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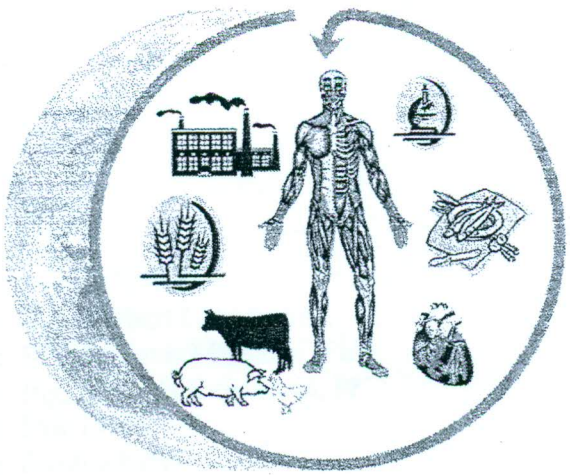

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ANTIBIOTIC RESISTANCE OF UBIQUITOUS BACTERIAL CENOSE ISOLATED FROM RECTAL SWABS OF CHICKEN

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Abstract

The aim of this study was to identified antibiotic resistance of ubiquitous bacterial cenose isolated from rectal swabs of chicken. The cultivation was done on a selective agars, for *Enterobacteriaceae* genera we used MacConkey agar and for *Enterococcus* spp. we used Slanetz-Bartley agar. Antibiotic susceptibility testing was done on the Muller-Hinton agar with this antibiotics: Streptomycin (S 10), Tetracycline (TE 30) and Ampicillin (AMP). For identification of this microorganisms we used ENTERO-test for *Enterobacteriaceae* genera and ENCOCCUS-test for *Enterococcus* spp. In this study, we determine that the highest resistance in *Enterococcus* spp. was to Streptomycin (86.66 %). The lowest resistance was to Tetracycline (46.66 %). We found *Enterococcus faecium* which resistant to Ampicillin and Tetracycline and *Enterococcus casseliflavus* which was resistant to Ampicillin by ENCOCCUS-test. In *Enterobacteriaceae* genera the highest resistance was to Tetracycline (80.00 %). The lowest resistance in this genera was to Ampicillin (0.00 %). From this genera, we found *Enterobacter cloacae*, *Escherichia coli* and *Raoultella ornithinolytica* which resistant to Streptomycin and Tetracycline. The use of antibiotics in commercial livestock increases resistance of bacteria against antibiotics. The results show that the bacteria can transfer resistance genes and their sporead not only in vertical but also horizontal way. Monitoring of resistance bacteria, we can track the presence of antibiotic use and choose the right antibiotic for treating diseases.

Key words: Antibiotic resistance, *Enterobacteriaceae* genera, *Enterococcus* spp., chicken

Introduction

Enterococcus are ubiquitous Gram-positive and *Enterobacteriaceae* genera bacteria are Gram-negative and that can be found in soil, food, and water while making up a significant portion of the normal gut flora of humans and animals (Murray, 1990). As some other bacteria of the gut cenose, *Enterococcus* spp. and *Enterobacteriaceae* are implicated in infectious diseases. They can cause urinary tract, intra-abdominal and pelvic infections, bacteremia, wound and tissue infections, and endocarditis (Kayser, 2003). The resistance of microorganisms to antibiotics is considered to be the major international public health problem and involves the fields of both human and veterinary medicine. It has been widely demonstrated that the use of antibiotics in animals can lead to the selection of resistant strains that colonize the intestines and are subsequently excreted, which may lead to contamination of the environment and of meats destined for human consumption (Nováková et.al., 2009). Endogenous bacterial cenose may play an important role as acceptor and donor of transmissible drug resistance genes (Davis, 1994; Sunde et.al., 1998). These microorganisms and their possible resistance determinants may be transmitted to humans if these foods are improperly cooked or otherwise mishandled (Bongers et.al., 1995; London et.al., 1994; Nijsten et.al., 1996).

The objective of our study was to determine and compare antibiotic resistance in ubiquitous bacterial cenose isolated from rectal swabs of chicken. Also determine species of ubiquitous bacteria from endogenous tract of chicken.

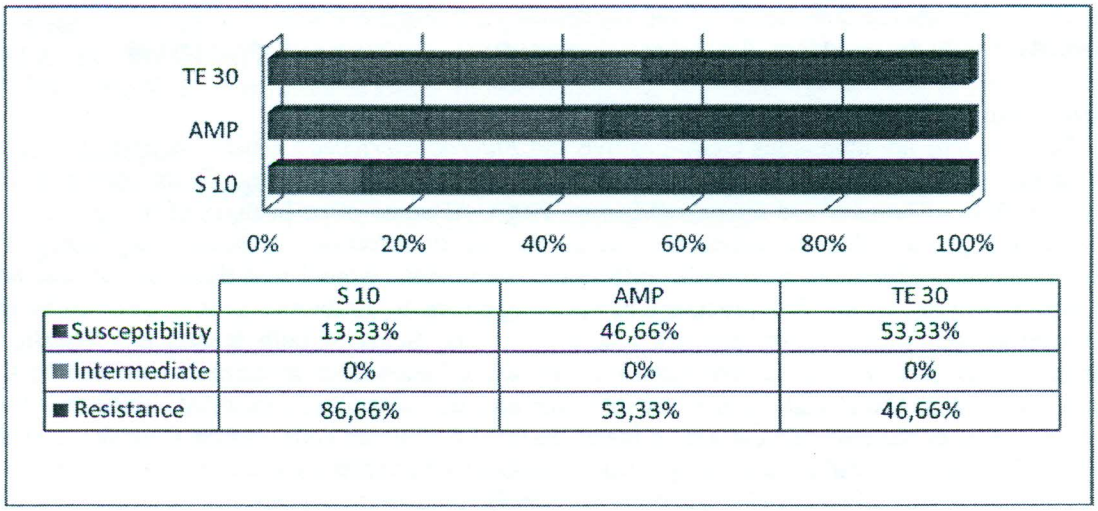
Material and methods

Antibiotics resistance study was done on *Enterococcus* spp. and *Enterobacteriaceae* isolated from rectal swabs of chicken. The bacterial strains were isolated from rectal swabs collected with a kit containing the swab (Copan Inovation, Brescia) and the transport in medium to laboratory. For cultivation of *Enterococcus* spp. Slanetz-Bartley agar (Biomark, Pune) and for *Enterobacteriaceae* Mac Conkey agar (Biomark, Pune) was used. Cultivation of these microorganisms was done during 24 hours for *Enterobacteriaceae* and 48 hours for *Enterococcus* spp. at 37 ± 1°C. The pure colonies were recultivation at the same conditions. The inoculum of *Enterobacteriaceae* and *Enterococcus* spp. 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 isolates was tested against: Streptomycin (S 10) 10μg/disk, Tetracycline (TE 30) 30 μg/disk and Ampicillin (AMP) 10μg/disk. We used disk diffusion methods (according to CLSI – Clinical and Laboratory Standards Institute) (**CLSI**). The incubation of strains with antibiotic disks was done on the Mueller-Hinton agar at the 37 ± 1°C. The interpretation of inhibition zones around the disk was according to CLSI 2004 Performance standards for antimicrobial susceptibility testing. The inhibition zones were controlled with reference strains *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212. Identification of strains was done by ENTEROtest 24 (Pliva, Lachema) and EN-COCCUStest (Pliva, Lachema).

Results and discussion

We studied antimicrobial drug resistance in commensal *Enterobacteriaceae* genera and *Enterococcus* spp. strains, which are considered a potentional reservoir for resistance genes in farms animal. On-farm reservoirs of resistant bacteria provide a potentional source for resistance gene transfer between bacteria as well as an environment for dissemination to new animals, environments and food products. Therefore, identifying these reservoirs and mechanisms of persistance will be a key to reducing the load of resistant bacteria in everywhere.

In our study, we identified resistant *Enterobacteriaceae* genera and *Enterococcus* spp. strains to antibiotics. *Enterococcus* spp. were the following results. The highest resistance was 86.66 % to S 10. The lowest resistance was 46.66 % to TE 30. The highest susceptibility was 53.33 % to TE 30 and the lowest susceptibility was 13.33 % to S 10. The other results are shown in figure 1.



	S 10	AMP	TE 30
Susceptibility	13,33%	46,66%	53,33%
Intermediate	0%	0%	0%
Resistance	86,66%	53,33%	46,66%

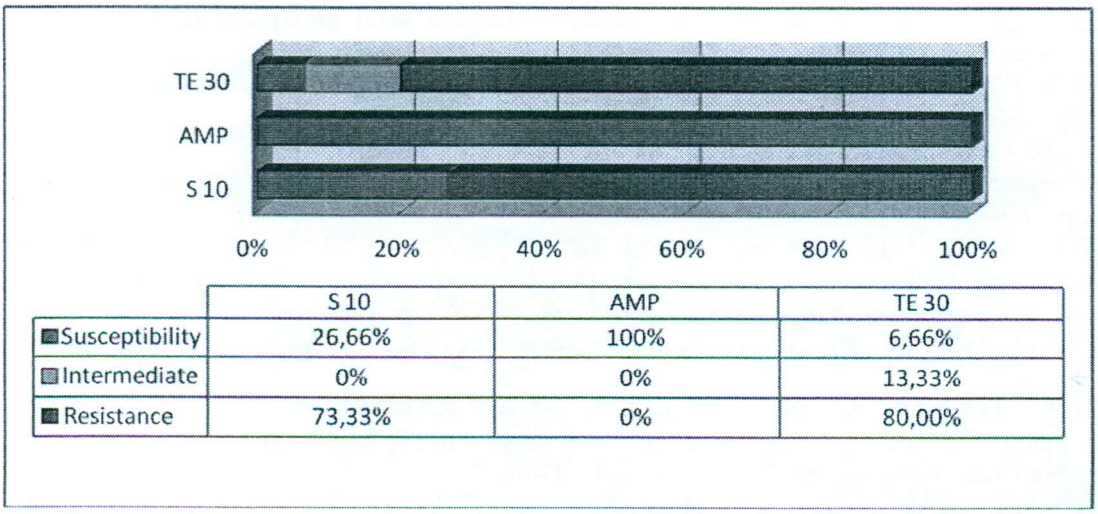
Figure 1 Antibiotic resistance profile of Enteroccus spp. isolated from rectal swabs of chicken

In this samples, we identified two strains, which were resistant to antibiotics we were used. We found *Enterococcus faecium* and *Enteroccus casseliflavus*. *Enterococcus faecium* was resistant to Ampicillin and Tetracycline. The similar results were A. Kasimoglu-Dogru et al. (2010) worth 39 % of resistant *Enterococcus faecium* to Tetracycline. *Enterococcus casseliflavus* was resistant to Ampicillin.

Table 1 Identified resistance strains of Enterococcus spp. isolated from rectal swab of chicken

Strains	Resistance to
Enterococcus faecium	Ampicillin, Tetracycline
Enterococcus casseliflavus	Ampicillin

Enterobacteriaceae genera were the following results. The highest resistance was 80.00 % to TE 30. The lowest resistance was 0.00 % to AMP. The highest susceptibility was 100.00 % to AMP. The lowest susceptibility was 6.66 % to TE 30. The intermediate resistance was 13.33 % to TE 30 only. Different results achieved J.M. Miranda et al. (2007) in their work. Resistance to Ampicillin was 48.30 % and susceptibility was 35.00 %. The other results are shown in figure no.2.



	S 10	AMP	TE 30
Susceptibility	26,66%	100%	6,66%
Intermediate	0%	0%	13,33%
Resistance	73,33%	0%	80,00%

Figure 2 Antibiotic resistance profile of Enterobacteriaceae genera isolated from rectal swabs of chicken

In this samples, we identified three strains, which were resistant to antibiotics we were used. We found *Enterobacter cloacae, Escherischia coli* and *Raoultella ornithinolytica.* J.M. Miranda et al. (2007) in their work indicated the similar species like in our work. For example, *Enterobacter* spp. (11 %) and *Escherischia* spp. (5 %). *Enterobacter cloacae* was resistant to Streptomycin and Tetracycline. *Escherischia coli* was resistant to Streptomycin and Tetracycline. *Raoultella ornithinolytica* was resistant to Streptomycin and Tetracycline too.

Table 2 Identified resistance strains of Enterobacteriaceae genera isolated from rectal swabs of chicken

Strains	Resistance to
Enterobacter cloacae	Streptomycin, Tetracycline
Escherischia coli	Streptomycin, Tetracycline
Raoultella ornithinolytica	Streptomycin, Tetracycline

Conclusion

The use of antibiotics in commercial livestock increases resistance of bacteria against antibiotics. We are create resistant strains, which causes the diseases. Such diseases are difficult to treat. The results show that the bacteria can transfer resistance genes and their spread not only in vertical but also horizontal. Monitoring of resistance bacteria, we can track the presence of antibiotic use and choose the right antibiotic for treating diseases. Though, antibiotic resistance is very different between strains and different from study to study.

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