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## Antibiotic resistance of lactic acid bacteria and staphylococci isolated from autochthonous Serbian cheeses

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### Introduction

Lactic acid bacteria play a significant role in food hygiene and technology. Their benefits are well known: positive impact on human health; anti-microbial effect on harmful bacteria and technological preferences in product's development. On the other hand, recent data clearly warn of the risk related to antibiotic resistance in "harmless" lactic acid bacteria, in particular the horizontal transfer of antibiotic resistance genes to pathogens. It is clear that commensal bacteria could also contribute to spreading genes resistant to pathogens (Mathur and Singh, 2005; Ruzauskas et al., 2009).

Due to the high prevalence of bovine mastitis and the associated antibiotic consumption, enrichment of antibiotic-resistant staphylococci is expected and has been observed. Penicillin, neomycin, gentamicin, chloramphenicol, tetracycline, erythromycin, and lincomycin resistant *S. aureus* and coagulase-negative staphylococci were noted in fermented food of animal origin (Perreten et al., 1998).

The aim of this study was to isolate LAB and staphylococci from traditional Serbian raw cow's cheese, and to investigate their susceptibility and resistance towards selected antimicrobials. Based on the results obtained, we were able to estimate the presence of resistant or multi-resistant strains in traditional foods and the level of potential risk for consumer health regarding the spread of antimicrobial resistance in the food chain.

### Materials and Methods

The studied LAB strains and staphylococci were isolated from manufacturing and ripening of autochthonous Serbian cheeses (traditionally made cheese without starters at different farmhouses). The isolates belonged to the dominant bacterial groups (lactobacilli and enterococci) and were isolated on agar plates of either Kanamycin Aesculine Azide (KAA) agar for enterococci or MRS agar for lactobacilli. Staphylococci were isolated according to procedure EN ISO 6888-1. In total, 19 strains of lactobacilli, 17 enterococcal strains and 83 strains of staphylococci were isolated.

Antibiotic susceptibility testing was accomplished with the commercial test BBL Sensi-Disc Antimicrobial Susceptibility Test Discs, performing the standard disc diffusion method (National Committee for Clinical Laboratory Standards, 1993). The isolated strains of LAB were tested for resistance to 11 antibiotics: erythromycin (15 µg), gentamicin (120 µg), tetracycline (30 µg), penicillin (10 IU), lincomycin (2 µg), fusidic acid (10 µg), neomycin (30 µg), vancomycin (3 µg), cefotaxime (30 µg), ofloxacin (5 µg), metronidazole (80 µg). The selected antibiotics for disc diffusion performing test in staphylococci population were: erythromycin (15 µg), gentamicin (120 µg), tetracycline (30 µg), penicillin (10 IU), lincomycin (2 µg) neomycin (30 µg), vancomycin (3 µg), chloramphenicol (30 µg), oxacillin (1 µg), ampicillin (10 µg), ciprofloxacin (5 µg). The level of antibiotic susceptibility for each isolate was reported as resistant, intermediate or sensitive according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The E test strips (AB Biodisk, Sweden) was used to evaluate the MICs for tetracycline, penicillin and erythromycin. MICs were read directly from the test strip according to the instructions of the manufacturer.



## Results

In order to illustrate the current situation of antibiotic resistance patterns in beneficial LAB, studies were first conducted to isolate lactic acid bacteria from autochthonous cheeses originated from Serbia, and secondly to evaluate their antimicrobial resistance patterns. Also, antibiotic resistance profiles of staphylococci isolated from the same samples were analyzed.

Out of 83 analyzed strains of staphylococci, 43 showed resistance to either tetracycline, penicillin or erythromycin, or combinations of those antibiotics by performing the disc diffusion test (results not shown). The resistant subpopulation of staphylococci was subjected to MIC's evaluation for penicillin and tetracycline where 17 strains were characterized by resistance to tetracycline and 20 isolates by resistance to penicillin. None of the isolates showed resistance to erythromycin. (Table 1.)

**Table 1.** Resistant population of staphylococci, lactobacilli and enterococci according to results of MIC evaluation for tetracycline, penicillin and erythromycin

| Antibiotic                        | MIC ( $\mu\text{g/mL}$ ) | Number of resistant strains |
|-----------------------------------|--------------------------|-----------------------------|
| <b><i>Staphylococci</i> spp.</b>  |                          |                             |
| tetracycline                      | $R \geq 1$               | 17                          |
| penicillin                        | $R \geq 0.125$           | 20                          |
| <b><i>Lactobacillus</i> spp.*</b> |                          |                             |
| tetracycline                      | $R \geq 8$               | 4                           |
| penicillin                        | $R \geq 2$               | 1                           |
| erythromycin                      | $R \geq 1$               | 3                           |
| <b><i>Enterococcus</i> spp.*</b>  |                          |                             |
| tetracycline                      | $R \geq 2$               | 4                           |
| penicillin                        | $R \geq 2$               | 1                           |
| erythromycin                      | $R \geq 4$               | 2                           |

\*Proposed breakpoints MIC ( $\mu\text{g/ml}$ ) according to FEEDAP (European Commission, 2008)

In total, 19 strains of lactobacilli were isolated and subjected to disc diffusion test to evaluate the antibiotic resistance to selected antibiotics. Out of 19, 11 strains were characterized by resistance to either tetracycline, penicillin or erythromycin, or combinations of those antibiotics (results not shown). According to results of MIC's evaluation, 4 strains showed resistance to tetracycline, 1 to penicillin, and 3 strains to erythromycin.

All 17 isolates of enterococci subjected to disc diffusion performance were characterized by resistance to either tetracycline, penicillin or erythromycin, or combinations of those antibiotics. By applying the MIC determination, 4 isolates showed resistance to tetracycline, 1 strain was characterized by resistance to penicillin, and 2 strains showed resistance to erythromycin.

Distribution of MIC's among the population of staphylococci, lactobacilli and enterococci showed high level resistance to selected antibiotics (tetracycline, penicillin, erythromycin) (results not shown).

## Conclusions

- Multiresistance and high level resistance to selected antibiotics among the population of LABs and staphylococci isolated from autochthonous Serbian cheeses were detected
- Further investigations of LAB populations and staphylococci necessary to obtain relevant data to be included in risk assessment related to traditional food products.

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