

Convergent acquisition of antibiotic resistance determinants amongst the *Enterobacteriaceae* isolates of the Mhlathuze River, KwaZulu-Natal (RSA)

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Abstract

The Mhlathuze River has become a major reservoir for antibiotic-resistant microbes and a wide pool of antibiotic resistant genes with the environmental isolates exists in this water system. The ability of commensal organisms to carry resistant genes of clinical importance and their ability to transfer such genes to other bacteria are of greater concern than phenotypic measurements. Forty-three *Enterobacteriaceae* isolates, which were capable of resisting more than 4 different classes of antibiotics, were used for the molecular characterisation of antibiotic resistances. It was found that 58% of these multiple antibiotic-resistant isolates possess the Class 1 integron. Amongst these 25 isolates with positive detection of the Class 1 integron, the beta-lactamase gene (*pse*) was the most common, being present in 44% of these integrons. The aminoglycoside-resistant gene was detected in 16% of these integrons and 24% of Class 1 integrons contained two genes coding for sulphonamide resistance and for quaternary ammonium compounds resistance. A high degree of genotypic diversity and the lack of correlation between antimicrobial resistance patterns and molecular types of the isolates suggest convergent acquisition of resistance determinants by genetically unrelated strains rather than epidemic spread of resistant isolates in the community. Of the tested environmental isolates, 56% transferred their plasmids as well as their antibiotic resistance profiles to the recipient cells. The possibility of transmission of resistant genes between bacteria (especially pathogenic) which invade human and animal populations within this river poses a health risk to the communities who are dependent on this river for water consumption.

Keywords: Mhlathuze River, antibiotic resistance, conjugation, *Enterobacteriaceae*, Class I integron

Introduction

The Mhlathuze River in northern KwaZulu Natal, South Africa, flows into the estuary at Richards Bay and supports a rapidly growing agricultural and industrial community (Steyl et al., 2000). The results of the microbiological evaluation of this river (Bezuidenhout et al., 2002; Lin et al., 2004a) show high levels of total and faecal coliform contamination. The majority of the *Enterobacteriaceae* isolates from this water system also carry antibiotic-resistant gene(s) especially the β -lactamase genes (Lin et al., 2004b). Ash et al. (2002) and Park et al. (2003) also reported that several rivers in the USA as well as in South Korea have become major reservoirs for antibiotic-resistant microbes. A strong correlation ($r=0.97$) of antibiotic-resistance profiles between the clinical and the environmental isolates was observed in this region suggesting a strong link between diarrhoeal incidence and water quality (Lin et al., 2004b).

Given the propensity of the *Enterobacteriaceae* family and clinically significant bacteria to acquire antimicrobial resistance, consistent surveillance of the agents commonly prescribed to treat infections arising from these organisms is imperative (Sahm et al., 2000). These organisms could create a widespread antibiotic-resistant gene pool. These genes could be transferred into human and animal disease organisms (Kruse and Sorum, 1994), which subsequently could generate significant health and economic impacts.

Antibiotic-resistant genes are carried on either chromosomes or mobile genetic elements e.g. plasmids; integrons, etc. These mobile gene elements with antibiotic- or metal-resistant genes can be transferred between different species through transformation, conjugation and transduction (Madigan et al., 2003). It is therefore important to track antibiotic-resistant genes in a variety of commensal, pathogenic and environmental bacteria in order to measure the environmental pool of resistance and to understand the ecology of antibiotic resistance (Aminov et al., 2001; Goni-Urriza et al., 2000). Aquatic (river) ecosystems are ideal since they may support a wide array of micro-organisms e.g. indicator organisms, faecal and total coliforms, pathogens, animal and human, commensals, human and animals as well as environmental bacteria.

Until recently, studies on antibiotic resistance were only concerned with the collection of phenotypic data. However, in order to study the environmental pool of resistant genes and to understand the molecular ecology of antibiotic resistance the development of genotyping tools is indispensable (Aminov et al., 2001).

Drug resistance especially multiple-drug resistance (MR) in enteric organisms is often associated with integrons. Integrons generally contain an integrase gene (*intI*) and a cassette integration site (*attI*), into which antibiotic-resistant gene cassettes have integrated. A gene cassette contains an antibiotic resistance gene and a 59 base pair (bp), a short inverted repeat element with a recombination site. Four classes of chromosomal and plasmid-borne integrons in gram-negative bacteria have been described (Bass et al., 1999). Class 1 integrons are the most common and have been found primarily in complete or truncated derivatives of the Mu- like transposon *Tn402*, which resides in a wide range of host plasmids or within *Tn21*. They generally contain the qua-

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