

EPIDEMIOLOGICAL SUSCEPTIBILITY OF *ENTEROCOCCUS* ISOLATED FROM POULTRY

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ABSTRACT

Epidemiological susceptibility (natural species susceptibility) of isolated enterococci in different farms of poultry all over the country was tested. Minimal inhibitory concentrations (MIC) to the 17 different antimicrobials were tested using “Sensititre” NARMS plates (TREK Diagnostic Systems). Forty six isolates were tested. Interpretation of results was done according to the epidemiological cut-off values from the database of EUCAST. Results showed that all tested isolates showed to be susceptible (MIC lower or equal to the epidemiological cut-off values of wild type strains) to linesolid and quinupristin/dalfopristin without reference of the enterococcus species. All tested strains had higher MIC than cut-off values to linkomycin. Frequent resistance was also to tetracycline (67 %), erythromycin (57 %), flavomycin (48 %), streptomycin (43 %) and nitrofurantoin (30 %). Just only few isolates had increased MIC to gentamicin, chloramphenicol, fluoroquinolones and vancomycin. The results showed that enterococci as a part of natural habitats in poultry farms had increased resistance to the different classes of antibiotics. Such resistance could be potentially hazardous from the point of carrying resistance genes and possible genetic transfer to zoonotic bacteria.

KEY WORDS: Enterococcus, epidemiological susceptibility, antimicrobials, poultry.

INTRODUCTION

Uncontrolled usage of antimicrobials is recognized as the most important factor that determines development and spreading of resistant microorganisms [2, 3]. Of great importance are the investigation works on the susceptibility of pathogenic bacteria in different regions or countries. According to EU Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, Member States must implement a monitoring programme that provides comparable data on the occurrence of antimicrobial resistance in zoonotic agents and, in so far as they present a threat to public health, other agents. A number of countries have national surveillance programmes to assess bacterial susceptibility to antimicrobials among zoonotic and commensal bacteria isolated from healthy and sick animals. 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 [9]. Resistance in commensal bacteria often is high to broad spectrum of antimicrobials. Those bacteria often left uncontrolled, because they do not cause any clinical signs in the aetiology of diseases. That helps them to survive in different conditions by pressure of different quantities and spectrum 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 intermediate sensitive to penicillin, ampicillin and glycopeptides [7]. Widespread resistance to chloramphenicol, macrolides, kanamycin, streptomycin and tetracycline was found among isolates of *E. faecalis* and *E. faecium* isolated from humans, broilers and pigs [1]. However resistance in different countries is not equal. It depends from geographical position, antimicrobial usage politics in the country and even from

selection of starting material for investigations. Investigations on antimicrobial resistance may be directed in different points, according to the aim of such investigations. Most studies are associated with testing of clinical susceptibility of certain antibiotics that are directly used for treatment of infections of humans and animals. Such testing is very useful for clinical practitioners when choice of certain antibiotics for treatment could be clarified. In that case the results are clearly interpreted and show that certain strain is susceptible intermediate susceptible or resistant. However the development of bacterial resistance could be started from low, inappreciable level of such development. In that case clinical tests often show that strain is susceptible, however some mutations or other mechanisms of resistance development could be started in certain strains. Those transmutations could be distinguished by testing of acquired microbiological or epidemiological resistance. Epidemiological resistance shows how certain strain of every separate species of bacteria differs from natural, specific for certain species resistance. The epidemiological natural susceptibility for every species of bacteria is determined centrally by European Committee for Antimicrobial Susceptibility Testing (EUCAST). Results of microbiological susceptibility testing are also important for molecular testing, because it more distinctly show about genome changing than the results of clinical testing. Such data are also important for epidemiologists for better understanding of spreading antimicrobial resistance globally.

The aim of this study was to determine frequency of epidemiological resistance of enterococci isolated from poultry origin.

MATERIAL AND METHODS

Clinical material was collected in different regions of the country with the aim to obtain representative samples from different farms of poultry in 2008. Slaughtered broilers and older hens were tested. Investigations were carried out according to design of preparing monitoring programmes described by OIE and pursuance of recommendations of some authors. Clinical material (faeces) was taken from slaughtered animals from intestines 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. Media were incubated for 48 hours at +35 °C.

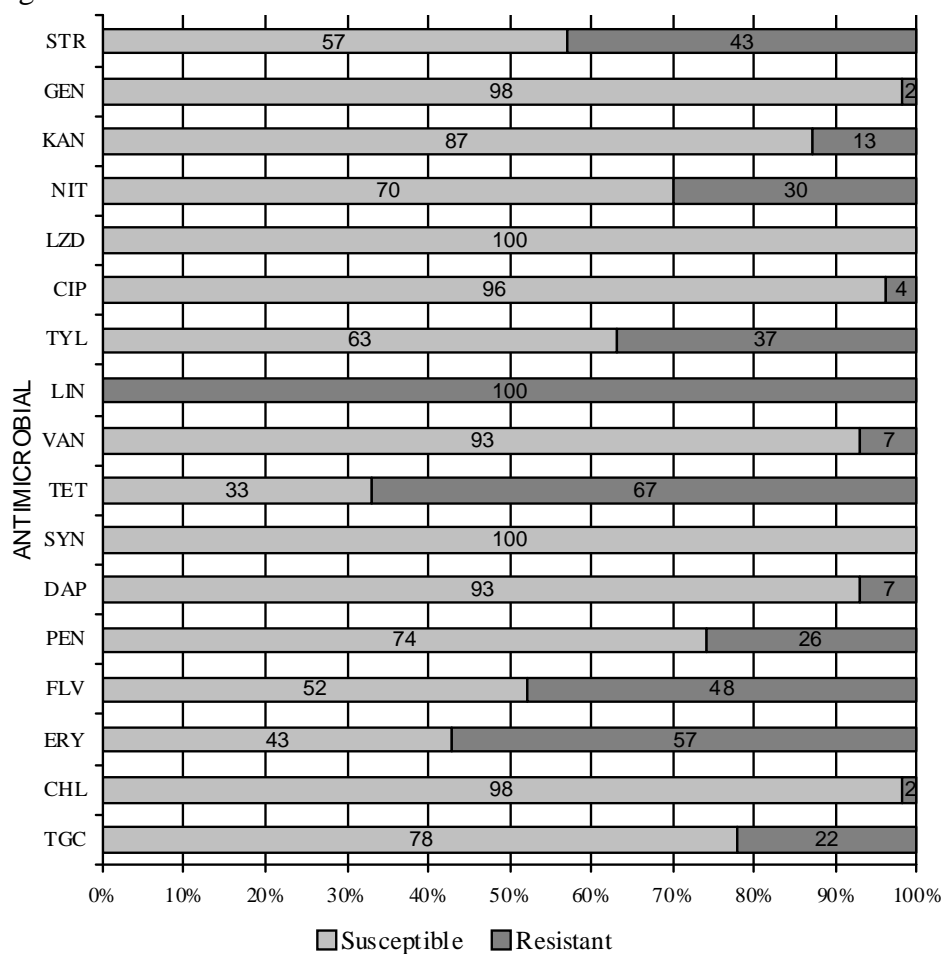
Control microorganisms such as *Enterococcus faecalis* ATTC 29212 were used for control of media and panels with antimicrobials. Identification was performed by typical growing characteristics on selective media and using identification system RapID STR (Remel, USA). Results were interpreted using computer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

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

RESULTS AND DISCUSSION

46 strains of *Enterococcus* were isolated from different poultry farms all over the country. Fifteen strains were identified as *E. faecalis* and 15 strains as *E. faecium*. Other *Enterococcus* spp. included *E. durans*, *E. gallinarum* and some other species. Recovery of enterococci in separate farms varied from 75 to 100 %, however just few strains from each farm were selected for testing with the aim to test different strains in different farms.

Epidemiological susceptibility according to cut-off values of all tested enterococci is shown in Figure 1.



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.

Figure 1. **Epidemiological susceptibility of all *Enterococcus* spp. strains isolated from poultry, %**

As could be seen from Figure 1, all tested isolates showed to be susceptible (MIC lower or equal to the epidemiological cut-off values of wild type strains) to linesolid and quinupristin/dalfopristin without reference of the enterococcus species. All tested strains had higher MIC than cut-off values to linkomycin. Frequent resistance was also to tetracycline (67 %), erythromycin (57 %), flavomycin (48 %), streptomycin (43 %) and nitrofurantoin (30 %). Just only few isolates had increased MIC to gentamicin (2 %), chloramphenicol (2 %), fluoroquinolones (4 %), daptomycin (7 %) and vancomycin (7 %). The most frequent resistance was demonstrated to those antimicrobials that are used in veterinary medicine. However susceptibility to certain antimicrobials that are used in huge quantities in poultry farms (fluoroquinolones) was also high. Our previous studies on clinical susceptibility of enterococci isolated from poultry showed that even 35 % of all tested enterococci were clinically resistant to fluoroquinolones [8]. This means that naturally enterococci are very susceptible to fluoroquinolones, however clinical potency of those compounds become less effective because of the acquired resistance. Highest resistance was also demonstrated to those compounds that are used in mass treatment of animals. Decreased susceptibility to

lincomycin, flavomycin and tylosin clearly demonstrates that statement. Data obtained by other authors also demonstrates that enterococci tend to be more resistant to antimicrobials used in mass medication treatment [5]. Some other studies also indicate high frequency of resistance to tetracyclines and macrolides [6]. In general dissimilarities in antimicrobial resistance patterns among enterococci recovered from different meat types may reflect the use of approved antimicrobial agents in each food animal production class [4].

The results showed that enterococci as a part of natural habitats in poultry farms had increased resistance to the different classes of antibiotics. Such resistance could be potentially hazardous from the point of carrying resistance genes and possible genetic transfer to zoonotic bacteria.

CONCLUSIONS

1. Enterococci from poultry origin frequently demonstrate decreased epidemiological susceptibility to different classes of antimicrobials.
2. The most frequent resistance was demonstrated to lincomycin (100 %), tetracycline (67 %), erythromycin (57 %), flavomycin (48 %), streptomycin (43 %) and nitrofurantoin (30 %). All tested strains had lower or equal MIC values according to epidemiological cut-off values, to linesolid and quinupristin/dalfopristin.

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