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Susceptibility of *Pseudomonas aeruginosa* isolates collected from river water in Japan to antipseudomonal agents

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HIGHLIGHTS

- ► Distribution of antibiotic resistance *P. aeruginosa* was investigated in rivers.
- ► Susceptibility of the *P. aeruginosa* to various antibiotics was determined by MIC.
- ► Less than 1% of all 516 isolates was resistant to imipenem.
- ► Antibiotic-resistant *P. aeruginosa* are likely to be widely distributed in rivers.

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ABSTRACT

Pseudomonas aeruginosa is responsible for a number of opportunistic and nosocomial infections. However, very little information is available on the ecology of *P. aeruginosa* in water environments and its association with antimicrobial resistance. In this study, the distribution of *P. aeruginosa* and the resistance of *P. aeruginosa* isolates to various antibiotics were investigated from two rivers, Kiyotake and Yae that flow through Miyazaki City, Japan. *P. aeruginosa* was distributed widely along the river basins with counts ranging from 2–46 cfu/100 mL. The susceptibility of *P. aeruginosa* isolates collected from the rivers to various antibiotics was examined by minimum inhibitory concentration. Multidrug-resistant *P. aeruginosa* strains were not observed or isolated from either river. However, one piperacillin-resistant *P. aeruginosa* was detected among a total of 516 isolates, and this isolate was also resistant to cefotaxime and showed intermediate resistance to cefitazidime. Less than 1% of all isolates ($n=5$) were resistant to imipenem, which is the most effective antibiotic against both Gram-negative and Gram-positive bacteria. However, all *P. aeruginosa* isolates were completely resistant to tetracyclines, which are the most commonly prescribed antibiotics. In advanced nations such as Japan where the majority of the population is urban and where medical services are widespread, antibiotic-resistant bacteria such as *P. aeruginosa* are likely to be widely distributed, even in apparently pristine rivers.

1. Introduction

Pseudomonas aeruginosa is a Gram-negative bacillus that causes a number of diseases in immunocompromised individuals such as infants, hospital inpatients, and the elderly. In recent years, *P. aeruginosa* has become an important health issue as it has been identified as being responsible for a number of opportunistic and nosocomial infections (Master et al., 2011). Because *P. aeruginosa* exhibits extremely high metabolic versatility and adapts to a variety of conditions, it can survive in a variety of habitats, for example in different types of soil (Hall et al., 1998), rivers (Baleux and Troussellier, 1989), lakes (Sherry, 1986), and wastewater (Wheater et al., 1980; Schwartz et al., 2006). Native resistance to drugs is the physiological characteristic of *P. aeruginosa*. It also tends to develop an acquired resistance to antibiotics, and many new forms of *P. aeruginosa* with acquired resistance have been generated due to the abuse of antibiotics (Master et al., 2011). Recently, a multidrug-resistant *P. aeruginosa* was detected with acquired resistance to all three known groups of antimicrobial agents most effective against the bacterium, including carbapenems, aminoglycosides, and fluoroqinolones (Sanders et al., 1984). The multidrug-resistant *P. aeruginosa* causes serious problems in the treatment of nosocomial infections. The field of medicine and epidemiology has accumulated a large amount of information on nosocomial clinical infections due to drug-resistant *P. aeruginosa* and on the susceptibility of *P. aeruginosa* to various antimicrobial agents (Al-Tawfiq, 2007; Gad et al., 2008; Inglis et al., 2010; Patzer and Dzierzanowska, 2007; Fujimura et al., 2009; Tsutsui et al., 2011). In addition, a number of molecular biological studies on the resistance-acquiring mechanism have been performed (Patzer et al., 2004; Ryoo et al., 2009), and appropriate measures for addressing nosocomial infections have been examined (Nagao et al., 2011).

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It is seldom possible to avoid all forms of illness while living an ordinary life and it is common to take multiple medications or apply a number of different ointments to treat the different disease symptoms experienced. Enteric bacteria are dramatically affected by the ingestion of such medication, and once excreted by defecation and urination are discharged into the wastewater system. Therefore, the components of the drugs ingested and the ointments applied are inevitably contaminated with sewage and domestic wastewater. Thus, drug-resistant bacteria may exist even in water environments, and the possibility of wide dispersion of such bacteria cannot be denied. Antibiotic-resistant *P. aeruginosa* isolates have been collected from drinking water [both from surface water (Vaz-Moreira et al., 2012) and natural mineral water sources (Legnani et al., 1999)]. Multidrug-resistant *P. aeruginosa* strains have also been isolated from river water (Pirnay et al., 2005), wastewater (Fuentefria et al., 2011), and underground water sources (Kaszab et al., 2010). Moreover, an NDM-1 positive *P. aeruginosa* resistant to all antibiotics was isolated recently from tap water in New Delhi, India (Walsh et al., 2011).

Waterfront areas such as riverbanks and other bathing places are used for recreation and for physical and spiritual refreshment. Thus, humans cannot exist without being in regular contact with water. A source of *P. aeruginosa* infection was rivers used for swimming, which illustrates the potential risk of infection by *P. aeruginosa* through the intermediary of water (Medema et al., 1996). Nevertheless, there is very little data available on the prevalence and distribution of drug-resistant *P. aeruginosa* in the river basins, even in case of a river flowing through a city particularly in Japan. Environmental protection and medical control of such pathogens will only be possible if sufficient information on the ecology of drug-resistant *P. aeruginosa* in water environments is collected and analyzed. As a first step in this regard, in the present study the distribution of *P. aeruginosa* and its antibiotic resistance were investigated from samples of two rivers, the Kiyotake River and Yae River, which flow through the urban area of Miyazaki City in Kyushu Island, Japan.

2. Materials and methods

2.1. Sampling

Samples were collected from two rivers: the Kiyotake River and Yae River (Fig. 1). These rivers flow through Miyazaki City, which was considered as a model of a general provincial city in this study. It has an approximate population of 400,000 and a developed infrastructure with sewerage systems or septic tanks used for wastewater treatment. The sampling dates and locations for each of the rivers surveyed are shown in Table 1. In view of the whole basin of the river, the sampling points of Kiyotake and Yae rivers were set from upstream to downstream and investigated once in 2010 and 2011, respectively. The Kiyotake River flows through a forest with some coppice areas dotted with settlements, farmland, and the city; it flows into the Hyuga-nada Sea, which is part of the Pacific Ocean in Japan. The Kiyotake River was sampled upstream of the coppice area, midstream in the urban area, and downstream in the estuary. The main water supply for the Yae River is domestic wastewater and/or treated sewage water. The Yae River was sampled upstream in the densely populated area and downstream in the estuary. At the sampling point, the surface water (0 to 0.3 cm depth) was sampled from the center of river cross-section using a sterilized bucket. The collected water samples were stored in sterile 5-L polyethylene bottles and immediately transported to the laboratory for microbial analysis and water quality tests. After sampling, microbial and water quality analyses were started within 4 h. pH, electrical conductivity, and turbidity were also determined using a pH meter (HM-30G; TOA DKK, Tokyo, Japan), conductivity meter (CM30S; TOA DKK), and turbidity meter (SEP-PT-706D; Mitsubishi Kagaku, Tokyo, Japan), respectively. In addition, the concentrations of total organic carbon (TOC) and total nitrogen (T-N) in the samples were determined as pollution parameters using a TOC analyzer (TOC Analyzer Wet Oxidation/NDIR Method Model, SIMADU Co., Japan).

2.2. Analysis and isolation of *P. aeruginosa* by membrane filtration

Membrane filtration (MF) using nalidixic acid cetrimide (NAC) agar plates (Nissui Pharmaceutical Co., Japan) was employed to isolate *P. aeruginosa*. A total of 10–500 mL of each water sample was filtered through a 0.45-μm pore membrane filter (47-mm diameter, sterile, mixed cellulose ester; Advantec, Tokyo, Japan) and incubated on NAC agar plates for 24 h at 37 °C. Fluorescent colonies with blue halos were counted as *P. aeruginosa* isolates. The number of *P. aeruginosa* isolated by MF was expressed as colony forming units (cfu) per 100 mL of water. A representative bacterial count for each sample isolated by MF was determined from the mean cfu of 10 replicate plates.

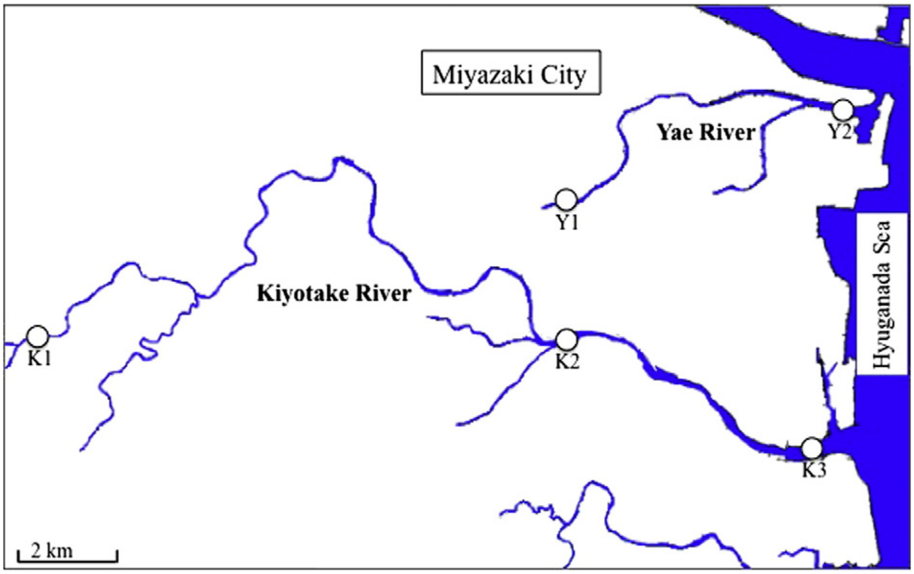

Fig. 1. The location of sampling points in the Kiyotake River and the Yae River in Miyazaki City, Japan. K1: upstream, K2: midstream, and K3: downstream in the Kiyotake River. Y1: upstream and Y2: downstream in the Yae River.