39.7%) to erythromycin, and 20 (27.4%) were resistant to ampicillin. Two isolates were resistant to neomycin and chloramphenicol and only 1 isolate was resistant to gentamicin (Table 1).

TABLE 1. Drug susceptibility of 73 isolates of *Vibrio cholerae* to various antibiotics.

| Antibiotic | Resistant Isolates of <i>Vibrio cholerae</i> | |
|--------------------------------|--|----|
| | No. | % |
| Trimethoprim/sulphamethoxazole | 67 | 92 |
| Streptomycin | 60 | 82 |
| Furazolidone | 45 | 62 |
| Tetracycline | 42 | 58 |
| Rifampicin | 40 | 55 |
| Erythromycin | 29 | 40 |
| Ampicillin | 20 | 27 |
| Neomycin | 2 | 3 |
| Chloramphenicol | 2 | 3 |
| Gentamicin | 1 | 1 |
| Ciprofloxacin | 0 | 0 |
| Norfloxacin | 0 | 0 |
| Ofloxacin | 0 | 0 |
| Nalidixic acid | 0 | 0 |
| Tobramycin | 0 | 0 |

Discussion

During the 1994 epidemic, 73 isolates of *Vibrio cholerae* O1 tested were ogawa type, biotype El Tor. Saudi Arabia has frequently been affected by these epidemics due to a strain introduced by southern Asians who were performing pilgrimage (Hajj). Biotype El Tor was commonly observed in other countries except Bangladesh where most of the strains isolated recently were changing their biotype from El Tor to classical type^[6]. Person-to-person transmission may be involved in the spread of cholera^[4]. During the pilgrimage season, more than two million Muslims from all over the world congregate for at least two weeks in Makkah, Saudi Arabia. Epidemiological studies have shown that water is an important vehicle for the transmission of cholera^[7]. Thus it is possible that the close contact among pilgrims near the main water supply helps the spread of in-

fection.

In Tanzania, multiple antibiotic-resistant *Vibrio cholerae* was thought to have been disseminated with the hospital and to the surrounding communities from crowded pediatric wards by person-to-person contact^[8].

A disturbing finding was that most of the *Vibrio cholerae* strains recovered in this study were resistant to commonly-used drugs, *i.e.*, trimethoprim/sulphamethoxazole, streptomycin, tetracycline, furazolidone, rifampicin, and ampicillin. The widespread occurrence of resistant strains clearly reflect the indiscriminate use of antibiotics in the treatment of a variety of infections. Multi-drug resistance of *Vibrio cholerae* has been reported from time-to-time^[3].

Tetracycline has been the antibiotic of choice in the treatment of cholera in both adults and children. The effectiveness of this drug is related to its enterohepatic circulation which continuously maintains an effective therapeutic level of the antibiotic in the intestine^[9]. The emergence of tetracycline-resistant strains of *Vibrio cholerae* have been reported from Kenya^[11], Thailand^[4], Bangladesh^[10], and India^[3]. Unfortunately, in the present study 57.5% of *Vibrio cholerae* strains were resistant to tetracycline.

After tetracycline, furazolidone is the drug of choice for cholera, especially for children and pregnant women^[11]. Following the emergence of chloramphenicol and tetracycline-resistant *Vibrio cholerae* in the Philippines, Kobari *et al*^[11] recommended the use of furazolidone, a nitrofurantoin derivative, as an alternate drug for the treatment of cholera. In Bangladesh, Siddique *et al*^[10] reported a higher incidence of furazolidone-resistant *Vibrio cholerae*. Our study showed a similar finding: that a large proportion (61.6%) of *Vibrio cholerae* isolates in Makkah were resistant to furazolidone. Thus, ciprofloxacin, ofloxacin, norfloxacin, nalidixic acid, tobramycin and, to a lesser extent, gentamicin, neomycin, and chloramphenicol ($\leq 3\%$ resistance) are alternative drugs of choice and can be used as an empirical therapy for the treatment of *Vibrio cholerae*.

It is hoped that this data will offer some guidance to physicians who have to deal with this infection in order to select the proper antibiotic(s) for the treatment of *Vibrio cholerae*.

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حساسية جرثومة الضمة الهيفية المفصولة في مكة للمضادات الحيوية

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المستخلص. لقد جرى تحديد قابلية ٧٣ ضمة هيفية لخمسة عشر مضاداً حيوياً باستخدام طريقة انتشار القرص وقد أبدت معظم العزلات مقاومة للمضادات: ٨, ٩١٪ للترايمثريم / سلفاميتكسازول، ٢, ٨٢٪. للستربتومايسين، ٦, ٦١٪ للفيورازولدون، ٥, ٥٧٪ للتتراسيكلين، ٨, ٥٤٪ للريفميسين، ٧, ٣٩٪ للإرثرومييسين، ٤, ٢٧٪ للأمبسلين وأقل من ٣٪ لكل من جنتاميسين، نيوميسين، كلورمفنكول. وكانت معظم الذريات حساسة للسبروفلكساسين، أفلكساسين، نورفلكساسين، نالدكسك أسيد والتبرميسين.