

Antibiotic Resistance of Enterococci and Coliform Bacteria in Dairy Products from Commercial Farms

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Abstract

The aim of this study was to determine the prevalence and antibiotic resistance of enterococci and coliform bacteria isolated from sheep and cows cheese from commercial farms. Susceptibilities of isolated enterococci and coliform bacteria were tested using the disk diffusion method. The bacteria were tested on antibiotics enrofloxacin, sulphonamides, tetracycline and streptomycin. All isolates of *Enterococcus* strains were resistant of all used antibiotics. The similar results were detected of coliform bacteria on tested antibiotics.

Keywords: antibiotic resistance, coliform bacteria, *Enterococcus* sp., sheep and cows cheese

1. Introduction

Lactic acid bacteria (LAB) are a group of Gram negative and mainly Grampositive anaerobic bacteria which excrete lactic acid as a main fermentation product into the medium.

LAB are important microorganisms situated in parts of the human and animal body (intestine, nasopharyngeal and vaginal mucosa), and in environments (mainly plants) where spontaneous fermentations of carbohydrate containing substrates occur. These spontaneous events developed into important food fermentations of plant material (sour dough, sauerkraut, etc.) and animal products (sausages, cheeses and sour milk from meat and milk, respectively) [1].

Such food has been and still is an important vehicle of living bacteria from animal and plant sources into the human body. At cell densities of 10^8 to 10^9 viable bacteria per gram, one portion (100 g) of fermented product delivers 10^{10} to 10^{11} viable microorganisms. Indeed, some LAB strains from animal and human intestinal microflora have been adopted as 'probiotic' food supplements including *Enterococcus faecium*, *Lactobacillus*

plantarum, *Lb. acidophilus*, *Lb. casei* subsp. *ramnosus*, and several *Bifidobacterium* and *Propionibacterium* species [12].

Strains intended for the use in food systems as starters or probiotics should be carefully examined for antimicrobial susceptibility, especially those isolated during the so called "post-antibiotic era". Lactic acid bacteria (LAB) from fermented products may act as a reservoir of antimicrobial-resistance genes that could be transferred to pathogens, either in the food matrix or, more importantly, in the gastrointestinal tract. The production of fermented dairy products from raw milk in antibiotic challenged environments may select antibiotic-resistant LAB harbouring transmissible resistance genes [5].

The evolution of antibiotic resistance in microbial communities is enhanced by horizontal transfer of resistance genes over species and genus borders by conjugative plasmids, transposons, the possession of integrand and insertion elements, as well as lytic and temperate bacteriophages. During 50 years of large scale antibiotic application several mechanisms of resistance against a particular antibiotic have evolved including

enzymatic inactivation of the antibiotic (e.g. by β -lactamases, aminoglycoside acetyl-, nucleotidyl- and phosphoryltransferases), restricted import of antibiotics (e.g. penicillin binding proteins), active export of antibiotics (e.g. by membrane inserted ATP-dependent efflux systems) or target modification (e.g. methylation of 23S rRNA, mutation of aminoacid sequence of topoisomerase) For certain pathogenic and potentially pathogenic bacteria like staphylococci and enterococci, the evolution of highly resistant clones has inaugurated a crisis in antibiotic resistance [7].

In fact, horizontal gene transfer is essential for bacteria to survive and adapt to new environments [9].

The aim of our study was to determine the prevalence and antibiotic resistance of enterococci and coliform bacteria isolated from sheep and cows cheese from commercial farms.

2. Materials and methods

The sensitivity study was done on enterococci and *E. coli* isolated from sheep and cows cheese from commercial farms. All the samples were placed in sterile plastic bags and kept at 4°C prior to analysis.

For cultivation of enterococci were used two selective media – Slanetz and Bartley medium and Pfizer Selective Enterococcus Agar (Biomark, Pune). For cultivation of *E. coli* McConkey Agar (Biomark, Pune) was used.

The cells from typical colonies were stained for morphology by the Gram method, and tested for catalase production.

The inoculum of enterococcal and *E. coli* strains was prepared by suspending of colonies from agar plates and the suspension was adjusted to equal a 0.5 McFarland standard. The sensitivity of all enterococcal and *E. coli* isolates was tested against: tetracycline (TET) 300 µg/disk, enrofloxacin (ENR) 5 µg/disk, compound sulphonamides (S3) 300 µg/disk and streptomycin (S) 10 µg/disk (according to the CLSI requirements) using the disk diffusion method. The incubation of strains was done at the temperature 35°C. The interpretation of inhibition zones around the disks was according to CLSI 2006 Performance standards for antimicrobial susceptibility testing [3]. The inhibition zones were controlled with the reference *Enterococcus*

faecalis ATCC 29212 and *Escherichia coli* ATCC 25922.

3. Results and discussion

We studied antimicrobial drug resistance in commensal *E. coli* and enterococci isolates, which are considered a potential reservoir for resistance genes in dairy products. They are not, however, considered “generally recognised as safe” (GRAS) and their presence is an indicator of faecal contamination. Indeed, they normally colonise the intestinal tract of humans and animals, although they are also known to be opportunistic pathogens responsible for a wide variety of human infections such as endocarditis, urinary and genital tract infections, meningitis and septicemia [10]

Enterococci may occur also in natural milk starter cultures, which are still widely used for the manufacture of variety of cheeses, mostly artisan cheeses, produced both in European countries from raw or pasteurised milk [6].

In our study, all isolates (*E. coli* and *enterococci*) were multiresistant (100%) to enrofloxacin (ENR), tetracycline (TET), streptomycin (S) and compound sulphonamides (S3). There were no significant correlations between groups.

The resistance of *Enterococcus* sp. strains on chosen antibiotics is showed in Figure 1.

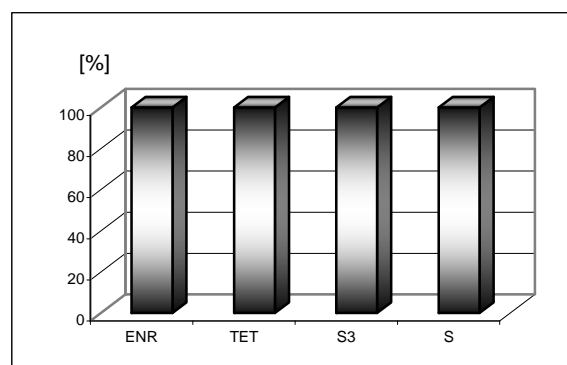


Figure 1. Antibiotic resistance profiles of *Enterococcus* sp. isolated from dairy products

On the basis of the several authors [2,4,10,11], although enterococci are present in raw milk due to faecal contamination from the animal, it is tempting to speculate that traditional raw milk cheeses should not be considered the main source of untreatable nosocomial enterococcal infections

of human. However, enterococci introduced with the cheeses can acquire additional resistance in the hospital environment. Enterococci are rather promiscuous bacteria capable of acquiring resistance genes from different bacterial species, with a surprising high frequency of gene transfer. Therefore, one needs to consider not only the epidemic spread of strains, but also the spread of the resistance-encoding transposons. In the same way, the dairy enterococcal population colonising the human gut can acquire resistance to antibiotics from enteropathogenic bacteria and can therefore have the same pattern of resistance to antibiotics as the clinical strains.

The data shown on the Figure 2 (Antibiotic resistance profiles of *E. coli* isolated from dairy products) indicate that the highest percentage of the resistance was exhibited against all chosen antibiotic (100%).

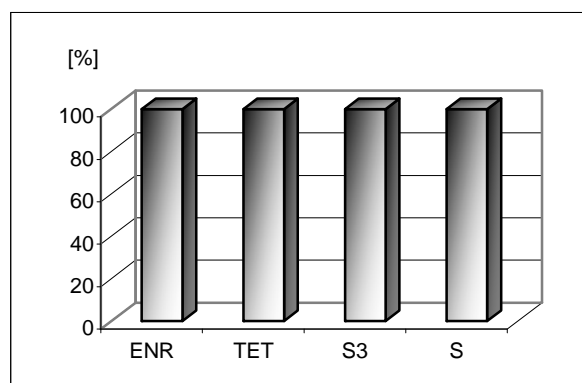


Figure 2. Antibiotic resistance profiles of *E. coli* isolated from dairy products

It has been reported that the selection and the maintenance of streptomycin - sulphonamides-tetracycline-resistant *Escherichia coli* may be due to environmental components independent of antibiotics selection [8].

4. Conclusions

A previous report showed on the high occurrence of antibiotic drug resistance in commensal *E. coli* and enterococci isolates, which are considered a potential reservoir for resistance genes in dairy products. The present findings suggest that the antibiotic pressure on LAB from the wide use of antibiotics, in veterinary medicine and agriculture for example, could be contributing to the dissemination of resistances into cheese-related ecological niches.

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