International Journal of Food Microbiology 145 (2011) 315–319

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short Communication

Characterization of antibiotic resistant enterococci isolated from untreated waters for human consumption in Portugal

Ana S. Macedo [a], Ana R. Freitas [b], Cristina Abreu [a,b], Elisabete Machado [a,b], Luísa Peixe [b], João C. Sousa [a], Carla Novais [a,b,*]

[a] CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 256, 4200-150 Porto, Portugal
[b] REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-033 Porto, Portugal

ARTICLE INFO

Article history:
Received 30 May 2010
Received in revised form 10 November 2010
Accepted 12 November 2010

Keywords:
Human drinking water
Antibiotic resistance
tet genes
erm(B)
aac(6´)-Ie-aph(2″)
Virulence

ABSTRACT

Untreated drinking water is frequently overlooked as a source of antibiotic resistance in developed countries. To gain further insight on this topic, we isolated the indicator bacteria *Enterococcus* spp. from water samples collected in wells, fountains and natural springs supplying different communities across Portugal, and characterized their antibiotic resistance profile with both phenotypic and genetic approaches. We found various rates of resistance to seven antibiotic families. Over 50% of the isolates were resistant to at least ciprofloxacin, tetracyclines or quinupristin–dalfopristin and 57% were multidrug resistant to ≥3 antibiotics from different families. Multiple enterococcal species (*E. faecalis*, *E. faecium*, *E. hirae*, *E. casseliflavus* and other *Enterococcus* spp) from different water samples harbored genes encoding resistance to tetracyclines, erythromycin or gentamicin [*tet*(M)-46%, *tet*(L)-14%, *tet*(S)-5%, *erm*(B)-22%, *aac(6´)-Ie-aph(2″)*-12%] and putative virulence factors [*gel*-28%, *asa*1-16%]. The present study positions untreated drinking water within the spectrum of ecological niches that may be reservoirs of or vehicles for antibiotic resistant enterococci/genes. These findings are worthy of attention as spread of antibiotic resistant enterococci to humans and animals through water ingestion cannot be dismissed.

1. Introduction

Providing populations with reliably safe drinking water is standard practice in developed nations, being recognized as a basic human right and a cost effective measure for controlling disease. Concerns about the microbial quality of water have traditionally been focused on the occurrence of pathogens and/or indicator bacteria (e.g. *Escherichia coli*, coliforms, enterococci) (Ashbolt et al., 2001). However, the global scientific community is increasingly concerned by descriptions of aquatic reservoirs of antibiotic resistant bacteria and/or genes. Both might be disseminated to humans, and so contribute to the decrease of therapeutic alternatives and to the evolution and emergence of new genetic platforms with clinical consequences along with aquatic autochthons flora (Baquero et al., 2008; Martinez, 2009). Better information on various aspects of water quality is considered an urgent requirement for both the general public and the drinking water industry.

In previous studies by our group it was clear that antibiotic resistant enterococci were present in various ecological niches, including in the feces of healthy humans from different Portuguese regions (Novais et al., 2004, 2005a,b, 2006). Although we previously established the importance of food products of animal origin for the dissemination of antibiotic resistance to healthy humans (Novais et al., 2006), other little studied reservoirs can also be contacted by people on a daily basis. For example, non-treated natural water is frequently used in rural Southern European countries for drinking, cooking, bathing and agriculture field irrigation. It is estimated that 50% of the extracted water in Mediterranean countries is used in these activities (European Environment Agency 2000). In Portugal, non-treated water is often employed for human drinking in several rural regions, along with or instead of the pipeline distributed treated water. Also, many public fountains are contaminated with bacteria and many wells, fountains, boreholes and springs are located in private properties. In these cases, the water sources are out of official sanitization control and can be located in proximity to reservoirs of antibiotic resistant bacteria and genes (e.g. septic tanks, livestock yards, liquid-tight manure storage, fertilizer storage). Although the consumption of contaminated water should be considered a public health concern, untreated human drinking water is often an overlooked source in the epidemiology of antibiotic resistance in Portugal and other developed countries. In these regions, data concerning enterococci resistant to antibiotics in the aquatic environment are mostly for residual waters and surface waters used for occasional recreation activities (Tejedor-Junco et al., 2001; Novais et al., 2005b; Kühn et al., 2005; Caplin et al., 2008; Łuczkiewicz et al., 2010). In this

* Corresponding author. Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, 256, 4200-150 Porto, Portugal. Tel.: +351 225074630; fax: +351 22 5074637.
E-mail address: cnovais@ufp.edu.pt (C. Novais).

0168-1605/$ – see front matter
doi:10.1016/j.ijfoodmicro.2010.11.024

Table 1
Antibiotic resistance profiles of enterococci of various species isolated from various sources of drinking water in Portugal.

Species	No. isolates	Type of samples (no.)	Environment (no. samples)	Region	Year	Antibiotic resistance profile. No.(%)												
						VAN	TEC	AMP	TET	MIN	ERY	Q/D	CIP	CHL	GEN	STR	NIT	LIN
E. faecalis	20	Fountain (3); spring (5); well (3); borehole (1)	Agriculture and residential area (2); agriculture and animal domestic production area (2); agriculture, animal domestic production and residential area (1); residential area (3); forest area (1); industrial area (1); NHNA (2)	North; Center	2006; 2008	0(0)	0(0)	0(0)	15(75)	15(75)	15(75)	15(75)[a]	9(45)	0(0)	6(30)	2(10)	0(0)	0(0)
E. faecium	12	Fountain (2); well (3), borehole (1);	Aquaculture production (1); agriculture area (2); agriculture and animal domestic production area (2); agriculture, animal domestic production and residential area (1), agriculture and residential area (1)	North	2006; 2008	0(0)	0(0)	0(0)	10(83)	10(83)	10(83)	2(17)	10(83)	2(17)	0(0)	0(0)	2(17)	0(0)
E. hirae	6	Spring (1), well (1)	Agriculture area (1), NHNA (1)	North; Center	2006	0(0)	0(0)	0(0)	3(50)	3(50)	1(17)	1(17)	0(0)	0(0)	0(0)	0(0)	1(17)	0(0)
E. casseliflavus	3	Well (2)	Agriculture and animal domestic or intensive production area (1); agriculture, animal domestic production and residual water treatment station area (1).	North	2008	0(0)	0(0)	0(0)	0(0)	0(0)	1(33)	1(33)	1(33)	0(0)	0(0)	0(0)	0(0)	0(0)
Enterococcus spp	35	Well (3); borehole (1); mine (3); spring (3)	Agriculture and animal domestic or intensive production area (1); agriculture, animal domestic production and residual water treatment station area (1); agriculture and animal domestic production area (1); agriculture area (2); forest area (1); residential (1) area; NHNA (3)	North; Center	2006; 2008	0(0)	0(0)	0(0)	10(29)	7(20)	16(46)	15(43)	27(77)	0(0)	3(9)	0(0)	12(34)	0(0)
Total	76					0(0)	0(0)	0(0)	38(50)	35(46)	43(57)	38(50)	47(62)	2(3)	9(12)	2(3)	15(2)0	0(0)

Abbreviations: NHNA = non-human, non-animal influence, VAN = vancomycin, TEC = teicoplanin, AMP = ampicillin, TET = tetracycline, MIN = minocycline, ERY = erythromycin, Q/D = quinupristin–dalfopristin, CIP = ciprofloxacin, CHL = chloramphenicol, GENT = HLR to gentamicin, STR = HLR to streptomycin, NIT = nitrofurantoin, LIN = linezolide.

[a] Natural resistance.