# Antibiotic Resistance of *Enterobacteriaceae* Species Associated with Faecal Bacterial Cenosis of Ducks

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**Abstract**
The aim of this study was monitoring of antibiotic resistance of *Enterobacteriaceae* genera isolated from rectal swabs of ducks during seven weeks. For the antibiotic susceptibility testing disk diffusion method was used. *Enterobacteriaceae* genera were tested against four antibiotics: ampicillin, tetracycline, streptomycin and chloramphenicol. For the detection and identification of each species, we used Chromogenic coliform agar, Triple Sugar Iron agar and biochemical test (ENTEROtest 24). The highest resistance was determined in isolates of *Enterobacteriaceae* genera to tetracycline (32.43%). To streptomycin and to ampicillin resistance reached 8.10% and to chloramphenicol 5.40%. From rectal swabs of ducks during seven weeks following species were isolated: *Escherichia coli*, *Yersinia enterolytica*, *Enterobacter aerogenes*, *Citrobacter freundii* and one non-enterobacteriaceae specie *Pseudomonas aeruginosa*. Each of this species were resistant to monitored antibiotics. Antibiotic resistant bacteria are a biological danger in relation to animal and human health.

**Keywords**: Antibiotic resistance, *Enterobacteriaceae*, faecal, ducks

## 1. Introduction

*Enterobacteriaceae* genera are ubiquitous Gram-negative bacteria which can be found in soil, food, and water while making up a significant portion of the normal gut bacterial cenose of humans and animals [1]. As some other bacteria of the gut bacterial cenose, *Enterobacteriaceae* are implicated in infectious diseases. They can cause urinary tract, intra-abdominal and pelvic infections, bacteremia, wound and tissue infections, and endocarditis [2]. 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 [3]. 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 [4,5]. Endogenous bacterial cenose may play an important role as acceptor and donor of transmissible drug resistance genes [6,7]. These microorganisms and their possible resistance determinants may be transmitted to humans if these foods are improperly cooked or otherwise mishandled [8-11] note that in recent years, accumulating problems with resistant bacteria, leading to predictions that we are back the period before the discovery of antibiotics. One of way around this problem is to introduce new antibacterial preparation which operates on a locking mechanism of virulence. More precisely, a type III (T3SS) secretion system. Also, one way to avoid this may be the introduction of different antibacterial preparation, which used [12,13] in their experiments. Infections caused by resistant strains of microorganisms causing costly treatment of animals and humans. Such infections prolong the pathological condition and if not treated with the right antibiotics may be increased mortality [14].

The objective of our study was to determine the antibiotic resistance in ubiquitous bacterial cenose

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isolated from rectal swabs of ducks during seven weeks, as well as determine species spectrum of ubiquitous bacteria from endogenous tract of ducks.

## 2. Materials and methods

Antibiotic resistance study was done in *Enterobacteriaceae* genera isolated from rectal swabs of ducks from conventional breeding from Slovakia. From ducks breeding farm were obtained 37 samples. Samples were collected in the first day, the first week, second week, third week, fifth week and in seventh week of raring. In our study, we identified strains which were resistant and susceptible to antibiotics. We compared antibiotic resistant in *Enterobacteriaceae* genera during seven weeks of breeding, including the first day. Bacterial strains were isolated from rectal swabs of ducks and collected with a kit containing the swab (Copan Inovation, Brescia) and transported in medium to laboratory Department of Microbiology, Faculty of Biotechnology and Food Science in Slovak University of Agriculture in Nitra. Samples were suspended in physiological solution. For cultivation of *Enterobacteriaceae* genera MacConkey agar (Biomark, Pune) was used. The pure inoculum of strains of *Enterobacteriaeceae* genera was prepared by suspending of colonies from the agar plates and suspension was adjusted to equal a 0.5 McFarland standard. The sensitivity of all strains of *Enterobacteriaceae* genera was tested against: tetracycline (TE 30) 30 µg.disc$^{-1}$, ampicillin (AMP 10) 10 µg.disc$^{-1}$, chloramphenicol (C 30) 30 µg.disc$^{-1}$ and streptomycin (S 10) 10 µg.disc$^{-1}$. We used disc diffusion methods according [15] (EUCAST – European committee on antimicrobial susceptibility testing). The incubation of strains was done at the temperature 37 °C. The interpretation of inhibition zones around the disc was according to EUCAST. The inhibition zones were controlled with the reference *Escherichia coli* ATCC 25922. Initial identification of strains of *Enterobacteriaceae* genera were done on the Triple sugar iron agar iso (Biolife, Italiana). Biochemical identification of strains of *Enterobacteriaceae* genera was done by ENTEROtest 24 (Pliva, Lachema). Evaluation of biochemical tests was done in identifying computer program TNW Lite 7.0 software (Pliva, Lachema). From the obtained data we calculated using statistical program STATGRAPHICS basic variation-statistical values like average, standard deviation, minimum, maximum, coefficient of variation and frequency of the size of inhibition zones.

## 3. Results and discussion

### Antibiotic resistance profile of *Enterobacteriaceae*

*Enterobacteriaceae* genera are very important group of bacteria for clinical microbiology, because this group includes facultative and obligate pathogens. Many scientists considered *Enterobacteriaceae* genera as a reservoir of resistant genes and frequent contaminant of food and water. Finally, resistant bacteria can be transfered to animal and human intestinal tract where bacteria can receive resistant genes.

In our experiment, we determined resistance of bacteria from *Enterobacteriaceae* genera to tetracycline (32.43%), to streptomycin and to ampicillin on the same level of 8.10% and to chloramphenicol (5.40%) from all samples irrespective of sampling time (figure 1).

Comparison of resistance depending on the age of ducks was as follows: in the first day immediately after hatching was resistance to tetracycline (33.33%), to streptomycin (33.33%), to chloramphenicol (16.66%) and to ampicillin (0.00%). A week later decreased resistance to tetracycline and streptomycin to 20.00% and in case of chloramphenicol and ampicillin 0.00%.

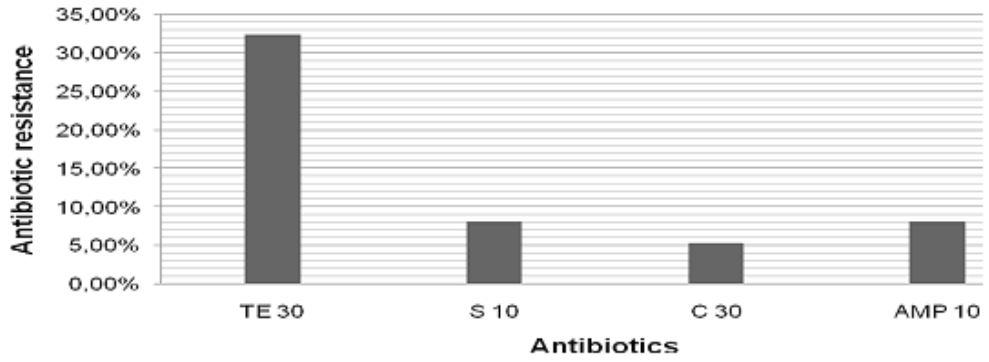


**Figure 1:** Antibiotic resistance profile of *Enterobacteriaceae* isolated from rectal swabs of ducks

In the second week of breeding resistance to tetracycline was again 33.33%, but zero resistance was detected to streptomycin, chloramphenicol and ampicillin. Resistance to tetracycline in the third week increased to 37.50% and resistance to streptomycin, chloramphenicol and ampicillin remained at 0.00% level. In the fifth week was resistance to tetracycline (33.33%), to streptomycin (0.00%), to chloramphenicol (16.66%) and in case ampicillin increased resistance to 50.00%. In the seventh week remained resistance to tetracycline (33.33%) and in cases streptomycin, chloramphenicol and ampicillin decreased or remained to 0.00%. Results are shown in the figure 2.

**Antibiotic resistance profile of individual species of isolated bacteria**

Most represented isolates among all samples were *Escherichia coli* isolates (n = 16). *Escherichia coli* was resistant to tetracycline (37.50%), to streptomycin (12.50%), to ampicillin (6.25%) and to chloramphenicol (0%). Tatsuya et al. [16] reported higher resistance in *E. coli* isolated from ducks. They determined that resistance to tetrycycline was 69.4%, to streptomycin 29.4%, to ampicillin 24.3% and resistance to chloramphenicol was 3.0%. Even higher values of resistance in *E. coli* isolated from ducks reported Tao et al. [17] in 2010. Their experiment shown that *Escherichia coli* was resistant to ampicillin (87.8%), to chloramphenicol (84.4%) and to tetracycline (96.6%). *Klebsiella pneumoniae* (n = 11) was the second most representation isolate in our experiment. From the eleven isolates, we determined that *Klebsiella pneumoniae* was resistant only to tetracycline (36.36%). Resistance of *Klebsiella pneumoniae* to the rest of monitored antibiotics was not detected. Also Shahid et al. [18] examined antibiotic resistance in *Klebsiella pneumoniae* but not from ducks. They determined that resistance to tetracycline was about 10%. Resistance to antibiotics has not been detected in case *Yersinia enterolytica* isolates (n = 4). In the all isolates of *Yersinia enterolitica* were 100% sensitive. In case of other bacterial strains detected, few numbers of these isolates not permitted to count resistance level.

These isolates includes *Enterobacter aerogenes* (n = 3), *Citrobacter freundii* (n = 2) and non *Enterobacteriaceae Pseudomonas aeruginosa* (n = 1). Ogasawara et al. [19] isolated multiresistant *Pseudomonas aeruginosa* from domestic animals, food and human, which was resistant to streptomycin (69%), to tetracycline (98%), to ampicillin (100%) and to chloramphenicol (100%).

**Spectrum of species and their resistance ratio**

The first collection of samples was done immediately after hatching. In the samples of the first day, we isolated four *Escherichia coli* isolates of which were two isolates resistant to tetracycline and one isolate to streptomycin. Also, we identified one colony of *Klebsiella pneumoniae*, which was in the first day of collection sensitive to antibiotics. The next strain, which we isolated from rectal swabs of ducks from the first day was one colony of *Citrobacter freundii*. It was resistant to streptomycin and chloramphenicol. After the first week we isolated three *Escherichia coli* isolates of which one isolate was resistant to tetracycline and streptomycin. One colony of *Klebsiella pneumoniae* was sensitive to antibiotics. One colony of *Citrobacter freundii* was a sensitive to antibiotics, which we used. After the second week we isolated one colony of *Escherichia coli*, which was sensitive to antibiotics.

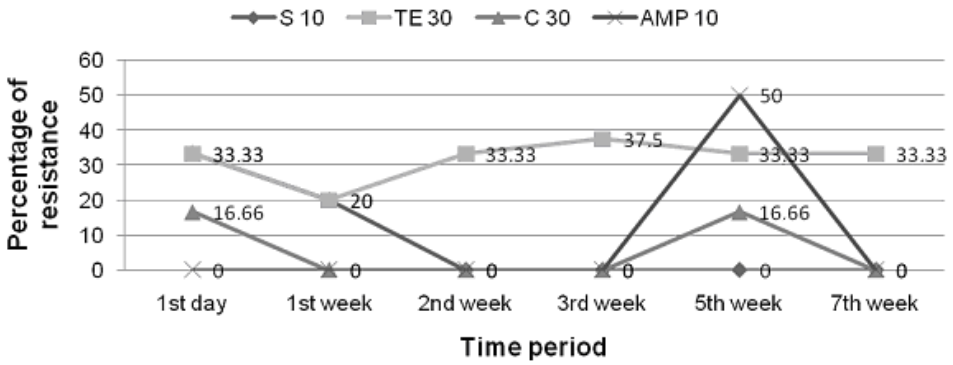


**Figure 2.** Development of antibiotic resistance of *Enterobacteriaceae* during seven weeks of rearing

Four colonies of *Klebsiella pneumoniae* was isolated in the second week of which two isolates was resistant to tetracycline. One colony of *Enterobacter aerogenes* was isolated from the second week too and resistance to antibiotics was not detected. In the third week we isolated three *Klebsiella pneumoniae* isolates of which two isolates were resistant to tetracycline. Also, in the third week were isolated four *Yersinia enterolitica*, which were sensitive to antibiotics. One colony of *Enterobacter aerogenes* was resistant to tetracycline in the third week. After the fifth week we isolated three *Escherichia coli* isolates of which one was resistant to tetracycline and ampicillin. One colony of *Klebsiella pneumoniae* was isolated of fifth week from rectal swabs of ducks, which was sensitive. One colony of *Enterobacter aerogenes*, which was isolated in the fifth week was resistant to ampicillin. Also, we isolated one colony of non *Enterobacteriaceae Pseudomonas aeruginosa*, which was resistant to tetracycline, chloramphenicol and ampicillin. In the seventh week we isolated five colonies of *Escherichia coli* of which two isolates was resistant to tetracycline. One colony of *Klebsiella pneumoniae* was sensitive to antibiotics. Other samples, which were not mentioned in the text were sensitive to antibiotics used. For better representation of results are shown in Table 1. Alan M. Fuge [20] in 2001 did similar isolates and identification from wild bird. He isolated and identified similar species like we from the intestinal tract of bird, including *Escherichia coli, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa and Yersinia* spp.

**Statistical evaluation of inhibition zones of *Enterobacteriaceae* genera**

For the statistical evaluation of the size of inhibition zones, we used statistical program STATGRAPHICS and calculated the basic statistical values. Average of the size inhibition zone of ampicillin was 20.65±4.76 mm with minimun 7 mm and maximum 30 mm. For the chloramphenicol was average of the size of inhibition zone 28.57±5.11 mm with minimum 9 and maximum 34 mm. In the case of streptomycin was average of the size of inhibition zone 18.51±3.38 mm with minimum 9 mm and maximum 24 mm. Average of the size of inhibition zone of tetracycline was 15.62±5.27 mm with minimum 7 mm and maximum 23 mm.

With the help of statistical programe STATISTICA histograms of frequency of the size of inhibition zones for each antibiotics were made. In these histograms is true that the more closely a range of frequency of the sizes of inhibition zones, the population of bacteria is more balanced in antibiotic resistance. The most frequent size of inhibition zone of ampicillin was about 20 to 22 mm. Frequency of inhibition zone coincides with the average (20.65 mm). The sizes of inhibition zones in relation to resistance were balanced. Results are shown in Figure 3. In the histogram of the size of inhibition zones of chloramphenicol is visible, that average (28.57 mm) is similar to most frequent size of inhibition zone (28-30 mm). This histogram of chloramphenicol was the most balanced. This means that bacteria were the most balanced in relation to antibiotic resistance too. The detail results are shown the Figure 4. The most frequent size of inhibition zones of streptomycin was about 18 and 20 mm. Frequency of inhibition zone coincides with the average (18.51 mm). The sizes of inhibition zones in

relation to resistance were not balanced, thus bacteria were not in terms of antibiotic resistance homogenous. Concrete results are shown in the Figure 5. Final histogram of tetracycline shows that average is not similar with the sizes inhibition zones, because it has two peaks of inhibition zones. This histogram is divided into two parts and the both parts are balanced. This fact indicates the balance between resistant bacteria and susceptible bacteria. The detail results are shown in the Figure 6.

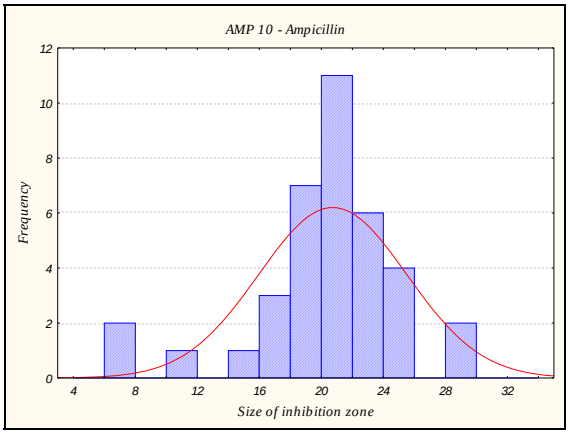


**Figure 3** Frequency of the size of inhibition zones and average (top of line) of ampicillin

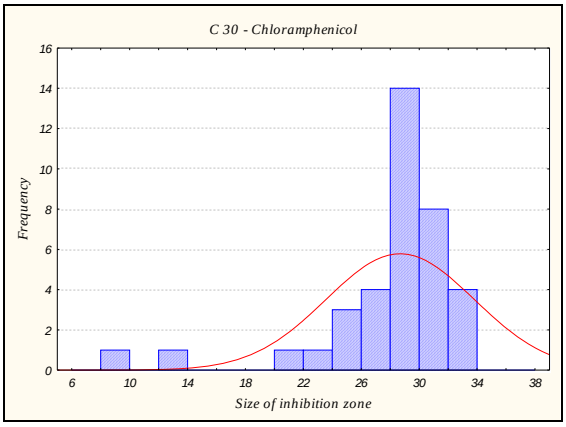


**Figure 4** Frequency of the size of inhibition zones and average (top of line) of chloramphenicol

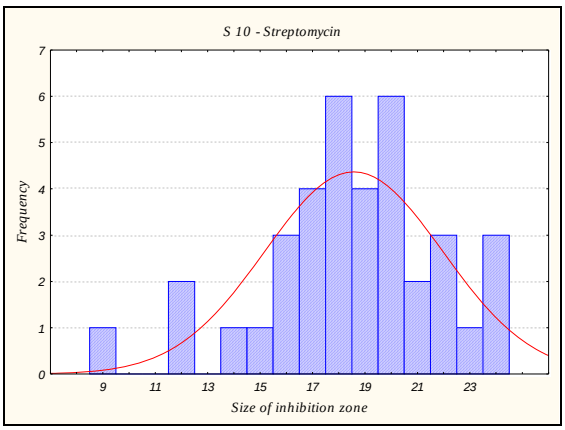


**Figure 5.** Frequency of the size of inhibition zones and average (top of line) of streptomycine

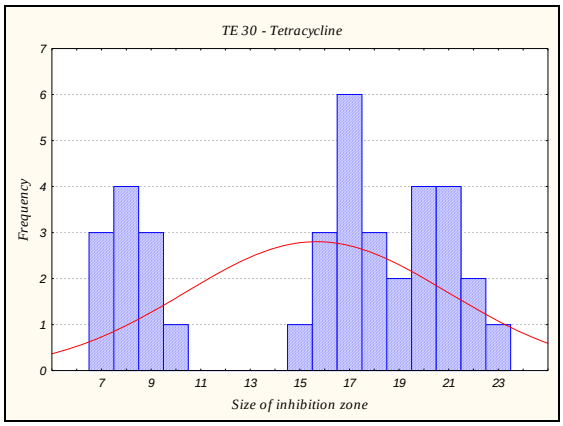


**Figure 6.** Frequency of the size of inhibition zones and average (top of line) of tetracycline

## 4. Conclusions

Antibiotic resistant genes in pathogens bacteria endanger animal and human health. Our results confirmed well known fact of loosing ability of resistance in bacteria, when not exposed to antibiotics. Specifically in the case of streptomycin. Some genes of resistance survive and spread in environment as visible from results for tetracycline. Also, from results can see that humans act very important role in transfer of resistant genes, because antibiotics were not used in this breeding. However, antibiotic resistance or resistant genes may rearing from external environment or animal food. Genes surviving in the environment are the most dangerous and can be transferred from environment to animal, then to food and finally to human. Infection, which can be causes of resistant bacteria is then difficult to treat and is very costly.

**Table 1** Presence of resistant and sensitive strains isolated from rectal swabs of ducks during seven weeks

| Isolated species | | Resistance and susceptibility of isolated strains ATB disk(number of resistant and sensitive cases/number of all ATB disk) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1st day | 1st week | 2nd week | 3rd week | 5th week | 7th week |
| *Escherichia coli* | R | TE30,S10 (3/16) | TE30, S10 (2/12) | n | n | TE30, AMP10 (2/12) | TE30 (2/20) |
| | S | C30, AMP10 (13/16) | C30, AMP10 (10/12) | TE30, S10, C30, AMP10 (4/4) | n | S10, C30 (10/12) | S10, C30, AMP10 (18/20) |
| *Klebsiella pneumoniae* | R | n | n | TE30 (2/16) | TE30 (2/12) | n | n |
| | S | TE30, S10 C30, AMP10 (4/4) | TE30, S10 C30, AMP10 (4/4) | S10, C30, AMP10 (14/16) | S10, C30, AMP10 (10/12) | TE30, S10 C30, AMP10 (4/4) | TE30, S10 C30, AMP10 (4/4) |
| *Yersinia enterolitica* | R | n | n | n | n | n | n |
| | S | n | n | n | TE30, S10 C30, AMP10 (16/16) | n | n |
| *Enterobacter aerogenes* | R | n | n | n | TE30 (1/4) | AMP10 (1/4) | n |
| | S | n | n | TE30, S10 C30, AMP10 (4/4) | S10, C30, AMP10 (3/4) | TE30, S10, C30 (3/4) | n |
| *Citrobacter freundii* | R | S10, C30 (2/4) | n | n | n | n | n |
| | S | TE30, AMP10 (2/4) | TE30, S10 C30, AMP10 (4/4) | n | n | n | n |
| *Pseudomonas aeruginosa* (non *Enterobacteriaceae*) | R | n | n | n | n | TE30, C30, AMP10 (3/4) | n |
| | S | n | n | n | n | S10 (1/4) | n |

Legend: S – susceptibility, R – resistance, n – no isolates, ATB – antibiotics (TE30 – tetracycline, S10 – streptomycin, C30 – chloramphenicol, AMP10 – ampicillin)

**Table 2** Statistical values of the size of inhibition zones

*Summary Statistical Values*

| | AMP 10 | C 30 | S 10 | TE 30 |
|---|---|---|---|---|
| Count | 37 | 37 | 37 | 37 |
| Average | 20.65 | 28.57 | 18.51 | 15.62 |
| Standard deviation | 4.76 | 5.11 | 3.38 | 5.27 |
| Coeff. of variation | 0.23 | 0.18 | 0.18 | 0.34 |
| Minimum | 7.00 | 9.00 | 9.00 | 7.00 |
| Maximum | 30.00 | 34.00 | 24.00 | 23.00 |
| Range | 23.00 | 25.00 | 15.00 | 16.00 |

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