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Antimicrobial resistance of *Enterococcus* spp. isolated from livestock in Lithuania

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ABSTRACT

105 isolates of *Enterococcus* spp. were selected as representative samples from different herds of pigs (n=25), cattle (n=46) and poultry (n=34). Antimicrobial susceptibility was determined according to epidemiological cut-off values. The highest number of strains demonstrated epidemiological resistance to lincomycin (78%), tetracycline, (65%), flavomycin (59%) and erythromycin (55%). The smallest number of strains was resistant to linezolid (1%) and tigecycline (2%). Six percent of all tested strains were epidemiologically resistant to ciprofloxacin, vancomycin and daptomycin. Cattle isolates showed the most frequent resistance to flavomycin (71%), lincomycin (54%), tetracycline (45%), streptomycin (40%) and erythromycin (40%). Enterococci isolated from pigs showed the highest resistance to tetracycline and lincomycin (92%), erythromycin (76%), kanamycin (56%) and streptomycin (52%). All strains isolated from poultry were epidemiologically resistant to lincomycin. The most frequent resistance of poultry strains was also demonstrated to tetracycline (72%), erythromycin (63%), streptomycin (50%), flavomycin (48%) and tylosin (47%). However all strains isolated from poultry were susceptible to chloramphenicol, quinupristin/dalfopristin, linezolid and bacitracin.

Key words: antibiotics, antimicrobial susceptibility, cut-off values

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Introduction

Uncontrolled usage of antimicrobials is recognized as the most important factor that determines the development and spread of resistant microorganisms (ACAR and RÖSTEL, 2001; BURCH, 2005; MORENO et al., 2000). It helps to select resistant bacteria and also breaks down the natural microflora in the gut of animals and humans (PIDDOCK, 1996). This has led to the emergence and dissemination of resistant bacteria and resistance genes in animals and humans. In both populations antibiotics are used for therapy and prophylaxis of infectious diseases (VAN DEN BOGAARD and STOBBERINGH 2000; BERŽANSKYTĖ et al., 2004). Commensal bacteria constitute a reservoir of resistance genes for pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens (LUKAŠOVA and ŠUSTAČKOVA, 2003). Resistance in commensal bacteria is often high to broad spectrum antimicrobials (GOLDSTEIN et al., 2001). Commensal bacteria are left uncontrolled, because they do not cause any clinical symptoms in the etiology of diseases. That helps them to survive in different conditions by pressure of different quantities and spectra of antimicrobials. Enterococci have been known to be resistant to most antibiotics used in clinical practice. They are naturally resistant to cephalosporins, aminoglycosides and clindamycin and may also be resistant to tetracyclines and erythromycin. They are intermediately sensitive to penicillin, ampicillin and glycopeptides (LUKAŠOVA and ŠUSTAČKOVA, 2003). Enterococci are known to acquire antibiotic resistance with relative ease and to be able to spread these resistance genes to other species (KUHN et al., 2000). Widespread resistance to chloramphenicol, macrolides, kanamycin, streptomycin and tetracycline has been found among isolates of *E. faecalis* and *E. faecium* isolated from humans, broilers and pigs (AARESTRUP et al., 2000).

Several antimicrobial agents frequently used in animals belong to the same class of antimicrobial agents as those that are important for use in therapy for some enterococcal infections in humans (e.g., ampicillin, gentamicin, and virginiamycin) (HEUER et al., 2006). Antimicrobial-resistant enterococci are prevalent in food animals and in food of animal origin (MAY et al., 2005; TEUBER, 2001) and thus may frequently be transferred to humans, either by ingestion of contaminated food or from the environment (DONABEDIAN et al., 2003; HEUER et al., 2006). Subsequent emergence of infections in humans caused by resistant bacteria that originate from the animal reservoir is of great concern. In the European Union, the use of antimicrobial agents (e.g., virginiamycin and avoparcin) as feed additives for growth promotion in food animals has been banned because of cross-resistance to antimicrobial agents used in human therapy of enterococcal and other bacterial infections (e.g., quinupristin/dalfopristin and vancomycin). So it is important to determine resistance to different classes of antimicrobials including ones used in veterinary medicine and also in human medicine.

The aim of this study was to determine the frequency of microbiological (epidemiological) resistance of enterococci isolated from food animals.

Materials and methods

Clinical material was collected in different regions of the country with the aim of obtaining representative samples from different farms of cattle, pigs and poultry. It was the first more obvious study in the country testing antimicrobial susceptibility of enterococci isolated from animals. For this reason different types of animal housing, different sizes of herds (but mostly large complexes) were included in this study with the aim of obtaining initial tentative results for the better understanding of common situations of antimicrobial resistance. Investigations were carried out according to the design of preparing monitoring programmes described by OIE and pursuance of the recommendations of some authors (AARESTRUP, 2004; BAX et al., 2001; FRANKLIN et al., 2000). Clinical material (faeces) was taken from live animals from the rectum using cotton swabs with transport media (Transwab, UK). Slanetz-Bartley Agar+TTC, Aesculine Bile Agar and Pfizer Selective Enterococcus Agar (Liofilchem, Italy) were used for inoculation of clinical material. The media were incubated for 48 hours at 35 ºC.

Control microorganisms such as *Enterococcus faecalis* ATTC 29212 were used for control of media and panels with antimicrobials. Identification was performed by typical growing characteristics on selective media and using the RapID STR identification system (Remel, USA). Results were interpreted using the ERIC computer programme (Remel).

For antimicrobial susceptibility testing panels of vetMIC E-cocci (SVA, Sweden) and Sensititre NARMS Enterococcus plates (TREK Diagnostic Systems, USA) were used using the microdilution method. McFarland standards (Remel, USA) and an electronic optical densitometer (Liap, Latvia) were used for preparing the suspension. Panels were inoculated and incubated according to the manufacturers' instructions. The minimum inhibitory concentration (MIC) was determined for each strain. Results of microbiological susceptibility were evaluated separately for each species using EUCAST database programme, according to cut-off values. The strains that had higher resistance according to the Wilde Type Epidemiological cut-off value were called "resistant" and those that had susceptibility lower or the same cut-off value-"susceptible".

Results

105 isolates of *Enterococcus* spp. were selected as representative samples from different herds of pigs (n = 25), cattle (n = 46) and poultry (n = 34). 50 isolates were identified as *E. faecalis*, 40 *E. faecium* and 15 strains depended to other species (mostly *E. hirae*). The microbiological susceptibility of all the *Enterococcus* spp. strains to different tested antimicrobials according to cut-off values is shown in Fig. 1.

As may be seen in Fig. 1. there were no tested antimicrobials absolutely "effective" against all tested isolates. The largest number of strains demonstrated resistance to lincomycin, (78%), tetracycline, (65%), flavomycin (59%) and erythromycin (55%). The

smallest number of strains were resistant to linezolid (1%) and tigecycline (2%). Six percent of all tested strains were epidemiologically resistant to ciprofloxacin, vancomycin and daptomycin.

The susceptibility of enterococci isolated from different animal species is shown in Figs. 2-4.

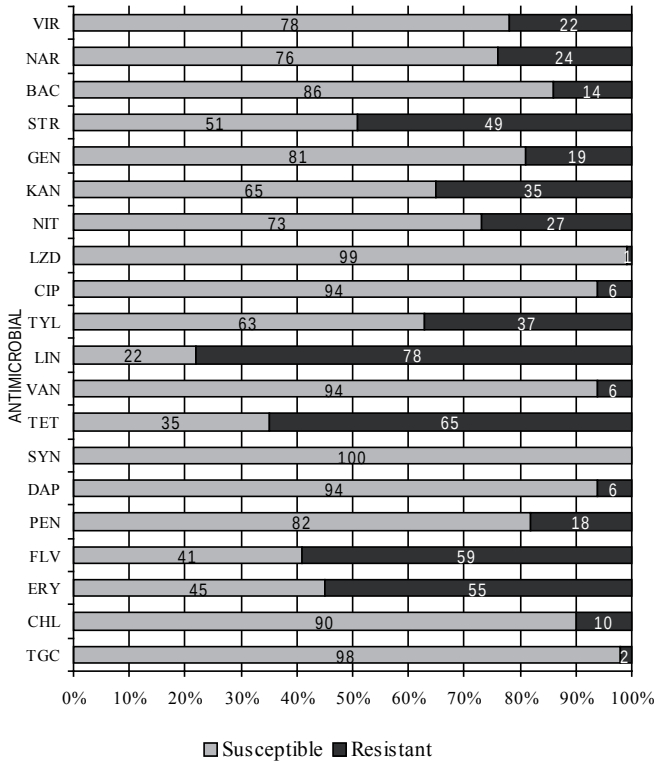

Fig. 1. Microbiological susceptibility of all tested *Enterococcus* spp. to antimicrobials,%. VIR-virginiamycin, NAR-narasin, BAC-bacitracin, STR-streptomycin, GEN-gentamicin, KAN-kanamycin, NIT-nitrofurantoin, LZD-linezolid, CIP-ciprofloxacin, TYL-tylosin, LIN-lincomycin, VAN-vancomycin, TET-tetracycline, SYN-quinupristin/dalfopristin, DAP-daptomycin, PEN-penicillin, FLV-flavomycin, ERY-erythromycin, CHL-chloramphenicol, TGC-tigecycline.

As may be seen in Fig. 2 cattle isolates showed the most frequent resistance to flavomycin (71%), lincomycin (54%), tetracycline (45%), streptomycin (40%) and erythromycin (40%). The lowest percentage of strains was resistant to linezolid, (3%), daptomycin (4%) and penicillin (4%). Eight percent of the strains demonstrated resistance to ciprofloxacin, vancomycin and chloramphenicol. All enterococci were susceptible to tigecycline.

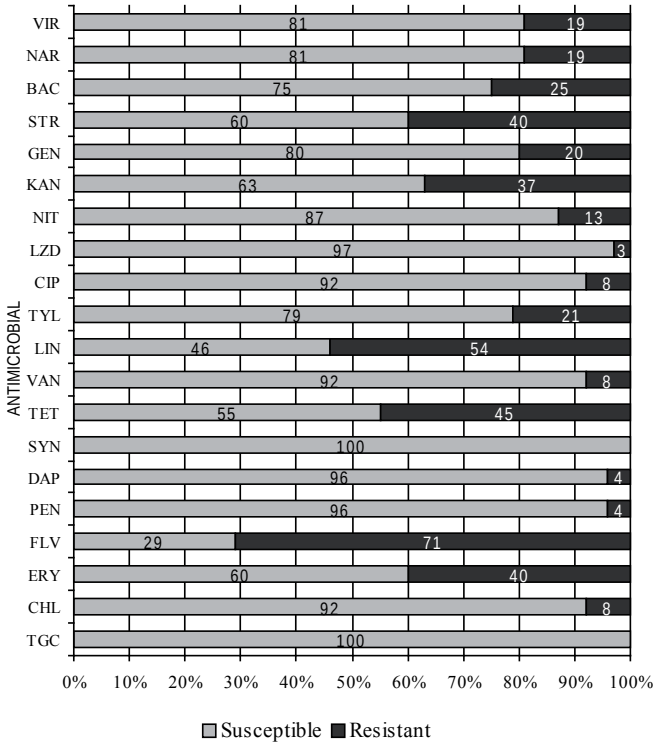

Fig. 2. Microbiological susceptibility of *Enterococcus* spp. strains isolated from cattle. VIR-virginiamycin, NAR-narasin, BAC-bacitracin, STR-streptomycin, GEN-gentamicin, KAN-kanamycin, NIT-nitrofurantoin, LZD-linesolid, CIP-ciprofloxacin, TYL-tylosin, LIN-lincomycin, VAN-vancomycin, TET-tetracycline, SYN-quinupristin/dalfopristin, DAP-daptomycin, PEN-penicillin, FLV-flavomycin, ERY-erythromycin, CHL-chloramphenicol, TGC-tigecycline.

As may be seen in Fig. 3 all strains of enterococci isolated from pigs showed natural susceptibility to linezolid and quinupristin/dalfopristin. The largest number of strains were demonstrated to be resistant to tetracycline and lincomycin (92%), erythromycin (76%), kanamycin (56%) and streptomycin (52%). Four percent of enterococci showed resistance to tigecycline, daptomycin and ciprofloxacin.

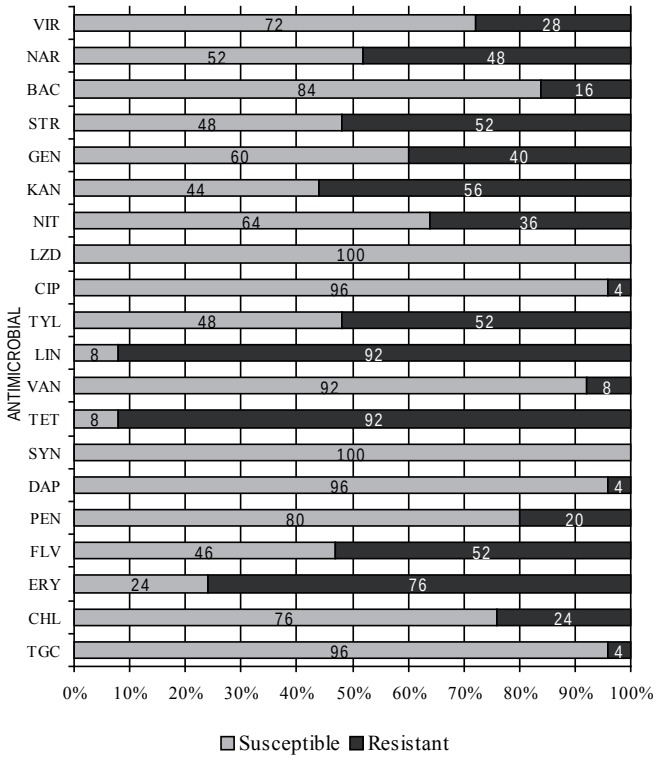

Fig. 3. Microbiological susceptibility of *Enterococcus* spp. strains isolated from pigs, %. VIR-virginiamycin, NAR-narasin, BAC-bacitracin, STR-streptomycin, GEN-gentamicin, KAN-kanamycin, NIT-nitrofurantoin, LZD-linesolid, CIP-ciprofloxacin, TYL-tylosin, LIN-lincomycin, VAN-vancomycin, TET-tetracycline, SYN-quinupristin/dalfopristin, DAP-daptomycin, PEN-penicillin, FLV-flavomycin, ERY-erythromycin, CHL-chloramphenicol, TGC-tigecycline.

As may be seen from Fig. 4, all strains of enterococci isolated from poultry were microbiologically resistant to lincomycin. Resistance was also most frequently demonstrated to tetracycline (72%), erythromycin (63%), streptomycin (50%), flavomycin (48%) and tylosin (47%). All strains were susceptible to chloramphenicol, quinupristin/dalfopristin, linezolid and bacitracin. Only a few isolates showed resistance to gentamicin (3%), tigecycline and ciprofloxacin (4%).

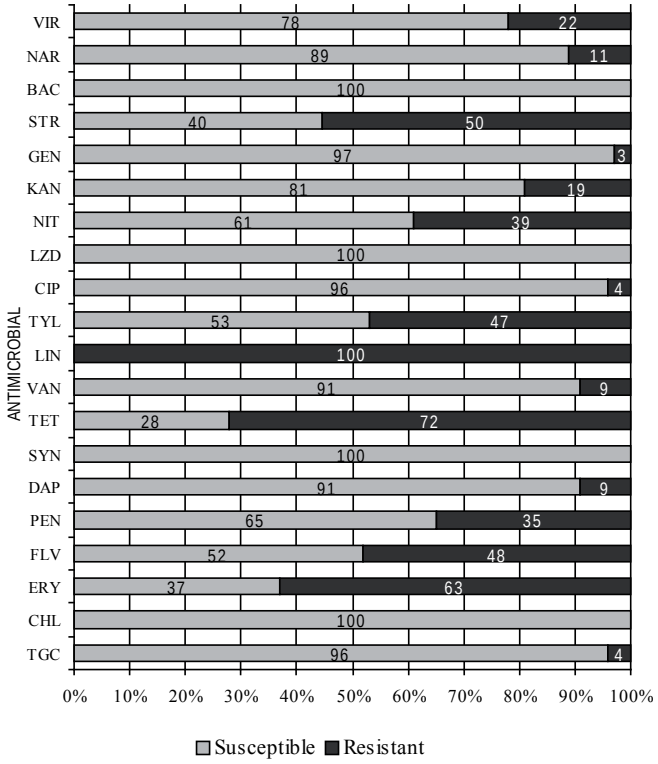

Fig. 4. Microbiological susceptibility of *Enterococcus* spp. strains isolated from poultry, %. VIR-virginiamycin, NAR-narasin, BAC-bacitracin, STR-streptomycin, GEN-gentamicin, KAN-kanamycin, NIT-nitrofurantoin, LZD-linesolid, CIP-ciprofloxacin, TYL-tylosin, LIN-lincomycin, VAN-vancomycin, TET-tetracycline, SYN-quinupristin/dalfopristin, DAP-daptomycin, PEN-penicillin, FLV-flavomycin, ERY-erythromycin, CHL-chloramphenicol, TGC-tigecycline.

Discussion

Microbiological susceptibility of microorganisms shows a natural species susceptibility to different molecules of antimicrobials. Every species adapts to its natural environment and does not express features that are not necessary for the security of its vital functions. Increased resistance to antimicrobials shows contacts with such antimicrobials and microorganisms. Moreover the level of resistance is also associated with antimicrobial pressure in the environment. Monitoring of microbiological resistance could be approached as an indicator of the intensive and extensive usage of different antimicrobials. This could be useful when the strategy and politics of antimicrobials usage are being developed in a country or region. The rapid economic progress that ensured after Lithuania joined the EU and opened its borders resulted in changes to the bacterial ecosystems in the agricultural environment. Epidemiological investigations into bacterial resistance show the current situation of resistance in the country. The results indicate that this situation is not favourable. Only a few antimicrobials show no influence on bacteria, especially those that have never been used in animal husbandry. Linezolid, tigecycline and combination of quinupristin and dalfopristin demonstrated the highest effectiveness in the test of antimicrobial susceptibility of enterococci. However they are used as an exception in human medicine and only in treating severe infections. Other antimicrobials that are used in human medicine or in animal treatment widely showed to be more often resistant in the test of epidemiological susceptibility. Enterococci showed to be very well adapted to the environment and demonstrated frequent resistance to old and widely used antimicrobials, such as tetracycline, linkomycin, erythromycin and of course to compounds used as feed additives or growth promoters. More than a half the tested strains demonstrated resistance to lincomycin, tetracycline, erythromycin and flavomycin. Some authors confirm that the use of antimicrobial agents in the modern farm industry has created a reservoir of resistant bacteria in food animals (AARESTRUP, 2001; KHACHATOURIANS, 1998; PIDDOCK, 1996).

This study also demonstrates that resistance to some antibiotics that have never been used in animal treatment (vancomycin) demonstrates the spread of resistant strains or genetic determinants among humans and animals. This statement has also been supported by other studies (KHACHATOURIANS, 1998; STOBBERINGH et al., 1999; WITTE, 2000). Some authors have suggested that glycopeptides, such as avoparcin, are responsible for the development of vancomycin-resistant enterococci in animals (BORGEN et al., 2000). When comparing antimicrobial resistance isolated from different species of animals (mammals and poultry) it may be said that there is a relationship between extensive usage of certain antimicrobials for those species. For example 97% of enterococci isolated from poultry were susceptible to gentamicin-an antibiotic that is used parenterally for mammals; however 40% of isolates from pigs were gentamicin resistant. All poultry

strains were susceptible to chloramphenicol. This antibiotic was banned for treatment of production animals in the country about 10 years ago; however 8% of the cattle and even 24% of the pigs had increased resistance to this antimicrobial. It is only possible to determine the reason for this increased resistance by assumption. One reason may be the use of the same class of compounds (for example florphenicol) in the treatment of animals; another reason may be that bacteria could stay resistant to certain antimicrobials for a very long time. Other reasons such as using inappropriate antibiotics in humans and possible environment contamination by resistant enterococci or illegal use in animal husbandry could be discussed as well.

The obtained results show the unsatisfactory situation regarding the antimicrobial resistance of commensal bacteria in animal farms. More attention must be paid to the prudent usage of antimicrobials because the influence of antimicrobials on ecological systems was clearly demonstrated in this study. Microbiological resistance could be the first warning that the bacterial population is resistant to different antimicrobials and the testing of clinical susceptibility to certain antibiotics in that area should be strongly recommended.

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SAŽETAK

Ukupno je 105 izolata bakterija *Enterococcus* spp. bilo odabrano kao predstavnici iz različitih uzgoja svinja (n = 25), goveda (n = 46) i peradi (n = 34). Osjetljivost prema antimikrobnim tvarima određivana je na osnovi epidemioloških graničnih vrijednosti. Većina izolata bila je otporna prema linkomicinu (78%), tetraciklinu (65%), flavomicinu (59%) i eritromicinu (55%). Najmanje izolata bilo je otporno prema linezolidu (1%) i tigeciklinu (2%). Šest posto svih pretraženih sojeva bilo je otporno na ciprofloksacin, vankomicin i daptomicin. Izolati iz goveda bili su najčešće otporni na flavomicin (71%), linkomicin (54%), tetraciklin (45%), streptomicin (40%) i eritromicin (40%). Enterokoki izdvojeni iz svinja bili su najotporniji prema tetraciklinu i linkomicinu (92%), eritromicinu (76%), kanamicinu (56%) i streptomicinu (52%). Svi izolati iz peradi bili su otporni prema linkomicinu. Sojevi iz peradi također su bili najčešće otporni prema tetraciklinu (72%), eritromicinu (63%), streptomicinu (50%), flavomicinu (48%) i tilozinu (47%). Međutim svi sojevi izdvojeni iz peradi bili su osjetljivi prema kloramfenikolu, kvinupristinu/dalfopristinu, linezolidu i bacitracinu.

Ključne riječi: antibiotici, antimikrobna osjetljivost, granične vrijednosti