Characterization and antimicrobial resistance of faecal enterococci from wild-living animals

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INTRODUCTION

The enterococci are widely distributed in air, water, sewage, soil and vegetation and primarily distributed in the gastrointestinal tract of humans and most warm blooded animals [1]. The Enterococcus genus consists of 34 species [2]. The most frequently widespread species are E. faecalis and E. faecium. Enterococci have become dangerous pathogens and one of the mechanisms of their pathogenicity is antibiotic resistance. The problem of antimicrobial resistance in enterococci is not only restricted to the clinical setting, but also to other environments. These resistant enterococci might act as reservoir of antimicrobial resistance genes that could be transmitted to other pathogenic bacteria [3].

RESULTS

One hundred and seventy bacterial isolates able to grow on bile esculin azide agar and to hydrolyze esculin were isolated from the faeces of wild-living animals and identified by MALDI-TOF analysis. An interesting finding was that the E. faecalis species is not always a dominant representative of the intestinal enterococcal microflora in wild animals. Instead, E. hirae (in forest shrew and red deer) an E. faecium (in owl) were found to be dominant. In beaver faeces E. casseliflavus species was found to dominate (Fig. 1). In studied isolates the most frequency resistance to streptomycin (76.02%) and kanamycin (56.64%) was detected and the highest frequency of resistant isolates was observed for E. faecalis species (Table 1). Higher occurrence of antibiotic resistance to all antibiotics tested in birds (Fig. 2) may be due to their rapid horizontal transport and migration of some species between many environments, or it may be associated with high levels of body temperature, which ranges between 40 - 42 °C.

Presence of selected antibiotic resistance genes (encoding for tetracycline and erythromycin resistance) was analysed. The tet(M) gene is frequently implicated in tetracycline resistance in enterococci. Thus, we confirmed that the tet(M) gene is distributed in different terrestrial environment what can lead to transmission of these antimicrobial resistance genes to wild-living animals (Fig. 4). Our results indicate, that erm(B) was the most frequently found resistance gene among the erythromycin-resistant enterococci tested (Fig. 3). On the other side, we observed a significantly higher frequency of mef(A) gene in mammals (13 isolates, 56.52%) compared to birds (3 isolates, 23.14%), %. Increased presence of mef(A) gene was mainly found in animals from mountain areas, and could be possibly correlated with the food requirements of these animals, who feed on plants rich of essential oils, and it has already been shown that these plant oils have antibacterial effect similar to antibiotics.

CONCLUSIONS

In this work, we studied the occurrence of enterococci in wild animals and their resistance to six antibiotics (ampicillin, erythromycin, chloramphenicol, kanamycin, streptomycin and tetracycline). By MALDI-TOF analysis, we found, that in most animals enterococcal population is dominated by E. faecalis, and in some wild-living animals predominant strains are E. faecium and E. hirae. The highest frequency of antibiotic resistance was observed in E. faecalis strains. Significantly higher frequency of resistant enterococci was observed in enterococci from birds compared to enterococci from mammals. Subsequently, we studied the presence of tetracycline resistance (tet(M), tet(L)) genes and macrolide resistance genes (erm(B), mef(A)). While animal enterococci show similar ratio of tet(M)/tet(L) genes as clinical isolates, higher ratio mef(A)/erm(B) genes were observed in wild animal enterococci. Enterococci of the intestinal tract of wild-living animals can serve as reservoir of antimicrobial resistant genes. These genes, or the resistant bacteria could be transmitted to other animals or even to humans.

REFERENCES


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Table 1. Antimicrobial resistance of 143 enterococcal isolates isolated from faecal samples of wild animals

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number (percentage) of enterococci of different species showing resistance to different antimicrobial agents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. faecalis (N=103)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>26 (62.24)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>35 (33.98)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>27 (26.21)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>64 (62.14)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>89 (86.40)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>52 (50.48)</td>
</tr>
</tbody>
</table>

Fig. 1. Percentage of enterococcal strains of the intestinal microflora of wild animals

Fig. 2. Comparison of antibiotic resistance in strains of enterococcal isolated from wild birds (n=40) and mammals (n=103)

Fig. 3. Percentage of erythromycin resistance genes erm(B), mef(A) in the isolates showing resistance to erythromycin (n=36)

Fig. 4. Percentage of the tetracycline resistance gene (tet(M), tet(L)) in the isolates showing tetracycline resistance (n=66)