

PREVALENCE OF ANTIMICROBIAL RESISTANCE IN COMMENSAL *ESCHERICHIA COLI* PRODUCING ESBL/AMPC ISOLATED FROM CAECAL SAMPLES OF SLAUGHTERED PIGS IN ROMANIA

PREVALENȚA REZISTENȚEI LA ANTIMICROBIENE A TULPINILOR COMENSALE DE *ESCHERICHIA COLI* SINTETIZATOARE ESBL/AMPC, IZOLATE DIN CONȚINUTUL CECAL AL PORCILOR ABATORIZAȚI ÎN ROMÂNIA

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ABSTRACT REZUMAT

The discovery of antibiotics is regarded as one of the greatest achievements of human civilization, but during the last years, the excessive use of antimicrobials in human and veterinary medicine has accelerated the emergence and the spread of resistant microorganisms. This situation is also worsened by the lack of investments in developing new efficient antibiotics. At the level of the European Union, the antimicrobial resistance (AMR) is a priority, and, following the enforcement of Decision 652CE/12/2013, harmonized monitoring and reporting norms were established in order to be implemented by the EU Member States in relation with the AMR of commensal and zoonotic bacteria, in the veterinary and human fields.

The monitoring of the antimicrobial resistance of *Escherichia coli* commensal strains isolated from caecal samples collected from slaughtered pigs was implemented for the first time in Romania, in 2015, by testing a number of 399 samples. The isolation of commensal extended spectrum beta-lactamases and cephalosporinases producing *E. coli* strains (TESBL/AmpC producing *E. coli*) was carried out using the MacConkey agar supplemented with cefotaxime. The identification was performed with classical bacteriological techniques, based on phenotypical tests. The ESBL/AmpC producing *E. coli* isolates were tested for antimicrobial susceptibility using the broth microdilution method, in accordance to SR ISO 20776-1:2007, and the minimal inhibitory concentrations (MIC) obtained were compared to the epidemiological cut-off values according to the interpretation criteria established by Decision 652CE / 12/2013.

The prevalence of commensal ESBL/AmpC producing *E. coli* strains isolated from the slaughtered

Descoperirea antibioticelor este considerată una dintre cele mai mari realizări ale civilizației umane, însă utilizarea excesivă a medicamentelor antimicrobiene în medicina umană și veterinară, în ultimii ani, a accelerat apariția și răspândirea microorganismelor rezistente, această situație fiind agravată și de lipsa investițiilor în dezvoltarea de noi antibiotice eficiente. La nivelul Uniunii Europene, rezistența antimicrobiană (RAM) reprezintă o prioritate, astfel că, în conformitate cu Decizia 652CE/12/2013, au fost stabilite norme de monitorizare și raportare armonizate, care se aplică de către Statele Membre, referitoare la RAM a bacteriilor zoonotice și comensale, în domeniile uman și veterinar.

În România, monitorizarea rezistenței antimicrobiene a tulpinilor comensale de *Escherichia coli* izolate din probe de conținut cecal, prelevate de la porcii abatorizați, s-a efectuat pe parcursul anului 2015, prin testarea unui număr de 399 de probe. Izolarea tulpinilor comensale de *Escherichia coli* sintetizatoare de beta-lactamaze cu spectru extins și cefalosporinaze (*E. coli* sintetizatoare ESBL/AmpC) s-a realizat prin utilizarea mediului MacConkey suplimentat cu cefotaxim, iar identificarea, prin tehnici bacteriologice clasice, pe baza testelor fenotipice (caracterele morfo-culturale și metabolice). Testarea rezistenței la antimicrobiene s-a efectuat prin metoda microdiluției în bulion, în conformitate cu standardul SR ISO 20776-1:2007, iar concentrațiile minime inhibitorii obținute au fost raportate la valorile limită epidemiologice, conform criteriilor de interpretare stabilite prin Decizia 652CE/12/2013.

Prevalența izolatelor de *E. coli* sintetizatoare ESBL/AmpC, din conținutul cecal al porcilor sacrificați prin abatorizare, a fost de 65,78% (223 probe), iar pe baza concentrației minime inhibitorii obținute la testarea pentru 21 antimicrobiene, acestea au fost rezistente la cefotaxim (100%), ampicilină (100%), cefepim (91,93%), ceftazidim (90,13%), sulfametoxazol (73,54%), tetraciclină (71,30%), trimetoprim (62,33

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strains were resistant to cefotaxime (100%), ampicillin (100%), cefepime (91.93%), ceftazidime (90.13%), sulfamethoxazole (73.54%), tetracycline (71.30%), trimethoprim (62.33%), ciprofloxacin (53.81%), chloramphenicol (41.70%), nalidixic acid (39.91%), gentamicin (20.63%), cefoxitin (20.63%), azithromycin (14.35%), colistin (3.59%), ertapenem (1.79%).

This survey shows that the pigs slaughtered in Romania during the year 2015 were colonized, in a proportion of more than 60%, with strains resistant to cephalosporins, sulfonamides, tetracyclines and fluoroquinolones. The results obtained will provide important insight into effects of using antimicrobials at animals. The study was funded by the National Sanitary Veterinary and Food Safety Authority of Romania and European Commission, on the basis of Grant Decision SI2.700679/26.02.2015.

Keywords: antimicrobial resistance, ESBL/AmpC producing *E.coli*, broth microdilution method, minimal inhibitory concentration

INTRODUCTION

The discovery of antibiotics is regarded as one of the greatest achievements of human civilization, but during the last years, the excessive use of antimicrobials in human and veterinary medicine has accelerated the emergence and the spread of resistant microorganisms. This situation is also worsened by the lack of investments in developing new efficient antibiotics (2,4).

There are studies have shown that antimicrobial resistance is an ever-evolving issue driven by antimicrobial usage pressure and pigs might be a reservoir of third-generation cephalosporin-resistant *E. coli* (6, 7). In a joint report published in 2015, the three sister agencies EFSA, ECDC and EMA concluded that the use of certain antimicrobials in animals and humans is associated with the occurrence of resistance to these antimicrobials (5, 10, 11). The use of commensal intestinal *E. coli* as indicator for the presence of resistance determinants in bacterial flora is considered a key component of surveillance programs both in food-producing animals and wildlife (6).

At the level of the European Union, the antimicrobial resistance (AMR) is a priority and, following the enforcement of Decision 652CE/12/2013, harmonized monitoring and reporting norms were established in order to be implemented by the EU Member States in relation with the AMR of commensal and zoonotic bacteria, in the veterinary and human fields (1).

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Această lucrare arată că, porcii sacrificați în România pe parcursul anului 2015, au fost colonizați în proporție de peste 60% cu tulpini rezistente la cefalosporine, sulfonamide, tetracicline și fluorochinolone.

Rezultatele obținute furnizează informații importante privind efectele utilizării antimicrobienele la animale. Studiul a fost finanțat în anul 2015 de către Autoritatea Națională Sanitară Veterinară și pentru Siguranța Alimentelor din România și Comisia Europeană, în baza Deciziei de Grant SI2.700679/26.02.2015.

Cuvinte cheie: rezistența antimicrobiană, *E. coli* sintetizatoare ESBL/AmpC, metoda microdiluției în bulion, concentrație minimă inhibitorie

MATERIAL AND METHODS

Antimicrobial resistance monitoring of commensal ESBL/AmpC producing *E. coli* has been implemented in pigs slaughtered in Romania. Caecal samples ($n = 399$) were randomly collected from slaughtered pigs, during 2015.

According EURL Antimicrobial Resistance, Denmark, for isolation and identification ESBL/AmpC producing *E. coli* used in laboratories, the method used was: Ig of caecal samples was added to 9 ml Buffered Peptone Water and incubated at 37 ± 1 °C for 18-22 h. The overnight culture was streak onto a MacConkey agar plate containing 1 mg/l cefotaxime and incubated for 18-22 hours at 44 ± 0.5 °C. Presumptive ESBL/AmpC producing *E. coli* colonies were colored in purple/red on the MacConkey CTX (Fig. 1). Up to three colonies were sub-culture by re-streaking onto MacConkey CTX and incubate at 37°C for 18-22 h.

Colonies showing typical *E. coli* morphology were confirmed biochemically (TSİ, MIU, ONPG) and a single isolate representing each sample was selected for antimicrobial resistance testing, with microbroth dilution method (3, 9).

The Minimum Inhibitory Concentrations (MICs) (mg/l) were determined for 21 antibiotics using Trek Sensititre 96-well plates, respectively two panels: first panel - ampicillin (AMP), cefotaxime (FOT), ceftazidime (TAZ), gentamicin (GEN), tetracycline (TET), sulfamethoxazole (SMX), trimethoprim (TMP), cipro-

floxacin (CIP), nalidixic acid (NAL), chloramphenicol (CHL), azithromycin (AZI), tigecycline (TGC), colistin (COL), meropenem (MERO) and second panel - cefotaxime (FOT), ceftazidime (TAZ), ceftazidime (FOX), cefepime (FEP), temocillin (TRM), meropenem (MERO), imipenem (IMI), ertapenem (ETP), cefotaxime+ clavulanic acid (F/C) and ceftazidime + clavulanic acid (T/C). *E. coli* ATCC 25922 was used as the quality control strain. The reading of the plates was done automatically with the BioMicV3 Microbiology system (Fig. 2).

The values obtained were compared to the epidemiological cut-off values developed by EUCAST (12) according to the interpretation criteria established by Decision 2013/652/EU and EFSA criteria for pheno-type (8).

RESULTS AND DISCUSSIONS

The samples were collected during 2015 from fattening pigs between 5-12 months, slaughtered in 26 slaughterhouses from 21 counties. Each sample was taken by veterinary officers from slaughter line, immediately after slaughter, and submitted to the laboratories, along with a set of information. The samples were processed in the labs no more than 48 hours from sampling.

The global prevalence of commensal ESBL/AmpC producing *E. coli* strains isolated from the pigs caeca was 65,78%, respectively 223 strains out of 339 samples (Fig. 3). Presumptive ESBL-positive strains were more frequent (78.9%) compared with presumptive AmpC-type cephalosporinase producers (16.14%), and only one strain was another phenotype (Fig. 4).

The prevalence of antimicrobial resistance in commensal producing ESBL/AmpC *E. coli* is shown in Table 1. Based on MIC obtained for 21 antimicrobials, these strains were resistant to cefotaxime (100%), ampicillin (100%), cefepime (91.93%), ceftazidime (90.13%), sulfamethoxazole (73.54%), tetracycline (71.30%), trimethoprim (62.33%), ciprofloxacin (53.81%), chloramphenicol (41.70%), nalidixic acid (39.91%), gentamicin (20.63%), ceftazidime (20.63%), azithromycin (14,35%), colistin (3.59%) and ertapenem (1.79%).

There are variable frequencies for beta-lactams, respectively from 1,79% (ETP) to 91,93% (FEP), excepting the values 100% for AMP and FOT due to isolation on MacConkey agar plate containing 1 mg/l cefotaxime (Fig. 5). For non-beta-lactams antimicrobials it was detected a high prevalence of resistance to sulfonamides (SMX - 73,54%), tetracyclines (TET -

71.30%), fluoroquinolones (CIP - 53.81%), low prevalence of resistance to polymyxins (COL- 3,59%) and no resistance for glycylicylines (TGC - 0%).



Fig. 1. Positiv lactose colonies of *E. coli* on MacConkey FOT agar

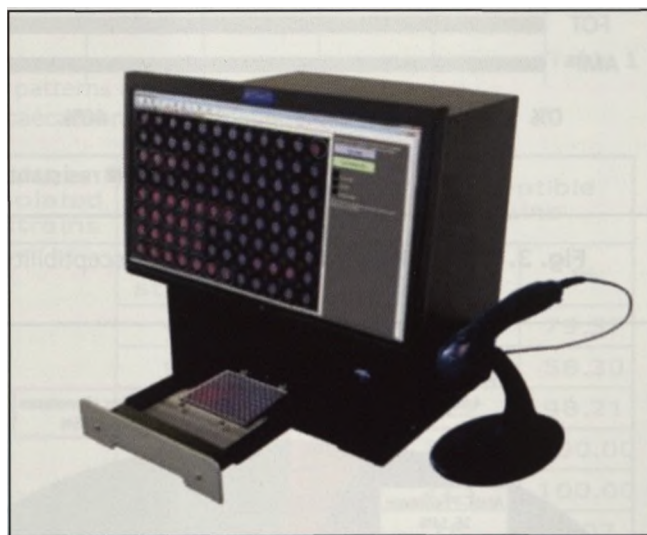


Fig. 2. BioMic V3 System

CONCLUSIONS

This is the first study in Romania fortesting anti-microbial resistance of commensal *E. coli* strains isolated from slaughtered pigs, and add our country to the worldwide landscape of extended spectrum betalactamases and AmpC-type cephalosporinases occurrence.

The prevalence of ESBL/AmpC producing isolates was high in swine caecal samples, respectively 65,78 %. This survey shows that the pigs slaughtered in Romania during the year 2015 were colonized, more than 60%, with strains resistant to cephalosporins, sulfonamides, tetracyclines and fluoroquinolones.

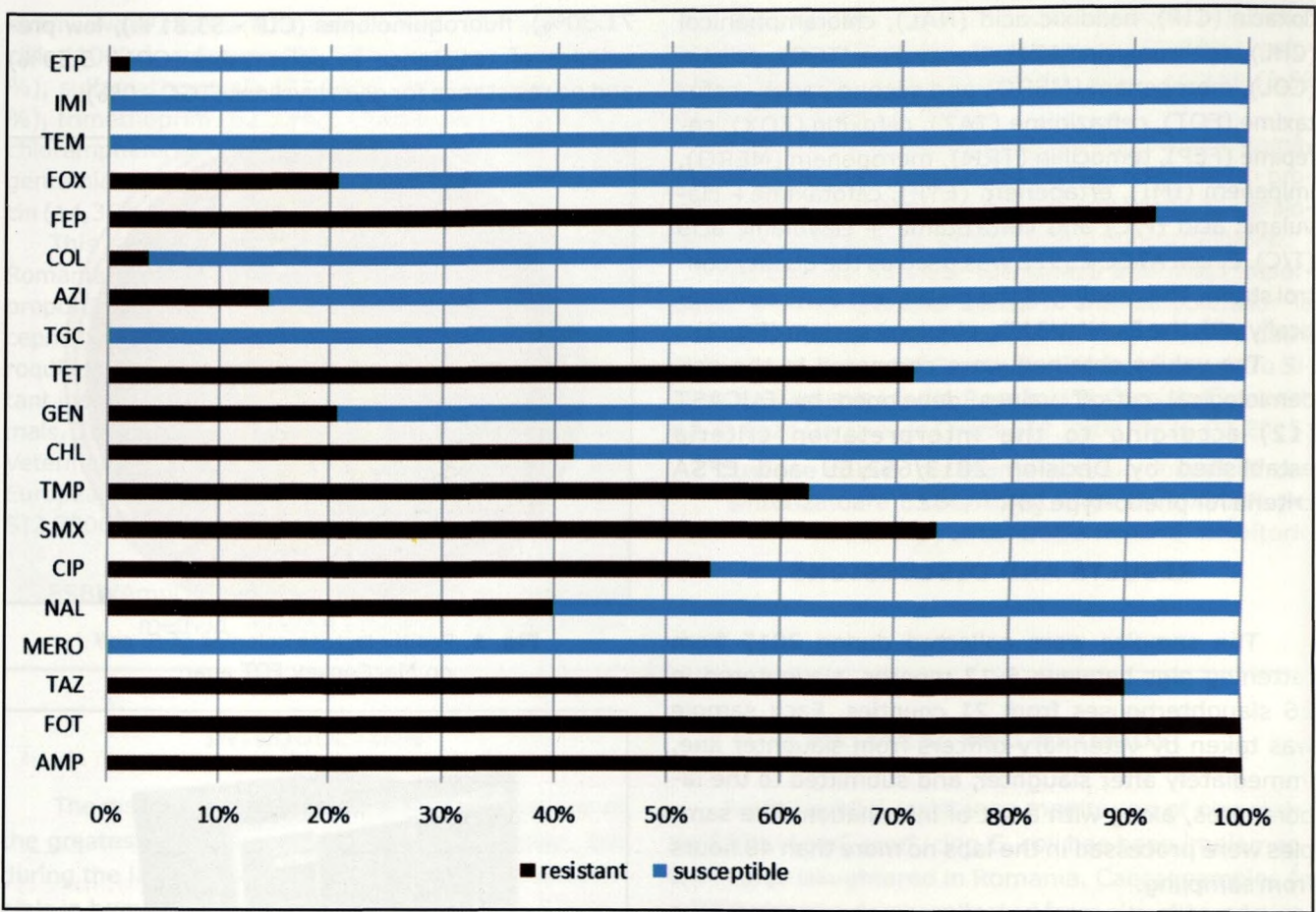


Fig. 3. Microbiological resistance and susceptibility in commensal *Escherichia coli* producing ESBL/AmpC

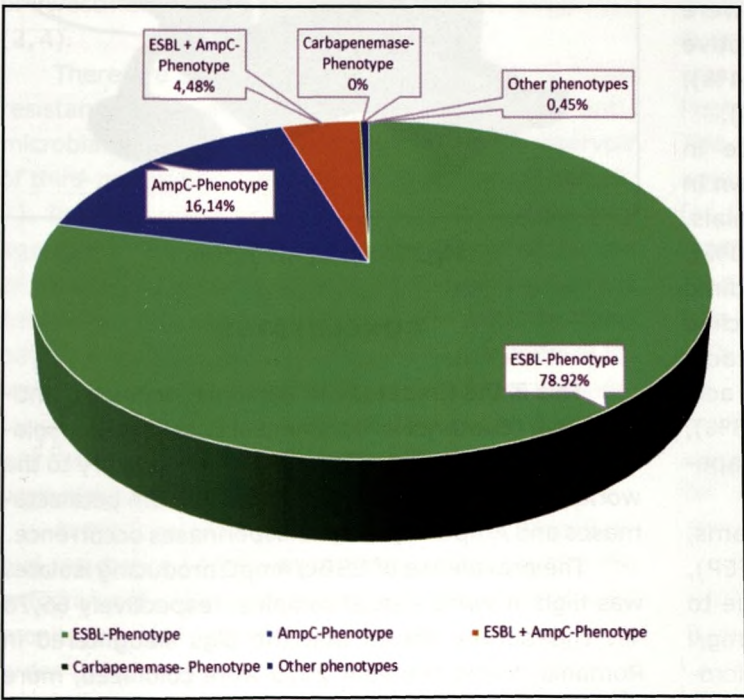


Fig. 4. *Escherichia coli* - ESBL/AmpC/Carbapenemase phenotypes

Our study indicates that slaughtered pigs are colonized with cephalosporin-resistant strains and will provide important insights into effects of using antimicrobials at animals. These results are important for further testing to better know the situation at the national level.

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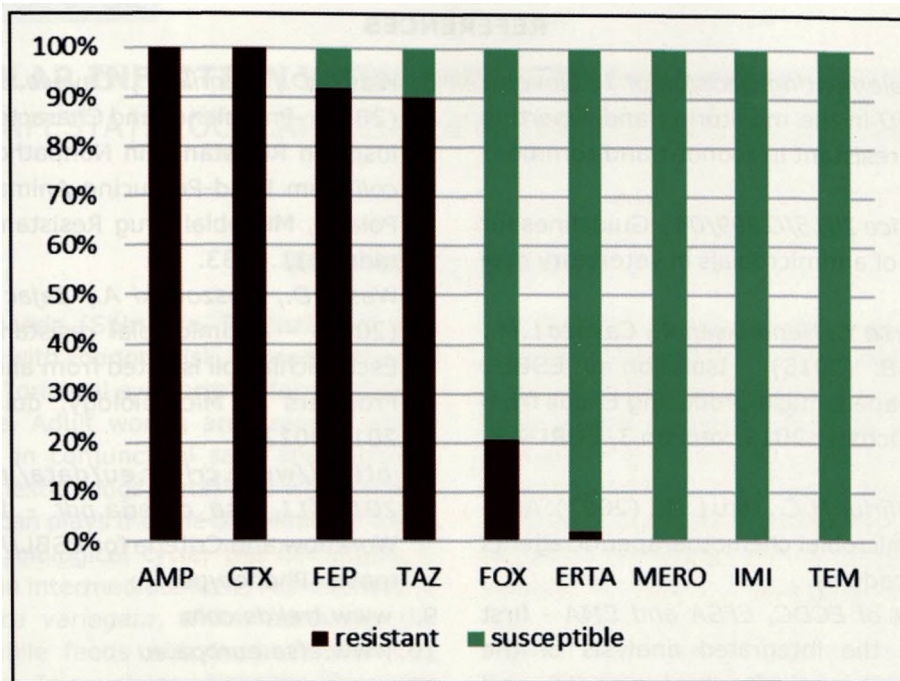


Fig. 5. Microbiological resistance and susceptibility to beta-lactams in commensal *Escherichia coli* producing ESBL/AmpC

Table 1

Antimicrobial susceptibility and resistance patterns in commensal *Escherichia coli* producing ESBL/AmpC isolated from caecal samples of slaughtered pigs

Antimicrobial subclass and generic names	No. samples	No. isolated strains	Resistant strains		Susceptible strains	
			No. strains	%	No. strains	%
Aminoglycosides - Gentamicin	399	223	46	20.63	177	79.37
Amphenicols - Chloramphenicol			93	41.70	130	58.30
Carbapenems - Ertapenem			4	1.79	219	98.21
Carbapenems -Imipenem			0	0.00	223	100.00
Carbapenems - Meropenem			0	0.00	223	100.00
Cephalosporins - Cefepime			205	91.93	18	8.07
Cephalosporins - Cefotaxime			223	100.00	0	0.00
Cephalosporins - Cefoxitin			46	20.63	177	79.37
Cephalosporins - Ceftazidime			201	90.13	22	9.87
Fluoroquinolones - Ciprofloxacin			120	53.81	103	46.19
Glycylcyclines - Tigecycline			0	0.00	223	100.00
Macrolides - Azithromycin			32	14.35	191	85.65
Penicillins - Ampicillin			223	100.00	0	0.00
Penicillins -Temocillin			0	0.00	223	100.00
Polymyxins - Colistin			8	3.59	215	96.41
Quinolones- Nalidixic acid			89	39.91	134	60.09
Sulfonamides-Sulfamethoxazole			164	73.54	59	26.46
Tetracyclines - Tetracycline			159	71.30	64	28.70
Trimethoprim			139	62.33	84	37.67

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