ANTIMICROBIAL RESISTANCE

Antibiotic resistance patterns of enterococci isolated from coastal bathing waters

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Recreational water should be considered a risk for enterococcal infections in regions with high utilisation and long exposure periods. A total of 1113 enterococcal isolates was obtained from 1670 bathing water samples from 120 bathing areas of seven prefectures in northern Greece. Enterococcus avium, E. raffinosus and E. faecium were the most prevalent species. Single, double and multiple antibiotic resistance patterns were observed in 33.5% 31.0% and 22.8% of the isolates, respectively. Resistance to erythromycin occurred most frequently, in 57.3% isolates, many of which also exhibited resistance to ciprofloxacin and rifampicin as well as high-level resistance to kanamycin and streptomycin. The results suggest that bathing water may contribute to the dissemination of uncommon enterococcal species that exhibit resistance to several antibiotics which are used to treat community-acquired infections.

Introduction

Enterococci are important human pathogens in both community and hospital infections and exhibit intrinsic resistance to various antibiotics. In recent years, enterococcal infections have become a major therapeutic challenge because of their increased incidence and the spread of strains that have acquired resistance to several antimicrobial agents [1]. There is increasing awareness of the occurrence of multiresistant enterococci, particularly in isolates from patients in hospital and in extended-care facilities [2–4]. However, little is known about the species distribution and the epidemiology of antibiotic resistance among enterococci isolated from environmental sources [5–7].

One source of resistant enterococci may be bathing waters. The presence in this environment of enterococcal strains that exhibit antibiotic resistance is of particular interest because of the possible link between community-acquired enterococcal infections and recreational activities [8, 9]. There have been few reports of the presence and antibiotic resistance of enterococci in water of bathing beaches [7, 10]. This study evaluated the antibiotic resistance patterns of enterococcal species cultured from waters of 120 bathing areas in Greece.

Materials and methods

Sampling

During the bathing season from May to October 1998, 1670 sea samples from 120 sampling points in seven prefectures in northern Greece were analysed for the presence of the standard pollution indicator bacteria and of enterococci, by use of the membrane filtration technique as recommended by the American Public Health Association [8]. All samples were transported on ice and analysed within 24 h of collection.

Microbiological examination

Slanetz and Bartley Agar (Oxoid) was used for the isolation of enterococci and distinct colony types were evaluated further. Individual isolates were subcultured on to brain-heart infusion agar plates and characterised as enterococci by additional tests (salt tolerance, growth on bile esculin agar, catalase activity). Enterococcal isolates were stored at −70°C in glycerol broth. Of the 1113 enterococcal isolates from 1670 samples, 316 were randomly selected for further study. Identification to species level was performed by the conventional test scheme proposed by Facklam and Collins [11], by the use of key tests prepared in-house for the discrimination of the three major groups of...
enterococci and the identification of the species within each group. The following test reactions were used: arabinoose, lactose, mannitol, raffinose, sorbitol, sucrose and succinate fermentation; arginine deamination; pyruvate utilisation; motility and pigmentation. Additional biochemical tests (ribose fermentation and tellurite reduction) were performed on lactose-negative isolates to distinguish *Enterococcus faecalis* from *E. solitarius* [12].

**Antibiotic susceptibility testing**

Enterococci were tested for their susceptibility to 10 antimicrobial agents: ampicillin, amoxicillin-clavulanate (2:1), ciprofloxacin, erythromycin, gentamicin, kanamycin, rifampicin, streptomycin, trimethoprim and vancomycin (purchased from commercial sources). Minimum inhibitory concentrations (MICs) were determined by the agar dilution method [13]. An inoculum of 10^4 cfu/spot from a log-phase broth culture was delivered by a multi-point inoculator on to Mueller-Hinton agar plates containing appropriate antibiotic concentrations. Interpretable criteria for susceptibility status were those of the National Committee for Clinical Laboratory Standards [13]. High-level resistance to gentamicin was defined as an MIC >500 mg/L and to kanamycin and streptomycin as an MIC >1000 mg/L.

**Results**

The results in terms of species identified are shown in Table 1. Ten different species were identified, of which *E. avium* was the most prevalent, accounting for 36 (27.2%) of the isolates. *E. raffinosus* was found in 74 instances (23.4%) and *E. faecium* in 63 instances (19.9%). The following were identified at lower frequencies: *E. casseliflavus* (24), *E. faecalis* and *E. pseudoavium* (22 each) and *E. durans* (15). *E. malodoratus* (4), *E. hirae* and *E. gallinarum* (3 each) were found sporadically.

Among the 316 enterococci, 276 (87.3%) exhibited resistance to one or more antibiotics. Altogether, 20 different profiles were recorded on the basis of their antibiotic resistance (Table 1). Single, double and multiple antibiotic resistance patterns were observed in 33.5%, 31.0% and 22.8% of the isolates, respectively. The most frequent pattern was resistance to both erythromycin and rifampicin (63 isolates), followed by resistance to erythromycin only (35 isolates) and by resistance to kanamycin or to rifampicin only (24 isolates each). Enterococci sharing these predominant antibiotic resistance profiles were geographically randomly dispersed (data not shown). Among the common enterococcal species, *E. avium*, *E. raffinosus* and *E. pseudoavium* were the species with the highest proportions of antibiotic-resistant isolates.

The quantitative susceptibilities of the enterococcal isolates examined are shown in Table 2 as the number of resistant isolates, the MIC range and the MIC50 and MIC90 values. Overall, resistance to erythromycin was commonest and was observed in 181 isolates (57.3%), whereas resistance to rifampicin was observed in 167 isolates (52.8%), to ciprofloxacin in 49 isolates (15.5%) and to trimethoprim in only four isolates (1.3%). High-level resistance to kanamycin was detected in 99 (31.3%) and to streptomycin in 31 (9.8%) of enterococcal isolates. Isolates that exhibited streptomycin resistance (18 of 31) were mostly also kanamycin-resistant. Resistance to ampicillin, amoxicillin-clavulanate, vancomycin and gentamicin at high level was not detected in any of the isolates examined. All three *E. gallinarum* isolates and most *E. casseliflavus* isolates (20 of 24) exhibited reduced susceptibility to vancomycin or were vancomycin intermediate (MIC 4–16 mg/L), suggesting the presence of the species-specific genes *vanC*-1 and *vanC*-2, respectively.

**Discussion**

Identification of enterococcal species in recreational waters may help to clarify their ecological and epidemiological characteristics. *E. faecalis* and *E. faecium* are thought to be more specific for man than other enterococcal species, and in comparison with other enterococcal species they were considered to survive longer once exposed to aquatic environments [14]. In previous reports, these two species were found to predominate among antibiotic-resistant enterococci isolated from various aquatic habitats [6, 10]. However, in this study, species such as *E. avium* and *E. raffinosus* – which usually occur at higher densities in the faeces of other warm-blooded animals such as seabirds and mammals – were isolated more frequently from Greek bathing waters. Consistent with these findings, *E. avium* was the most prevalent species detected in samples from freshwater bathing sites in north-west England [7].

Although clinical isolates of *Enterococcus* spp. in our region show a higher frequency of antibiotic resistance [4], the results of this survey indicate that enterococcal isolates exhibiting resistance to a variety of antimicrobial agents may also be isolated from the aquatic environment. In Greece, bathing water should be considered a potential risk for community-acquired infections because of the high utilisation and the long exposure periods [8, 9]. The high percentage of erythromycin-resistant enterococci in the coastal environment was of particular interest, because macrofides are frequently used in the community for the empirical treatment of infectious diseases, as well as for the treatment of enterococcal infections, especially when allergy to penicillins is suspected. Earlier findings suggest that the emergence of erythromycin resistance in streptococci might be the result of prior
### Table 1. Antibiotic resistance patterns among *Enterococcus* spp. isolated from bathing waters

<table>
<thead>
<tr>
<th>Antibiotic resistance pattern</th>
<th>E. <em>avium</em> (n = 86)</th>
<th>E. <em>raffinosus</em> (n = 74)</th>
<th>E. <em>faecium</em> (n = 63)</th>
<th>E. <em>casseliflavus</em> (n = 24)</th>
<th>E. <em>faecalis</em> (n = 22)</th>
<th>E. <em>pseudoeuroium</em> (n = 17)</th>
<th>E. <em>durans</em> (n = 15)</th>
<th>E. <em>malodoratus</em> (n = 4)</th>
<th>E. <em>hirae</em> (n = 3)</th>
<th>E. <em>gallinarum</em> (n = 3)</th>
<th>Total (n = 36)</th>
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Cp, ciprofloxacin; Er, erythromycin; Kn, kanamycin; Rf, rifampicin; St, streptomycin; Tm, trimethoprim.
exposure to the drug [15]. The overuse of erythromycin and other macrolides in the community setting may be responsible for the resistance rates observed in this study. Fluorinated quinolones are also used frequently in community-acquired infections in this region and a considerable percentage of enterococci was found to exhibit ciprofloxacin resistance. On the contrary, trimethoprim resistance was rarely detected in the study environment, although the agent is prescribed in a wide variety of enterococcal infections in the community, such as urinary tract infections. It is also of interest that, although most clinical isolates of E. faecium in Greece are ampicillin-resistant [4], the E. faecium isolates from Greek coastal waters were ampicillin-susceptible and therefore may have had a different origin.

Aminoglycosides are frequently used in combination with cell-wall-active antibiotics for severe enterococcal infections. Acquired high-level aminoglycoside resistance may be caused by various aminoglycoside-modifying enzymes and predicts a failure of synergy between cell-wall-active agents and the aminoglycoside to which the organism is highly resistant [1]. A previous report has shown that high-level resistance to gentamicin is rarely detected among enterococci isolated from the aquatic environment [10]. In the present study, none of the enterococcal isolates was found to exhibit high-level resistance to the drug. However, a considerable percentage of isolates exhibited high-level resistance to streptomycin, an aminoglycoside that continues to be an important drug used in synergic combinations to treat serious enterococcal infections. The presence of kanamycin-resistant isolates is also of interest, as resistance to this drug predicts the failure of amikacin in such combinations [1].

Potential reservoirs in the environment for antibiotic-resistant enterococci have already been demonstrated [16]. The findings of the present study suggest that bathing waters may contribute to the dissemination of antibiotic-resistant enterococci. The resistance rates to erythromycin and other antimicrobial substances imply that susceptibility trends of enterococci from aquatic environments should be monitored carefully. Detailed genetic analysis is in progress to identify possible epidemiological linkage among antibiotic-resistant enterococci isolated from recreational waters and clinical infections.

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