Ankara Ecz. Fak. Derg.
32 (3) 151-157, 2003

J. Fac. Pharm, Ankara
32(3)151-157,2003

# OCCURENCE AND ANTIBIOTIC SUSCEPTIBILITY OF MOTILE *AEROMONAS* SPP. OF UNTREATED WELL WATER

# KUYU SULARINDA HAREKETLİ AEROMONAS VARLIĞI VE ANTİBİYOTİK DUYARLILIĞI

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**ABSTRACT**

*Drinking untreated water samples collected from Ankara, Turkey were analysed for the presence of motile Aeromonas species. In this study, 166 of untreated water samples were examined and total of 66 motile Aeromonas were isolated . A. hydrophila, A. caviae and A. sobria were present in all wells investigated, forty (60.60 %) A. hydrophila, 19 (28.78 %) A. sobria, 7 (10.60 %) A. caviae were isolated in water samples. The most of the strains isolated were A. hydrophila. minimal tests such as oxidase, motility, sensitivity to the Vibriostatic agent 0/129, fermentation, and gas production from glucose, $H_2S$ production from cysteine, esculin hydrolysis and growth in KCN broth were sufficient to classify the majority of Aeromonas strains into species. Aeromonas strains were tested for haemolytic activity, and antibiotic susceptibility. All of the 66 Aeromonas strains, study were resistant to ampicilline and erytromicin,but susceptible to ciprofloxacin, cefazolin, cefixime, ceftriaxone, trimetoprim and sulphamethoxazole and gentamicin.*

*Key Words: Motile Aeromonas* spp., *incidence, antibiotic susceptibility, untreated well water*

**ÖZET**

*Ankara'dan toplanan içme suyu olarak kullanılan kuyu suları hareketli Aeromonasların varlığı yönünden analiz edildi. Bu çalışmada 166 kuyu suyu incelendi ve toplam 66 hareketli Aeromonas izole edildi. 40 A. hydrophila (% 60.60) , 7 A. caviae (10.60 %), 19 A. sobria (% 28.78) izole edildi. Suşların çoğunluğunu A. hydrophila oluşturmaktaydı. Aeromonas suşlarının tür tayininde oksidaz, hareket, vibriostatik ajana (O/ 129) karşı duyarlılık, fermentasyon ve glikozdan gaz üretimi, sisteinden H2S oluşumu, esculin hidrolizi ve KCN besiyerinde üreme gibi testler en etkili minimal testler olarak kullanıldı. Aeromonas suşları hemolitik aktivite ve antibiyotik duyarlılık yönünden test edildi. Aeromonas suşlarının 66 adedi (% 100) ampisilin ve eritromisine dirençli bulunurken bütün suşlar ciprofloksazin , sefazolin.sefixim, seftriakson, trimetoprim ve sulfametoksazol duyarlı bulundu.*

***Anahtar Kelimeler;*** *Hareketli aeromonas türleri, bulunma sıklığı, antibiyotik duyarlılığı, kuyu suları*

## INTRODUCTION

Members of the genus *Aeromonas* are Gram negative rods, oxidase positive, facultative anaerobic and belong to the family *Vibrionaceae.* (1,2,3). Bacteria of the motile *Aeromonas* group are common inhabitants of aquatic environments (Specifically,Aerom0na.y *hydrophila, A.sobria and A.caviae)* (2,4). Organisms of the hydrophila group occur widely in the environment, especially in water(5). They are found in a wide range of aquatic systems (6, 7). Several studies have reported that the untreated drinking water is the most probable manner of acquiring these organisms. Drinking water and food are reservoirs of aeromonads, and therefore may be important sources of human infections (8).

They have been implicated as the causative agents of diarrhaea, wound infections, and septicemiae of humans. Contaminated drinking water has been shown to be a vehicle of human gastroenteritis (9,10), because *Aeromonas* spp. are distributed widely in aquatic environments, there is considerable speculation about their role as contaminants. Water has an important role in direct transmission of motile *Aeromonas* spp. which may also contaminate foods.

Therefore, seafood, particularly fish, contaminated with these species could expand risks of foodborne diseases (12).

Aeromonads are considered to be opportunistic pathogens capable of producing disease only in weakened populations of fish or as secondary invaders in fish suffering from other diseases. Burke *et al.* have reported that haemolytic activity is strongly associated with enterotoxin production in members of the *Aeromonas* genus (13).

The purpose of this study was to invastigate the presence of motile *Aeromonas* spp. in drinking untreated well water in Ankara area and the ability to produce haemolysin and antibiotic susceptibility.

## MATERIALS and METHODS

### Standard strains

Standard strains *A.hydrophila Her* 1209 CI and *A.hydrophila Her* 1210 (ATCC 7966) were obtained from the Microbiology Department of University of Laval, Canada.

**Water samples**

Onehundredsixtysix untreated drinking water samples were collected from Ankara, Turkey (during March and June 2002). They were collected in 100 ml sterile flasks and transported immediately in an ice bath to the laboratory and processed within one hour. Water samples were filtered through 0,45 u*m* pore sized membrane filters, and than the membranes were transferred into 10 ml of sterile Alkaline Peptone Water (APW, pH= 8.4). Samples were first enriched in APW and incubated at 30°C for 24 h. Later, a loopful of the APW was streaked on to *Pseudomonas- Aeromonas* Agar (GSP) (Merck) containing ampicillin (Sigma Chemical CO., St.Louis, Mo: A-9393) (l0mg/L). All plates were incubated aerobically at 30 °C for 24h. and examined for the formation of yellow- honey colored colonies in 2-4 mm diameter and these colonies were subcultered on Trypticase Soy Agar and incubated at 30°C for 24h Gram negative, oxidase positive , motile organism were further tested for the following chracteristics; oxidation and fermentation of glucose, resistance to 2,4- diamino- 6,7- diisopropylpteridine (Vibriostatic agent 0/129, Sigma), production of gelatinase and DNase, reduction of nitrat, growth in the presence of KCN, and Voges Proscauer reaction, aesculin hydrolysis, gas production from glucose and acid from arabinose (Table 1).

Table 1 shows the biochemical patterns and species identification of the *Aeromonas* isolates.

**Table 1.** Biochemical reactions of presumptive aeromonas (14)

| *Aeromonas* spp. | VP | AE | GAS | ARA |
|---|---|---|---|---|
| *A. hydrophila* | + | + | + | + |
| *A.caviae* | - | + | - | +/- |
| *A.sobria* | +/- | - | +/- | - |

VP, Voges- Proskauer reaction; AE, aesculin hydrolysis; GAS, gas production from glucose; ARA, acid from arabinose

Isolates were classified as *A. sobria, A. hydrophila*, and *A. caviae* by the criteria of *Aeromonas* spp.(14) were tested in the commercially prepared identification system API E 20 and in certain conventional tests to determine the accuracy and speed of identification of *Aeromonas* to the genus and to the species level. API 20E test kits were read after 18 to 24 h at 37° C and reexamined at 48 h if definitive results were not obtained.

**Haemolysin production**

Haemolysin was detected on trypticase soy agar plates containing 5 % sheep blood at 37 °C for 48 h. Beta haemolytic zones in 2 mm or more around the colonies were regarded as the sing of positive haemolysis.

**Antibiotic susceptibility**

The antimicrobial agents ampicillin (10/xg), erytromicin (15 μg) , cefazolin (30 μg), ciprofloxacin (5 μg), cefixime (5 μg), ceftriaxone (30 μg), and trimethoprim-sulphamethoxazole (25 μg) and gentamicin (10 μg) were tested on Mueller Hinton Agar plates at 30°C by the standard disc method (15).

**RESULTS and DISCUSSION**

Motile *Aeromonas* species were isolated to be prevalent in untreated drinking well water in Ankara and the results of this study are given in Table 2.

Sixtysix *Aeromonas* species were isolated from 166 (39.75 %) water samples. This strains were identified as being *A. hydrophila* 40 (60.60 %), *A. sobria* 19 (28.78%), *A. caviae* 7 (10.60 %).

**Table 2.** Incidence of A. *hydrophila, A. sobria, A. caviae* from untreated drinking well water

| Number of tested sample | Number (%) of samples *Aeromonas* | *A.hydrophila* | *A.sobria* | *A. caviae* |
|---|---|---|---|---|
| 166 | 66 (39.75) | 40 (60.60) | 19 (28.78) | 7 (10.60) |

In this study, *A. hydrophila* was the most common species and rare *A.sobria* inuntreated well water. In many publications, researches have also shown the similiar results of these microorganisms is possible in untreated water samples (11, 16).

In our scheme for the isolation of motile *Aeromonas* we propose the utilization of GSP agar containing ampicillin (10 mg/1). Ampicillin is an important factor that increases the selectivity of this medium since *Aeromonas* strains are generally resistant to this antibiotic.

Another important factor associated with GSP agar is the yellowish tonality of colonies produced from the utilization of starch present in the medium by *Aeromonas strains.*

In this research, sixtysix of 166 samples analysed (% 39.75) were positive for *Aeromonas* spp.. These results are similiar to those of Barnhart *et al.* (16), who isolated motile *Aeromonas* from water samples (% 52). Millership *et al.* (10) have tested 100 samples of drinkable water from various wells, 16 strains were identified as *A. caviae* and 21 as *A. sobria.*

Altwegg (17) have showed that the most frequently isolated from the water samples in their study was *Aeromonas cavia* . This species were the predominant in water with high levels of fecal pollution. In fact, *A. caviae* is the species most frequently isolated for human feces in Europe.

(17). Altwegg (17) indicated that *A. caviae* was the predominant species in waters with high faecal pollution, while *A.hydrophila* was more abundant in drinking waters. Our results agree with those reported by Altwegg (17).

**Table 3.** Antibiotic susceptibility of motile *Aeromonas* spp. isolates from untreated well water

| Antibiotic | Concentration *(Hg 1* disk) | *A. hydrophila* n=40 | A *sobria* n=19 | A. *caviae* n=7 |
|---|---|---|---|---|
| Ciprofloxacin | 5 | 40 (% 100.00) | 19 (% 100.00) | 7 (% 100.00) |
| Cefazolin | 30 | 27 (% 67.50) | 15 (% 78.94) | 7 (% 100.00) |
| Cefixime | 5 | 33 (% 82.50) | 16 (% 84.21) | 6 (% 85.00) |
| Ceftriaxone | 30 | 35 (% 87.50) | 17 *(%* 89.47) | 7 (% 100.00) |
| Ampicilline | 10 | R | R | R |
| Erytromycin | 15 | R | R | R |
| Trimethoprim+ Sulfamethoxazole | 1.25+23.75 | 31 (% 77.50) | 16 (% 84.21) | 7 (% 100.00) |
| Gentamicin | 10 | 40 (% 100.00) | 19 (% 100.00) | 7 (% 100.00) |

*n: number of isolates*

*R: resistance*

All of the 66 *Aeromonas* strains were resistant to ampicillin and erytromycin . All strains were, however, susceptible to ciprofloxacin, cefazolin, cefixime, ceftriaxone, trimetoprim and sulphamethoxazole and gentamicin.

These results are similiar to those reported by Rahim and Aziz (18) who found mesophilic *Aeromonas* spp. were susceptible to third generation cephalosporin, cefepim, gentamicin, and resistance to ampicillin and erytromicin. Barnhart *et* al.(16) reported that the

resistance of *Aeromonas* strains to ampicillin and cephalotin. According to these results, ciprofloxacin, gentamicin and third generation cephalosporin are suitable drugs that can be used in the *Aeromonas* infections.

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**Received: 01.07.2003**

**Accepted: 09.09.2003**