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Susceptibility to rifaximin of *Vibrio cholerae* strains from different geographical areas

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Four hundred and eight clinical strains of *Vibrio cholerae* isolated from different geographical areas and with different antimicrobial resistance patterns were tested for susceptibility to rifaximin, a non-absorbable antibiotic active *in vitro* against Gram-negative bacteria. The MICs ranged from 0.5 to 4 mg/L for all strains. These values and the pharmacokinetic properties suggest rifaximin as an attractive antimicrobial agent for cholera.

Keywords: rifamycin derivative, antimicrobial activity, cholera

Introduction

Vibrio cholerae O1 El Tor biotype is the causative organism of the current seventh cholera pandemic. In addition to O1 strains of the El Tor biotype, strains of *V. cholerae* O139 and specific strains of *V. cholerae* O1 classical biotype are also responsible for cholera outbreaks in Asian countries.[1]

Antimicrobial agents in the treatment of cholera cases are often recommended for reduction of symptomatology and vibrio excretion in the environment. The antimicrobials traditionally used have been tetracycline, trimethoprim–sulfamethoxazole, erythromycin and furazolidone.

Rifaximin, a rifamycin derivative, acts by binding irreversibly to the β-subunit of the bacterial DNA-dependent RNA polymerase and inhibits RNA synthesis. Susceptibility testing of enterobacterial pathogens associated with traveller's diarrhoea has suggested that rifaximin could be considered as a potential agent in the treatment of intestinal bacterial infections.[2] In this study, we investigated the *in vitro* activity of rifaximin against *V. cholerae* strains isolated from different outbreaks in different regions and in different years.

Materials and methods

Sources and characterization of V. cholerae strains

A total of 408 *V. cholerae* strains were analysed in this study (Table 1). Of these, 359 *V. cholerae* O1 El Tor strains were isolated from clinical cases representative of outbreaks which occurred in Africa from 1985 to 1999, 12 El Tor strains were from outbreaks in Central and South America, and 32 strains from outbreaks in South Europe. Three clinical strains of *V. cholerae* O139 isolated in India and two reference O1 strains of the classical biotype were also included.

Rectal swabs, stool samples, or both (in Cary-Blair transport medium) were plated on thiosulphate-citrate-bile salts-sucrose (TCBS) (Oxoid, Milan, Italy) agar and incubated at 37°C for 18–24 h. Part of each specimen was enriched in alkaline peptone water pH 8.5 (Oxoid, Milan, Italy) and then plated on TCBS agar. Well-isolated suspect colonies were picked to Kligler iron agar slants and tested for urease and oxidase production (Oxoid, Milan, Italy). Isolates giving typical reactions were biochemically characterized by the API 20E system with additional tests for *Vibrio* spp. O-antigen groups were serologically identified by using commercially available antisera. *V. cholerae* isolates were also tested by agglutination with chicken erythrocytes and polymyxin B susceptibility. Original stock cultures of isolates were kept in 20% glycerol Luria-Bertani broth at –70°C.

Biotypes and genetic relationships of strains were identified by DNA-based typing methods. Fingerprints of genomic DNA were obtained using PCR to generate amplified polymorphic DNA (APD). The primers were six oligonucleotides selected from enterobacterial repetitive consensus sequences,[3] from *V. cholerae* repetitive DNA sequences,[4] and from the CTXϕ phage genome of *V. cholerae*.[5] Each strain was characterized by the combination of the six APD patterns. Combinations were compared and different APD clusters were identified to establish a reference framework to assess clonal relatedness.

Antibiotic susceptibility testing

The antibacterial susceptibility pattern of each isolate was determined by the disc diffusion method.[6] The antimicrobial discs were used at the following concentrations: ampicillin (10 μg), chloramphenicol (30 μg), doxycycline (30 μg), kanamycin (30 μg), streptomycin (10 μg), spectino-

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Table 1. Clonal relatedness and antimicrobial resistance pattern of 408 *V. cholerae* strains of different geographical origins

Area (no. of strains) and year of isolation	No. of different clones (serotype, biotype)	Resistance pattern (no. of strains)/ geographical distribution	Reference
Africa (359) 1985, 1993, 1994, 1995, 1998, 1999	16 (O1, El Tor)	AMP SMX SPT STR TMP (131)/eastern Africa AMP DOX KAN SMX SPT STR TET (57)/eastern Africa AMP KAN SMX SPT STR TMP (2)/southern Africa DOX SMX SPT STR TET TMP (6)/eastern and southern Africa SMX SPT STR TMP (74)/eastern and southern Africa SPT (89)/northern, eastern and southern Africa	9, this work
Europe (32) 1973, 1994	2 (O1, El Tor)	SMX SPT STR TET TMP (28)/southern Europe SPT (4)/southern Europe	this work
America (12) 1991, 1994	2 (O1, El Tor)	SMX SPT STR (2)/central America SPT (10)/central and southern America	this work
India (5) 1992 1948, 1964	1 (O139) 2 (O1, Classical)	SMX SPT STR TMP (3) SPT STR (1) SPT (1)	10 10

AMP, ampicillin; DOX, doxycycline; KAN, kanamycin; SPT, spectinomycin; STR, streptomycin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim.

mycin (10 μg), tetracycline (30 μg), sulfamethoxazole (25 μg) and trimethoprim (5 μg) (Oxoid, Milan, Italy). MICs of rifaximin and tetracycline (Sigma, Milan, Italy), were tested for each isolate according to NCCLS guidelines[7] with final concentrations ranging from 0.0125 to 256 mg/L. Rifaximin was a gift from Alfa Wassermann SpA, Bologna, Italy (Batch i/2096c). *Escherichia coli* ATCC 25922 was used as a quality control strain.

Results and discussion

The clonal relatedness and the antimicrobial resistance patterns of the 408 *V. cholerae* O1 strains tested are shown in Table 1.

Genetic diversity was assessed by PCR fingerprinting with selected primers generating amplified polymorphic genomic DNA. Clonal relationships were determined by comparison and analysis of combinations of the amplified DNA patterns. On this basis, all strains were clustered into 23 clones. The 359 strains isolated in Africa from 1985 to 1999 consisted of 16 clones. The European and American strains were classified in two clones, respectively, and the Indian strains in three clones.

Tests for antimicrobial susceptibility revealed six patterns within the African strains. The large group of El Tor strains resistant to ampicillin, sulfamethoxazole, spectinomycin, streptomycin, trimethoprim (131 strains) and the group resistant to ampicillin, doxycycline, kanamycin, sulfamethoxazole, spectinomycin, streptomycin, tetracycline (57 strains) isolated from East African countries in 1985, 1998 and 1999 belonged to four and two clones, respectively. Two susceptibility patterns were found in the European and American strains, respectively.

All strains were resistant to spectinomycin (100% of isolates) and the vast majority were resistant to streptomycin (74%) and sulfamethoxazole (74%). Resistances to trimethoprim and ampicillin were present in 60% and 47% of all strains, respectively, corresponding to 81% and 63% of the multidrug-resistant isolates. A significant percentage of isolates were also resistant to tetracycline (22%), doxycycline (15%) and kanamycin (14%).

Table 2. MIC_{50}s and MIC_{90}s (mg/L) of rifaximin and tetracycline among *V. cholerae* O1 El Tor strains[a] distributed by geographic area

No. of strains/ geographic area	Rifaximin		Tetracycline	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
359/Africa	2	4	2	64
32/Europe	4	4	16	16
12/America	2	2	1	2

[a]The MIC range of RFX and TET for the three strains of V. cholerae O139 and the two strains of V. cholerae O1 classical biotype was 1–2 mg/L for both antimicrobials.

The distribution of the MICs of rifaximin and tetracycline for the *V. cholerae* El Tor isolates by geographic area is given in Table 2. MICs of tetracycline were determined for comparison between rifaximin and a traditionally used antimicrobial. Tetracycline showed lower activity for the African isolates (MIC_{90} = 64 mg/L) than for the European (MIC_{90} = 16 mg/L) and American (MIC_{90} = 2 mg/L) isolates. No differences in the MIC_{50}s (2–4 mg/L) and MIC_{90}s (2–4 mg/L) of rifaximin were observed among the three groups of O1 El Tor strains.

The MIC values of rifaximin for *V. cholerae* El Tor were far lower than those found among other enteric bacterial pathogens isolated from cases of traveller's diarrhoea (MIC_{50} = 16 mg/L and MIC_{90} = 32 mg/L).[2]

Rifaximin is a poorly absorbed antibiotic and in pharmacokinetic studies, high levels of rifaximin (up to 8000 μg/g) were detected in faeces of patients and healthy volunteers after 3 days of a daily dosage of 800 mg.[8] The MICs of rifaximin for all *V. cholerae* strains analysed are well below the levels of faecal concentrations which rifaximin

can reach *in vivo*. These findings indicate rifaximin as a candidate antimicrobial agent to be tested in cholera patients.

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