Healthy Farms in Slovakia: Antibiotic Sensitivity of *Escherichia coli* Isolated from Rectal Swabs of Chicken and Ram

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Abstract

The aim of this experiment was determine of antibiotic resistance profile of *Escherichia coli* isolated from rectal swabs of chicken and ram from two different conventional breeding from Slovakia. For the antibiotic susceptibility testing disk diffusion method was used. A tested bacterium, Escherichia coli was exposed against four antibiotics: amikacin, gentamycin, tetracycline and chloramphenicol. For the identification of this strain, we used Chromogenic coliform agar, Triple sugar iron agar and biochemical test (ENTEROtest 24). For genetic identification of *Escherichia coli* Step One Real Time PCR with using special primer was used. Was determined that antibiotic resistance in *Escherichia coli* was not found. Was found susceptibility in all cases of *Escherichia coli* isolates. Antibiotic resistance is a biological danger. Bacteria, which we study, are considered to reservoirs of resistant genes and they are facultative and obligate pathogens. If these pathogen bacteria cause diseases those these diseases are difficult to treat. In this study, we determined that we have healthy farms in Slovakia too. In this farm antibiotic was not use and we do not determined any resistance to antibiotics, which we used in experiment.

Keywords: Antibiotic resistance, *Escherichia coli*, chicken, ram, rectal swabs

1. Introduction

Antibiotic resistance is significant health, social and economic problem at this time. Antibiotic resistance of bacteria is biological risk, which increases morbidity and mortality of animals and humans [1]. Keyser [2] note that in recent years accumulating problems with bacteria that are resistant to antibiotics occur. It is leading them to predictions that we return to the time before the discovery of antibiotics. One of the possibility could be the introducing of different antibacterial preparation, which used [3, 4] in their experiments. Most technologies in the production and food processing reduced the incidence of pathogens including resistant bacteria to antibiotics. Experimental monitoring confirmed that the treatment of food technology based on damage to cell membranes and enzymes may help to generate and transfer of antibiotic resistance [5-7]. The health safety of foods [8, 9], including meat is an integral part of consumers policy and health [10]. The use of antimicrobial agents in any venue, including therapeutically in human and veterinary medicine, or as prophylaxis for growth

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promotion in animal husbandry, ultimately exerts selective pressure favorable for the propagation of antimicrobial-resistant bacteria [11]. Resistant bacteria from the intestines of food animals may be transferred to retail meat products resulting from fecal contamination during various stages of the slaughter process (e.g. evisceration) and subsequent handling of animal tissue [12]. Endogenous bacterial flora may play an important role as acceptor and donor of transmissible drug resistance genes [13, 14]. *Escherichia coli* is commonly found in the intestinal tract of humans and animals [4, 15] and can also be implicated in human and animal infectious diseases [16]. Animal food products are an important and frequent source of *E. coli* as fecal contamination of carcasses at the slaughterhouse. These microorganisms and their possible resistance determinants may be transmitted to humans if these foods are improperly cooked or otherwise mishandled. The level of antibiotic resistance in *E. coli* represents a useful indicator of the resistance dissemination in bacterial populations. There are some reports in which antibiotic susceptibility of *E. coli* isolates from healthy humans [17, 18, 19] or animals [14, 20, 21, 22, 23] have been studied, but in few cases comparative results have been shown [14, 24] or isolates from foods analyzed.

The aim of this experiment was determine of antibiotic resistance profile of *Escherichia coli* isolated from rectal swabs of chicken and ram from two different conventional breeding from Slovakia.

2. Materials and methods

Collection of the samples and isolation of *Escherichia coli*

The samples were obtained from rectal swabs of chicken and ram from two different conventional farms in Slovakia. From these conventional farms it was obtained twenty samples of rectal swabs of chicken and twelve samples of rectal swabs of ram. The samples were collected by sterile cotton swabs (Copan Inovation, Brescia) and transported to the laboratory (SUA in Nitra, Department of Microbiology). *Escherichia coli* isolations were performed by a conventional plating method. The first step was done on the MacConkey agar (Biomark, Pune). Incubation was performing for 24 hours at 37°C±1. After incubation on the MacConkey agar, we used Chromogenic coliform agar (Biolife, Italy) and we chose the streak plate (four-ways) method for obtaining the pure colonies. Incubation was conducted for 24 hours at 37°C±1. This step was repeating until we had completely cleaned culture of *Escherichia coli*. After the incubation and identification it was isolated from each samples three pure colonies of *Escherichia coli*, so that it was sixty pure colonies from rectal swab of chicken and thirty-six pure colonies from rectal swab of ram.

The biochemical identification of *Escherichia coli*

Method on the Triple sugar iron agar (Biolife, Italy) for the basic biochemical identification of *Escherichia coli* and ENTEROtest 24 (Pliva-Lachema, Brno), including TNW Lite 7.0 identification software (Pliva-Lachema, Brno) for more detailed biochemical identification was used. Preparation of identification plates of ENTEROtest 24 was done inside the Laminaire box (ADS Laminaire, Le Pre-Saint Gervais) to ensure the high sterility, less risk of contaminations from air and for precise results. Working procedure of ENTEROtest 24 is described into the manufacturer manual.

The isolation of DNA from *Escherichia coli*

The pure colonies of *Escherichia coli* were subjected to DNA isolation using PrepSEQ™ Rapid Spin Sample Preparation Kit (Applied Biosystem, USA). Complete working procedure is described into the manufacturer manual.

General Sample Preparation Protocol

Sample of 750 μL was loaded onto the spin column and microcentrifuged for 3 minutes at maximum speed (12000 rpm). Supernatant was discarded and 50 μL of Lysis Buffer was added to the pellet. Samples were incubated for 10 minutes at 95°C. The samples after incubation were added to cool for 2 min at room temperature. Then were added 250 μl of water to samples. After, the samples were centrifuged one minute at maximum speed (12000 rpm).

Identification of *Escherichia coli* by Real time PCR

PCR reaction in Step ONE® Real time PCR for a genetic identification of *Escherichia coli* was

used. In PCR reaction specific primer was used, which was designed by Hsu and coworkers [25]. Also, their PCR protocol was followed.

Primers:
Emdh1: 5´- ACTGAAAGGCAAACAGCCAAG - 3´ (1123–1144),
Emdh2: 5´- CGTTCTGTTCAAATGGCCTCAGG - 3´ (1514–1492).

Molecular weight of the expected PCR product is 392 bp. The correct lenght of PCR product was evaluated by electrophoresis gel and it was visualised.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by disk diffusion method according Eucast [26]. Antibiotic disks were used (Oxoid, England). The pure inoculum of strain of *Escherichia coli* were prepared by suspending of colonies from the agar plates in physiological solution and the suspension was adjusted to equal a 0.5 McFarland standard. We streaked 100 µl suspensions to plates surface and we spreaded over surface of agar thoroughly. Antimicrobial susceptibility testing was performed according to the manufacturer's instructions. The following antimicrobials were tested: amikacin (AK 30) 30 µg.disk^{-1}, gentamycin (CN 10) 10 µg.disk^{-1}, tetracycline (TE 30) 30 µg.disk^{-1} and chloramphenicol (C 30) 30 µg.disk^{-1}. The incubation of strains was performing for 16-18 hours at the temperature 37 °C. The interpretation of inhibition zones around the disk was done according to EUCAST 2009. The inhibition zones were controlled with the reference of *Escherichia coli* ATCC 25922. Statistical evaluation of inhibition zones around the disk was done by STATGRAPHICS.

3. Results and discussion

We studied antibiotic resistance in *Escherichia coli*, which are considered to be potential reservoirs for resistant genes in animal farms. Farm reservoirs of resistant bacteria provide potential sources for resistant genes transfer between bacteria as well as an environment for dissemination to new animals, environment and food products. Finally, pathogenic bacteria can get into the human body and cause diseases, which is difficult to treat. Therefore, identifying these reservoirs and mechanisms of persistence could be a key to reducing the load of resistant bacteria everywhere.

In our experiment was studied antibiotic resistance profile of *Escherichia coli* isolated from rectal swabs of chicken and ram from two conventional farms from Slovakia. We determined that antibiotic resistance profile in *Escherichia coli* was not found. We found susceptibility in all cases of *Escherichia coli* isolates. The highest susceptibility was found in *Escherichia coli* isolates isolated from rectal swabs of chicken and ram. Susceptibility to amikacin was 100 %, gentamycin (100 %), chloramphenicol (100 %) and to tetracycline (100 %). Results are shown into Figure 1.
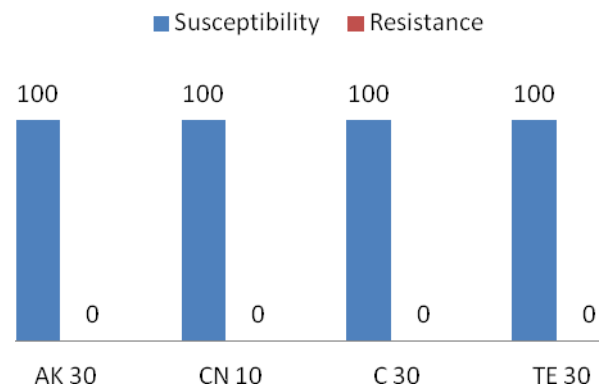

Figure 1. Antibiotic resistance profile in *Escherichia coli* isolated from rectal swabs of chicken and ram

Conversely, [27] found resistant *Enterobacteriaceae* genera to gentamycin and to chloramphenicol only 6.7%. During recent years, several studies have reported the antimicrobial resistance of some *Enterobacteriaceae* genera isolated from poultry, such as *Escherichia coli* [24, 28-33]

Table 1 Summary statistics for inhibition zones of *E. coli* isolated from rectal swabs of chicken

	AK 30	C 30	CN 10	TE 30
Count	60	60	60	60
Average	20.4	26.8	22.05	26.0
Standard deviation	0.875595	1.61933	1.14139	0.666667
Coeff. of variation	4.29213%	6.04227%	5.17639%	2.5641%
Minimum	19.0	24.0	20.5	25.0
Maximum	22.0	29.0	24.0	27.0
Range	3.0	5.0	3.5	2.0

Table 2 Summary statistics for inhibition zones of *E. coli* isolated from rectal swabs of ram

	AK 30	C 30	CN 10	TE 30
Count	36	36	36	36
Average	20.25	26.6667	22.0	26.1667
Standard deviation	0.758288	1.50555	0.707107	0.752773
Coeff. of variation	3.74463%	5.64579%	3.21412%	2.87684%
Minimum	19.0	25.0	21.0	25.0
Maximum	21.0	28.0	23.0	27.0
Range	2.0	3.0	2.0	2.0

The several researches like [34-37], who researched antibiotic resistance in *E. coli*, respectively in *Enterobacteriaceae* genera isolated from different products have argued, that results of antibiotic resistance vary from study to study.

Statistical evaluation of inhibition zones, we determined that the greatest variability was in the samples from rectal swabs of chicken, when we used chloramphenicol (6.04 %). Minimum and maximum values of inhibition zones ranged from 24 to 29 mm and average of inhibition zone was 26.8 mm. The lowest variability was in the samples from rectal swabs of chicken, when we used tetracycline (2.56 %). Minimum and maximum values of inhibition zones ranged from 25 to 27 mm and average of inhibition zone was 26 mm. Others variation-statistical values are shown in the Table 1.

The greatest variability was in the samples from rectal swabs of ram, when we used chloramphenicol (5.64 %). Minimum and maximum values of inhibition zones ranged from 25 to 28 mm and average of inhibition zone was 26.66 mm. The lowest variability was in the samples from rectal swabs of ram, when we used tetracycline (2.87 %). Minimum and maximum values of inhibition zones ranged from 25 to 27 mm and average of inhibition zone was 26.16 mm. Others variation-statistical values are shown in the Table 2.

4. Conclusion

Using of antibiotics in livestock farming cause that more and more obligatory and facultative pathogens are resistant to various antibiotics used commercially. Our experiment results show that antibiotics was not use in this breeding and rearing were not introduced into the external environment also, that is positive information for end consumers. Results confirm that antibiotic resistance was not detected in *Escherichia coli* isolates. It is very important in commercial breeding to observe of sanitation and hygiene conditions. Meats and eggs are end products, which are also used in human food chain. If bacteria not contain resistant genes, which are part of food or contaminants, then it means good news for end consumers. Because, if coliforms bacteria including *Escherichia coli* are resistant to undesirable reproducing, then it may cause consumers infections and diseases, which are then difficult to treat. For diseases caused by resistant bacteria are antibiotic unnecessary and useless. Therefore, the monitoring of resistant bacteria is needed to reduce or eradicate this global problem.

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