Serotypes of *Vibrio cholerae* Non-O1 Isolated from Water Supplies for Human Consumption in Campeche, México and their Antibiotic Susceptibility Pattern

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The presence of Vibrio cholerae *non-O1 in water supplies for human consumption in the city of Campeche and rural locality of Bécal was investigated.* V. cholerae *non-O1 was detected in 5.9% of the samples obtained in deep pools of Campeche. Studies conducted in Bécal and neighbourhood of Morelos in Campeche indicated that collected samples harbored* V. cholerae *non-O1 in 31.5% and 8.7% respectively. There was a particular pattern of distribution of V.* cholerae *non-O1 serotypes among different studied regions. Accordingly,* V. cholerae *non-O1 serotype O14 predominated in the deep pools of Campeche and together with* V. cholerae *non-O1, O155 were preferentially founds in samples taken from intradomiciliary faucets in the neighbourhood of Morelos. Samples from Bécal predominantly presented the serotype O112. 60% and 53.8% of all studied strains of* V. cholerae *non-O1 proved to be resistant to ampicillin and carbenicillin. 3.1%, 7.7% and 6.2% presented resistant to doxycycline, trimethoprim-sulfamethoxazole and erythromycin respectively. The study showed the necessity of performing a strong epidemiologic surveillance for emergence and distribution of* V. cholerae *non-O1.*

Key words: *Vibrio cholerae* non-O1 - serotypes - antibiotic susceptibility - water supplies - Campeche - México

Vibrio cholerae serogroup non-O1 are autochthonous bacteria of aquatic environments (Morris 1990) and right from the 1950s and 1960s has been identified in outbreaks of gastroenteritis in different parts of the world (McIntyre et al. 1965, Aldová et al. 1968, El-Shawi & Thewaini 1969, Kamal 1971). Currently it is recognized that non-O1 serogroup includes approximately 150 serotypes and play an important role as an aethiological agent of gastrointestinal illness ranging from mild watery diarrhea to febrile enteritis with bloody diarrhea. In contrast to *V. cholerae* O1, *V. cholerae* non-O1 causes extraintestinal infections (Sanyal 1992); but gastroenteritis is the most common clinical manifestation (Morris 1990, WHO 1993).

In October 1992, an epidemic of cholera-like illness caused by a *V. cholerae* serogroup non-O1 broke in Madras, India. The causative agent was found to be a new serogroup of *V. cholerae,* defined as O139, with the synonym Bengal, in recognition of the origin of this strain (Shimada et al. 1993, Ramamurthy et al. 1993). This strain has also been responsible for epidemic outbreaks of cholera in Bangladesh, southern Asia and U.S.A. (Albert et al. 1993, Bhattacharya et al. 1993, CDC 1993, Gonzalez et al. 1993) with possible disemination to Latin America throughout imported cases. Before of this epidemics of *V. cholerae* non-O1, O139 only *V. cholerae* O1 was considered capable of producing epidemics of cholera.

In Peru, cholera appeared in January 1991 and spreaded quickly in Latin America (PAHO 1995). The disease evolved in explosive epidemics, the largest recorded since the beginning of the seventh pandemic in Indonesia in 1961. The first reported cases of cholera in the state of Campeche, located in southeast of México, were registered in August 1991 and because of a number of environmental conditions determined by economic, sociologic and educational factors, this disease spread quickly and from 1992 became one of the federal states with more reported cases of this disease, registering the first places in morbility and mortality rates in México (DGE 1993, 1995, Valdespino 1994).

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From 1992 up to now city of Campeche has experienced several outbreaks of cholera and from July to September, 1993 Bécal, rural locality of Campeche, registered an important number of cases of this disease. The purpose of this study was the characterization of serotypes and the antibiotic susceptibility pattern of *V. cholerae* non-O1 strains isolated from water supplies for human consumption before, during and after those outbreaks of cholera.

MATERIALS AND METHODS

This study was performed in three different steps. The first one was from August 1992 to July 1993 and included seven samplings of water coming from 35 deep pools that provide water to the city of Campeche. The second step was carried out during August to December 1993 and included two collections of samples from the distribution network placed in the rural locality of Bécal. In addition, were examined intradomiciliary deep pools and rainwater cistern systems which are commonly used in this region for human consumption. The third step was performed during July 1994 to February 1995 and included two water monitorings of neighbourhood of Morelos in the city of Campeche.

Testing of total residual chlorine concentration - Presence of total residual chlorine in water samples was evaluated by the ortotoluidine method (Rodier 1990). Chlorine concentration studied varied from 0 to 3 ppm.

Isolation - The Spira swab (Spira & Ahmed 1981) and double enrichment in alkaline peptone water (Chandra 1992) methods were used for the isolation of *V. cholerae*. In both cases were employed the TCBS agar (thiosulfate-citrate-bile-sucrose agar). The water samples were storaged and transported at 4ºC and processed within the next 2 hr of their collection.

Identification - Yellow colonies from TCBS agar were submitted to the standard series of biochemical media used for identification of members of the *Vibrionaceae* family (Holt et al. 1994). The serological identification was determined by slide agglutination test using polyvalent O1 and type-specific Inaba and Ogawa antisera (Difco) and by coagglutination test employing monoclonal antibody anti-*V.cholerae* O1 adsorbed to heat-killed *S. aureus* (New Horizons Diagnostics Co.). *V. cholerae* serogroup O1 and non-O1 were used as controls.

Serological classification of V. cholerae non-O1 - Polyvalent antisera for serological classification of different *V. cholerae* non-O1 serotypes were used. The reference strains of *V. cholerae* non-O1 serotypes O1 to O83 were kindly provided by Dr Bernard Rowe, Head of Enteric Pathogens Central Laboratory of Public Health, London, England, and those reference strains from serotype O84 to O140 and O155 were provided by Dr T Shimada from the National Institute of Health, Tokyo, Japan. Antigens used for serological classification were obtained by heating bacterial cultures at 100ºC for 1 hr and adjusted with tube 3 of McFarland nephelometer; 1:100 serum dilutions were used in a proportion of antigen/sera (V/V) of 50 ml in 96 wells microtitration plates, incubated at 50ºC for 24 hr. Sera that agglutinated with this antigen preparation was serially diluted twice to know the titer and so determinate identity of each serotype (Sakazaki & Donovan 1984, Shimada et al. 1994).

Antimicrobial susceptibility testing - All *V. cholerae* non-O1 isolates were tested for antibiotic susceptibility to 15 antibiotics (Difco) by Kirby-Bauer method (NCCLS 1988) and strains were recorded as susceptible, intermediate, moderately susceptible or resistant.

RESULTS

The results indicate that in 41.1% (14/34) and 80.7% (67/83) of the samples collected from intradomiciliary faucets of rural locality of Bécal and neighbourhood of Morelos a range of 0.5-1.5 ppm and 0.3-3 ppm of total residual chlorine was detected, respectively, by the ortotoluidine method. In the case of deep pools in Morelos and Bécal, 76.9% (10/13) and 12.5% (1/8) of samples collected presented a range of 1-3 ppm. Samples obtained both from deep pools in Campeche and from the distribution tanks, intradomiciliary deep pools and rainwater cistern systems in the studied areas did not contained chlorine.

In Table I it is shown the frequency of isolation of *V. cholerae* non-O1 strains in different water sources. 5.9% (13/222) of water samples taken from deep pools of the city of Campeche, and 31.5% (17/54) and 8.7% (9/104) of those water samples from Bécal and Morelos respectively harbored *V. cholerae* non-O1.

Biochemical features of isolated strains were very similar to those reported by Holt et al. (1994). It has been reported that *V. cholerae* do not perform esculin hydrolysis, however, 67.7% (44/65) of those strains isolated can indeed to hydrolize this compound at 0 and 1% of sodium chloride. In addition, 3.1% (2/65) of bacterial strains grew in nutrient agar with 8% of NaCl.

A total of 65 strains belonging to 29 different serotypes were identified during the period of study. In Table II it is shown the distribution and frequency of *V. cholerae* non-O1 serotypes in relation to the total of bacterial strains isolated in the studied regions. The 35.7% (5/14) of samples analyzed from deep pools in Campeche contained *V. cholerae* non-O1 serotype O14. In the neighbourhood of Morelos 33.3% (3/9) and 22.2% (2/9) of samples taken from intradomiciliary faucets harbored *V. cholerae* serotypes O155 and O14 respectively.

Only in one out of three deep pools of Bécal was isolated *V. cholerae* non-O1 serotype O112 in all the tested samples. Additionaly, in 66.7% (2/3) of those samples were isolated bacterias that biochemically behave as *V. cholerae* non-O1 but did not agglutinate with any of the 141 anti-*V. cholerae* non-O1 polyvalent sera (anti-*V. cholerae* non-O1 serotype O1 to O140 and O155) availables. In 80% (8/10) of tested samples of intradomiciliary faucets was detected *V. cholerae* non-O1 serotype O112.

The water samples taken from the intradomiciliary deep pools in Bécal registered the presence of *V. cholerae* non-O1 serotypes O58, O38, O8 and O4. It was demonstrated the presence of *V. cholerae* non-O1 serotype O98 in rainwater cistern systems.

The pattern of susceptibility of 65 isolated strains of *V. cholerae* non-O1 with 15 different antibiotics is shown in Table III. More than 67.7% (44/65) of the isolated strains were susceptible to the aminoglycosides, cephalosporins, tetracyclines, chloramphenicol, nitrofurantoin and trimethoprim-sulfamethoxazole. In contrast, 50.8% (33/65) of all strains showed resistance to two or more drugs. 49.2% (32/65) of these microorganisms were resistant to ampicillin and carbenicillin, while 10.8% (7/65) were resistant only to ampicillin and 4.6% (3/65) exhibited resistance only to carbenicillin. The rate of resistance to these substances was higher than to the other drugs. In addition, 3.1% (2/65), 7.7% (5/65) and 6.2% (4/65) of *V. cholerae* non-O1 strains presented resistance to the doxycycline, trimethoprim-sulfamethoxazole and erythromycin respectively.

TABLE I

Frequency of isolation of *Vibrio cholerae* non-O1 from water supplies for human consumption

Place	Water sources studied	No. of water sources studied	Water source with bacteria		
			No.	%	No. of strains
City of Campeche	Deep pool	35	10	28.6	20
	Intradomiciliary faucet	1	1	100.0	1
Neighbourhood of Morelos, city of Campeche	Deep pool	1	0	0.0	0
	Intradomiciliary faucet	45	8	17.7	11
	Rainwater cistern system	4	0	0.0	0
Rural locality of Bécal	Deep pool	3	1	33.3	7
	Distribution tank	1	0	0.0	0
	Intradomiciliary faucet	18	10	55.6	19
	Intradomiciliary deep pool	3	3	100.0	4
	Rainwater cistern system	5	1	20.0	3

TABLE II

Distribution of *Vibrio cholerae* non-O1 serotypes

Place	Serotype	Strains number	%
City of Campeche	O5	1	4.8
	O7	1	4.8
	O14	5	23.6
	O29	1	4.8
	O30	1	4.8
	O35	2	9.5
	O37	2	9.5
	O39	1	4.8
	O57	1	4.8
	O80	1	4.8
	O85	2	9.5
	O126	2	9.5
	O?	1	4.8
Neighbourhood of Morelos, city of Campeche	O14	2	18.2
	O35	1	9.1
	O41	1	9.1
	O89	1	9.1
	O93	1	9.1
	O97	1	9.1
	O123	1	9.1
	O155	3	27.2
Rural locality of Bécal	O4	1	3.0
	O7	2	6.2
	O8	1	3.0
	O9	1	3.0
	O16	1	3.0
	O32	1	3.0
	O38	1	3.0
	O58	1	3.0
	O98	3	9.1
	O107	1	3.0
	O112	17	51.6
	O?	3	9.1

TABLE III

Antimicrobial susceptibility of *Vibrio cholerae* non-O1

Antibiotic	R		I		MS		S	
	No.	%	No.	%	No.	%	No.	%
Amikacin 30 mcg	2	3.1	5	7.7	1	1.5	57	87.7
Ampicillin 10 mcg	39	60.0	0	0.0	18	27.7	8	12.3
Carbenicillin 100mcg	35	53.8	0	0.0	18	27.7	12	18.5
Cephalothin 30 mcg	2	3.1	0	0.0	18	27.7	45	69.2
Cefotaxime 30 mcg	0	0.0	0	0.0	15	23.1	50	76.9
Ceftriaxone 30 mcg	0	0.0	0	0.0	11	16.9	54	83.1
Chloramphenicol 30 mcg	0	0.0	0	0.0	0	0.0	65	100.0
Gentamicin 10 mcg	0	0.0	2	3.1	0	0.0	63	96.9
Netilmicin 30 mcg	0	0.0	0	0.0	0	0.0	65	100.0
Nitrofurantoin 300 mcg	1	1.5	4	6.2	0	0.0	60	92.3
Trimethoprim-Sulfamethoxazole 25mcg	5	7.7	1	1.5	1	1.5	58	89.3
Pefloxacine 5 mcg	0	0.0	28	43.1	0	0.0	37	56.9
Erythromycin 15 mcg	4	6.2	59	90.7	0	0.0	2	3.1
Doxycycline 30 mcg	2	3.1	3	4.6	0	0.0	60	92.3
Tetracycline 30mcg	0	0.0	19	29.2	0	0.0	46	70.8

R: resistant; I: intermediate; MS: moderately susceptible; S: susceptible.

DISCUSSION

Based in the results of the present study it is possible to conclude that the water distribution system plays an important role as transmission vehicle for *V. cholerae* non-O1. Considering the presence of *V. cholerae* non-O1 in the studied sources, it is possible to assume that this organism might be responsible of a proportion of diarrheal diseases among the population of the city of Campeche and the rural locality of Bécal.

It is important to observe that 29 *V. cholerae* non-O1 serotypes found in this study display a clear pattern of geographic distribution. As can be observed in the Table II *V. cholerae* non-O1, O112 was located only in Bécal, and *V. cholerae* non-O1, O14 and O155 were exclusively located in the city of Campeche. Regarding to those serotypes with a less frequent presentation; those present in Bécal are different to the ones found in the city of Campeche with only one exception that corresponded to *V. cholerae* non-O1, O7. *V. cholerae* non-O1, O14 and O35 were present in samples taken from city of Campeche and neighbourhood of Morelos, nonetheless the serotypes of the remaining strains were different.

Additionaly, 9.1% (3/33) and 4.8% (1/21) of those strains isolated in Bécal and the city of Campeche, respectively belong to a particular *V. cholerae* non-O1 strain which do not corresponded to any of those 141 serotypes that were studied in this work and might represent an autoctonous uncharacterized serotypes.

The presence of 2 or 3 different serotypes of *V. cholerae* non-O1 were detected in 21.4% (3/14), 22.0% (2/9) and 29.4% (5/17) of samples collected from the city of Campeche, neighbourhood of Morelos and the rural locality of Bécal respectively. This indicate that coexistance of different serotypes of *V. cholerae* non-O1 frequently happens within the same habitat.

Nonetheless that a high percentage of strains isolated during the development of this study displayed susceptibility to a diverse group of antibiotics, it is important to observe that *V. cholerae* non-O1 strains presented an important resistance to doxycycline, trimethoprim-sulfamethoxazole and erithromycin. Additionaly, a hight percentage of those strains registered an intermediate susceptibility to erithromycin and tetracycline. This susceptibility pattern represent a significant information because they are the antibiotics that WHO recommends for the treatment of cholera. In México during 1991, 3.2% of clinical isolates of *V. cholerae* O1 presented resistance to tetracycline and 1.5% to doxycycline. During 1992 this multiresistance decreased to 1.4% and from 1993 to 1995 only 0.1% of *V. cholerae* O1 strains registered resistance to the antibiotics mentioned; nonetheless the strains isolated conserved their resistance to ampicillin (Gutiérrez-Cogio 1995). In México, there is not available information about antibiotic susceptibility pattern of environmental isolates of *V. cholerae* non-O1. However, according to the results obtained in this study it is possible to conclude that the environmental *V. cholerae* non-O1 strains are significantly more resistant to antibiotics than the clinical *V. cholerae* O1 strains. This acquired an important significance, because of phenomenon of acquired plasmid-mediated resistance that might occur in a habitat where *V. cholerae* serogroup O1 and non-O1 coexists. Studies performed in Tanzania and Bangladesh reported strains of *V. cholerae* with plasmid-mediated resistance to a wide range of antibiotics (Mhalu et al. 1979, Threlfall & Rowe 1982).

Dalsgaard et al. (1995) reported that of 23 environmental *V. cholerae* non-O1 strains, 78.3% exhibited resistance to tetracycline and 8.7% were resistant to ampicillin and chloramphenicol. These results differ from the results of the present study. Nevertheless, the resistance to trimethoprim/sulfamethoxazole and erythromycin were similar in both studies. It is possible that variations in the antibiotic susceptibility pattern could be connected with the origin and habitat conditions of the *V. cholerae* non-O1 strains and with a variety of antibiotics used in differents parts of the world.

The sudden emergence of *V. cholerae* non-O1, O139 as ethiologic agent of epidemic cholera represents an important shift in the epidemiology of this disease. The evidence suggests the *V. cholerae* O1 El Tor gave rise to O139 by adquisition of novel DNA (Colwell 1996). Studies have shown that DNA sequence that determine the antigenic properties of the O139 cell surface is also present in two *V. cholerae* non-O1 strains with serotypes O69 and O141 (Colwell 1996) and additionaly that *V. cholerae* non-O1 serotypes O22 and O155 posses somatic (O) antigen factors in common with O139 strain (Mukhopadhyay et al. 1995). Cravioto et al. (1994) proved that the Bengal strain is genetically related to *V. cholerae* O1 El Tor Ogawa isolated in México, and two other non-O1 strains (serotypes O22 and O30). In the present study *V. cholerae* serotypes O30 and O155 were detected in samples analyzed in the city of Campeche. Considering these finding, it is not possible to establish a line of division between *V. cholerae* O1, O139 and the bacterial strains belonging to serogroup non-O1. It is now evident that these microorganisms have acquired an increasing importance, particularly in relation to medical relevance and ecological distribution.

The fact that the serogroup O139 emerged as responsible of epidemic cholera raised the possibility that such an event can occur once again, in relation to *V. cholerae* non-O1 serotypes prevalents in the Americas. It is recommended a strong epidemiologic surveillance in particular regarding emergence and distribution of *V. cholerae* non-O1 strains both in environmental samples and clinical specimens. Additional support to these study is found in the fact that *V. cholerae* non-O1 has been isolated in patients with diarrhoea in México (Gutiérrez-Cogio 1995). In addition, the relatively high prevalence of multiple antibiotic resistance of *V. cholerae* non-O1 strains recovered from water samples indicate the necessity of performing a epidemiologic surveillance of the pattern of antibiotic susceptibility.

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