Virulence factors and antibiotic susceptibility of Aeromonas spp. from drinking water reservoirs (Faskia) in Tripoli-Libya

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Isolation, virulence factors and antibiotic susceptibility of *Aeromonas* spp. from drinking water reservoirs (Faskia) in Tripoli-Libya

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ABSTRACT

Introduction: *Aeromonas* spp. have been associated with bacteremia, wound infections and gastroenteritis. A number of virulence factors, including hemolysin production and mannose-resistant haemagglutination (MRHA) have been associated with their pathogenicity.
Materials and Methods: Samples of drinking water obtained from water reservoirs (Faskia) in Tripoli were examined for the presence of *Aeromonas*. Using standard microbiological procedures, isolated *Aeromonas* were identified to the species level and tested for hemolysin production, MRHA and susceptibility to antibiotics.
Results: Of 56 water samples examined, 41 (73.%) were positive for *Aeromonas* spp. Speciation of *Aeromonas* isolates resulted in 10 (24.4%) being *A. hydrophila*, 10 (24.4%) *A. caviae*, 9 (22%) *A. sobria*, and 12 (29.2%) other *Aeromonas* spp. All (100%) aeromonads were positive for hemolysin production and 34% showed MRHA. Of the isolated *Aeromonas* spp., 75.6% were resistant to ampicillin, 34.1% to augmentin, 7.3% to amikacin, 36.6% to chloramphenicol, 2.4% to ciprofloxacin, 7.3% to nalidixic acid, 2.4% to tetracycline and 4.8% to trimethoprim-sulphamethoxazole.
Conclusion: Potentially pathogenic antibiotic-resistant *Aeromonas* spp. are very common in drinking water from water reservoirs (Faskia) in Tripoli.

Key words:
Aeromonas, Drinking water, Haemagglutination, Antibiotic resistance, Libya.

INTRODUCTION

Members of the genus *Aeromonas* are Gram-negative, oxidase-positive, facultative anaerobic bacilli in the family *Aeromonadaceae* (1). The taxonomy of the genus *Aeromonas* is in a state of change with new species still being added. More than 14 hybridization groups or genospecies and 14 phenospecies are described in the last edition of Bergy`s Manual (2). Only *A. hydrophila, A. veroni* biovar sobria (*A. sobria*) and *A. caviae* are commonly isolated from clinical, food and water sources in developing countries (3),

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which is similar to what has been reported from developed countries (4).

These organisms are widely distributed in aquatic environments and are important pathogens of fish and other aquatic animals. In developing countries, *Aeromonas* spp. have been associated with a wide spectrum of infections that include gastroenteritis, wound infections, septicemia and lung infections (3).

A number of virulence factors have been related to the pathogenicity of *Aeromonas* spp. These include exotoxins (hemolysins, cytotoxins, enterotoxins), hemagglutinins, adhesins and the ability to invade tissue in culture (5-7). Studies have shown that hemolysin produced by *Aeromonas* spp. is strongly associated with enterotoxic and cytotoxic activity (8).

Several studies have demonstrated a strong association of haemagglutination activity and enteropathogenicity of *Aeromonas* isolated from clinical and environmental sources (6, 9, 10). Ghenghesh *et al*. (11) found a significant association between *Aeromonas* isolated from children with diarrhoea and mannose-resistant haemagglutination (MRHA).

High rates of resistance to commonly used, cheap oral antimicrobial agents among *Aeromonas* spp., particularly those isolated from clinical sources and to a lesser extent from foods and water have been reported from several developing countries (12). Gastrointestinal infections with *Aeromonas* spp. are generally self-limiting and usually do not require treatment with antimicrobial agents. However, there is a dearth of information on the treatment of extraintestinal infection due to aeromonads in developing countries (3). Such infections are usually associated with high mortality rates particularly in the immunocompromised individuals (13,14).

Drinking of untreated water is believed to be the most probable manner of acquiring *Aeromonas* spp. (15,16). In the course of surveying the bacteriological quality of different sources of drinking water in Tripoli city we noticed that the few water samples obtained from water reservoirs (also known locally as Faskia) were of unacceptable quality (Ghenghesh KS., unpublished observation). Faskia is an underground reservoir to collect rain water used for drinking. This type of water reservoir is common in Libyan cities particularly in Tripoli. Usually the inhabitants clean the roofs of their houses before the start of the rainy season (during the month of October). The first rain fall is usually not collected and used to clean the roof of the house. Thereafter rain water is collected in the underground reservoirs that are constructed close to the houses. It is common locally not to treat the water in Faskia with chlorine and to use it mainly for drinking.

The present study was carried out to isolate *Aeromonas* spp. from drinking water reservoirs (Faskia) in Tripoli, Libya, to determine the virulence factors of the isolated *Aeromonas*, and to determine the susceptibility of the isolated organisms to the commonly used antimicrobial agents.

MATERIALS AND METHODS

Samples: From October 2003-July 2004, 56 water samples were obtained from 56 water reservoirs (Faskia) in Tripoli. Water samples were collected aseptically in sterile containers, placed in an ice chest and processed within two hours of collection.

Microbiology: Water samples were examined for coliform and *Escherichia coli* counts by the five-tube most probable number method (17). For isolation of *E. coli* a loopful from each tube positive for *E. coli* was plated onto MacConkey agar (MA) and incubated at 37^{o}C overnight. For isolation of *Aeromonas* spp. samples were enriched in alkaline peptone water (APW, pH 8.6) and incubated at 37^{o}C. After overnight incubation, a loopful from APW was plated onto ampicillin blood agar (ABA, 10 mg/L) and incubated at 37^{o}C overnight. Suspected colonies from MA and ABA were identified biochemically using standard microbiological procedures (18) and API20E (bioMerieux, France). Speciation of *Aeromonas* species was carried out as reported previously (19).

Isolated *Aeromonas* were tested for hemolysin production by streaking on 5% blood agar plates and incubated at 37°C overnight. Aeromonads were also tested for agglutination of human RBCs in the absence of mannose [mannose-sensitive haemagglutination (MSHA)] and in the presence of mannose [mannose-resistant haemagglutintion (MRHA)] as described by Evans *et al*. (20). Susceptibility to antimicrobial agents was carried out by the disc diffusion method (21).

RESULTS

The coliforms MPN of the 56 water samples examined ranged between 0.0 - > 3.8 x 10^2/100ml (mean=1.1 x 10^2) and the *E. coli* MPN ranged between 0 - > 1.1 x 10^2/100ml (mean=1.1 x 10^2). *Aeromonas* spp. were detected in 41 (73.2%) and *E. coli* in 15 (26.8%) water samples examined. Speciation of the isolated aeromonads resulted in 10 (24.4%) being *A. hydrophila*, 10 (24.4%) *A. caviae*, 9 (22%) *A. sobria*, and 12 (29.2%) other *Aeromonas* species (See Table 1).

All (100%) *Aeromonas* isolates produced hemolysin on blood agar. Of the isolated *Aeromonas* 20 (48.8%) showed mannose-sensitive hemagglutination (MSHA) and 14 (34.1%) showed mannose-resistant hemagglutination (MRHA). Of the 41 *Aeromonas* isolates examined 31 (75.6%) were resistant to amplicillin, 14 (34.1%) to amoxicillin clavulanic acid, and 1 (2.4%) to ciprofloxacin. Table 2 shows the resistance profile of the isolated aeromonads to antimicrobial agents.

DISCUSSION

In the present work, 56 drinking water samples from underground water reservoirs known as Faskia in Tripoli were examined. *Aeromonas* spp. and fecal *E. coli* were detected in more than 73% and more than 26% of the samples, respectively. Recently, a study from Tripoli examined drinking water samples taken from 50 mosques and reported rates of 18% and 14% for *Aeromonas* spp. and *E. coli*, respectively (22). A previous study carried out in Tripoli reported the isolation of *Aeromonas* spp. from 48% of 1000 drinking water samples from wells with the predominance of *A. hdrophila* (59%), followed by *A. caviae* (27%), A. sobrai (11%) and other *Aeromonas* spp. (3%) (19). In the present investigation *A. hydrophila*, *A. caviae* and *A. sobria* were detected at approximately similar rates being 24%, 24% and 22%, respectively. Several studies reported that *A. hydrophila* is the predominant species in freshwater and municipal drinking water supplies (23 -25). Similar to our findings, others reported no significant differences in the

Table 1. *Aeromonas* spp. isolated from drinking water reservoirs (Faskia) in Tripoli, Libya

Species	No (%) positive
A. hydrophila	10 (24.4)
A. caviae	10 (24.4)
A. sobria	9 (22)
Aeromonas spp.	12 (29.2)
Total	41 (100)

Table 2. Resistance of *Aeromonas* spp. isolated from drinking water reservoirs (Faskia) to antimicrobial agents.

Antimicrobial agent	No (%) resistant (n=41)
Ampicillin	31 (75.6)
Amoxicillin + clavulanic acid	14 (34.1)
Amikacin	3 (7.3)
Ciprofloxacin	1 (2.4)
Nalidixic acid	3 (7.3)
Tetracycline	1 (2.4)
Trimethoprim -sulpha-methoxazole	2 (4.8)

isolation rates of the *A. hydrophila*, *A. caviae* and *A. sobria* from fresh water (26,27).

In the present study, haemolytic activity using human RBCs was observed in all (100%) of the 41 *Aeromonas* isolates examined. Sharma *et al*. (27) tested 30 *Aeromonas* isolates from the River Narmada in India. They found 14 (47%) of them positive for hemolysin production. Ghenghesh *et al*. (19) detected hemolytic activity in more than 50% of 171 *Aeromonas* strains from well water in Tripoli. We detected MRHA in 34% of *Aeromonas* spp. isolated from drinking water reservoirs in Tripoli. Gibotti *et al* (26) reported that 29 (91%) of 32 *Aeromonas* isolates from fresh water tested for haemagglutination with human RBCs showed MRHA. These differences in the detection rates of hemolysin production and MRHA may be due to the source of *Aeromonas* spp. and geographical location.

Several studies from Libya reported rates of resistance among *Aeromonas* spp. isolated from drinking water ranging between 89 - 100% to ampicillin, 0 - 5% to tetracycline, 0 - 22% to trimethoprim-sulphamethoxazole (19,22). Similar to these studies aeromonads from drinking water reservoirs in Tripoli showed resistance rates within the ranges reported previously with respect to tetracycline and trimethoprim-sulphamethoxazole. Although the rate of resistance to ampicilin among aeromonads isolated in our study was lower than previously reported it is still considerably high at 76%.

Resistance to ciprofloxacin has never been reported previously for *Aeromonas* spp. from drinking water in Libya. We detected only one (2.4%) *Aeromonas* isolate resistant to this drug. Ciprofloxacin is a fluoroquinolone and widely used in the treatment of urinary, respiratory and gastrointestinal tract infections among other infectious diseases. Nearly a decade ago we reported the isolation of *Aeromonas* resistant to ciprofloxacin from chicken carcases in Tripoli (28). Isolation of multiple-resistant *Aeromonas* species (including the quinolones) from freshwater in other countries has been reported previously (29). The findings of the present investigation warrant the need to take proper measures to prevent the introduction of these organisms, that are resistant to these drugs, to water sources used by humans.

Up until mid-nineties of last century, Libya`s supply of water came mainly from underground aquifers or desalination plants on the coast. Both these sources of water were of poor quality and sometimes undrinkable. In September 1996 a major stage of the Great Man-Made River project was inaugurated (http://www.mathaba.net/info/ggmr-2.htm). This project provides Tripoli and the surrounding region with fresh water pumped from sub-Saharan aquifers. However, many houses, particularly in the outskirts of Tripoli and other Libyan cities are not yet connected to this project. Inhabitants of such houses are mainly dependent on wells and rain-water reservoirs as their main sources of fresh water for drinking, cooking and for others uses. The findings of the present study have demonstrated that more than 25% of water samples from Faskia were positive for fecal *E. coli* which makes such reservoirs unacceptable as sources of drinking water. In addition, potentially enteropathogenic antibiotic-resistant *Aeromonas* spp. were very common in drinking water obtained from Faskia in Tripoli, Libya. This may pose a hazard to users of this source of drinking water particularly to the very young, the old, and the immunocompromised. The Health and Water Authorities in Tripoli and in other Libyan cities should educate the public with respect to the risks that might arise from using untreated water sources for drinking.

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