ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERIA FROM CANINE FAECES

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Abstract

The present study has focused on the occurrence of Enterococcus sp., Lactobacillus sp. and Staphylococcus sp., detected in faeces of healthy dogs, and their resistance to commonly used antibiotics. The average of the total count of enterococci reached $5.9 \log_{10} + 1.2$, that of lactobacilli $6.9 \log_{10} + 1.6$, and of staphylococci $2.4 \log_{10} + 1.1$. Among antibiotics tested, the highest resistance of the isolates was found to penicillin G. Most effective antimicrobial agents against canine staphylococci were gentamicin and vancomycin. Ampicillin and chloramphenicol were effective against enterococci and lactobacilli. Moreover, enterococci were sensitive to vancomycin.

Key words: dogs, faeces, bacteria, resistance to antibiotics.

Lactic acid bacteria (LAB) are a broad group of Gram-positive, non-sporing rods and cocci, usually non-motile, that ferment carbohydrates and form lactic acid as the major end-product (1). They have a role as commensals on mucosal surfaces and skin and inhabit the digestive tract of many animal species and humans (18). Numerous species of LAB can be detected in the digestive tract (19), but their prevalence and distribution vary according to the animal species with which they are associated. In general, LAB belong to the organisms which first colonize the digestive system of animals. However, the counts of these bacteria usually decrease with age, particularly due to their lower ability to adhere to the intestinal mucous membrane (12).

Many LAB possess probiotic properties and are widely used in probiotic preparations. Therefore, intestinal population may be supplemented with lactic acid bacteria from dairy or probiotic products. Administering the LAB has also been recommended for canine use. However, only few studies concerning probiotics have been performed with dogs (4, 23).

On the other hand, certain species or strains of LAB (most often of staphylococci and enterococci) are involved in a wide variety of diseases in animals (14). Diarrhoea associated with enterococcal colonization of the mucosal surface of the small intestine has been reported in foals, piglets, rats, a pup and calves (13). In these cases, the isolates were identified as Ent. durans except in rats, where it was identified as Ent. hirae. Members of various staphylococcal species, especially Staphylococcus aureus, Staph. hyicus and Staph. intermedius, can cause pyogenic infection in different locations (e.g. skin, mammary gland, ears, joints, internal organs) in animals (9). A significance of Lactobacillus sp. in pathogenic processes in animals is still unclear. However, its passing from the intestine to local tissues was detected (6). A prerequisite of LAB infections, it must be mentioned, is often impaired host health. Above mentioned infections are frequently treated with antibiotics, and consequently, antibiotic resistance and/or acquired resistance have developed (11). Although great attention is directed at present time especially to animal production, the anti-microbial resistance in companion animals is potentially important because pets are present at home and have close contact with humans. So, in addition to complicate therapy in these animals, a zoonotic potential must be considered.

Studies concerning the antimicrobial resistance of bacteria from animals often include pathogenic isolates from sites of infection while knowledge about resistance in normal bacterial flora; i.e. not associated with infections is only limited. Therefore, the resistance of LAB isolated from faeces of healthy dogs to antimicrobial agents was investigated.

Material and Methods

Isolation of bacteria. Faeces were collected from sixteen healthy dogs; among them ten were of ten breeds and six mixed-breeds, at average age of 2 years (in the range from 3 months to 6 years); 7 were females and 9 males. Dogs receiving antibiotic treatment were excluded. Faeces were placed in a sterile plastic bag, diluted in ratio 1:10 in sterile saline solution (pH 7.0) and then homogenized. One hundred µl of homogenate was used to inoculate selective bacteriological media. The following media were used: M-Enterococcus agar
(Becton and Dickinson, Cockeysville, USA), Mannitol Salt Agar (Becton and Dickinson), and MRS agar (Merck, Germany). After incubation at 37°C for 48 h, the total counts of bacteria expressed in colony forming units per ml/g (CFU) were counted. Numbers of CFU are expressed as log_{10}/g faeces. The results are given as arithmetical means ± SD.

One hundred and thirty-seven isolates of lactic acid bacteria (52 isolates of enterococci, 47 of lactobacilli and 38 of staphylococci) were tested for their resistance to antibiotics. Most of enterococci (40 isolates) were identified by tDNA-intergenic PCR according to Welsh and McClelland (20) followed by capillary electrophoresis and interpreted as described by Baele et al. (2). Eleven isolates were identified as Ent. faecium, 2 isolates as Ent. hirae and 2 isolates belonged to the species Ent. faecalis. Other strains were not specified.

Antibiotic resistance. Susceptibility testing was performed by disk diffusion method on Columbia agar plates enriched with 5% of defibrinated sheep blood (Becton and Dickinson) using the following antibiotic disks (Becton and Dickinson): ampicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), vancomycin (30 µg), erythromycin (15 µg), lincomycin (10 µg), novobiocin (30 µg), gentamicin (10 µg), penicillin G (10 IU), kanamycin (30 µg), novobiocin (30 µg). The plates were incubated at 37°C for 24 h. The strains were classified as resistant by comparing the sizes of the inhibition zone. Staph. aureus ATCC 25923 and Ent. faecalis ATCC 29212 were used as the control strains.

Results

The total count of staphylococci reached 2.4 log_{10} ± 1.1 CFU per gram, that of enterococci 5.9 log_{10} ± 1.2, and of lactobacilli 6.9 log_{10} ± 1.6.

The results of testing the resistance to antibiotics are given in Table 1. None of the dogs had received antibiotic 6 months prior to faecal collection. As shown in the table, resistance to most of the antibiotics tested was observed. Staphylococci exhibited certain resistance to 7 of 9 antibiotics tested. Resistance to penicillin G and tetracycline was the highest, followed by that to ampicillin, lincomycin and erythromycin. The active antimicrobial agents against staphylococci were gentamicin and vancomycin. Enterococcal isolates were mostly resistant to penicillin G, tetracycline and erythromycin. They remained most sensitive to ampicillin, chloramphenicol and vancomycin. The strains of lactobacilli showed some resistance to 6 of 7 antibiotics tested, i.e. to penicillin G, lincomycin, novobiocin, erythromycin, tetracycline and vancomycin. Ampicillin and chloramphenicol were active towards lactobacilli.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Staphylococcus sp.</th>
<th>Enterococcus sp.</th>
<th>Lactobacillus sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>31.6</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>7.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>18.4</td>
<td>26.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>5.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>26.3</td>
<td>ND</td>
<td>40.4</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>42.1</td>
<td>59.6</td>
<td>54.6</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>36.8</td>
<td>26.9</td>
<td>6.4</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.0</td>
<td>0.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>ND</td>
<td>ND</td>
<td>34.0</td>
</tr>
</tbody>
</table>

Discussion

The level of enterococci observed (5.9 log_{10} CFU/g) was similar as earlier reported by Zentek et al. (23) for the ileum and faeces of dogs. The count of lactobacilli (6.9 log_{10} CFU/g) was slightly lower compared to count detected (9.3 log_{10} CFU/g) by Strickling et al. (16). However, many various factors may affect amount of microorganisms in the digestive tract of animals, such as diet, age, housing conditions, etc. There is a lack of studies concerning quantitative detection of staphylococci in faeces of dogs, although one of their reservoir is the anus (5).
All tested bacteria could potentially cause disorders and/or diseases under special conditions such as multiplying or low immunological status. The presented antibiotics are the most frequently used agents to treat those types of disorders. However, when pets are continually exposed to antibiotic treatment for longer period, then it can induce their antibiotic resistance. Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. However, other factors such as environmental stress, age, crowding, and management seem to contribute to the occurrence of antibiotic resistance among normal bacterial flora (15).

In our study, staphylococci showed resistance to antibiotics in similar proportions as in the study of Normand et al. (11). Sensitivity of staphylococci to gentamicin presented here is in agreement with other reported studies (21). Gentamicin was the most active antimicrobial agent also against staphylococci isolated from the saliva of healthy cats (10). Vancomycin remained effective against canine staphylococci and enterococci despite some evidence of detection of vancomycin resistant enterococci in pigs and poultry receiving avoparcin (7). As for lactobacilli, only 6.4% resistance to vancomycin was observed, that is in some species of lactobacilli (L. rhamnosus, L. casei, L. paracasei), chromosomally encoded (3). Antibiotics belonging to the penicillin group are the most commonly used antimicrobial agents in veterinary medicine. This correlates with our findings of high resistance to penicillin (enterococci 59.6%; staphylococci 42.1%; lactobacilli 54.6%). But ampicillin was found to be still effective against enterococci and lactobacilli (0.0% resistance). Surprisingly, all the isolates of enterococci and lactobacilli were sensitive to chloramphenicol and only 7.9% of staphylococci were resistant. However, resistance of LAB from pets to chloramphenicol is commonly low (11).

These results were obtained only from a limited number of strains and there is a need to enrich their amount. To ensure the future efficacy of antimicrobials in animals as well as in humans, it is necessary to minimize the emergence and spread of antimicrobial resistance by the reduction of antibiotic use in a population. Some of isolates of enterococci and lactobacilli showed probiotic potential that was presented in another study (16).

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