Egyetemi doktori (PhD) értekezés tézisei

Epidemiology, antimicrobial resistance and virulence profiles of avian enteropathogenic *Escherichia coli* (EPEC).

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Introduction

E. coli is a widely distributed, mostly commensal bacterium which take part in the construction of the normal gut microbiota and maintaining its harmony. However, some *E. coli* strains are pathogens which can infect humans and animals as well.

There are two groups of *E. coli* depending of place of their infection, namely extraintestinal and intestinal. Extraintestinal group consists of meningitis-associated *E. coli* (MNEC), uropathogenic *E. coli* (UPEC) and avian pathogenic *E. coli* (APEC). Intestinal group comprises enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli (*EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC) and adherent-invasive *E. coli* (AIEC).

EPEC, EHEC, ETEC, EAEC and EIEC may have veterinary importance or can cause infection from the intestinal pathogenic *E. coli* groups. These pathotypes of *E. coli* were described in poultry in the last few years and articles shared more or less information about them (Alonso et al., 2011; Dorigeraee et al., 2016; Krause et al., 2005; Lee et al., 2009; Wang et al., 2017).

These intestinal pathogenic *E. coli* strains may have zoonotic potential and it can mean public-health risk from food producing poultry. EPEC are very important group from the aforementioned pathotypes which was first written as human enteral pathogen causing infantile diarrhea (Neter, 1965). All EPEC strains have close relationship with the EHEC strains which are important zoonotic bacteria. EHEC strains can be constructed from EPEC strains by infection with stx toxin gene carrying bacteriophages (Tóth et al., 2003). Therefore, the differentiation of the members of these two pathogroups is important in case of consideration of their possible outcome of the caused infection.

The EPEC group is not uniform. It contains two subtypes based on their patomechanism where the capability of bundle forming pilus (bfp) production is crucial. Typical EPEC strains (tEPEC) have ability to produce bfp and atypical EPEC strains (aEPEC) cannot harbor the gene for bfp production. Therefore, the aEPEC

strains use another type of adhesins to connect themselves to the intestinal cells. Typical EPEC strains mainly infect humans and rabbits. Nonetheless, aEPEC strains are widespread in animal species like in humans. Therefore, aEPEC can be the source of infection of humans and animals as well. In the last few years the infection by tEPEC decreased sharply and its position was replaced by the aEPEC caused diarrhoea. Therefore, the aEPEC became an important human infective pathogroup both in the developing and developed countries.

Atypical EPEC strains were researched in several animal species in the past and scientists found connection between the aEPEC infection and the sickness outcome in dogs and cats from companion animals. Researcher also investigated these strains as a possible human infective bacterium where the close contact between humans an companion animals can enhance this opportunity.

In sheep the aEPEC strains can also be isolated frequently, but their occurrence decreased during the their grow up. Sheep can often carry EHEC-like strains which may originate from aEPEC strains with infection by shiga toxin harboring bacteriophage. Hungarian research group verified this possibility in an *ex vivo* study (Tóth et al., 2003). The aEPEC strains can be isolated from pig diarrhoea cases as well where aEPEC strains use special adhesins and their frequency can be enhanced by predisposing factors according to the Hungarian studies (Malik et al., 2006; Malik et al., 2012).

Scientist found similar results worldwide investigating poultry. They recorded frequent and diverse distribution of aEPEC which can be a source of virulence factors and antibiotic resistance genes and may serve their transmission (Szmolka et al., 2012; Malik et al., 2017).

Our aims

There is scarce information about the frequency and distribution of EPEC, especially aEPEC in Hungary. In poultry there were no datas or investigation about them before our research.

Therefore, our aim was to inspect the existence of intestinal *E. coli* pathotypes in mostly kept poultry species (chicken, turkey, duck, goose, pigeon). Furthermore, we would like to increase the accessible knowledge about the poultry based on our result.

Thus, we wanted to investigate during our PhD work these next few questions:

1. Which *E. coli* pathotypes were harbored by chicken using own isolates and reference samples.
2. Identifying the phenotype (antibiotic resistance and serogroup) and phylogenetic origin of possible intestinal pathogenic *E. coli*.
3. Testing the antibiotic resistance of potential pathogenic bacteria beside recording their frequency as well.
4. We would like to conduct a comparative study about carried intestinal pathogenic *E. coli* in five mainly kept poultry focusing on the possible influence of bird age.

In the first study we researched broiler chicken where aEPEC strains were isolated first in Hungary and they were multidrug-resistant.

In the next stage we made comparison between five poultry species, especially focusing on the age as influencer. In that study we could make the first aEPEC description from goose in the world and we first mentioned aEPEC strains in turkey and pigeon in Hungary.

Material and methods

Cotton swab samples were collected from bird caecum and carcasses (broiler) in slaghterhouses (chicken, turkey, duck, goose) and in Nébih-ÁDI. Cloaca samples were gained from live poultry. The reference broiler *E. coli* collection of Nébih-ÁDI were used for testes as well.

Normal bacterium isolation was performed on agar plate (Bromothymol-blue, MacConkey) surfaces from cottons swab samples. Pure bacterium culture were gained by serial inoculation of one lactose positive colony. Isolates were verifyed as *E. coli* by MALDI-TOF or biochemical testes.

Serotipisation of probable pathogenic *E. coli* strains were completed with O and H antigen specific immunsera with agglutination test in National Public Health Center, Hungary.

Antibiotic resistance test were fulfilled on Mueller-Hinton agar against 15 antibiotics according to the CLSI (Clinical and Laboratory Standards Institute) recommendation. The mobilized colistin resistance gene were checked in all strains in PCR with previously designed and written primer for *mcr-1* gene detection.

Virulence gene identification and phylogenetic classification of pathogenic *E. coli* strains were also performed by PCR.

Statistical analysis of our results were done with 95% confidence interval and predicted frequency calculation, Fisher's exact test and ANOVA.

Results

In our study *eae* (intimin) gene was identified frequently in *E. coli* strains which gene is typical for EPEC. Other virulence factors of intestinal pathogenic *E. coli* was not recorded at all. All EPEC strains were verified as atypical EPEC according to the absence of EAF plasmid and its carried *bfpA* (bundle forming pilus) gene. However, all aEPEC strains had *tir* (translocated intimin receptor) gene which has an important role in the patomechanism of EPEC strains. Mobilized colistin resistance gene (*mcr-1*) was not identifiable in any *E. coli* isolates.

Atypical EPEC strains appeared with high frequency in samples from slaughterhouse (28% - 35 aEPEC) and Nébih-ÁDI (30% - 48 aEPEC). The B2 phylogenetic group had high representation among aEPEC strains (25.5%) in contrast with non-EPEC strains (2.5%) and the two groups difference was found significant with Fisher's exact test. Serogroups of boriler aEPEC stains were diverse between flocks (O108 and O14). Nonetheless, it was uniform in the same flock. Number of antibiotic resistance pattern was 15 in slaughterhouse sample and 41 in Nébih-ÁDI sample. Multidrug-resistance was very common with 94% (slaughterhouse) and 98% (Nébih-ÁDI) prevalence. The isolates had very wide antibiotic resistance spectrum and they had in some cases almost 100% or exactly 100% frequency. (Figure 1.)

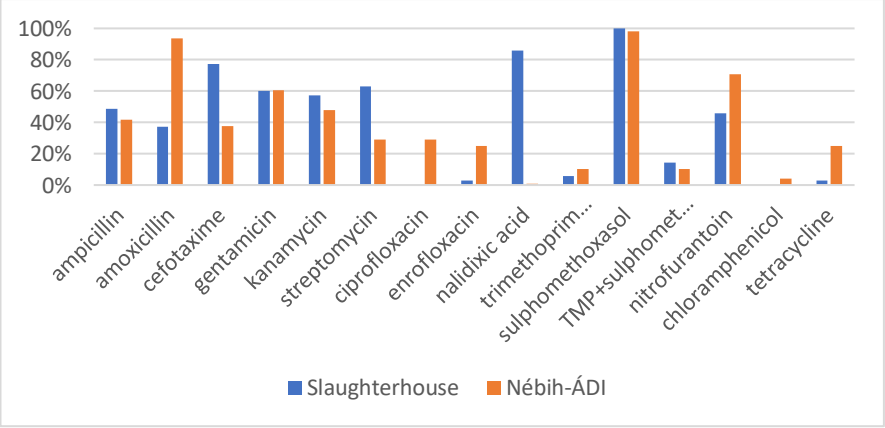

Figure 1. Frequency of antibiotic resistance among aEPEC strains.

Only 7 aEPEC strains were isolated studying 319 samples in five poultry species. These *E. coli* isolates originated from diverse age groups and different keeping purpose flocks from slaugtherhouses, backyard flocks and dead poultry. (Table 1.)

Table 1. Age distribution of the samples collected and number of samples positive to *eae* gene.

	Ages	Pigeon	Chicken	Duck	Goose	Turkey	Overall
Diagnostic Directorate	0-1 week		14	27			41
	1-6 weeks			4	26	*2/4	34
	7-16 weeks				13		13
	15 weeks			4			4
	17 weeks-6 months				3		3
	6 months	*1/1					1
	6-12 months				11		11
	Over 1 year		15	1			16
	Sum	**1**	**29**	**36**	**53**	**4**	**123**

	Ages	Pigeon	Chicken	Duck	Goose	Turkey	Overall
Backyard	nestlings	*2/12					**12**
	3-4 months	8					8
	6 months	4					4
	2-3 years	10	13				23
	Sum	**34**	**13**				**47**

	Ages	Pigeon	Chicken	Duck	Goose	Turkey	Overall
Slaughter-house	14 weeks			51			51
	16 weeks				*2/48		48
	20 weeks					50	50
	Sum			**51**	**48**	**50**	**149**

	Total	**35**	**42**	**87**	**101**	**54**	**319**

*n/n means: *eae* (intimin) positive sample(s)/all sample(s)

Results, serotypes and antibiotic resistance pattern of 7 aEPEC strains from turkey, goose and pigeon were summarized in Table 2.

Table 2. Antibiotic resistance patterns, phylogenetic and serogroups of aEPEC isolates.

Species	Age	ECOR	Serotype	Antibiotic resistance pattern
Turkey	4 weeks	B1	O NT : NM	AMC, AMP, CHL, CIP, ENR, FOX, KAN, NAL, NIT, SMX, STR, SXT, TET, TMP
Turkey	4 weeks	F	O76 : NM	AMC, AMP, CHL, CIP, ENR, NAL, SMX, STR, SXT, TET, TMP
Goose	16 weeks	B2	O145 : H SP	AMC, AMP, NAL, NIT, SMX, STR, SXT, TET, TMP
Goose	16 weeks	B2	O145 : H SP	AMC, AMP, CIP, ENR, NAL, NIT, SMX, STR, SXT, TET, TMP
Pigeon	nestlings	B1	O109 : H21	AMP, NIT, SMX
Pigeon	nestlings	B1	O109 : H21	AMC, SMX
Pigeon	6 months	B1	O NT : H35	AMC, AMP, SMX, STR

Abbreviations: ECOR (phylogenetic group); Serotype: NT (not typable), NM (not moving), SP (spontaneous agglutianion); AMC (amoxicillin-clavulanate), AMP (ampicillin), CHL (chloramphenicol), CIP (ciprofloxacin), ENR (enrofloxacin), FOX (Cefoxitin), KAN (kanamycin), NAL (nalidixic acid), NIT (nitrofurantoin), SMX (sulphomethoxasol), STR (streptomycin), SXT (sulphomethoxasol+trimethoprim), TET (tetracycline), TMP (trimethoprim)

Discussion

There was no information about the poultry aEPEC in Hungary before our research. However, we demonstrated it with high frequency in broilers and in representative *E. coli* collection too. Our findings could prove that aEPEC strains can often harbor several antibiotic resistance genes. In addition , there was no data about the aEPEC existance in goose (*Anser anser domestica*) in the literature. Therefore, our description was the first mention of aEPEC in goose in the world. There were also no information about aEPEC in turkey and pigeon in Hungary which was demonstrated first by us.

The possible effect of bird age, their sickness or their keeping purpose on aEPEC distribution were first examined in poultry and we assumed their influence on aEPEC prevalence based on the recent literature datas as well.

However, we published several new and not existing information about poultry EPEC in prestigious peer reviewed journals. But our research could not be complete because of limited available resources. Therefore, we plan to continue the study with intimin typization, *in vitro* and *in vivo* pathogenocity examination and testing the species specificity of aEPEC isolates in the future.

Nonetheless, we believe that these findings and future results can establish our participation in hungarian and international microbiological researches. In addition, we hope that this work and future results will provide usefull information for teaching (epidemiology and food-hygiene) as well.

New Scientific Results

E. coli strain collection with 437 isolates was established as our precomposed aim. All *E. coli* strains were tested for virulence genes of intestinal *E. coli* pathotypes. High number of aEPEC strains were identified.

We can draw a conclusion based on the characterization of these aEPEC strain:

1. Atypical EPEC strains were first isolated from chicken, turkey and pigeon in Hungary.

2. Atypical EPEC strain from goose (*Anser anser domestica*) was first mentioned and characterized in the literature.

3. The possible effect of bird age on aEPEC distribution was revealed first.

4. Frequent B2 phylogenetic group (25%) representation of aEPEC strains from broilers was first demonstrated.

5. Various and frequent antibiotic resistance was described among aEPEC strains beside diverse serogroups.

6. Poultry species can influence of antibiotic resistance of aEPEC strains.

7. We assume that poultry carry mainly aEPEC and the other pathotypes can come from contamination of other meat sources.

List of publications used for dissertation

Adorján A, Makrai L, Mag T, Jánosi S, Könyves L, Tóth I (2020) High frequency of multidrug-resistant (MDR) atypical enteropathogenic *Escherichia coli* (aEPEC) in broilers in Hungary. Front Vet Sci 7:511 doi:10.3389/fvets.2020.00511 Scientometric data: D1 – Impact factor: 3.412

Adorján, A, Makrai, L, Könyves, L, Tóth, I (2021) Enteropathogenic *Escherichia coli* (EPEC). Mini review. MÁL 143(7):429-438 Scientometric data: Q4 – Impact factor: 0.22

Adorján A, Thuma Á, Könyves L, Tóth I (2021) First isolation of atypical enteropathogenic *Escherichia coli* from geese (*Anser anser domestica*) and first description of atypical EPEC from turkeys and pigeons in Hungary. BMC Vet Res 17(1):263 doi:10.1186/s12917-021-02968-w Scientometric data: Q1 – Impact factor: 2.741

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