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Short communication

Diversity of *Salmonella* spp. and fungi in northern Greek rivers and their correlation to fecal pollution indicators

M. Arvanitidou*, K. Kanellou, D.G. Vagiona

Department of Hygiene, Medical School, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

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Abstract

The prevalence and diversity of *Salmonella* spp., filamentous fungi, and yeasts and their correlation with fecal pollution indicators (total coliforms, fecal coliforms, enterococci) and total heterotrophic bacteria counts were investigated in 95 water samples from the northern Greek rivers Aliakmon and Axios. *Salmonella* spp. were isolated in 27.4% of the samples and a total of 19 serotypes were identified. The frequency of *Salmonella* isolation was higher in the Axios (36.8%) than in the Aliakmon (21.0%) river. Significantly ($P<0.001$) more *Salmonella* spp. were recovered during warm (41.4%) than cold (5.4%) months. *Salmonella*-positive samples showed significantly higher counts of total heterotrophic bacteria and coliforms. Filamentous fungi were isolated from 98.9% and yeasts from 17.9% of the samples with respective mean counts of 2.36×10^3 and 1.28×10^2 cfu/100 mL. Totals of 23 genera of filamentous fungi and 3 genera of yeasts were identified. The most frequent filamentous fungi were *Penicillium* and *Aspergillus*, while *Candida* was the most prevalent yeast. A significant ($P<0.001$) positive correlation of the fecal pollution indicators was demonstrated only for filamentous fungi. The results of this study indicate that these rivers may be potential pathways for human and other animal contamination with *Salmonella* spp., filamentous fungi, and yeasts, which contribute to the pollution of marine waters and the surrounding environment.

Keywords: Fungi; Indicator bacteria; River water; *Salmonella*; Yeasts

1. Introduction

Information on the prevalence and diversity of *Salmonella* serotypes in surface waters is essential for the epidemiology and the ecology of these species. More than 2300 *Salmonella* serovars have been identified worldwide, but only a limited set of them prevail within clinical and environmental isolates (Baird-Parker, 1990; Baudart et al., 2000). *Salmonella* spp. are constantly found in environmental samples, because they are excreted by humans, pets, farm animals, and wild life. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in natural waters. It has been suggested that the survival capacity of environmental strains may depend on species and pollution sources (Polo et al., 1998; Baudart et al., 2000).

In untreated recreational water *Salmonella* spp. can cause gastroenteritis, but some serotypes also cause typhoid and paratyphoid fever (Baird-Parker, 1990). It has been shown that the bacterial load of *Salmonella* spp. in rivers and coastal areas is very important for public health and environmental issues (Baudart et al., 2000). International organizations recommend to their member countries the microbiological studies correlating the density of bacterial indicator organisms with the presence and density of pathogens such as *Salmonella* (UNEP/WHO, 1996).

Fungi are natural inhabitants of soil and water and in the past were considered harmless saprophytes or

*Corresponding author. Fax: +30 2310 999701.
E-mail address: vayona@med.auth.gr (M. Arvanitidou).

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opportunistic pathogens (Niemi et al., 1982; Nagy and Olson, 1983; Rosenzweig et al., 1986). However, in the past decade, fungi have emerged as a frequent cause of infections in immunocompromised patients and have increased in importance as pathogens (Jarvis, 1995). Fungal transmission in man may be either endogenous or exogenous and may cause outbreaks (Muittari et al., 1980; Macher and Girman, 1990; Vukovic et al., 1995). However, despite the widespread occurrence of fungi, little attention has been given to their presence and significance in aquatic environments (Pitlik et al., 1987; Arvanitidou et al., 1999). In untreated recreational water, skin infections have been associated with *Candida albicans*, *Pityrosporum furfur*, and *Microsporum canis* (Philipp, 1991).

Waterborne disease can be traced primarily to the contamination of water with fecal matter. Because of the difficulties of monitoring water for all of the known pathogens that are transmitted by waterborne routes, microbiological pollution is confined to non-pathogenic indicator bacteria. Comparisons, in recreational waters, between indicators to determine which correlates best with human pathogenic bacteria, such as *Salmonella*, have been conducted by few research teams, and there is general agreement that *Salmonella* is present at high densities of indicator organisms (Efstratiou et al., 1998; Polo et al., 1998). Pruss (1998) reviewed studies on uncontrolled waters, such as seas, lakes, and rivers, to evaluate the health risks caused by poor microbiological quality of recreational water. Most studies reported a dose-related increase of health risk in swimmers with an increase in the indicator bacteria count in recreational waters. In seawater samples, moderate positive correlation was also found between fecal pollution indicators and *C. albicans* (Robertson and Tobin, 1983; Efstratiou et al., 1998) or the total counts of yeasts and fungi (Arvanitidou et al., 2002).

Recreational water quality, in the European Community (EC) countries, is assessed according to the EC Bathing Water Directive with regard to mandatory values of indicator bacteria (10,000 cfu/100 mL for total coliforms, 2000 cfu/100 mL for fecal coliforms, and 500 cfu/100 mL for enterococci), which must be followed, and guide values (500 cfu/100 mL for total coliforms and 100 cfu/100 mL for fecal coliforms and enterococci), with which the Member States are encouraged to comply (EEC, 1976). In the present study, total coliforms, fecal coliforms, enterococci, and total heterotrophic bacteria counts were estimated in parallel with the prevalence of *Salmonella* spp. and with the enumeration of yeasts and filamentous fungi in the northern Greek rivers Aliakmon and Axios. The correlation of fungi and *Salmonella* spp. with the pollution indicator bacteria was also investigated.

2. Materials and methods

2.1. Sampling

The Aliakmon river is the longest river in Greece with a total length of about 310 km, originating from northwestern Greece and discharging into the Thermaikos Gulf, where Thessaloniki, the second biggest city of the country, is located. The Axios river is about 300 km long, originates from New Yugoslavia, and crosses the former Yugoslav Republic of Macedonia; the lowest 75 km flows through Greece, discharging into the Thermaikos Gulf. The estuaries of the Axios river form an extended delta, which is protected by the Ramsar Convention. The Convention on Wetlands, mostly known as the Ramsar Convention, is an intergovernmental treaty which provides the framework for national action and international cooperation for the conservation and wise use of wetlands and their resources. The present study serves the scopes of this convention as the extended delta of the Axios river is an ecosystem extremely important for waterbirds, for biodiversity conservation in general, and for well-being of humans.

The average sampling frequency was estimated weekly throughout the study period. Samples were taken about 25 cm below the surface of water and processed within approximately 6–8 h of collection (APHA, 1995). Water temperature was measured at the sampling sites.

2.2. Isolation procedure

Salmonella spp. were isolated, using a qualitative method, after concentrating 1000 mL of water through 0.45-μm membranes which were then preenriched in buffered peptone water at 37 °C for 24 h. Enrichment followed in selenite broth (Difco) at 37 °C for 24 h and in Rappaport–Vassiliadis broth (Oxoid) at 43 °C for 48 h (APHA, 1995). The identification of *Salmonella* spp. was performed from *Salmonella–Shigella* agar (Difco) and brilliant green desoxycholate agar (Oxoid) as selective media. Representative numbers of suspicious colonies were verified by standard biochemical and serological procedures (APHA, 1995).

The pour plate method was used to estimate the number of heterotrophic bacteria (plate count agar, 37 °C, 48 h), and the membrane filter technique was applied for total coliforms (m-Endo medium, 37 °C, 24 h), fecal coliforms (m-Fc agar, 44 °C, 24 h), and enterococci (Slanetz and Bartley agar, 37 °C, 48 h) (APHA, 1995).

The membrane filter technique was employed for the isolation and enumeration of yeasts and fungi (APHA, 1995). A volume of 100 mL and serial dilutions 1/10, 1/100, and 1/1000 of the samples was filtered, in duplicate, through 0.45-μm pore-size membrane filters. The