

8. Summary

Bacteria *Escherichia coli*, belonging to *Enterobacteriaceae* family, represent permanent and dominant microflora of mammal and birds' intestinal tract, colonise large intestine directly after birth, stay there through the whole life of the organisms. They exist inside the body and perform many important functions, for example, they take part in producing vitamins from groups: B, C, K and folic acid, they help in food digestion, and slow down the growth of pathogenic bacteria in the host organism. However, numerous external factors, such as: stress, inappropriate diet change, lack of disinfection and hygiene and antibiotic therapy can affect the physiological imbalances of intestinal microflora, contributing to the proliferation of pathogenic *E. coli* strains, and consequently to the development of the disease called Colibacillosis.

The majority of studies in domestic and foreign literature refers to the pathogenic *E. coli* strains isolated from people, cattle and pigs, however, only few publications refer to APEC (*Avian Pathogenic Escherichia coli*) strains, responsible for the development of infections in birds called as colibacillosis. APEC are responsible for the occurrence of pericarditis, airsacculitis, persistent diarrhea, omphalitis, septicemia and many other diseases, posing the cause of significant economic losses in poultry farming, especially turkey, chicken, duck and goose.

Numerous studies concerning pathogenic *E. coli* in poultry showed that they belong to serological groups: O1, O2, O8, O15, O18, O78, O115, among which the most frequent were: O1, O2, O78, resulting in colibacillosis caused by APEC. The pathogenic ability of strains in poultry depends on their invasiveness and ability to produce toxins. Toxinogenic strains cause the poisoning of the organism through the production of numerous toxins in intestines, for example, toxins: LT-I, ST-I, ST-II, fimbriae: 41, F4, F6, F18, F5, intimin, and also Shiga toxins, coming in two varieties: Stx1 and Stx2, resulting in hemorrhagic enteritis. Due to the mechanism of pathogenic effect and virulence markers within pathogenic *E. coli* strains we distinguish: enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli*/Enterohemorrhagic *E. coli* (STEC/EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC, EA_gEC), diffusely adhering *E. coli* (DAEC), adherent-invasive *E. coli* (AIEC).

Antibiotic therapy is traditionally used in the treatment of infections caused by APEC strains, however, its efficiency is minimal, because the phenomenon of drug resistance

among microorganisms is commonly increasing. Moreover, within the last two decades the increasing number of microorganisms is becoming multiresistant (Multiple Drug resistance - MDR). Particular bacteria strains are resistant to several antibiotic groups at the same time. One of the most epidemiologically and clinically important mechanisms of resistance to medicines in *E. coli* strains, is producing extended-spectrum beta lactamases (ESBL), decaying penicillin, I-III generation of cephalosporins, monobactams and showing poor activity against cephamycins.

With a view to significant threat resulting from the occurrence of multi-resistance among bacteria causing colibacillosis in poultry, the aim of this doctoral dissertation was isolation and complex phenotypic and genotypic characterization of *E. coli* strains, isolated from different species of poultry emphasizing serological classification of examined strains and their resistance to particular antibiotics.

In the first part of the study seventy-three *E. coli* strains coming from different species of dead birds were isolated on microbiological media. Next, macroscopic evaluation of isolates, and tests for the presence of catalase enzyme were conducted. Then, the morphology of single cells in preparations dyed according to Gram method was examined. Species affiliation of the examined isolates was additionally confirmed by the method of mass spectrometry MALDI-TOF MS. There was conducted serological classification of the examined *E. coli* strains using the monospecific test sera O1, O2, O78 for somatic antigen O. Next, antibiotic sensitivity was assessed in relation to obtained bacterial isolates using the method of MIC (Minimal Inhibitory Concentration), applying commercial antibiogram plates containing 25 antibiotics in different concentration. Genetic analysis of pathogenic *E. coli* strains concerning occurrence of the genes coding virulence factors (toxins: Stx1 and Stx2, fimbriae: F41, F4, F5, F6, F18, intimin, LT-I, ST-I, ST-II) and antibiotic-resistance genes (including: beta-lactams, aminoglycosides, tetracyclines, phenicols, trimethoprim and sulfonamides) by the PCR and multiplex PCR methods was also conducted.

In terms of morphology, we used Columbia Agar with sheep blood and isolated big, shiny, grey *E. coli* colonies, dying Gram-negative pink and producing catalase enzyme. Moreover, species affiliation of all 73 strains was confirmed using the method of mass spectrometry MALDI-TOF MS. The conducted analysis showed that the highest percentage of strains belonged to serotype O78, next to serotype O2, however, the most rare was serotype O1. The analysed *E. coli* strains had different antibiotic resistance. The most effective antibiotics working against examined *E. coli* strains were: cefquinome, ceftiofur, gentamicin, colistin, cephalexin and neomycin. Moreover, all of the tested isolates of *E. coli* were resistant to:

nafcillin, penicillin G, erythromycin, tylosin, tylvalosin, lincomycin and tiamuline. In the population of the examined *E. coli* strains there were multi-drug-resistant isolates, showing resistance to at least 8 out of 25 tested antibiotics. It has been proved that the most common gene of resistance was *dhfrV*. Moreover, genes coding toxins Stx1 were detected in 3 isolates, and those coding fimbriae F18 were found in 2 isolates.

Poultry infected with pathogenic *E. coli* strains pose the threat also for human. Even 10-50 bacteria, present in our meal can lead to the infection threatening one's health or life. Pathogenic *E. coli* strains coming from chicken meat colonise large intestine through toxins, destroy intestinal villi, kidney cells, and can also be the cause of hemolytic uremic syndrome (HUS).

To sum up, based on the results obtained we should emphasise the necessity to carry out the research concerning detailed identification and characterisation of *E. coli* type bacteria with the special emphasis on their virulence and drug-resistance, which will undoubtedly to widen knowledge of the possibility to limit the prevalence of the risks to animals and poultry consumers.