ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA ENTERITIDIS ISOLATED FROM PORK AND POULTRY

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ABSTRACT
Antimicrobial susceptibility of Salmonella enteritidis was tested. Salmonella isolates were obtained from pork and poultry meat. Susceptibility was tested by agar diffusion method according to NCCLS guidelines. Isolates from pork showed the highest resistance to tetracycline 48.9 %, less to chloramphenicol 18.4 % and florfenicol 14.3 %. Isolates from poultry showed the highest resistance to ampicillin 12.7 %, less to tetracycline 10.2 % and sulphamethoxazole/trimethoprim 10.2 %. Isolates from pork showed a high resistance to tetracycline was still frequent, while Salmonella susceptibility pattern from poultry was changed.

INTRODUCTION
Salmonella has been consistently associated with foodborne illnesses and an economic burden in most countries of the world. More than seventy thousand notified cases of salmonellosis occur each year in Germany, and most of them are attributed to food-borne transmission. The main sources are foods of animal origin, such as poultry, eggs, milk, beef and pork. In addition, fruit and vegetables have been implicated as vehicles in Salmonella transmission. Contamination can occur at multiple steps along the food chain [7, 1]. Increased application of antibiotics in both veterinary and human medicine is believed to be largely responsible for the emergence of drug resistant bacteria. The emergence and spread of antimicrobial resistant pathogens, among them Salmonella, has become a serious health hazard worldwide. The routine practice of giving antimicrobial agents to domestic livestock as means of preventing and treating diseases, as well as promoting growth, is an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to humans by the food chain [10]. Diarrhea is an important problem to unhealthy sanitary conditions. Furthermore, the development of antibiotic resistance in enteropathogens, including Salmonella spp., has increased the problem. Resistance to some β-lactam antibiotics, tetracycline, chloramphenicol, or trimethoprim is reported with increasing frequency [6]. Being easily spread among the population, they can cause an epidemic outbreak, especially if sanitary conditions are not optimum [5]. Another health concern is the discovery that antibiotic resistance can be transferred through R-factor plasmid vectors, from commensal to pathogenic bacteria.

The aim of this study was to determine the incidence of antimicrobial resistance in Salmonella strains from pork and poultry.

MATERIALS AND METHODS.
Isolates from food (carcasses and products) were collected and identified by LVA Veterinary institute and National veterinary laboratory during 12 month project (2006-2007). Salmonella strains identification was performed using EnteroPluri Tests (Liofilchem, Italy) and API20E strips (bioMerieux, France). Serotyping was performed according to the Kaufman-White scheme with antisera from Sanofi (France). Only epidemiologically unrelated strains were selected. Susceptibility was tested in LVA Veterinary institute. The agar diffusion method according to NCCLS guidelines [8] was applied for antimicrobial susceptibility testing of Salmonella isolates. Mueller Hinton Agar II (BBL, LAB39) was used
in order to perform this test. Discs were supplied by Oxoid (Basingstoke, UK). Results were scored susceptible, intermediate or resistant according to NCCLS criteria [8]. Escherichia coli (ATCC 25922) was used as a reference strain. The respective quantities (µg) of active compounds were: ampicillin (AMP), 10; ceftiofur (EFT), 30; cefalotin (KF), 30; ciprofloxacin (CIP), 5; chloramphenicol (C), 30; florfenicol (FFC), 10; gentamicin (CN), 10; nalidixic acid (NA), 30; norfloxacin (NOR), 10; sulphamethoxazole/trimethoprim (SXT), 23.7/1.25; neomycin (N), 10; tetracycline (TE), 30.

RESULTS AND DISCUSSION

Even though pork accounts for 50 % of the total meat consumption in Lithuania, reports of outbreaks of salmonellosis in humans linked to pork consumption are rare. Information on the potential role of commercial swine production in dissemination drug resistant strains in Lithuania is very limited.

As shown in figure 1, isolated Salmonella was most resistant to tetracycline (48.9 %), chloramphenicol (18.4 %), and florfenicol (14.3 %). Salmonella strains resistant to fluoroquinolones were not detected. Tetracycline has been widely used for the treatment purpose for many years in Lithuanian pig farms. High resistance to tetracycline was detected in previous studies during 1998-2003. Resistance to tetracycline was 55 %. Only Choleraesuis serotype was tested, but 92 % of isolated Salmonella from pigs depended on serovar S. Choleraesuis [9]. Salmonella resistance to tetracycline is high in other European Union countries: 36 % in Denmark, 38.3 % in Belgium, 84 % in Great Britain, and 83.3 % in Spain [4, 3, 2].

In spite of chloramphenicol prohibition for about 10 years, resistance to this antimicrobial agent still occurs. Some farming units are quite old, so some kind of persistence of resistant bacteria strains is possible. The frequency of resistance to individual antibiotics among isolates from poultry was different to that among pork isolates (Figure 2). The rates of resistance to sulphamethoxazole/trimethoprim, tetracycline, ampicillin and chloramphenicol were 10.9 %, 10.9 %, 12.7 % and 7.2 % respectively. None of the isolates was resistant to florfenicol, cefalotin, ciprofloxacin, norfloxacin and gentamicin. Resistance to nolidixic acid
was low. Susceptibility trends of *Salmonella* isolates from poultry are changed. In 1998-2003 *Salmonella* were resistant to tetracycline 20%, nalidixic acid 16 % and streptomycin 12 %. [8].

![Figure 2. Susceptibility of *Salmonella* strains isolated from poultry, (n=55).](image)

*Salmonella* resistance tendency is also similar to other EU countries. In Denmark 31 % of *Salmonella* poultry isolates are resistant to tetracycline, 39 % to ampicillin. In Belgium 17.5 % resistant to tetracycline, 25.2 % to ampicillin, 18.8 % to nalidixic acid. In Spain 33.9 % *Salmonella* isolates showed resistance to tetracycline, 34.8 % to ampicillin, 44.6 % to chloramphenicol. High resistance to ampicillin and tetracycline could be influenced by intensive usage of these antimicrobials. They have a wide spectrum of action, with a low side effect. But oral administration leaves possibility that concentration of antimicrobial could be at low level, and this is one of most important factors for resistance development.

**CONCLUSIONS**

1. *Salmonella* isolates from pork showed the highest resistance to tetracycline 48.9 %, less to chloramphenicol 18.4 % and florfenicol 14.3 %. High resistance to tetracycline is possible due to a long time use of it and resistant bacteria persistent on farms.

2. *Salmonella* isolates from poultry was most resistant to ampicillin 12.7 %, less to tetracycline 10.2 % and sulphanmethoxazole/trimethoprim 10.2 %.
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REFERENCES
8. NCCLS. Performance standarts for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Villanova, USA. 2002.
9. Modestas Ruzauskas, Marius Virgailis, Vytautas Spakauskas. Serological diversity and antimicrobial resistance of *Salmonella* isolated from different sources in Lithuania. Veterinarski Arhiv 2005. 75 (3), 211-221,