

**ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROTOXIGENIC STRAINS OF *ESCHERICHIA COLI* ISOLATED FROM WEANED PIGS IN CROATIA**

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*The E-test method was employed to assess the sensitivity of 114 Escherichia coli, isolates to 10 antimicrobials. All E. coli isolates were obtained from weaned piglets that died with clinical signs of diarrhea. E. coli isolates showed highest rate of resistance to oxytetracycline, streptomycin and sulphametoxazole/trimethoprim (89%, 74% and 50% of isolates, respectively), whereas 68% of the isolates were resistant to 4 or more antimicrobials. The high sensitivity rate of (97 %) to colistin is attributed to the fact that colistin has been used in veterinary medicine in Croatia only last 2-3 years. All strains were sensitive to cefotaxime. The high multidrug resistance of E. coli strains isolated from large swine agglomerations most probably was due to the obsolete farming technology where economically profitable productivity could hardly be maintained without the systemic use of antibiotics administered in the critical steps of production.*

*Key words: antimicrobial susceptibility, E. coli, weaned pigs*

#### INTRODUCTION

Antimicrobial resistance has emerged in the past few years as the major problem and many programs have been set up for its surveillance in human and veterinary medicine. These programs are directed to pathogens that are important in human and veterinary medicine and also to bacteria that cause zoonosis. Indicator bacteria of the normal intestinal flora of animals are also subjected to antimicrobial resistance testing (Lanz *et al.*, 2003).

Large pig farms present the majority of intensive pig breeding in Croatia. These farms have between 600 and 5000 sows per farm with the proportional number of boars for artificial insemination, resulting in keeping a great number of animals in a relatively small area. The majority of farms are farrowing – finishing units, based on the all-in-all-out principle (Bilić and Habrun, 1996).

Such farms have a high prevalence of so-called breeding diseases caused by opportunistic bacteria (Bilić and Habrun, 1996). Although a novel subunit and polyvalent vaccines have recently been developed, there is no efficient immunoprophylaxis for all bacterial diseases occurring in intensive pig breeding.

This is why the use of antibiotics for prevention and therapy of some important bacterial diseases remains necessary.

*Escherichia coli* are the most common etiological agent of diarrhea in pigs after weaning. The causative agents are usually enterotoxigenic *E. coli* that colonize the small intestine and produce one or more enterotoxines. Typically a treatment consists of a broad spectrum of antimicrobials, although the resistance to such drugs has greatly increased over the last years (Bischoff *et al.* 2002).

The purpose of this study was to demonstrate antimicrobial resistance of enterotoxigenic strains of *E. coli* isolated from weaning pigs with diarrhea on large pig breeding farms in Croatia.

#### MATERIAL AND METHODS

During the two year period, in 9 large pig breeding farms in Croatia, 10 to 14 isolates of *E. coli* from dead pigs were collected. All *E. coli* isolates (n=114) were obtained from the small intestine from the weaned piglets that died with clinical symptoms of diarrhea. The isolates were identified applying API 32E biochemical gallery (BioMerieux, France). The hemolytic activity of *E. coli* isolates was tested on 5% sheep blood agar. The hemolytic strains containing F4, F6 and/or F18 fimbriae were included. Fimbriae were determined by the method of agglutination using commercial antisera.

All collected isolates were stored in Luria-Bartrani broth with glycerin at  $-70^{\circ}\text{C}$  until sensitivity testing.

The minimal inhibitory concentration (MIC) was determined by the use of E-test (AB Biodisk, Sweden). The test is an expansion of the disk diffusion method with the same agar and inoculum preparation. The antibiotic content of the strip was graded, and the concentration was printed linearly along the strip. The test was performed according to the manufacturer's instructions. The MIC for the following antimicrobials was determined: amoxicillin, amoxicillin + clavulanic acid, cefotaxime, colistin, enrofloxacin, gentamicin, spectinomycin, streptomycin, tetracycline, and sulfamethoxazole + trimethoprim.

Antimicrobial agent concentrations ranged from 0.002 to 32  $\mu\text{g}/\text{mL}$  (enrofloxacin, and thrimethoprim + sulfamethoxazole), from 0.016 to 256  $\mu\text{g}/\text{mL}$  (amoxicillin, amoxicillin + clavulanic acid, cefotaxime, gentamicin, and tetracycline), and from 0.064 to 1024  $\mu\text{g}/\text{mL}$  (colistin, spectinomycin, and streptomycin). According to CLSI (M31 A3), Mueller-Hinton agar was used as a culture medium (Merck 1.05435), while *E. coli* ATCC 25922 was used as a control strain.

#### RESULTS AND DISCUSSION

MIC 50 and MIC 90 values for the isolates are displayed in Table 1. The percentage of sensitive, intermediary sensitive and resistant strains is shown in Table 2.

Table 1. Detection of minimal inhibitory concentration (MIC mg/L) in 114 isolates of enterotoxigenic strains of *Escherichia coli*

Antimicrobial	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC Range
Amoxicillin	8.0	>256	1.0 - >256
Amoxicillin + clavulanic acid	3.0	04.0	0.38 - 24
Cefotaxime	0.016	0.064	0.012 – 0.125
Colistin	0.094	0.125	0.064 - 512
Enrofloxacin	0.047	12.0	0.016 - >32
Gentamicin	0.50	12.0	0.019 - >256
Streptomycin	32.0	96.0	0.5 - 96
Spectinomycin	32.0	256.0	8 - 1024
Tetracycline	64.0	192.0	1.0 - >256
Sulfamethoxazole/trimethoprim	3.0	>32.0	0.016 - >32

Table 2. The percentage of isolates of *E. coli* with the following categorical interpretation<sup>1</sup>

Antimicrobial	S*	I	R
Amoxicillin	68		33
Amoxicillin + clavulanic acid	95		5
Cefotaxime	100		
Colistin	97		3
Enrofloxacin	74	5	21
Gentamicin	57	11	32
Streptomycin	26		74
Spectinomycin	58		42
Tetracycline	11		89
Sulfamethoxazole/trimethoprim	50		50

<sup>1</sup> According to recommendations on CLSI M31 A3 2008, for cefotaxime  
CSLI M100-S18 (2008) and for streptomycin (Sunde *et al.* 2005)

\* S – sensitive; I – intermediary sensitive, R – resistant

Enterotoxigenic *E. coli* is the most common etiologic agent of diarrhea in the weaning pigs. The treatment of enteric *E. coli* infections in swine usually includes the broad-spectrum of antibiotics (Bishoff *et al.* 2002). In the present study, the sensitivity of 114 *E. coli* isolates to 10 antimicrobials was tested (Table 1). The highest resistance rate was to tetracycline (89%), streptomycin (74%) and sulfamethoxazole/trimethoprim (50%). All the isolates were susceptible to cefotaxime and 97 % of isolates were susceptible to colistin (Table 1 and 2).

Multidrug resistance was frequently observed, with 84.2% of *E. coli* isolates resistant to 3 or more antimicrobials tested and 68.4% were resistant to 4 or more antibiotics (Table 3). Tetracyclines, aminoglycosides and sulfonamides are widely used in swine production for the treatment and prevention of bacterial diseases and therefore, a high resistance rate to these antimicrobial classes was not unexpected. The swine *E. coli* isolates showed a pattern similar to other clinical veterinary *E. coli* strains in terms of their increased resistance to tetracycline, sulfamethoxazole / trimethoprim and gentamicin (Coates and Hoopes, 1980; Libal and Gates, 1982; Nijsten *et al.*, 1996).

Table 3. Multiple drug resistance of *E. coli* strains

(from 10 tested antimicrobials)	No of drug resistance	
	No. of resistance strains	% of resistance strains
0	6	5.2
1	12	10.5
3	18	15.8
4	54	47.4
5	18	15.8
6	6	5.2

A steady decrease has been observed in the sensitivity to enrofloxacin, (74 % of strains were sensitive). The previous studies conducted in Croatia (1990) revealed that *E. coli* was not resistant to fluoroquinolones, and in 1996, 92.5% of *E. coli* isolates collected from pigs was susceptible to enrofloxacin (Habrun *et al.*, 1997).

The high sensitivity rate (97%) to colistin is attributed to the fact that colistin has been used in veterinary medicine in Croatia only in last 2-3 years. A more common use of colistin in intensive pig breeding for the treatment and metaphylaxis of colibacillosis probably may result in an increased resistance to this antimicrobial in the future.

The high multidrug resistance of *E. coli* strains isolated from large swine agglomerations most probably was due to the obsolete farming technology where profitable productivity could hardly be maintained without the systemic use of antibiotics, administered in the critical steps of production. In order to decrease antibiotic resistance, large pig farms should improve their breeding technology and food quality and reduce antibiotics as prophylaxis and in treatment of colibacillosis.

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#### ANTIMIKROBNA OSETLJIVOST ENTEROTOKSIGENIH SOJEVA BAKTERIJE *ESCHERICHIA COLI* IZOLOVANIH OD ZALUČENE PRASADI U HRVATSKOJ

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#### SADRŽAJ

Pomoću E-testa ispitana je osetljivost 114 izolata bakterije *Escherichia coli* prema 10 antimikrobnih lekova. Svi izolati *E. coli* bili su uzorkovani od zalučene prasadi koje je uginula sa kliničkim znakovima dijareje. Izolati *E. coli* imali su najveći stepen rezistencije prema oksitetraciklinu (89%), streptomycinu (74%) i sulfametoksazolu/trimetoprimu (50%). Na više od 4 testirana antimikrobna leka

bilo je rezistentno 68% izolata. Najveća osetljivost utvrđena je prema kolistinu (97%) najverojatnije zbog toga što se kolistin intenzivnije koristi u svinjarskoj industriji u Hrvatskoj poslednje 2-3 godine. Svi sojevi su bili osetljivi prema cefotaksim. Visok stepen multirezistencije *E. coli* izolovane iz velikih svinjarskih farmi najverojatnije je uzrokovana zastarelom tehnologijom na većem broju farmi zbog čega je ekonomski profitabilnu proizvodnju teško održati bez sistemskog korišćenja antimikrobnih lekova u kritičnim fazama proizvodnje.