Serogroups, K1 antigen, and antimicrobial resistance patterns of *Aeromonas* spp. strains isolated from different sources in Mexico

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A total of 221 strains of Aeromonas *species isolated in Mexico from clinical (161), environmental (40), and food (20) samples were identified using the automated system bioMérieux-Vitek®. Antisera for serogroups O1 to 044 were tested using the Shimada and Sakazaki scheme. The K1 antigen was examined using as antiserum the O7:K1C of* Escherichia coli. *Besides, we studied the antimicrobial patterns according to Vitek AutoMicrobic system.*

Among the 161 clinical strains 60% were identified as A. hydrophila*, 20.4% as* A. caviae*, and 19.25% as* A. veronii *biovar sobria. Only* A. hydrophila *and* A. veronii *biovar sobria were found in food* (55 *and 90% respectively) and environmental sources (45 and 10% respectively). Using "O" antisera, only 42.5% (94/221) of the strains were serologically identified, 55% (121/221) were non-typable, and 2.5% (6/221) were rough strains. Twenty-two different serogroups were found, O14, O16, O19, O22, and O34 represented 60% of the serotyped strains.*

More than 50% of Aeromonas *strain examined (112/221) expressed K1 encapsulating antigen; this characteristic was predominant among* Aeromonas *strains of clinical origin. Resistance to ampicillin/sulbactam and cephazolin was detected in 100 and 67% of* Aeromonas *strain tested for their susceptibility to antibiotics. In conclusion, antibiotic-resistant* Aeromonas *species that possess the K1 encapsulating antigen and represent serogroups associated with clinical syndrome in man are not uncommon among* Aeromonas *strains isolated from clinical, food and environmental sources in Mexico.*

Key words: *Aeromonas* - serogroups - resistance patterns - K1 antigen

The genus *Aeromonas* is composed of a large number of different taxa. Currently this group is included in the family *Aeromonadaceae*, at least 17 genome-species are recognized in the genus (Martin-Carnahan & Joseph 2005). However, *A. hydrophila, A. caviae*, and *A. veronii* biovar sobria are considered human pathogens since they are related with intestinal and extraintestinal infections, including septicemia, wounds, and respiratory tract disease (Janda 1991). Previous studies have demonstrated that the genus *Aeromonas* is the second cause of gastroenteritis in children and the fifth in adult patients (Janda 2001).

Serologic typing is based on the presence of specific somatic "O" antigen. The *Aeromonas* genus has 96 serogroups: the 45 serogroups proposed by Sakasaki and Shimada (1984) include rough strains obtained from mesophilic *Aeromonas* strains and 52 serogroups from different strains isolated in England, Peru, Brazil, and Australia (Thomas et al. 1990). In these serologic schemes, several important serogroups have been defined, such as the O11 serogroup, which is related to extraintestinal infections including septicemia, meningitis, and peritonitis, whereas the O34 group has been found responsible for wound infections, and the O16 serogroup related to gastroenteritis (Merino et al. 1996, Janda 2001, Figueras 2005). A capsular polysaccharide has been found in serogroups O11 and O34 and this structure has been reported to play a role in the pathogenecity of virulent strains of *A. salmonicida* (Martínez et al. 1995). Therefore, serological typing methods are useful to relate characteristic *Aeromonas* spp. serogroups with virulence and several diseases. Epidemiological markers, including prevalence and geographical distributions, provide knowledge about the sources of the pathogenic characteristics and transmission mechanisms of infections produced by the *Aeromonas* genus.

In other microorganisms, the antigen structure has been used in epidemiological studies to define routes of transmission or relationships with pathogenicity. *E. coli* is serogically heterogeneous but has been classified based on its antigens, and specific serogroups can be associated with reproducible and certain clinical syndromes. These studies have been used to establish which strains are present in different countries (Sarff & McCraken 1975, Nataro & Kaper 1998). Although *Aeromonas* is associated with diseases in very young humans and in older inmunocompromised patients who often require antimicrobial therapy, reports about the susceptibility of these

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organisms to antimicrobial agents are limited in our country. The antibiotic susceptibility patterns may serve as important therapeutic and diagnostic guidelines and preestablish epidemiological markers, since few studies have focused on the isolation and characterization of *Aeromonas* species in Mexico (Castro-Escarpulli et al. 2002a,b).

The aim of this study was to determine the serogroups distribution, presence of the K1 antigen, and resistance patterns to antimicrobial agents exhibited by *Aeromonas* strains isolated from different sources in Mexico.

MATERIALS AND METHODS

Bacterial strains - Included in the study 221 strains: 161 from clinical specimens (140 from human feces and 21 from urine), 20 from food samples, and 40 environmental sample (16 freshwater and 24 sediments).

Biochemical identification - The strains were identified using the Vitek AutoMicrobic system (Vitek ASM, bioMérieux® Vitek, France) and complementary biochemical tests: production of acetylmethylcarbinol (Voges Proskauer), esculin hydrolysis, salicin fermentation, and gas production from glucose.

Serotyping - Serological typing was performed according to the antigenic scheme pattern proposed by Sakasaki and Shimada (1984). Antisera were prepared at the Facultad de Medicina, UNAM, Mexico, as previously reported (Thomas et al. 1990), using reference strains kindly provided by Dr B Rowe, Head of Enteric Pathogens Central Laboratory of Public Health, London, England.

K1 antigen determination - Five colonies from those grown on Tripticase soy agar (TSA, Oxoid, UK) were suspended in 3 ml of Tripticase soy broth (TSB, Oxoid), adjusted to 0.5 with the McFarland standard ($1.5\text{-}10^8$ cells ml^{-1}) and then 5 µl of this suspension was added to antiserum-agar (0.5 mg/ml of capsular anti-K1 was mixed with 1/10 v/v TSB plus 1.5% agarose). After incubation at 37°C for 24 h, presence of a precipitation reaction zone surrounding the colonies indicated the presence of the K1 antigen. *E. coli* O7:KC1, *E. coli* O5:H4, *A. hydrophila* ATCC 7966^T, *A. caviae* ATCC 15468^T, and *A. veronii* biovar sobria ATCC 35624^T strains were used as controls (Sarff & McCraken 1975).

Antimicrobial susceptibility testing - The resistance pattern of all strains to different antimicrobial agents was determinate by the Vitek AutoMicrobic system (Vitek ASM, bioMérieux® Vitek, France) with the GNS-110 card, containing: amikacin (2-32 µg/ml), ampicillin (0.5-32 µg/ml), ampicillin/sulbactam (2-32 µg/ml), cephazolin (4-64 µg/ml), cefotaxime (6-24 µg/ml), cefotetan (4-64 µg/ml), ceftazidime (4-64 µg/ml), ceftriazone (16-128 µg/ml), ciprofloxacin (1-4 µg/ml), gentamicin (0.5-8 µg/ml), imipenem (4-8 µg/ml), ofloxacin (1-10 µg /ml), piperacillin (8-64 µg/ml), tircarcillin/clavulanic acid (16-64 µg/ml), tobramycin (0.5-8 µg/ml), and trimethoprim/sulfamethoxazole (40-160 µg/ml). *A. hydrophila* ATCC 7966^T, *A. caviae* ATCC 15468^T, and *A. veronii* biovar sobria ATCC 35624^T strains were used as controls.

RESULTS

Identification of 221 *Aeromonas* strains to species level resulted in 65.2% being *A. hydrophila*,19.2% *A. veronii* bt sobria and 14.9% *A. caviae*. In clinical samples, *A. hydrophila* was the predominant species (60.2%), followed by *A. caviae* (20.5%), and *A. veronii* bt sobria (19.3%). *A. hydrophila* and *A. veronii* bt sobria were identified in 55 and 45% of the food samples and in 90 and 10% of the environmental samples, respectively. *A. caviae* was not found in food and environmental samples. Distribution of *Aeromonas* species according to sources of isolation is shown in Table I.

Of the *Aeromonas* strains serotyped with "O" serogroup antisera, 42.5% (94/221) were typable, 55% (121/221) were non-typable and 2.5% (6/221) were rough strains. Twenty-two different serogroups were found with O14, O16, O19, O22, O34 representing 60% of the typable strains. The O14 (6/74), O16 (7/74), O19 (6/74), O22 (38/74), O34 (7/74) serogroups were most commonly associated with the 74 typable clinical *Aeromona* strains; whereas serogroup O22 (38/94) was observed in *Aeromonas* strains from the three different sources. Distributions of serogroups with respect to species and sources of isolation in shown in Table II.

Capsular antigen - Fifty-one percent (112 of 221) of all *Aeromonas* strains reacted positively with the antiserum O7KC1 of *E. coli*. The K1 antigen was observed in 81.2% clinical, 12.5% environmental, and 6.4% food samples depicting *Aeromonas* strains (Figure).

Antimicrobial susceptibility testing - Testing the 221 *Aeromonas* strains against the 16 different antibiotics revealed that 100% were resistant to ampicillin and ampicillin/sulbactam. The clinical strains were resistant to cephazolin (67%), cefotaxime (3%), cefotetan (2%), ceftazidime (2%), ceftriaxone (2%), ciprofloxacin (2%), gentamicin (1%), imipenem (1%), ofloxacin (1%), piperacillin (2%), tircarcillin/clavulanic acid (1%),

TABLE I

Distribution of *Aeromonas* species by isolation origin

Species	No. (%) positive for			
	Clinical (n = 161)	Food (n = 20)	Environmental (n = 40)	Total (n = 221)
A. hydrophila	97 (60.2)	11 (55)	36 (90)	144 (65.2)
A. veronii biovar sobria	31 (19.2)	9 (45)	4 (10)	44 (19.9)
A. caviae	33 (20.4)	0	0	33 (14.9)

n: number strains

TABLE II
Distribution of *Aeromonas* "O" serogroups with respect to species and origin

	No. (%) positive for							
	Clinical (n = 74)			Environmental (n = 14)		Food (n = 6)		Total (%)
Serogroups	*A.h*	*A.c*	*A.v.s*	*A.h*	*A.v.s*	*A.h*	*A.v.s*	
02	1							1 (1)
08	2	1						3 (3)
09							1	1 (1)
010	1							1 (1)
013	1							1 (1)
014	4	1	1					6 (6)
015	2	1						3 (3)
016	6		1					7 (7)
019	5	1						6 (6)
020	1							1 (1)
022	12	6	5	11		2	2	38 (41)
025	1	1			1			3 (3)
026		1				1		2 (2)
027	1							1 (1)
029		1						1 (1)
033	1							1 (1)
034	4	3						7 (7)
035	1	1	1					3 (3)
037	1	1		1				3 (3)
039					1			1 (1)
044	1	1						2 (2)
045	2							2 (2)
Total	47(50)	19(20)	8(9)	12(13)	2(2)	3(3)	3(3)	94(100)

n: number of serogrouped strains; *A.h: A. hydrophila, A.c: A. caviae, A.v.s: A. veronii* biovar sobria.

tobramycin and trimethoprim/sulfamethoxazole (3%), and susceptible to amikacin.

Aeromonas strains from environmental and food samples were resistant to cephazolin (35 and 55%, respectively) and all strains were susceptible to amikacin, cefotaxime, cefotetan, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, ofloxacin, piperacillin, tircarcillin/clavulanic acid, tobramycin, and trimethoprim/sulfamethoxazole.

K1 immunoprecipitation around colonies of *Aeromonas* on TSA agar plus capsular anti-K1.

DISCUSSION

Distribution of the identified *Aeromonas* strains showed that *A. hydrophila* species (144 of 221) was the most frequently distributed among the group of strains coming from the three different sources (clinical, environmental, and food). These results are similar to those published by Janda et al. (1996) in which the most frequent species was *A. hydrophila* independently from the source. The 33 identified strains as *A. caviae* were all from clinical but not environmental or food sources. These results are accordance with previous studies, which described that *A. caviae* is the most common aeromonad isolated from diarrheic stool specimens (Mokracka et al. 2001, Castro-Escarpulli et al. 2002a,b). Infections caused by *Aeromonas* might come from exposure to environmental or food sources, but its geographical localization, hygienic and sanitary conditions, and other factors related with pathogenic characteristics should not be ignored (Janda 1991). The antigen diversity of the *Aeromonas* genus has suggested that its serological typing, based on the somatic antigen, is useful (Sakazaki & Shimada 1984, Thomas et

al. 1990). Nevertheless, these schemes are not species-specific. *Aeromonas* strains isolated in Mexico belonged to the O14, O16, O19, O22, and O34 serogroups. The high incidence observed for the O34 serogroup agrees with previous studies performed with *Aeromonas* strains isolated from different origins (Janda et al. 1996, Misra et al. 1989, Merino et al. 1991). This serogroup has been associated with human infections, such as septicemia (Martínez et al. 1995).

There are no previous reports about the O22 serogroup as related with the incidence or isolation of *Aeromonas* strains. In the United States and India, serogroups O11 and O16 are considered the most frequent. In the present work, O22 serogroup was the most frecuent serogroup of the typable *Aeromonas* strains isolated from differences sources in Mexico. This finding provides evidence that the distribution of serogroup among *Aeromonas* strains could be related with their geographic localization (Kokka & Janda 1990).

The presence of the polysaccharide K1 capsular antigen was mainly related with 81.2% of the *Aeromonas* strains from clinical sources. The K1 capsular antigen in *Aeromonas* has been related with biochemical and structural distribution of the capsular antigen belonging to group II (K1) of *E. coli*. This is important since some authors consider that the capsular antigen is related to serum complement resistance and participates in the adhesion in cell lines, as well as exhibiting anti-phagocytic capacity (Zhang et al. 2002).

The three species identified showed 100% resistance to ampicillin and ampicillin/sulbactam; while *A. hydrophila* and *A. veronii* bt sobria showed resistance to cefazolin (60 and 55%, respectively). These correlated with previous reports in which the resistance had been determined to cephalosporins with similar patterns as those observed in *Aeromonas* strains from clinical and environmental origins (Overman & Janda 1999, Castro-Escarpulli et al. 2002a).

McNicol et al. (1980) reported that 57% of the environmental *Aeromonas* isolates recovered in Chesapeake Bay and areas surrounding Dacca and the Matlab region of Bangladesh were resistant to multiple antibiotis. In our study, no antibiograms similar to those described above were obtained. These differences might be related to the source or recovered *Aeromonas* species, the method of isolation, the frequency of use of certain antimicrobial agents in a specific geographic area, or to other unknown factors. Our results show that the second and third generation cephalosporins and quinolones (ciprofloxacin and ofloxacin) are among the drugs with the best antimicrobial effect against *Aeromonas* spp.

The present work indicates that in Mexico there are many serogroup differences among *Aeromonas* spp., evidencing that the distribution of strains is related with their geographic localization. In conclusion, antibiotic-resistant *Aeromonas* species that possess the K1 encapsulating antigen and represent serogroup associated with clinical syndromes in man are not uncommon among *Aeromonas* strains isolated from clinical, food, and environmental sources in Mexico.

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