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THE OCCURRENCE OF *AEROMONAS* IN DRINKING WATER, TAP WATER AND THE PORSUK RIVER

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ABSTRACT

The occurrence of *Aeromonas* spp. in the Porsuk River, public drinking water and tap water in the City of Eskisehir (Turkey) was monitored. Fresh water samples were collected from several sampling sites during a period of one year. Total 102 typical colonies of *Aeromonas* spp. were submitted to biochemical tests for species differentiation and of 60 isolates were confirmed by biochemical tests. Further identifications of isolates were carried out first with the VITEK system (BioMe˜rieux) and then selected isolates from different phenotypes (VITEK types) were identified using the DuPont Qualicon RiboPrinter® system. *Aeromonas* spp. was detected only in the samples from the Porsuk River. According to the results obtained with the VITEK system, our isolates were 13% *Aeromonas hydrophila,* 37% *Aeromonas caviae*, 35% *Pseudomonas putida*, and 15% *Pseudomonas acidovorans.* In addition *Pseudomonas* sp., *Pseudomonas maltophila*, *Aeromonas salmonicida*, *Aeromonas hydrophila*, and *Aeromonas media* species were determined using the RiboPrinter® system. The samples taken from the Porsuk River were found to contain very diverse *Aeromonas* populations that can pose a risk for the residents of the city. On the other hand, drinking water and tap water of the City are free from *Aeromonas* pathogens and seem to be reliable water sources for the community.

Key words: *Aeromonas* spp, drinking water, river water, tap water, Riboprinter

INTRODUCTION

The importance of detection of *Aeromonas* spp. has increased in recent years due to their emergent human pathogenic properties (9). *Aeromonas* spp. can cause septicaemia, wound infections (10, 18) and diarrhoeal illness (7).

Aeromonas are Gram-negative, oxidase-positive, facultative anaerobic, glucose fermenting, rod-shaped bacteria of the family *Aeromonadaceae* (8). *Aeromonas* spp. are common aquatic microorganisms that occur in irrigation water, river water, brackish water, freshwater, spring water, surface water and groundwater, estuarine and sea water, chlorinated and non-chlorinated drinking water (5, 6, 12, 13, 24) and in some countries, in bottled mineral water (17). All phenospecies are found in sewage-contaminated water (2). The prevalence and distribution of *Aeromonas* in aquatic environments, its role as a contaminant for drinking water supplies and potential for pathogenicity mediated by mesophilic *Aeromonas* are all of great public health concern (3, 6).

Residents of the City of Eskisehir in Turkey use treated water not for drinking but for cleaning, washing, and various recreational purposes as tap water from the Porsuk River. Therefore, the safety of the treated water from the Porsuk River is very important for the residents of the City. Lack of information about the incidence of motile *Aeromonas* in

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Eskisehir tap water and the Porsuk River as well as in drinking water should be great concern because their possible occurrence in such waters could be a potential source of infection for the residents.

In this study, the occurence of *Aeromonas* species in the Porsuk River, tap water obtained from the Porsuk River after treatment and drinking water of the City of Eskisehir was investigated.

MATERIALS AND METHODS

Collection of Water Samples

Samples were collected from 12 different sites on the Porsuk River at monthly intervals during the period of a year. Tap water samples were collected from houses, business centers and hospitals, randomly selected different parts of the City of Eskisehir. Drinking water samples sold in plastic bottles were purchased from a variety of retailers in the city.

Water samples from the river and tap water were brought to the laboratory in 500 ml sterile glass bottles. In order to inactivate chlorine, sterile sodium thiosulphate solution was added (13.2 mg/l). The samples were immediately stored under ice-cold conditions and microbiological analyses were performed within 3 h of collection.

Isolation and Identification of the Strains

10 ml of water were inoculated in 90 ml of peptone water with 1% NaCl (w/v) at pH 8.6 adjusted with sodium hydroxide. After incubation at 37 °C for 24–48 h, the cultures were streaked on Glutamate Starch Phenol Red agar (GSP) and incubated at 37 °C for 24–48 h (3). Colonies of presumptive *Aeromonas* spp. surrounded by yellow zone on GSP medium were purified and maintained on nutrient agar slants at 4 °C after subculturing several times. Their identifications were confirmed on the basis of the results of the following tests: Gram stain test, oxidase test, the oxidation/fermentation test, a test to determine the resistance to vibriostatic agent O/129. Additional tests (to detect gas production from glucose, growth in KCN broth, H_2S production from cysteine, esculin hydrolysis, and the suicide phenomenon) were performed for identification at the species level according to *Bergey's Manual of Determinative Bacteriology* 9th ed. (21). Motility was determined after 18 h in motility test medium (14). The production of hemolysin was assayed by recording the lysis of sheep erythrocytes in agar plates (5% sheep blood in brain heart infusion agar). Plates were incubated aerobically for 48 h at 30°C. All isolated strains were analyzed for their Cephalothin resistance according to the Kirby-Bauer test by using Mueller-Hinton agar.

Further identification of isolates which are possible *Aeromonas* spp. was performed with VITEK Gram-Negative Identification Panel (VITEK, BioMérieux). VITEK GNI cards were processed according to the manufacturer specifications. At least random two isolates from each VITEK profile type was further studied by the RiboPrinter Microbial Characterization System (Qualicon Inc., Wilmington, DE). Automated ribotyping was performed according to the manufacturer's protocol. To summarize, the automated process begins by lysing cells and cutting the released DNA into fragments with a restriction enzyme (*Eco*RI) and the restriction fragments were separated by electrophoresis and then transferred to a nylon membrane. Ribosomal DNA was hybridized to a chemiluminescent-labeled *E. coli* rRNA operon (rrnB) probe. The chemiluminescent patterns were electronically imaged and analyzed with the RiboPrinter Microbial Characterization System computer. The generated Riboprinter® patterns were analyzed with dendogram based on Unweighted Pair Group Method using arithmetic Averages (UPGMA) and Pearson correlation coefficients.

RESULTS AND DISCUSSION

In this study, we isolated a total number of 102 possible *Aeromonas* colonies from 144 Porsuk River, tap water and drinking water samples on the basis of morphological characters on the GSP agar. These isolates were characterized with physiological and conventional biochemical tests. According to the test results, only 60 typical *Aeromonas*

isolates which all belong to the samples from the Porsuk River were determined and all positive isolates were Gram negative, motile, rod shaped and facultative anaerobes. The isolates were oxidase and catalase positive and did not tolerate NaCl concentrations higher than 3.0% (Table 1).

There is no reported study about the Riboprinter identification of *Aeromonas* isolated from water sources. In our study, VITEK and Riboprinter systems were employed to identify of *Aeromonas* isolates from water samples. *A. hydrophila*, *A. caviae*, *Pseudomonas putida* and *Pseudomonas acidovorans* strains were identified with biochemical tests and VITEK system. The number of *A. hydrophila* and *A. caviae* isolates obtained from different sites of the Porsuk River in different seasons in given in Table 2. These identification results are nearly concordant with those obtained with RiboPrinter Microbial Characterization System (Qualicon). *Aeromonas* and *Pseudomonas* genera were determined with the each system. Ribotyping profiles of some strains and standards are shown in Fig 1. In addition, the homology among the isolates was indicated using UPGMA.

Table 1. Biochemical characterization of *Aeromonas* and *Pseudomonas* spp. isolated from the Porsuk River

	Isolates		
Tests	***Aeromonas hydrophila*** **(8 isolates)**	***Aeromonas caviae*** **(22 isolates)**	***Pseudomonas* sp.** **(30 isolates)**
Motility	+	+	+
Oxidase	+	+	+
Catalase	+	+	+
KCN	+	+	+
H_2S from gelatin cysteine throsulfate	+	-	-
Urea	-	-	-
Voges-Proskauer	+	-	-
Lysine decarboxylase	+	-	+
Arginine dihydrolase	+	+	+
Ornithine decarboxylase	-	-	+
D-glucose (gas)	+	-	v
D-Sorbitol	-	-	-
Salicin	+	+	-
Glycerol	+	+	+
L-Arabinose	+	+	+
Cellobiose	-	+	-
Sucrose	+	+	-
D-Trehalose	+	+	-
Adonitol	-	-	-
Citrate	+	+	+
D-Mannitol	+	+	-
Esculin hydrolysis	+	+	-
Growth in NaCl	-	-	-
O/129 (R)	+	+	+
Cephalothin	R	R	R

v: variable
R: resistant

Table 2. *Aeromonas* isolates obtained from different sites of the Porsuk River in different seasons in the city of Eskisehir

	A. hydrophila	***A. caviae***
Winter	3	3
Spring	-	4
Summer	-	8
Autumn	5	7

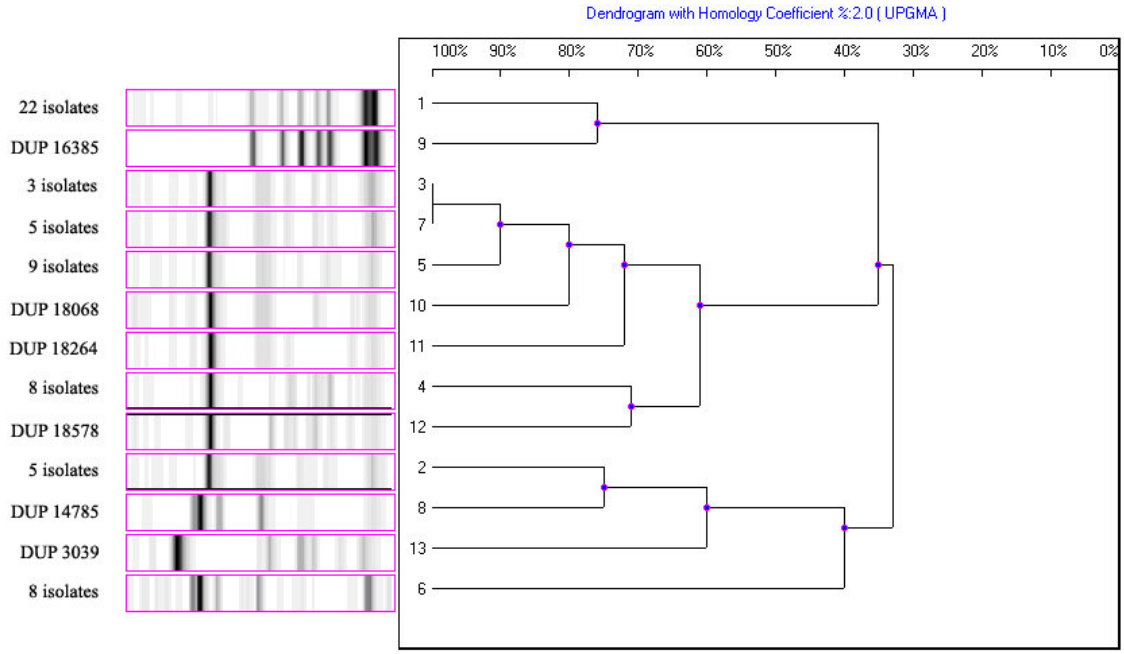

Figure 1. Ribotyping profiles of some isolates (1-7) and standards (8-13) placed the library of riboprinter system (DuPont)
1) *Pseudomonas species* (S=0,96: RG= ECORI-S-1), 2) *Aeromonas salmonicida* (S=0,82: RG= ECORI-S-3), 3) *Aeromonas hydrophila* (S=0,83: RG= ECORI-S-4), 4) *Aeromonas media* (S=0,76: RG= ECORI-S-5), 5) *Aeromonas salmonicida* (S=0,84: RG= ECORI-S-3), 6) *Pseudomonas maltophilia* (S=0,80: RG= ECORI-S-7), 7) *Aeromonas hydrophila* (S=0,84: RG= ECORI-S-4), 8) *Pseudomonas maltophila* DUP 14785, 9) *Pseudomonas species* DUP 16385, 10) *Aeromonas hydrophila* DUP 18068, 11) *Aeromonas salmonicida* DUP 18264, 12) *Aeromonas media* DUP 18578, 13) *E coli* DUP 3039
(S: Similarity, RG: Ribogrup)

The Riboprinter (DuPont-Qualicon, Wilmington, DE) is an automated ribotyping system that produces and analyzes ribotyping patterns of bacteria. Therefore, this method has the advantage of automation and standardization. In addition, the system reduces the time necessary to characterize or identify an organism to only 8 h after isolation, compared to days or even weeks for other techniques.

Aeromonas species occurred most frequently (82.53% of the isolates) during dry seasons from June to October in the Porsuk River. *A. hydrophila* only recovered during autumn and winter seasons. Lower isolation frequency noticed during the spring season. It was previously reported that higher numbers of *Aeromonas* were recovered during summer (6, 12).

The presence of *A. hydrophila* in drinking or tap water

should be concern for the public health. However, we have the results that there is no *Aeromonas* spp. in the tap and drinking water in the City of Eskisehir. In turn, the presence of *Aeromonas* spp. in the Porsuk River has a potential risk in terms of the residence health in the city. Because the Eskisehir tap water is as long as obtained from the Porsuk River, the health risk will be there for possible presence of *Aeromonas* spp in tap water. Furthermore, because of the irrigation of vegetables and watering gardens, and green places with water obtained from the Porsuk River, the potential of hazard is always there.

Wide distribution of motile *Aeromonas* spp. has been previously reported in the aquatic environment (5, 7, 12, 19, 20, 23) and our results obtained support this finding. The present study also revealed that 76% of *Aeromonas* isolates exhibited beta hemolytic activity. Beta hemolysin has been reported as a virulence factor in motile aeromonads (15, 16).

Aeromonas species principally associated with gastroenteritis are *A. caviae*, *A. hydrophila*, and *A. veronii* biovar *sobria*. *A. caviae* is particularly associated with young children especially under 3 years of age. Many studies reported the isolation of several species of *Aeromonas* from patients with gastroenteritis which has been extensively reviewed (1, 9, 11). The health significance of detecting mesophilic aeromonads in public water supplies is not well understood: no clearly defined point-source outbreak has been documented and establishing epidemiological links is difficult. Therefore, it is thought that Riboprinter microbial characterization system may provide to get correct results in the water analysis in short time.

World Health Organization (WHO) has reported the isolation ratio of *A. hydrophila* from drinking water to be 1.0–27% and the infectious dose to be $>10^{10}$ (4). Midilli (20) have isolated *Aeromonas* strains from 87.7% of environmental water samples and 40% of drinking waters in Istanbul. Mete *et al.* (19) have isolated eleven (2.4%) *Aeromonas* strains from 449 tap water samples around the City of Denizli in Turkey. These data are quite higher than the WHO's water quality standards.

Our data meet WHO's water quality standards. However, it has been reported that *Aeromonas* bacteria found in municipally treated drinking water possess a wide variety of virulence-related genes (23). This finding suggests that municipally treated drinking water could be a source of potentially pathogenic *Aeromonas* bacteria. We have analyzed only hemolytic activities as virulence factor of our isolates. Most of them were found to be hemolytic. Actually, further studies should be done in order to detect other virulence factors.

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