Journal of Antimicrobial Chemotherapy (2007) **60**, 662–664
doi:10.1093/jac/dkm257
Advance Access publication 11 July 2007

JAC

Antimicrobial resistance of *Vibrio cholerae* O1 serotype Ogawa isolated in Manhiça District Hospital, southern Mozambique

Inácio Mandomando[1,2]*, Mateu Espasa[1], Xavier Vallès[1], Jahit Sacarlal[1,3], Betuel Sigaúque[1,2], Joaquim Ruiz[4] and Pedro Alonso[1,4]

[1]Centro de Investigação em Saúde da Manhiça (CISM), Maputo, Mozambique; [2]Instituto Nacional de Saúde, Ministério da Saúde, Maputo, Mozambique; [3]Faculdade de Medicine, Universidade Eduardo Mondlane, Maputo, Mozambique; [4]Centre for International Health Research (CRESIB), Hospital Clinic/IDIBAPS, Universitat de Barcelona, Spain

Received 20 March 2007; returned 27 April 2007; revised 4 June 2007; accepted 20 June 2007

***Objectives*: To describe the antimicrobial susceptibility profile of isolated *Vibrio cholerae* O1 serotype Ogawa recovered from patients admitted to the cholera facility in the Manhiça District Hospital (MDH), Mozambique.**

***Methods*: Rectal swabs were collected from patients with complaints symptomatic of cholera admitted to the MDH cholera facility. Samples were processed for *V. cholerae* isolation at the Centro de Investigação em Saúde da Manhiça (CISM) and identified by biochemical reaction. Serotypes were determined by slide-agglutination antisera. Susceptibilities were determined by disc diffusion.**

***Results*: Seventy-seven isolates were examined for their resistance profile. High incidences of antimicrobial resistance were found for chloramphenicol (57.9%), co-trimoxazole (96.6%) and tetracycline (97.3%). Quinolone resistance remained low (4.2%).**

***Conclusions*: Although *V. cholerae* infections in Africa do not usually require antimicrobial treatment, strains in rural Mozambique show high incidences of resistance to readily available drugs. When appropriate, quinolones or third-generation cephalosporins can be used as treatment options.**

Keywords: susceptibility profile, cholera, diarrhoea

Introduction

Since the seventh pandemic caused by *Vibrio cholerae* biotype El Tor began in Indonesia in 1961, most regions of the world continue to report cholera outbreaks.[1] Cholera is most common in rural areas or communities where sanitation conditions and water supply are problematic.[2] In 1997, a cholera epidemic affected most countries of eastern, central and southern parts of Africa, including Mozambique. Nearly 80% of cases reported worldwide to WHO were from Africa (~118 000 cases), and Africa had the highest case fatality rate, 4.9%, compared with 1.3% in the Americas and 1.7% in Asia.[1] In Mozambique the epidemic started in August 1997, reaching an estimated 9000 cases and 259 deaths.[1] Cholera continues to be endemic in Mozambique, concentrated mainly in Beira and Maputo cities, with many cases occurring during the rainy season.[3]

In many African countries, *V. cholerae* O1 serotype Ogawa has been the strain most frequently isolated and associated with cholera outbreaks.[4–6] As with many other diarrhoeal diseases, cholera can be managed by re-hydration alone, but antimicrobial therapy can help to shorten the course of disease and to break the transmissibility cycle during epidemics. Antimicrobial therapy is also recommended for severe or chronic cases of diarrhoea. However, the increase in antimicrobial-resistant strains of bacteria causing diarrhoeal disease is concerning, especially in developing countries.[7,8] Tetracycline derivatives are the first-line drug for cholera treatment in many of these countries, however, *V. cholerae* strains resistant to this antimicrobial and others, including ciprofloxacin, have been reported.[8] In rural areas of Mozambique, data on antimicrobial resistance are scarce, particularly for *V. cholerae* strains. This study aimed to describe the antimicrobial susceptibility profiles of *V. cholerae* O1 serotype

*Correspondence address. Manhiça Health Research Centre, Street 12, Manhiça Village, PO Box 1929, Maputo, Mozambique. Tel: +258-21-810-002; Fax: +258-21-810-181; E-mail: inacio.mandomando@manhica.net, imandomando2004@yahoo.com.br

Ogawa strains isolated from patients admitted to the cholera facility at the Manhiça District Hospital (MDH) during two rainy seasons.

Patients and methods

Population and study site

Samples were systematically collected from patients with diarrhoea admitted to the MDH cholera facility during the two cholera outbreak periods (rainy seasons November 2002–April 2003 and November 2003–April 2004). The MDH is a 110 bed referral health facility for Manhiça District, a rural area of Maputo Province, southern Mozambique [the locations of Mozambique and Manhiça District can be seen in Figures S1 and S2, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)]. The characteristics of the area have been described in detail elsewhere.[9] Briefly, the climate is subtropical with two distinct seasons: a warm, rainy season between November and April, and a cool and dry season during the rest of the year. Manhiça has ~140 000 inhabitants, who are mostly subsistence farmers or workers in two large sugar- and fruit-processing factories.

The Manhiça Health Research Centre (Centro de Investigação em Saúde da Manhiça—CISM) is adjacent to the MDH and has been conducting continuous demographic surveillance for vital events and migrations since 1996. Approximately 70 000 individuals are followed by a demographic surveillance system.

Sample collection and laboratory methods

Rectal swabs were collected from patients admitted to the cholera facility and immediately placed in Cary–Blair transport medium (Selecta, Johannesburg, South Africa), and kept in boxes with cold packs until their processing (within 4 h) for *V. cholerae* isolation at CISM. Swabs were cultured onto thiosulphate citrate bile sucrose and MacConkey, and morphological colonies compatible with *Vibrio* were characterized by oxidase test and agglutinated with antiserum (Difco, Detroit, MI, USA) for serotype determination. As a quality control, 10% of isolates were confirmed as *V. cholerae* together with ATCC 2459 (*V. cholerae*) strain though biochemical tests with API20E (BioMerieux, Marcy-l'Etoile, France).

Antimicrobial susceptibility

Antimicrobial susceptibility to ampicillin, ceftriaxone, chloramphenicol, nalidixic acid, tetracycline and co-trimoxazole was tested using the disc diffusion method (Mast Diagnostics, Merseyside, UK). Isolates that showed full or intermediate resistance to nalidixic acid were also tested for ciprofloxacin. Interpretative categories of resistance (susceptible, intermediate or full resistance) were determined according to the Clinical and Laboratory Standards Institute (CLSI).[10]

Data management and statistical methods

Data were entered and analysed in Statistical Package for the Social Sciences (SPSS) for windows version 11.0 (SPSS, Chicago, IL, USA). A susceptibility percentage for each antimicrobial tested was calculated dividing the number of susceptible isolates by the total number of tested isolates.

Results and discussion

During the two surveillance periods (November 2002–April 2003 and November 2003–April 2004), 179 patients were admitted to MDH cholera facility. Rectal swabs were collected in ~83.8% (150/179) of patients and cultured for *Vibrio* isolation. *V. cholerae* were recovered in 60.7% (91/150) samples. All isolates were *V. cholerae* O1 serotype Ogawa. Among the isolates, antimicrobial susceptibility data were available for 77 strains. Resistance to chloramphenicol was 57.9%, co-trimoxazole 96.6% and tetracycline 97.3% (Table 1). The incidence of strains resistant to ampicillin and nalidixic acid was low, 12.2% and 4.2%, respectively. Chloramphenicol intermediate resistance was considerably high, 28.9% (Table 1). No isolates were resistant to ceftriaxone or ciprofloxacin.

Data generated in the present study suggest the presence of five main susceptibility profiles (Table 2). Taking into account the incidence of tetracycline resistance, the susceptibility profiles of the isolates suggest the possibility that three main clonal strains were circulating during the study period, however, this assumption could not be proven because no specific molecular studies designed to analyse clonal relations were done.

Our data show that tetracycline, one of the main antimicrobials used for cholera treatment, was not effective *in vitro* for *V. cholerae* strains, and the incidence of resistance observed in this study is higher than previously reported in this country.[5] In Mozambique, doxycycline is used as the first-line drug for cholera treatment when antimicrobials are recommended. The results obtained suggest the need to re-evaluate this treatment

Table 1. Antimicrobial susceptibility profile of *V. cholerae* O1 serotype Ogawa isolated in Manhiça District Hospital, Mozambique

	Susceptible		Intermediate		Resistant	
Antimicrobial[a]	*n*	(%)	*n*	(%)	*n*	(%)
Ampicillin ($n = 74$)	61	(82.4)	4	(5.4)	9	(12.2)
Chloramphenicol ($n = 76$)	10	(13.2)	22	(28.9)	44	(57.9)
Co-trimoxazole ($n = 58$)	2	(3.4)	00	00	56	(96.6)
Tetracycline ($n = 75$)	00	00	2	(2.7)	73	(97.3)
Nalidixic acid ($n = 72$)	68	(94.4)	1	(1.4)	3[b]	(4.2)

[a]All strains ($n = 71$) tested with ceftriaxone were susceptible.
[b]These strains were susceptible to ciprofloxacin.

Table 2. Main susceptibility profiles of *V. cholerae* O1 serotype Ogawa

Profiles	AMP	TET	CHL	SXT	NAL	CRO
Profile 1 ($n = 18$)	S	R	R	ND	S	S
Profile 2 ($n = 10$)	S	R	S	R	S	S
Profile 3 ($n = 10$)	S	R	I	R	S	S
Profile 4 ($n = 5$)	S	R	R	R	ND	S
Profile 5 ($n = 5$)	R	R	R	R	S	S

AMP, ampicillin; TET, tetracycline; CHL, chloramphenicol; SXT, co-trimoxazole; NAL, nalidixic acid; CRO, ceftriaxone.
S, susceptible; I, intermediate; R, resistant; ND, not done.

regimen because of the cross-resistance between tetracycline and doxycycline.

Resistance to co-trimoxazole was similar to that reported in a prior study.[5] The high incidence of resistance is alarming because co-trimoxazole is widely used to prevent opportunistic infections in HIV patients,[11] and sulfadoxine/pyrimethamine, a related antimicrobial that has been demonstrated to produce cross-resistance, is commonly used for malaria treatment.

The high incidence of chloramphenicol-resistant strains (57%) is also notable. This can be partially explained by its frequent usage as a first-line treatment for severe diseases in children under 2 years old and for infectious diarrhoea (when antimicrobials are recommended) in children over 2 months old. Other reports also found *V. cholerae* Ogawa strains resistant to chloramphenicol, and a similar incidence of resistance was observed in other pathogens causing diarrhoea in previous study conducted in the area.[12]

In contrast to a previous study on diarrhoeal aetiology that reported a high incidence of resistance to ampicillin for other bacteria causing diarrhoeal disease,[12] most isolates of *V. cholerae* Ogawa were susceptible to this antimicrobial. Similarly, most isolates were susceptible to nalidixic acid (68 out of 72; 94.4%). Even though the *V. cholerae* included in the present study were susceptible to ampicillin, the use of this antimicrobial should be reserved for other infections or in cases of resistance to quinolones. Instead, nalidixic acid can be used for empirical treatment.

Vibrio strains resistant to fluoroquinolones have been reported previously, especially in Asia,[8] and a continuous rise in the incidence of strains resistant to quinolones among diarrhoea-causing pathogens during the last few years has been observed in Asia.[8,13] Despite this, fluoroquinolones and ceftriaxone remain active against diarrhoeal bacterial agents including *Vibrio* strains circulating in Mozambique.[5,6,12] However, a significant limitation of fluoroquinolones is their controversial use in children. On the other hand, safer alternatives such as the third generation of cephalosporins are very expensive, and their supply is limited.

In conclusion, antimicrobial surveillance is important to monitor the emergence and trends of bacterial resistance to antimicrobials. Our data suggest that although *V. cholerae* infections do not usually require treatment with antimicrobials, strains in rural Mozambique show a high incidence of resistance to readily available drugs. Moreover, first-line antimicrobial treatment should be revised. When appropriate, quinolones or third-generation cephalosporins can be used as treatment options.

Acknowledgements

We thank the Manhiça Health District Authorities, and special thanks to Mariano Sitaúbe of the bacteriology laboratory at the CISM for supporting us in culturing and identifying *Vibrio* strains. CSI is also supported by RICET and RCESP networks. Joaquim Ruiz is the recipient of grants CP05/00130 and PI06/0204 from the Fondo de Investigaciones Sanitarias, Spain.

This project was supported by Fundació Clinic and Agencia Espanhola de Cooperacion Internacional (AECI).

Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1. WHO. Cholera in 1997. *Weekly Epidemiol Record* 1998; **73**: 201–8.

2. Rao S, Pai M, Iyanar A *et al*. Sanitation for rural communities: first win the people's support. *World Health Forum* 1997; **18**: 262–5.

3. WHO. Report on cholera control programme in Mozambique. *WHO Global Task Force on Cholera Control. Geneva: WHO*, 2003.

4. Acosta CJ, Galindo CM, Kimario J *et al*. Cholera outbreak in southern Tanzania: risk factors and patterns of transmission. *Emerg Infect Dis* 2001; **7**: 583–7.

5. Folgosa E, Mastrandrea S, Cappuccinelli P *et al*. Molecular identification of pathogenicity genes and ERIC types in *Vibrio cholerae* O1 epidemic strains from Mozambique. *Epidemiol Infect* 2001; **127**: 17–25.

6. Dalsgaard A, Forslund A, Sandvang D *et al*. *Vibrio cholerae* O1 outbreak isolates in Mozambique and South Africa in 1998 are multiple-drug resistant, contain the SXT element and the aadA2 gene located on class 1 integrons. *J Antimicrob Chemother* 2001; **48**: 827–38.

7. Okeke IN. Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect Dis* 2005; **5**: 481–93.

8. Garg P, Sinha S, Chakraborty R *et al*. Emergence of fluoroquinolone-resistant strains of *Vibrio cholerae* O1 biotype El Tor among hospitalized patients with cholera in Calcutta, India. *Antimicrob Agents Chemother* 2001; **45**: 1605–6.

9. Nhacolo AQ, Nhalungo DA, Sacoor CN *et al*. Levels and trends of demographic indices in southern rural Mozambique: evidence from demographic surveillance in Manhica district. *BMC Public Health* 2006; **6**: 291.

10. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard M2-A9*. CLSI, Wayne, PA, USA, 2006.

11. Chintu C, Bhat GJ, Walker AS *et al*. Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial. *Lancet* 2004; **364**: 1865–71.

12. Mandomando I, Macete E, Ruiz J *et al*. Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of Southern Mozambique. *Am J Trop Med Hyg* 2007; **76**: 522–7.

13. Sarkar K, Ghosh S, Niyogi SK *et al*. *Shigella dysenteriae* type 1 with reduced susceptibility to fluoroquinolones. *Lancet* 2003; **361**: 785.