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Antimicrobial resistance of *Escherichia coli* and *Klebsiella* spp. conventionally sampled from factory-farmed chickens – clinical submissions

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Abstract

Introduction. The article discusses the antimicrobial resistance of poultry-isolated bacteria in the Wielkopolska region of Poland.

Materials and method. From August 2014 – June 2016, antibiotic resistance screening tests were performed involving 4,496 samples of *Escherichia coli* and 84 samples of *Klebsiella* spp., and the following antibiotics: amoxicillin, amoxicillin with clavulanic acid, colistin, doxycycline, enrofloxacin, florfenicol, neomycin, norfloxacin, spectinomycin, and trimethoprim with sulfamethoxazole. The research used broth the microdilution method and CLSI standards.

Results. During the investigation period of 22 months a growing percentage of *E. coli* isolates showed antibiotic resistance to amoxicillin, amoxicillin with clavulanic acid, colistin, enrofloxacin, neomycin, norfloxacin, spectinomycin, and trimethoprim with sulfamethoxazole. Resistance to doxycycline and florfenicol decreased. The most efficient antibiotics against *E. coli* were colistin (84.64 %), neomycin (80.62 %), and amoxicillin with clavulanic acid (73.05 %). *Klebsiella* samples were the most susceptible to neomycin (85.71 %), colistin (84.52 %), and trimethoprim with sulfamethoxazole (73.81 %).

Conclusions. Antibiotic resistance of pathogenic micro-organisms, such as *Escherichia coli* and *Klebsiella* spp., is a serious problem both for poultry producers and for public health protection. Low efficiency of numerous antibiotic groups forces reflection on limiting the use of medicines in food-producing animals.

Key words

drug resistance, Escherichia coli, public health, Klebsiella

INTRODUCTION AND OBJECTIVE

In the last decade, the antimicrobial resistance (AMR) of bacteria causing food-borne illnesses has become a vital problem for public health. Zoonotic agents can be transmitted to humans directly or through animal source foods [1]. Antibiotic-resistant non-pathogenic microorganisms are also dangerous because resistance genes may be transferred to the natural animal and human microbiota through mechanisms such as a horizontal gene transfer [2].

In modern livestock production, antibiotics are commonly used for therapeutic purposes, and for many years they also served to prevent bacterial diseases or to stimulate animal growth [3]. After implementation of European Regulation (EC) 1831/2003 of 1 January 2006 on additives for use in animal nutrition, antibiotic growth stimulators were banned in the EU. The objective of the regulation was to slow down the increasing drug resistance of bacteria; however, intensive poultry production still largely relies on antibacterial drugs because due to frequent infections related to rearing they are indispensable in treating poultry diseases. The purpose of the mentioned strategy is to reduce bird mortality on farms and, thereby, to achieve profitability in production. The low immunity of chickens, high density of birds in sheds, and poor hygiene standards make it virtually impossible to raise a flock without bacterial infections that must be treated with drugs [4]. This is confirmed by data submitted by farm managers on food chain information declarations that accompany birds transported to slaughterhouses. The continued use of antibiotics in farm animals, including poultry, results in selective pressure exerted on microorganisms. A side-effect of excessive antibiotic therapy may be the spread of resistant strains in the environment [5], and data on the use of antibacterial preparations in livestock clearly show a growing trend. Currently, 7,982 tonnes of antibiotics are administered annually to pigs, cattle, and poultry in the European Economic Area. At the same time, 3,399.8 tonnes of antibiotics are used to treat bacterial diseases occurring in humans [6].

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The widespread use of antibacterial drugs and growing antimicrobial resistance against some groups of substances such as carbapenems, cephalosporins and fluoroquinolones, make it necessary to reach for such old-generation antibiotics as colistin [7]. Research on the susceptibility of microorganisms to antibacterial drugs may help in selecting adequate active ingredients and determining their dosage for flock treatment [8].

Currently, Poland is one of the largest global producers of poultry for slaughter. Within the last 15 years, Polish poultry production has increased rapidly, thus enhancing the share of Polish products on foreign markets. In 2014, production exceeded 2 million tonnes, one-third of which was exported [9]. Intensive poultry farming, if proper hygiene conditions and biosecurity measures are lacking, may increase the risk of the transfer of pathogenic microorganisms to poultry houses. This may result in lower safety and inferior quality of the animal products. Avian pathogenic *Escherichia coli* strains are often isolated. The presence of *Klebsiella* spp. is particularly dangerous due to its potential pathogenicity for humans.

For many years, *Escherichia coli* has been considered a common cause of infections in chickens, contributing to substantial economic losses [10]. Avian pathogenic *Escherichia coli* (APEC) strains are part of the natural enteric microbiota of healthy birds. With predisposing factors such as stress, high bird density, unfavourable conditions in chicken houses, and immuno-suppression related to viral diseases, these bacteria may secondarily cause disease symptoms. APEC is responsible for respiratory tract infections, multiple organ infections, navel infections and cellulitis. Yolk sac infections are most common and occur due to the pathogen presence on the egg surface and are fatal to embryos and chicks under 3 weeks old. The most important APEC-related diseases are respiratory tract and air sac inflammations which may evolve into bacteraemia, and subsequently cause symptoms of polyserositis. Oviduct infections can result in decreased laying performance [11].

The *Klebsiella* genus comprises pathogens that cause diseases both in humans and animals. In the majority of cases, they are opportunistic bacteria that may potentially infect the urinary system or lungs in humans [12, 13]. *Klebsiella pneumoniae* is one of the pathogens of the respiratory system responsible for high mortality in broiler chicks and hens [14, 15]. Numerous studies have confirmed the possibility of the transmission of ESBL-producing *Klebsiella* spp. strains from broiler chicken meat to humans [16, 17, 18]. The carcasses that pose a potential threat of infection to humans are most probably contaminated during the slaughtering process [19].

The objective of the study presented iss to determine the drug resistance of pathogenic *Escherichia coli* and *Klebsiella* spp. strains isolated from poultry in the Wielkopolska region of Poland between August 2014 – June 2016.

MATERIALS AND METHOD

The material used in the study comprised *Escherichia coli* and *Klebsiella* spp. strains isolated by Veterinary diagnostic laboratory LAB-VET Sp. z o. o., obtained from clinical samples provided for research as part of routine microbiological monitoring. The samples for analysis were submitted on a regular basis between August 2014 – June 2016. They were taken from changed organs such as the liver, spleen, heart and lungs of chickens from commercial farms located in Western Poland. The material was then inoculated onto the following media: Columbia Agar with 5% Sheep Blood (OXOID, Germany), MacConkey Agar (OXOID), and Brilliant Green Agar (OXOID). The incubation was carried out under aerobic conditions at 37 °C ± 1 °C for 18–24 hours. Suspicious colonies were streaked onto Nutrient Agar (OXOID) medium and incubated at 37 °C ± 1 °C for 18–24 hours for further identification. After the incubation, oxidase tests with Oxidase Reagent (BioMerieux) and Microbact 12A (OXOID) tests were performed.

The drug susceptibility tests were carried out using a broth microdilution method on Sensititre/POLBV (Thermo) plates coated with variable concentrations of antibiotics that were subsequently read using the Vizion instrument (Thermo Scientific). The following antibiotics were used: amoxicillin (AML), amoxicillin with clavulanic acid (AMC), colistin (CT), doxycycline (DO), enrofloxacin (ENR), florfenicol (FFL), neomycin (NEO), norfloxacin (NOR), spectinomycin (SPC), and trimethoprim with sulfamethoxazole (SXT).

The tests were performed on colonies of the investigated strains after 24 h incubation. The bacteria were suspended in 4 ml of demineralized water (Thermo) using a sterile cotton swab to obtain a 0.5 McFarland suspension. Density was assessed using the Sensititre Nephelometer (Thermo). Ten microliters of the bacterial suspension were transferred into 11 ml of Mueller-Hinton Broth (Thermo). The resulting inoculum was applied onto a POLBV plate, 50 μL into each well, using SensititreAutoInoculator (Thermo). Incubation was carried out at 37 °C ± 1 °C for 18–24 hours. The Sensititre plates were then read with SWIN software using a Vizion instrument (Thermo). The results were interpreted according to CLSI standards (VET01 Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals) [20]. The results then underwent statistical analysis. A chi-squared test revealed significant differences between the percentages of *Escherichia coli* and *Klebsiella* spp. strains resistant to the investigated antibiotics. The analysis included aggregated data collected throughout the study for the investigated antibiotics which, represented by the percentages of resistant strains, were pooled together. Results with $p < 0.05$ were deemed significant. Statistical significance for the investigated drugs is presented in Table 4 (*Escherichia coli)* and Table 5 (*Klebsiella* spp.). The results were processed using Statistica software by Kamsoft S.A. [PLC].

RESULTS

Drug resistance of *Escherichia coli* strains. Approximate number of *E. coli* samples for individual antibiotics ranging from 4,496 (trimethoprin with sulfamethxazole) to 4,481 (spectinomycin) were investigated (Tab. 1). The number of isolated *E. coli* strains confirmed responsibility of the species for a significant share of infections in the chickens. Assessment of all samples identified colistin as the most effective antibiotic. As many as 84.64% and 80.62% of the investigated *E. coli* strains were susceptible to neomycin and amoxicillin with clavulanic acid, respectively. Among the fluoroquinolones, enrofloxacin (34.64 %) was less effective than norfloxacin, to which half of the bacteria strains (51.11 %) were susceptible. The combination of trimethoprim with

Table 1. Antibiotic susceptibility of *Escherichia coli* strains. n – number of samples, S – susceptible strains, S% – percentage of susceptible strains, R – resistant strains, R% – percentage of resistant strains, I – intermediate strains, I% – percentage of intermediate strains

Antibiotic	n	I	I%	R	R%	S	S%
Amoxicillin	4495	89	1.98	3310	73.64	1096	24.38
Amoxicillin with clavulanic acid	4493	438	9.75	773	17.20	3282	73.05
Colistin	4491	0	0	690	15.36	3801	84.64
Doxycycline	4493	534	11.89	2340	52.08	1619	36.03
Enrofloxacin	4495	751	16.70	2187	48.65	1557	34.64
Florfenicol	4495	1985	44.16	1715	38.15	795	17.69
Neomycin	4495	2	0.04	869	19.33	3624	80.62
Norfloxacin	4492	172	3.83	2024	45.06	2296	51.11
Spectinomycin	4481	2690	60.03	1751	39.08	40	0.89
Trimethoprim with sulfamethoxazole	4496	5	0.11	2087	46.42	2404	53.47

sulfamethoxazole (53.47 %) and doxycycline (36.03 %) showed a similar effect. The efficacy of the remaining antibiotics was low or very low. Spectinomycin showed a bactericidal activity against 0.89% of bacteria. Amoxicillin and florfenicol were effective in 24.38% and 17.69% of cases, respectively. For amoxicillin, amoxicillin with clavulanic acid, colistin, enrofloxacin, neomycin, norfloxacin, spectinomycin, and trimethoprim with sulfamethoxazole, a growing percentage was observed of antibiotic-resistant samples in consecutive quarters of the study. The trend was not observed only for doxycycline and florfenicol, where the percentage of resistant samples decreased throughout the duration of the study (Tab. 2).

For *Escherichia coli*, significant differences ($p < 0.05$) were observed between amoxicillin and all the other investigated antibiotics. Amoxicillin with clavulanic acid was similarly effective as colistin ($p > 0.05$). No statistical significance was noted in the number of resistant strains when colistin was compared with neomycin, and doxycycline with enrofloxacin, norfloxacin, spectinomycin and trimethoprim. Also, the differences in the biocidal properties of enrofloxacin, florfenicol, norfloxacin, spectinomycin and trimethoprim were insignificant. Comparison of florfenicol with norfloxacin, spectinomycin, and trimethoprim, as well as of norfloxacin with spectinomycin and trimethoprim, showed no significant discrepancies. The same was observed for the comparison of spectinomycin with trimethoprim.

Table 3. Antibiotic susceptibility of *Klebsiella* spp. strains. n – number of samples, S – susceptible strains, S% – percentage of susceptible strains, R – resistant strains, R% – percentage of resistant strains, I – intermediate strains, I% – percentage of intermediate strains

Antibiotic	n	I	I%	R	R%	S	S%
Amoxicillin	84	1	1.19	82	97.62	1	1.19
Amoxicillin with clavulanic acid	84	15	17.86	37	44.05	32	38.09
Colistin	84	0	0	13	15.48	71	84.52
Doxycycline	84	5	5.96	29	34.52	50	59.52
Enrofloxacin	84	13	15.48	30	35.71	41	48.81
Florfenicol	84	29	34.52	46	54.77	9	10.71
Neomycin	84	0	0	12	14.29	72	85.71
Norfloxacin	84	0	0	24	28.57	60	71.43
Spectinomycin	84	54	64.29	29	34.52	1	1.19
Trimethoprim with sulfamethoxazole	84	0	0	22	26.19	62	73.81

Drug resistance of *Klebsiella* spp. strains. The study investigated 84 samples of *Klebsiella* spp. isolated from the internal organs of dead birds. Neomycin (85.71% susceptible), colistin (84.52%), trimethoprim with sulfamethoxazole (73.81%) and norfloxacin (71.43%) proved to be the most effective in the treatment of *Klebsiella* spp. infections. Doxycycline (59.52%) and enrofloxacin (48.81%) were less effective; resistant isolates amounted to 34.52% and 35.71%, respectively, and intermediate ones to 5.96% and 15.48%, respectively. Amoxicillin and spectinomycin (both 1.19%) were the least effective. The presence of clavulanic acid increased the efficacy of amoxicillin to 38.10%. Florfenicol demonstrated a bactericidal activity only against 10.71% of strains (Tab. 3).

Only 2 out of 84 *Klebsiella* spp. strains (2.38%) were resistant to all antibiotics investigated in the study. It was found that 8.33% of strains susceptible to only one antibiotic, 9.52% of the strains susceptible to 2, and 7.14% to 3 drugs at the same time. The overview presented above shows that 27.37% of the investigated *Klebsiella* spp. strains were resistant to 7 out of 10 investigated drugs, of which 51.19% were susceptible to half of the substances used. As in the case of *Escherichia coli*, a statistically significant difference ($p < 0.05$) was demonstrated for the comparison of amoxicillin with any of the other investigated antibiotics. Amoxicillin with clavulanic acid compared to doxycycline, enrofloxacin and florfenicol, did not show a statistically significant ($p < 0.05$) difference regarding the percentage

Table 2. Number of antibiotic-resistant *Escherichia coli* strains in the consecutive quarters between August 2014 and June 2016

	Amoxicillin [%]	Amoxicillin with clavulanic acid [%]	Colistin [%]	Doxycycline [%]	Enrofloxacin [%]	Florfenicol [%]	Neomycin [%]	Norfloxacin [%]	Spectinomycin [%]	Trimethoprim with sulfamethoxazole [%]
Q3 2014	71.34	20.2	16.94	56.68	39.74	36.81	19.22	38.76	37.46	43
Q4 2014	61.63	13.02	12.09	46.98	38.37	34.19	11.86	34.19	31.16	38.6
Q1 2015	64.51	10.31	10.49	51.92	38.29	33.39	16.26	35.31	29.37	39.51
Q2 2015	75.7	14.29	11.35	59.68	49.4	41.66	18.83	44.19	35.78	47.13
Q3 2015	77.92	20.35	18.3	64.67	53.94	53.15	19.56	48.58	44.64	50.32
Q4 2015	80.29	25.27	20.25	65.59	61.29	52.51	26.88	53.23	49.82	50.9
Q1 2016	74.45	14.58	15.2	35.27	49.84	27.27	18.03	48.9	38.56	47.96
Q2 2016	75.44	20.1	18.34	34.93	49.28	23.6	21.69	49.12	41.31	48.01

Table 4. Evaluation of statistical significance between the percentage of *Escherichia coli* strains resistant to the tested antibiotics. P<0.05 indicates pairs of antibiotics that show statistically significant difference in the resistance rate among *Escherichia coli*. Amoxicillin (AML), amoxicillin with clavulanic acid (AMC), colistin (CT), doxycycline (DO), enrofloxacin (ENR), florfenicol (FFL), neomycin (NEO), norfloxacin (NOR), spectinomycin (SPC), and trimethoprim with sulfamethoxazole (SXT).

E. coli	AML 73.64%	AMC 17.20%	CT 15.36%	DO 52.08%	ENR 48.65%	FFL 38.15%	NEO 19.33%	NOR 45.06%	SPC 39.08%	SXT 46.42%
AML 73.64%		p<0.001	p<0.001	p<0.01	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
AMC 17.20%	p<0.001		p= 0.6997	p<0.001	p<0.001	p<0.001	p= 0.7128	p<0.001	p<0.001	p<0.001
CT 15.36%	p<0.001	p=0.6997		p<0.001	p<0.001	p<0.001	p= 0.4515	p<0.001	p<0.001	p<0.001
DO 52.08%	p<0.01	p<0.001	p<0.001		p= 0.6714	p<0.05	p<0.001	p= 0.3220	p= 0.0649	p= 0.3961
ENR 48.65%	p<0.001	p<0.001	p<0.001	p= 0.6714		p= 0.1167	p<0.001	p= 0.5709	p= 0.1543	p= 0.6710
FFL 38.15%	p<0.001	p<0.001	p<0.001	p<0.05	p= 0.1167		p<0.01	p= 0.3151	p= 0.8845	p= 0.2517
NEO 19.33%	p<0.001	p= 0.7128	p= 0.4515	p<0.001	p<0.001	p<0.01		p<0.001	p<0.01	p<0.001
NOR 45.06%	p<0.001	p<0.001	p<0.001	p= 0.3220	p= 0.5709	p= 0.3151	p<0.001		p= 0.3900	p= 0.8871
SPC 39.08%	p<0.001	p<0.001	p<0.001	p= 0.0649	p= 0.1543	p= 0.8845	p<0.01	p= 0.3900		p= 0.3167
SXT 46.42%	p<0.001	p<0.001	p<0.001	p= 0.3961	p= 0.6710	p= 0.2517	p<0.001	p= 0.8871	p= 0.3167	

Table 5. Evaluation of statistical significance between the percentage of *Klebsiella spp.* strains resistant to the tested antibiotics. P<0.05 indicates pairs of antibiotics that show statistically significant difference in the resistance rate among Klebsiella spp. Amoxicillin (AML), amoxicillin with clavulanic acid (AMC), colistin (CT), doxycycline (DO), enrofloxacin (ENR), florfenicol (FFL), neomycin (NEO), norfloxacin (NOR), spectinomycin (SPC), and trimethoprim with sulfamethoxazole (SXT)

Klebsiella	AML 97.62%	AMC 44.05%	CT 15.48%	DO 34.52%	ENR 35.71%	FFL 54.76%	NEO 14.29%	NOR 28.57%	SPC 34.52%	SXT 26.19%
AML 97.62%		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
AMC 44.05%	p<0.001		p<0.001	p= 0.1930	p= 0.2482	p= 0.1198	p<0.001	p<0.05	p= 0.1930	p<0.01
CT 15.48%	p<0.001	p<0.001		p<0.01	p<0.001	p<0.001	p= 0.8408	p<0.05	p<0.01	p= 0.0540
DO 34.52%	p<0.001	p= 0.1930	p<0.01		p= 0.8825	p<0.01	p<0.001	p= 0.3631	p=1.0000	p= 0.1669
ENR 35.71%	p<0.001	p= 0.2482	p<0.001	p= 0.8825		p<0.01	p<0.001	p= 0.2906	p= 0.8825	p= 0.1263
FFL 54.76%	p<0.001	p= 0.1198	p<0.001	p<0.01	p<0.01		p<0.001	p<0.001	p<0.01	p<0.001
NEO 14.29%	p<0.001	p<0.001	p= 0.8408	p<0.001	p<0.001	p<0.001		p<0.01	p<0.001	p<0.05
NOR 28.57%	p<0.001	p<0.05	p<0.05	p= 0.3631	p= 0.2906	p<0.001	p<0.01		p= 0.3631	p= 0.6347
SPC 34.52%	p<0.001	p= 0.1930	p<0.01	p=1.0000	p= 0.8825	p<0.01	p<0.001	p= 0.3631		p= 0.1669
SXT 26.19%	p<0.001	p<0.01	p= 0.0540	p= 0.1669	p= 0.1263	p<0.001	p<0.05	p= 0.6347	p= 0.1669	

of resistant strains. The same was established for colistin compared to neomycin. The comparison of doxycycline with enrofloxacin, norfloxacin, spectinomycin and trimethoprim also proved to be insignificant ($p > 0.05$). The differences between the percentage of strains resistant to enrofloxacin and norfloxacin, spectinomycin or trimethoprim, were not significant ($p > 0.05$). Norfloxacin, compared to spectinomycin and trimethoprim, as well as spectinomycin compared to trimethoprim, did not provide significant differences concerning the percentage of resistant strains ($p > 0.05$).

Among the investigated substances, only amoxicillin showed statistically significant differences, i.e. a higher number of resistant strains compared with any of the other investigated antibiotics. This was established for both *Klebsiella* spp. and *Escherichia coli* strains.

DISCUSSION

Escherichia coli. To a certain extent, the results revealed an overlap with data published by the EFSA stating that commensal *Escherichia coli* strains isolated from poultry in the same period show the highest resistance to ampicillin (58.7%) and ciprofloxacin (65.7%). According to an EFSA report, *E. coli* was isolated from broilers, fattening turkeys and their meat, but not from changed organs such as liver, spleen, heart and lungs of chickens. The current study demonstrates resistance to another fluoroquinolone – enrofloxacin, affecting 65.4% of cases. Nevertheless, norfloxacin (48.89% susceptible strains) proved to be much more efficient than ciprofloxacin [21].

On a European scale, ampicillin proved to be much more efficient against *Escherichia coli* than amoxicillin (1.19% susceptible strains), but less efficient than amoxicillin with clavulanic acid (73.05% susceptible strains). However, taking into account only studies conducted on Polish samples, the results for ampicillin (72.6%) were similar to those of amoxicillin with clavulanic acid. Every fifth *E. coli* strain investigated by the EFSA was resistant to chloramphenicol (21.6%), which proved to be more effective than its derivative, florfenicol (82.31% of resistant strains) [21]. In a study carried out in Egypt in 2018, all E. coli strains isolated from heart, kidney, liver and lungs were multi-drug resistant, and 80% of the samples were resistant to ampicillin. The tested strains were also resistant to kanamycin (85%), oxytetracycline (85%), enrofloxacin (75%), and chloramphenicol (65%) [22]. Data from a national monitoring programme in Poland showed a high resistance of commensal *E. coli* isolated from broilers to ampicillin (86%), ciprofloxacin (81.9%), nalidixic acid (73.1%), tetracycline (66.7%), and sulfamethoxazole (66.7%), and increasing resistance to ampicillin

A study conducted in India by Hussain et al. [23] indicated that strains isolated from poultry and its meat most often showed resistance to tetracycline (84%), ciprofloxacin (70%), trimethoprim with sulfamethoxazole (45%) and gentamicin (32%), while resistance to chloramphenicol was rare (only 8%).

A similarly high share of tetracycline-resistant isolates was detected in turkey (78%) and chicken (34%) meat from different farming systems in the USA, in which the study showed no significant differences between standard, organic and RWA farming, and resistance prevalence was twice as high in turkey than in chicken meat. Additionally, over 50% of turkey meat samples demonstrated multi-drug resistance. The multi-drug resistance significantly correlated with the farming systems, and multi-resistant *E. coli* strains occurred more often in flocks treated with antibiotics than in RWA flocks [24]. Almost all of 50 APEC strains from Nepal tested by Subedi et al. were resistant to ampicillin (98%) [25]. APEC resistance to antibiotics commonly used in poultry is noticeably more frequent in countries where ASW was used for many years or is still acceptable, and where supervision of drug use is insufficient and access to the drugs is widespread [25, 26, 27]. Multi-drug resistance observed in majority of APEC strains for many years and often antibiotic therapy in chicken flocks turned out ineffective. The strains were usually resistant to sulfamethoxazole (93%), tetracycline (87%), streptomycin (86%), gentamicin (69%), and nalidixic acid (59%) [28]. These findings have been confirmed in more recent studies. Subedi et al. demonstrated that 98% of broiler APEC strains were resistant to ampicillin, 90% to sulfamethoxazole, and 62% to doxycycline [25]. Azam et al. claimed that the majority of *E. coli* strains isolated from dead chickens showed resistance to ampicillin (98.6%), tetracycline (97.3%), and ciprofloxacin (72%) [29]. An equally high resistance prevalence was confirmed in various studies conducted on different continents [30] (Tab. 6).

Considering that antibiotics that are commonly administered to livestock in Poland, the drug resistance of *E. coli* is higher than the European average. This is particularly true for penicillins and fluoroquinolones, where the number of resistant strains is by 14% and 22% higher than the European average [21, 31].

***Klebsiella* spp.** The investigated *Klebsiella* spp. strains showed very high resistance to beta-lactam antibiotics. Other authors proved the possibility of transmitting ESBL-producing *Klebsiella* spp. strains and plasmids from broiler chicken meat to humans [6, 17, 31]. In the current study, as many as 27.37% of samples were resistant to 7 out of 10 investigated antibiotics. This points to the conclusion that meat is a very probable route of transmitting antibiotic-resistant strains (including those resistant to many antibiotic groups) to humans [32].

In a study conducted by Wu et al. in 2016, 96.7% of the investigated *Klebsiella* spp. strains demonstrated resistance to at least 3 of the investigated antibiotics, and 91.1% were resistant to at least 3 beta-lactams. Additionally, 96.7% of the bacteria produced extended-spectrum beta-lactamases (ESBL). Almost half (48.81%) of the strains were resistant to more than 3 antibiotics. According to Wu at al., commercial chicken meat is a considerable source of ESBL-producing *Klebsiella* strains, which makes it even clearer that the rational use of antibiotics in animals for slaughter should be taken very seriously [28].

CONCLUSION

Recent studies on antibiotic resistance showed that only rational antibiotic therapy may help to slow down the increasing resistance trend. Improving zoohygienic conditions on farms, ensuring biosecurity in nurseries, raising the immunity of chickens, and using narrow-spectrum antibiotics in the case of a disease may, to some extent, slow down the evolution of multidrug-resistant strains of pathogenic bacteria. Importantly, such bacteria pose a threat not only to birds but also to employees of farms and slaughterhouses, as well as, indirectly, to meat.

This study identified amoxicillin with clavulanic acid, colistin, and neomycin as the most effective antibiotics against *E. coli* infections in chickens for slaughter, whereas *Klebsiella* isolates demonstrated the highest susceptibility to neomycin, norfloxacin, trimethoprim with sulfamethoxazole, colistin and doxycycline. It is worth remembering that some of these antibiotics, such as colistin and fluoroquinolones, are considered critically important in medicine, and strains of bacteria resistant to these substances have been found. Moreover, antibiotics such as spectinomycin and amoxicillin were characterized by low efficacy in both groups researched. In the case of *E. coli,* a small percentage of strains were also sensitive to florfenicol, amoxicillin, enrofloxacin and doxycycline.

Both of the critically important antibiotics in medicine and those with low efficacy against the investigated bacteria should be reduced to necessary cases in order not to increase the pool of antibiotic resistant strains.

The results presented prove that drug resistant strains of pathogenic bacteria pose a serious challenge to modern human and veterinary medicine. Bacteria responsible for common poultry infections are resistant to antibiotics routinely used in many countries. Extensive use of antibiotics reinforces the conclusion that in the future the scale of this phenomenon will continue to increase.

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