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Review

Sensitivity of *Escherichia coli* strains isolated from pigs in the period 2006 - 2015 to antimicrobial drugs: review

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Abstract

The paper provides a chronological review and analysis of the sensitivity test results for pathogenic strains of *Escherichia coli* isolated from pigs to the most commonly used antimicrobial drugs (AMD) in veterinary medicine for the period of 2006 – 2015.

The sensitivity of *E. coli* strains expressed in percentages from 51 to 100% in 2006-2010 was found to be within the range of 9-10 (37.5-47.6%) AMD, and in 2011- 2015 in the range of 5-11 (average 33.3%) AMD. The resistance of *E. coli* strains expressed in percentages from 51 to 100% in the first 5 years (2006 - 2010) rangedwithin 7-8 (33.3%) AMD, and during the period of 2011-2015 was within the range of 5 to 11 (33.3 - 41.7%) AMD.

Keywords: pigs, *E. coli*, antimicrobial drugs, sensitivity

Резюме

В статията се прави хронологичен преглед и анализ на резултатите от изпитванията за чувствителност на изолирани от прасета патогенни щамове *Escherichia coli* към най-често употребявани във ветеринарномедицинската практика антимикробни средства (АМС) за периода 2006 – 2015 г.

Установено е, че чувствителността на щамовете *E. coli*, изразена в степен от 51 до 100% през 2006 – 2010 година се движи в границите на 9-10 (37.5-47.6%) от АМС, а през периода 2011-2015 г. в границите на 5-11 (средно 33.3%) от АМС. Резистентността на щамовете *E. coli*, изразена в степен от 51 до 100% през първите 5 години се движи в рамките на 7-8 (33.3%) от АМС, а през периода 2011-2015 г. в границите от 5 до 11 (33.3-41.7%) от АМС.

Установено е, че през периода 2006-2015 г. се запазва тенденцията за повишаване на резистентността на щамовете *E. coli*, изолирани от прасета, спрямо регистрираната през периода 2003-2005 г., към амоксицилин, ампицилин, спектиномицин, стрептомицин, окситетрациклин, доксициклин, тилмикозин, еритромицин, линко-спектин, тиамулин и налидиксова киселина.

За първи път резистентни *E. coli* щамове към апрамицин, тилмикозин, тилвалозин, налидиксова киселина, флумеквин, енрофлоксацин и пефлоксацин, са регистрирани през 2006-2010 г. и към цефквином, цефотаксим, колистин и флорфеникол през 2011-2015 г..

Анализират се евентуалните причини и се препоръчват мерки за недопускане и ограничаване разпространението на резистентни *E. coli* щамове.

Introduction

Definition of colinfections

The group of colinfections combines several clinically manifested diseases and subclinically occurring infections, most commonly affecting newborn and growing animals and humans, caused by various members of the species *Escherichia coli* (Dimitrov, 1988; Bertschinger *et al*., 1992; Popova, 2009).

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Colibacteriosis (CB), such as colienteritis and edema disease of pigs

Colibacteriosis is one of the most widely spread diseases in suckling and growing pigs in all countries with advanced pig breeding. The disease is widespread both in traditional and industrial pig farms and causes great economic damage, entailing serious problems for the sub-sector (Yordanov, 1982; Dimitrova, 2009; Dragoycheva *et al*., 2011; Yordanov, 2014; Dimitrova *et al*., 2014; Petkova and Yordanov, 2016; Petkova, 2017).

Out of 501 samples from pigs with gastroenteritis in the period 2003-2005, Dimitrova (2009) obtained 245 isolates in the assay, 22.8% of which were *E. coli*.

In the next period (2006-2010), Dragoycheva *et al*. (2011) examined 335 samplesfrom pigs with diarrhea and in 129 cases (38.5%) isolated enteropathogenic (EPEC), enterotoxigenic (ETEC) and enterohaemorrhagic (EHEC) *E. coli* strains, more often from industrial complexes (45.2 %) and less often from traditional farms (37.8%) and private farms (36.6%), the average rate rising from 31.9% in 2006 to 58.6% in 2009. Lyutskanov (2012; 2013) and Petkova *et al*., (2014) found that the incidence of coli-infections in intensive pig production in 2012-2014 was 100%, and Petkova (2017) reported that in the period 2011-2015, coli infections in pigs in our country were caused by members of 12 hemolytic and non-hemolytic serogroups, of which strains O157, O149, O20 and O74 were dominant, and strains O8, O9, O74 and O78 were accepted as new, reported in 2014.

Use of antimicrobial agents

In cases of CB in pigs, the antibiotics streptomycin, neomycin, apramycin, spectinomycin and chloramphenicol were used in the past with good effect, but as some *E. coli* strains acquired resistance against them, the aminoglycosides gentamicin, kanamycin and amikacin; amphenicols - florfenicol and thiamphenicol; quinolones - nalidixic acid, oxolinic acid and flumequine became more relevant in the following years (Courvalen, 1994; Dunlop *et al*., 1998; Yordanov *et al*., 1999; Plumb, 2002; Popova and Dimitrov, 2001; Yordanov *et al*., 2005; Sčuca, 2005; Popova, 2009; Vestič, 2012), and second- and third-generation fluoroquinolones active against a large number of gram-negative and gram-positive bacteria: ciprofloxacin, norfloxacin, pefloxacin, enrofloxacin (accepted as the "gold standard"), marbofloxacin and orbifloxacin (Kantardzhiev, 1998; Dimitrova *et al*, 2005; Popova and Todorov, 2008; Dimitrova, 2009; Dimitrova *et al*., 2011; Popova, 2013; Dimitrova *et al*., 2014). There is no resistance to fluoroquinolones just yet or recorded resistance is very low, which is why they are referred to as strategic therapeutics (Georgiev *et al*., 2010; Lyutskanov, 2012; Fraile, 2013; Yordanov and Dimitrova, 2014; Petkova and Yordanov, 2016). In pig farms with permanent CB, in addition to the therapeutic course, metaphylactic course of therapy is applied using medicated (antibiotic) premixes, such as Colistin-premix, Nipoxim-premix, Fenivex-premix, Floron-premix, Oxytetracycline-premix, Tetramutin-premix, etc. ffor treatment of whole batches of pigs (Yordanov, 2014; Dimitrova, 2009; Lyutskanov, 2013; Dimitrova *et al*. 2016; Petkova, 2017).

Occurrence of resistant *E. coli* strains

The occurrence of resistant *E. coli* strains is associated, on the one hand, with the frequency of occurrence of genetically modified individuals (mutations) and, on the other hand, with the widespread use of certain antimicrobial drugs (AMD) (Dimitrov *et al*., 1984; Courvalen, 1994). Many of the agents used have already exhausted their capabilities due to the rapidly evolving resistance of microorganisms, which requires particular care in their selection (Dunlop *et al*., 1998; Habrun, 2010). According to Drumev (2001) and Friendship and Fraile (2013), both the poor choice of antimicrobial agents (AMCs) and antibiotic prophylaxis without a definite indication contribute to the development of resistance in microorganisms.

Antimicrobials used in productive animals in Europe are often the same, or belong to the same classes as those used in human medicine. Therefore, antimicrobial resistance (AMR) is a major undesirable side effect of AMD use both in humans and animals. In this regard, some *E. coli* strains are an example of zoonotic bacteria that can be ingested by humans with food of animal origin (Close *et al*., 2000; Zaharieva and Vassileva, 2019). According to Bosch (2004), even with the best management, pigs remain susceptible to bacterial infections, so the use of AMD will remain an integral part of their control, but this requires control of circulating *E. coli* strains and changes in their susceptibility to AMD.

In any event, the use of AMD should be based on the proven sensitivity of the isolated pathogenic strains to them (Drumev, 2001; Dimitrova, 2009; Georgiev *et al*., 2010; Lyutskanov, 2012; Yordanov and Dimitrova, 2014; Dimitrova *et al*., 2014; 2016). According to Mos *et al*. (2010), the disk diffusion method (DDM) is reliable in antibiotic testing, easily feasible and effective, and its results are compa-

rable to those of minimal inhibition concentration (MIC).

Results of *E. coli* susceptibility tests in previous periods

At the beginning of the new century, Dimitrova (2009) examined the susceptibility of *E. coli* strains isolated from pigs from different farms during the period 2003-2005, finding a sensitivity higher than 50% to: gentamicin 77.8%, sulfamethoxazole + trimethoprim (SMZ+T) 75%, pefloxacin 66.7%, kanamycin and streptomycin 55.6% and flumequine 50%. At the same time, resistance over 50% was demonstrated to erythromycin and linco-spectin 100%,oxytetracycline 80%, tylosin and tiamulin 75%, spectinomycin and nalidixic acid 60%, and doxycycline and tilmicosin 50%.

In a comparative susceptibility test for 16 AMD with 32 *E. coli* strains isolated from pigs in 2005, Yordanov *et al*. (2005) found that 75% of the strains were sensitive and 18.7% were moderately sensitive to enrofloxacin. Similar sensitivity (81.2% and 6.3%) was reported only for gentamicin and to some extent for kanamycin and flumequine. Significantly high resistance to other agents was found: amoxicillin 100%, streptomycin 73.4%, tetracycline 66.6%, ampicillin 65%, spectinomycin 60%. It was confirmed that there was no cross-resistance between enrofloxacin and representatives of other antibiotic groups, as some of them, considered to be effective until recently, have been reported to have high resistance.

Gin *et al*. (2015) found that AMR was often detectable among *E. coli* isolates in cases of post-weaning diarrhea of pigs (PWD) in France. The highest percentage of resistant ETEC isolates was found to amoxicillin (58.97%) and spectinomycin (48.08%), followed by apramycin (38.46%), neomycin (37.18%) and gentamicin (14.74%). Only 1.92% of the isolates were resistant to ceftiofur and none to amikacin and enrofloxacin. Increasinglylowersensitivity to colistin has been reported. In fattening pigs, the highest levels of AMR were found for tetracycline (52.1%), sulfamethoxazole (SMZ) (42.4%), ampicillin (38.5%) and trimethoprim (32.2%), with only 39.2% of *E. coli* isolates fully sensitive to the range of AMD tested.

Purpose of the review.

We set out to do a chronological review and analysis of the results of investigations on *E. coli* pathogenic strains isolated from pigs in Bulgaria for sensitivity to the most commonly used antimicrobial agents in the period 2006 - 2015.

Results and Discussion

Survey for the period 2006 – 2010

In a pig-breeding complex with intensive exploitation of the breeding herd

Forty-one *E. coli* strains from 6 serogroups, relevant to both the complex and the country, were isolated from suckling and weaned pigs (Dimitrova *et al*., 2014). The isolated strains were tested for susceptibility to 20 AMD. A sensitivity of more than 50% to 10 AMDs (47.6%), respectively to aminoglycoside agents (amikacin 75-60%; gentamicin and kanamycin 62.5-60%; streptomycin and apramycin 50%); fluoroquinolones (enrofloxacin 62.5-100% and pefloxacin 62.5-80%); quinolones (nalidixic acid and flumequine 50%); and SMZ+T 50-50% was recorded. High resistance, over 50%, was reported against 7 (33.3%) AMC: ampicillin 75-80%; oxytetracycline 50-60%; doxycycline – 75-60%; tylosin 62.5-60%; erythromycin 60-60%, spectinomycin 62.5-60%, and streptomycin 50% (Tables 1 and 2).

The analysis of the data revealed that, with the exception of amikacin and kanamycin, the strains were resistant against all other AMD. For the first time, strains resistant to nalidixic acid, flumequine, pefloxacin and enrofloxacin were reported. These results are close to those found by Dimitrova *et al*. (2011) and are in line with the opinion of Drumev (2001) that both the poor choice of AMD and antimicrobial prophylaxis without definite indication contribute to the emergence of resistance in microorganisms.

In industrial pig complex

Forty-seven *E. coli* strains isolated from industrially farmed pigs between 2006 and 2010 showed high sensitivity to 9 (37.5%) antibiotics in susceptibility testing conducted by Dimitrova *et al*. (2011) with 24 AMD: florfenicol (87.5%), enrofloxacin (76%), pefloxacin (78.7%), cefquinome (71.4%), gentamicin (68.3%), flumequine (62.5%), kanamycin (55.6%), amikacin (51.9%) and SMZ+T (52.9%); average sensitivity to: apramycin (80%), tilmicosin (53.1%), ampicillin (44.8%), oxytetracycline (44.4%), colistin (41%) and amikacin (40.7%), and the highest resistance to 8 (33.3%) of the agents: linco-spectin (100%), tylvalosin (95.4%), oxytetracycline (87.9%), tylosin (80%), doxycycline (68.42%), spectinomycin (60%) and nalidixic acid (60 %).No resistance to enrofloxacin, florfenicol, and cefquinome was recorded.

The data are largely in line with the findings of Yordanov *et al*. (2005) and it is noteworthy that

Table 1. Sensitivity of *E. coli* strains, isolated from pigs in 2006 – 2015 in degree from 51 % to 100% to used AMD

№	Antimicrobial drugs	2005 - 2009	2006 - 2010	2006 - 2010	2010 - 2011	2012 - 2013	2011 - 2013	2011 - 2013	2012 - 2014	2014	2014 - 2015	2014 - 2015	2015
		Dim. + 2014	Dim. + 2011	Lut. 2013	Dim. + 2014	Dim. + 2013	Petkova 2017	Yord. + 2016	Dim. + 2016	Petkova + 2014	Yordanov + 2016	Petkova 2017	Petkova 2017
1	Amikacin	+	+		+	+	+	+	+	+	+	+	+
2	Apramycin	+				+	+	+	+				
3	Gentamicin	+	+	+	+	+	+	+	+	+	+	+	+
4	Kanamycin	+	+		+	+	+	+	+	+	+	+	+
5	Spectinomycin			+									
6	Streptomycin	+											
7	Amoxicillin			+									
8	Ampicillin												
9	Oxytetracycline			+									
10	Doxycycline												
11	Cefquinome		+		+		+	+	+				
12	Cefotaxime				+	+	+	+	+				
13	Erythromycin												
14	Tylosin												
15	Tilmicosin												
16	Tylvalosin												
17	Linco-spectin			+									
18	Colistin			+									
19	Florfenicol		+	+	+	+	+	+	+		+	+	+
20	Nalidixic acid	+											
21	Flumequine	+	+	+		+							
22	Enrofloxacin	+	+	+	+	+	+	+	+		+	+	+
23	Pefloxacin	+	+		+		+	+	+		+	+	
24	Ciprofloxacin					+			+				+
25	Norfloxacin						+	+		+	+	+	+
26	Tiamulin												
27	SMZ + T	+	+			+	+	+	+	+	+	+	
	Num.	10	9	9	8	10	11	11	11	5	8	8	7
	%	47.6	37.5	81.8	66,7	58.8	45.8	45.8	42.3	20	33.3	33.4	26.9

Dim. + - Dimitrova *et al*. (2014)

some ETEC strains in pigs are no longer sensitive to tetracyclines, macrolides and linco-spectin. What is striking is the rather high percentage (53.1%) of medium-sensitive strains to tilmicosin, unlike the other members of the group, which is in support of what was already established by Dimitrova (2009). The information about high resistance (60%) to nalidixic acid and spectinomycin is disturbing.

In industrial pig complexes and pig farms from different regions

In studies on colibacteriosis in suckling and growing pigs in intensive pig production, Lutskanov (2013) tested the susceptibility of *E. coli* strains isolated in the period 2006-2010 to 11 of the most commonly used AMD, and found that with the exception of doxycycline, to all others, it ranged from 66.9% to 96.7%, as follows: amoxacillin and

Table 2. Resistance of *E. coli* strains, isolated from pigs in 2006 – 2015 in degree from 51 % to 100% to used AMD

№	Antimicrobial drugs	2005 - 2009	2006 - 2010	2006 - 2010	2010 - 2011	2012 - 2013	2011 - 2013	2011 - 2013	2012 - 2014	2014	2014 - 2015	2014 - 2015	2015
		Dim. + 2014	Dim. + 2011	Lut. 2013	Dim. + 2014	Dim. + 2013	Petkova 2017	Yord. + 2016	Dim. + 2016	Petkova + 2014	Yordanov + 2016	Petkova 2017	Petkova 2017
1	Amikacin												
2	Apramycin	+									+		
3	Gentamicin												
4	Kanamycin												
5	Spectinomycin	+	+		+		+	+			+	+	+
6	Streptomycin				+	+		+	+				
7	Amoxicillin					+	+		+	+	+	+	+
8	Ampicillin	+			+		+	+	+	+	+	+	+
9	Oxytetracycline	+	+		+	+	+	+	+	+	+	+	+
10	Doxycycline	+		+	+		+	+	+	+	+	+	+
11	Cefquinome										+	+	+
12	Cefotaxime									+			+
13	Erythromycin	+			+		+	+	+				
14	Tylosin	+	+		+		+	+	+	+	+	+	+
15	Tilmicosin	+	+			+	+	+	+	+	+	+	
16	Tylvalosin		+										
17	Linco-spectin		+										+
18	Colistin									+	+	+	+
19	Florfenicol												
20	Nalidixic acid		+								+	+	+
21	Flumequine										+		+
22	Enrofloxacin												
23	Pefloxacin												
24	Ciprofloxacin												
25	Norfloxacin												
26	Tiamulin					+	+	+	+	+	+	+	+
27	SMZ + T												
	Num.	8	7	1	7	5	9	9	9	9	13	11	12
	%	40	29.2	9.1	33.3	29.4	37.5	37.5	37.5	36	54.2	42.3	46.1

gentamicin 75.6%, neomycin 78%, spectinomycin 66.9%, tetracycline 83.4%, colistin 93.1% , flumequine 86.4, enrofloxacin 87.9%, and florfenicol 83.4%. The data differ from the results of Yordanov *et al*. (2005) and Dimitrova (2009), which may be due both to the origin of the isolates and to the antimicrobial agents and regimens used in the respective farms.

Survey for the period 2011 – 2015

In pig complex with total replacement of the breeding herd

In 2010, in pig-breeding complexes with intensive exploitation, after total replacement of the breeding herd with gilts from only one reproductive base (RB), 10 *E. coli* strains were isolated from two serogroups, one of which was new, and in 2011

19 strains were isolated from 4 serogroups, 3 of which were new to the complex (Dimitrova *et al*., 2014). Of the 12 (66.7%) *E. coli* strains tested, 8 (72.5%) were more than 50% sensitive to: amikacin 80-100%; gentamicin 40-100%; kanamycin 40-85.7%; cefquinome 80-85.7%; cefotaxime 60-85.7%; florfenicol 80-42.9%; enrofloxacin and pefloxacin 40-100%. Evidently, with the exception of florfenicol, sensitivity to the rest of AMD was increased. Over 51% of the strains were resistant to 7 kinds (58.3%) of AMD: streptomycin 60-42.9%; spectinomycin 60-14.2%; ampicillin 80-71.4%, oxytetracycline and doxycycline 60-42.9%; erythromycin 80-71.4% and tylosin 50-42.5%. It is evident that the resistance of all strains reported in 2013 was reduced compared to that established for 2012, with more than 50% for ampicillin and erythromycin alone. These results are likely to have been achieved through the repair of the breeding herd and the replacement of circulating strains, as well as the measures implemented for this purpose recommended by Binek *et al*. (1994), Courvalen (1994), Drumev (2001) and Fraile (2013).

In a semi-industrial pig farm with import of replacement pigs and fattening pigs from Western Europe in 2012-2013

Dimitrova *et al*. (2013) isolated from healthy and diseased pigs 37 strains of *E. coli* from 7 serogroups, of which O8, O157, O136 and O20 were dominant. When tested for sensitivity to 17 AMD, a sensitivity of 51 to 100% was found to 10 (58.8%) AMD: aminoglycosides (amikacin, kanamycin, gentamicin, apramycin), quinolones (flumequine), fluoroquinolones (enrofloxacin, ciprofloxacin), amphenicols (florfenicol) and SMZ+T, which corresponds to their pharmacological characteristics but also shows some decrease due to their widespread use during that period. Resistance data show that out of 17 AMD, one was found in 9 (53%) of them, and more than 50% in 5 kinds (294%) of AMD: oxytetracycline (90%), streptomycin (90%), amoxicillin (85%), tilmicosin (70%) and tiamulin (70%). Oxytetracycline, colistin and especially streptomycin (90%) are considered to be an indicator of acquired resistance.

Resistance of E. coli strains in weaning pigs from different regions in 2011-2015

In a longer study on the sensitivity of *E. coli* strains isolated from pigs in the period 2011-2015, Petkova (2017) found that out of the 24 AMD used, the numberand the corresponding percentage of susceptible and resistant strains varied in different years. In 2011, isolates were 51%-100% sensitive to 7 (29.2%) AMD: gentamicin, kanamycin, amikacin, florfenicol, enrofloxacin, pefloxacin and SMZ+T, and resistant to 5 (20.1%) AMD; in 2012, they were sensitive to 11 (45.8%) of the agents: gentamycin, kanamycin, apramycin, amikacin, cefquinome, cefotaxime, florfenicol, enrofloxacin, pefloxacin, norfloxacin and SMZ + T, and resistant to 8 (33.3%); in 2013, they were sensitive to 11 (45.8%) AMD: gentamicin, kanamycin, apramycin, amikacin, cefquinome, cefotaxime, florfenicol, enrofloxacin, pefloxacin, norfloxacin and SM + T, and resistant to 7 (29.2%). In 2014, they were sensitive to 6 (25%) of the AMD: gentamicin, amikacin, florfenicol, enrofloxacin, norfloxacin and SMZ + T, and resistant to 9 (37.5%), and in 2015 sensitive to 7 (29.2%) AMD: gentamicin, kanamycin, amikacin, florfenicol, enrofloxacin, pefloxacin and norfloxacin, and resistant to 11 (45.8%).

In summary, from 2011 to 2015, isolated *E. coli* strains were sensitive to 20 (83.3%) of the 24 AMD used, but 51%-100%-resistant only to 8 kinds (33.3%): gentamicin, kanamycin, amikacin, florfenicol, enrofloxacin, pefloxacin, norfloxacin and SMZ + T, of which amikacin, kanamycin and SMZ + T are rarely used in veterinary medicine. At the same time, there was resistance to 22 (91.7%) AMD, of which 51%-100%-resistant to 10 (41.7%): ampicillin, amoxicillin, spectinomycin, oxytetracycline, doxycycline, erythromycin, tylosin, tilmicosin, nalidixic acid and tiamulin, which is 8.4% more than the sensitive and is a worrying trend in the development of antimicrobial resistance of pathogenic *E. coli* in pigs. The data confirm the findings of Gavrocič *et al*. (2011) that 50% of strains isolated from pigs show multiple resistance and are in line with the findings by Courvalent (1994) and Popova (2013) that there is an increasing resistance of some of the strains to AMD. The opinion of some authors (Drumev, 2001; Dunlop *et al*., 1998; Georgiev *et al*., 2010) confirms that, in addition to genetic changes, the poor selection of antimicrobial agents and their unnecessary use for no definite indication or in inappropriate doses and courses contribute to the emergence of resistance in bacteria.

Surveys for 5 consecutive years, 2011 – 2015

Sensitivity studies of 62 intestinal *E. coli* strains isolated from pigs conducted by Yordanov *et al*. (2016) showed that in 2011 the strains were most sensitive to 7 AMD: enrofloxacin (100%), pefloxacin and florfenicol (80%), followed by gentamicin, kanamycin, amikacin and SMZ + T (60%). The highest rates of resistant strains were found to

6 AMD: ampicillin (80%), oxytetracycline, doxycycline, erythromycin, tylosin, and spectinomycin (60%). In 2012, the *E. coli* strains tested were sensitive to 9 of the agents: amikacin (93.3%), norfloxacin, cefquinome, SMZ + T and florfenicol (80%), followed by kanamycin (73.3%), gentamicin, enrofloxacin and pefloxacin (66.6%). Most frequently, *E. coli* continued to be resistant to 8 AMD: ampicillin, oxytetracycline, erythromycin and spectinomycin (80%); tiamulin (70%) and doxycycline, tilmicosin and streptomycin (60%). The established resistance, though still low (6.7%), to the members of the quinolone and fluoroquinolone groups and, for the first time, to florfenicol, is of concern because these data differ from older results by Yordanov *et al*. (1999) about the absence of strains resistant to flumequine and florfenicol. These results confirm the opinion of Binek *et al*. (1994) that some antibacterial agents have exhausted their capabilities and there is a need to fight in two directions: against the possibility of emergence of resistance and against already existing resistance (Drumev, 2001; Georgiev *et al*., 2010; Vestič, 2012; Fraile, 2013). The data for 2013 show increased sensitivity of *E. coli* to 9 of the AMD used: amikacin (100%), gentamicin, kanamycin, enrofloxacin, pefloxacin and cefquinome (85.7-88.2%); to cefotaxime and apramycin (65%) and retention of norfloxacin (80%), which is most likely due to the imposed restriction on the use of some of them. Some decrease in sensitivity was found for SMZ + T (70.5%) and florfenicol (64.6%), which in turn appeared to be the replacements of the first ones. The strains were most resistant to 7 (30.5%) of the AMD: amoxicillin (80%), ampicillin, erythromycin, oxytetracycline and tiamulin (70 - 71.4%) and to a large extent (58.7%) to tilmicosin and spectinomycin, which on the one hand, with the exception of spectinomycin, is explained by their general characteristics and, on the other, by the widespread use of tetracycline agents. Close (2000) is of the opinion that this is a natural and inevitable phenomenon, as well as that there is a need to test pathogenic *E. coli* strains for susceptibility, to select appropriate therapies and metaphylactics, and to refrain from using certain AMD for a specified period of time (Dimitrova, 2009; Lyutskanov and Urumova, 2010; Yordanov and Dimitrova, 2014; Dimitrova *et al*., 2011, 2014). In 2014, a decrease in *E. coli* strains sensitive to the AMD was reported. All tested strains were only sensitive (100%) to amikacin. Sensitivity to SMZ + T (77.8%), gentamicin, and florfenicol (61%) and enrofloxacin and norfloxacin (50%) remained unchanged to some extent. In regard to quinolones and fluoroquinolones, the reported sensitivity is less than 50%. Inversely, resistant strains were found to 18 of the agents, for 9 of them (39.1%) it was from 51% to 100%, including amoxicillin, oxytetracycline, tiamulin, ampicillin, tilmicosin, colistin, tylosin, doxycycline and spectinomycin. The rate of resistance to: amoxicillin, oxytetracycline, ampicillin, and tiamulin (71.4-88.9%), as well as to tilmicosin, doxycycline, spectinomycin and tylosin (58.3-66.7%) increased. Against this background, the established resistance to florfenicol (5.6%) may be considered low, but the fact that 33.6% of the strains already have an average sensitivity to it suggests a negative trend in this process. The findings for 2015 show that of the 24 AMD only 12 kinds (50%) of them were active against *E. coli* to varying degrees. Only 7 kinds (29.2%) of them were reported with higher sensitivity: enrofloxacin, florfenicol, amikacin and kanamycin (85.7%) and gentamicin, pefloxacin and norfloxacin (71.4%- 57.1%). Resistant strains were identified to 16 of the agents, with 11 (45.8%) ranging from 51% to 93.9%, confirming what was expressed by Gavrocič *et al*. (2011) that multiple AMD resistance was established in 50% of the strains isolated from pigs.

In pigs from different pig farms and pig-breeding complexes

In a study by Dimitrova *et al*. (2016), 43 *E. coli* strains isolated from pigs in the period 2012-2014 were tested for sensitivity to 26 AMD. As a result, sensitivity was found to 22 (84.6%) of them, but only to 11 (42.3%) it was ranging from 51% to 100%, including to: amikacin 94.1%, ciprofloxacin 80%, kanamycin 73.7%, enrofloxacin 72.7%, SMZ + T 67.9%, gentamicin 67.5%, florfenicol 67.4%, cefotaxime 66.7%, cefquinome 60.7%, apramycin 58.1% and pefloxacin 52.2%. Of the remaining 15 AMD, 11 (42.3%) were active in only 1% to 10% of the cases. Resistant strains were established against 22 of the AMD, of which 9 were 51%-80.7% resistantto: amoxicillin 80.7%, erythromycin 80%, ampicillin 72.2%, oxytetracycline 72.2%, tiamulin 70%, doxycycline 64.7%, streptomycin 63.1%, tilmicosin 62.2% and tylosin 52.9%.

In weaned pigs

In their studies on *E. coli* infections in weaned pigs, Petkova *et al*. (2014) found that *E. coli* strains isolated in 2014 were more than 50% sensitive to only 5 (20%) AMD: - amikacin (100%), kanamycin (80%), gentamicin (77%), CMZ+T (71.4%) and norfloxacin (66.7%); also moderately sensitive to

5 (20%): pefloxacin (66.7%), florfenicol (61.5%), cefuroxime (60%), flumequine and nalidixic acid (50%) and resistant to 9 (36%) AMD: amoxicillin, ampicillin and tetracycline (100%), cefquinome (80%), oxytetracycline, colistin (76.9%), tiamulin (71.4), and tylosin and tilmicosin (60%). These findings, compared with the results of Dimitrova *et al*. (2013), showed that 100% sensitivity of *E. coli* strains was observed only to amikacin, whereas to gentamicin, enrofloxacin, pefloxacin, kanamycin and cefuroxime, it decreased from 5.7% to 33%, which is alarming. Moreover, as compared to 2013, resistance to tetracycline and macrolide agents increased from 17.1% to 27.1%, and to ampicillin and erythromycin - by 28.6%, which, according to Drumev (2001) and Yordanov and Dimitrova (2014), contributes to their widespread and often unjustified use in the treatment and metaphylactics of many bacterial and viral diseases.

In weaned and growing pigs

Petkova (2017) studied the sensitivity of *E. coli* strains isolated from weaned and growing pigs in 2015 to 26 currently used AMD, finding that the strains exhibited sensitivity to a total of 16 AMD, of which 51-100% sensitivity to 7 (26.9%): amikacin, kanamycin, gentamicin, florfenicol, norfloxacin, enrofloxacin and ciprofloxacin. Resistant strains were established to 23 (88.5%) AMD, of which more than 50% to 13 (50.0%): amoxicillin, ampicillin, spectinomycin, oxytetracycline, doxycycline, cefquinome, cefotaxime, tylosin, linco-spectin, colistin, nalidixic acid, flumequine and tiamulin. The test data show that the sensitivity, compared to the results of Dimitrova *et al*. (2013), is observed only to amikacin, whereas for gentamicin, enrofloxacin, kanamycin, and cefuroxime, it is severely reduced, which is alarming. In addition, resistance to tetracyclines, macrolides, linco-spectin and some aminoglycosides increased from 17.1% to 28.6%, which, according to Drumev (2001), Yordanov and Dimitrova (2014), is most often due to their widespread and not always justified application for therapy and metaphylactics of certain bacterial and viral diseases. The high sensitivity to ciprofloxacin (100%), amikacin (97.4%) and kanamycin (77.8%) is attributed to both their pharmacological characteristics (Popova, 2009; Georgiev *et al*., 2010) and to their limited use in veterinary medicine. Overall, these results are largely consistent with the data of Gin *et al*. (2015), whohave come to believe that antimicrobial resistance is often detectable among *E. coli* isolates in cases of post-weaning diarrhea in pigs.

Conclusion

A review and analysis of the sensitivity and resistance of *E. coli* strains isolated from pigs in the period 2006-2015 to the most commonly used AMD shows that:

The sensitivity of *E. coli* strains in the first 5years ranges from 51% to 100%, to 9 to 10 kinds (37.5-47.6%) of AMD: amikacin, gentamicin, kanamycin, spectinomycin, florfenicol, flumequine, enrofloxacin, pefloxacin and SMZ+T., and in the second 5 years to 5–11 (avg. 33.3%) AMDs, including: amikacin, apramycin, gentamicin, kanamycin, cefquinome, cefotaxime, florfenicol, enrofloxacin, pefloxacin, ciprofloxacin, norfloxacin and SMZ+T., from 2011 to 2014 the sensitivity decreased markedly from 10 to 5 AMD, in 2014 increased to 11 , and again decreased to 8 AMD in 2015.

The resistance of *E. coli* strains isolated from pigs to the most commonly used AMD, ranging from 51% to 100% in the first 5 years (2006-2010) was manifested against 7-8 kinds (33.3%) of AMD: apramycin, spectinomycin, ampicillin, oxytetracycline, doxycycline, erythromycin, tylosin, tilmicosin, tylvalosin, linco-spectin and nalidixic acid, and in the second 5 years (2011-2015) was against 5 to 11 kinds (33.3-41.7) of AMD, while in 2011, 2012 and 2013 was in the range of 5-8 AMD, and in 2014 and 2015 it increased to 11 , with higher resistance to amoxicillin, cefquinome, cefotaxime, nalidixic acid, colistin, tiamulin, flumequine and streptomicin.

It was found that in the period 2006-2015 the tendency of increased resistance of *E. coli* strains isolated from pigs to the existing AMD remained unchanged in the period 2003-2005 to amoxicillin, ampicillin, spectinomycin, streptomycin, oxytetracycline, doxycycline, tilmicosin, erythromycin, linco-spectin, tiamulin and nalidixic acid.

For the first time, in the years 2005-2010, resistant *E. coli* strains isolated from pigs were recorded chronologically to nalidixic acid, flumequine, apramycin, spectinomycin, pefloxacin and enrofloxacin, and in 2011-2015 to ampicillin, tilmicosin, tylvalosin, colistin, florfenicol, cefquinome and cefuroxime.

The present review on the susceptibility of *E. coli* strains pathogenic to pigs demonstrates the need for awareness among human and veterinary doctors, the public and farmers to take steps to prevent the emergence and spread of resistant bacteria that have become a challenge to humanity. The implementation of new methods for monitoring AMR, the reasonable and justified use and control of the use of AMD should be a mandatory part of the AMR strategies.

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