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Research Article

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A Retrospective Study about Antibiotic Susceptibility of the Bacteria Isolated from Broiler Chicken Mortality Referred to Veterinary Laboratory of Kazerun City

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Abstract

Background and aim: Bacterial infections are one of the important causes of casualties and financial losses in the poultry industry. Despite the key role of antibiotics in the treatment of these infectious diseases, a significant increase in antibiotic resistance is considered a threat to societies. Several studies have investigated the antibiotic resistance levels of the different pathogenic bacterial strains in poultry. This study aimed to investigate the bacterial causes of death in chicken farms in Kazerun City and also to evaluate the drug sensitivity of these bacterial strains.
Materials and Methods: The samples were obtained from the clinically-affected broiler chickens from 118 industrial poultry farms around Kazerun City during 2021-2022. The samples were cultured on MacConkey agar and eosin methylene blue (EMB) agar culture media. Then, the antibiotic sensitivity of the samples that were positive for *Escherichia coli* was evaluated by different antibiotics (enrofloxacin, erythromycin, oxytetracycline, tylosin, gentamicin, doxycycline, danofloxacin, difloxacin, sultrim, florfenicol, flumequine, fosbac, chlortetracycline, colistin, lincospectin, and neomycin).
Results: Among the 118 samples, 106 samples were positive for gram-negative bacteria. 100 of the 106 gram-negative samples were positive for *Escherichia coli*. All the *Escherichia coli*-positive samples were resistant to neomycin, chlortetracycline, flumoquine, gentamicin, and tylosin. Also, 99% and 97% of the *Escherichia coli*-positive samples were resistant to lincospectin and difloxacin, respectively. The highest antibiotic sensitivity of the samples was found to fosbac (94%) and Colistin (90%).
Conclusion: Due to the increase in resistance against common antibiotics, alternative methods are needed to slower drug resistance development and also reduce their resulting mortality. Biosecurity and using acidifiers and probiotics are among the ways to reduce bacterial infections and their need for antibiotic consumption.

Keywords: *Antibiotic, Antibiotic Resistance, Poultry, Broiler chickens, Escherichia coli*

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Introduction

Since the discovery of antibiotics, they have saved millions of lives (Gottfried, 2005). Many animals have also been cured by antibiotics (Arsène *et al.,* 2022). Some antibiotics have also been used as animal growth promoters (Hughes & Heritage, 2004). However, their misuse and overuse have led to a major threat to both human and animal lives, called antibiotic resistance (Ventola, 2015). Antibiotic-resistant strains evolve in a way that they protect themselves from the antibacterials' effects (Munita & Arias, 2016). Also, multi-drug resistance has been widely observed among various bacterial species (El-Mokhtar *et al.,* 2019). As a result, a surge in resistant strain-related deaths is achieved globally *(Murray et al.,* 2022). In the poultry industry, resistant strains cause economic losses (due to the increased treatment costs and consequent mortality rates); in addition, zoonotic antibiotic-resistant strains are also capable of causing infections in their consumers (Hossain *et al.,* 2021).

Escherichia coli (*E. coli*) is a gram-negative bacteria from *the Enterobacteriaceae* family that acts as one of the major causes of economic loss in the poultry industry (Bhusan Das, 2020). Some pathogenic strains of *E. coli* (extraintestinal pathogenic *E. coli*s) cause colibacillosis in poultry, which is manifested by various signs, including subacute pericarditis, septicemia, perihepatitis, omphalitis, yolk sac infection, enteritis, and peritonitis (Bhusan Das, 2020; Lutful Kabir, 2010). Furthermore, extraintestinal pathogenic *E. coli*s that are zoonotic bacterial strains are divided into various strains, e.g. avian-pathogenic *E. coli* (APEC), uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), and sepsis-associated *E. coli* (SEPEC) (Mitchell *et al.,* 2015). Consumption of poultry meat with these bacterial strains leads to food-borne diseases in humans (Ali *et al.,* 2020). In addition to their role in human and poultry diseases, these strains contain some plasmids which contain antibiotic-resistance genes (Sun *et al.,* 2017). These plasmids are easily transferred horizontally among the species; hence, these resistant bacteria can cause antibiotic resistance in other bacterial species in human bodies (Sun *et al.,* 2017). Additionally, poultry manure is used to fertilize agricultural soils (Marutescu *et al.,* 2022). Poultry manure is a source of many bacterial species (Marutescu *et al.,* 2022). If the manure contains resistant bacterial strains, these bacteria can infect crops and finally reach the animals and humans that feed from these crops (Marutescu *et al.,* 2022).

Regarding the high mortality rates in commercial chicken farms, finding the responsible infectious agents and their antimicrobial resistance/sensitivity is of great importance. Hence, this study aims to evaluate the etiologic microbial cause(s) of broiler chicken mortalities and their treatment options in Kazerun City.

Materials and Methods

Farm selection and sampling

This study was retrospective. It was conducted on clinically-affected broiler chickens from 118 chicken farms which referred to Kazerun veterinary laboratory from 2021 to 2022.

Necropsy was done and samples were collected from the infected organs, especially from the liver, heart, and air sac.

Isolation and identification of bacterial isolates

Then, we mixed the samples from each farm. The mixture of each farm was cultured on MacConkey and eosin methylene blue (EMB) agar culture media. Then, these media were incubated at 37 °C for 24-48 hours. These media are specific for gram-negative bacteria. The positive result of the MacConkey and EMB agar tests were determined by their respective yellowish and metallic-green discoloration. Colonies of the positive culture media were obtained to make 0.5 McFarland media for antibiogram tests (Das *et al.,* 2017).

Antibacterial susceptibility tests

For evaluating the antibiotic sensitivity of the bacteria, an antibiogram test was done. Mueller-Hinton agar media and various antibiotic discs (including enrofloxacin, erythromycin, oxytetracycline, tylosin, gentamicin, doxycycline, danofloxacin, difloxacin, sultrim, florfenicol, flumequine, fosbac, chlortetracycline, colistin, lincospectin, and neomycin) were used for conducting antibiogram test. Then, the antibiogram plates were incubated at 37 °C for 24 hours. We used "Padtan teb disks". According to the halo diameter around the discs, the antibiotic susceptibility of the bacteria were determined, using Padtan teb catalog. The 3+, 4+, or 5+ halo diameters indicate high antibiotic sensitivity of the bacterial agents; whereas, 1+ and 2+ results are in favor of intermediate sensitivity against antibiotics. 0 means that the bacteria is resistant to the tested antibiotic (figure 1) (Bauer, 1966; Hooja *et al.,* 2016).

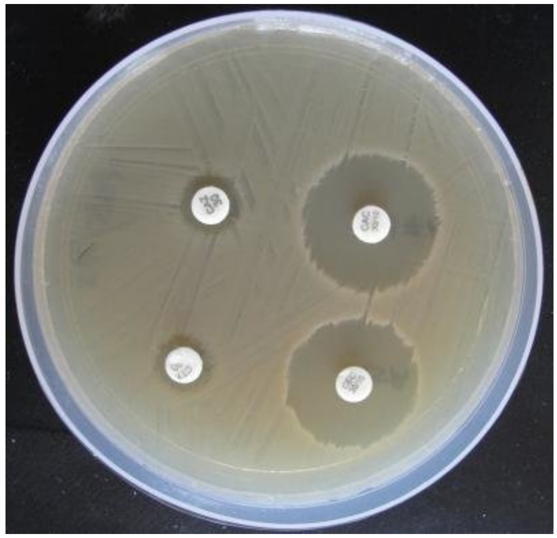

Figure 1. A sample of antibiogram test and different clear zones around each disc.

Results

From the 118 tested cases, 106 cases were positive for gram-negative bacteria. 100 of the 106 cases were *E. coli* positive. Then, the results of the antibiogram tests of the *E. coli* samples are shown in Table 1. The percentage of the resistant, sensitive, and semi-sensitive strains against each antibiotic is shown. All the tested strains were resistant to neomycin, chlortetracycline, flumequine, gentamicin, and tylosin. The majority of the strains were also resistant to lincospectin, difloxacin, danofloxacin, and erythromycin; whereas, the majority of them were sensitive to colistin and fosbac.

Antibiotic	ENR	ERY	OTC	TYL	GEN	DOX	DAN	DIF	Sultrim	FFL	FLU	FOS	CTC	CST	LS	NEO
Resistant strains' percentage	30	72	26	100	100	21	90	97	52	15	100	6	100	10	99	100
Percentage of the strains with intermediate sensitivity	38	25	41	-	-	37	5	2	10	42	-	19	-	1	1	-
Sensitive strains' percentage	32	3	33	-	-	42	5	1	38	43	-	75	-	89	-	-

Table 1. The prevalence of the resistant, semi-sensitive, and sensitive strains among the cases of our study against different antibiotics. Table abbreviations: Enrofloxacin=ENR, erythromycin=ERY, oxytetracycline=OTC, tylosin=TYL, gentamicin=GEN, doxycycline=DOX, danofloxacin=DAN, difloxacin=DIF, florfenicol=FFL, flumequine=FLU, fosbac=FOS, chlortetracycline=CTC, colistin=CST, lincospectin=LS, and neomycin=NEO

Discussion

As poultry products are the most important sources of food with reasonable costs, their production has an important role in the food industry worldwide (Ali and Hossain, 2012; Miller *et al.,* 2022). Due to the high mortality rates of commercial chicken farms, biosecurity, supervision of chicken farms, and disease control are crucial (Ngongolo & Chota, 2022; Rimi *et al.,* 2017). One of the useful approaches in combating diseases is using antibiograms (Jones *et al.,* 2013). Antibiograms are valuable tools that specify the bacterial agents' antibiotic sensitivity and help in deciding the best treatment options, as in Jones et al,'s study, the infected broiler breeder chickens with similar clinical signs were caused by different bacterial agents with different antibiotic susceptibilities (Jones *et al.,* 2013). In this study, we found that 84.7% of the cases were infected with *E. coli* strains and then we evaluated the antibiotic susceptibility of the *E. coli* strains that were obtained from the clinically-affected broiler chickens. Das et al, in 2015 found that 47% of the yolk sac and liver infections were caused by *E. coli* (Das *et al.,* 2017). Also in Ibrahim et al,'s study, 53.4% of the sick chickens were infected by *E. coli* strains (Ibrahim *et al.,* 2019). 66.6% of the post-mortem examined chickens in Lambie et al,'s study were also infected by *E. coli* strains (Lambie *et al.,* 2000).

Da Costa et al, found that 80.7%, 47.5%, and 18.4% of the *E. coli* strains were resistant to tetracycline, cotrimoxazole, and enrofloxacin, respectively (Da Costa *et al.,* 2008). These results were concomitant to our results, in which we found 100%, 52%, and 30% resistance against chlortetracycline, sultrim, and enrofloxacin. Al-Arfaj et al, found high antibiotic resistance in *E. coli* strains, in a way that 81% and 64.84% of the strains were resistant to erythromycin and gentamicin, respectively, compared to our results that the *E. coli* strains' resistance against erythromycin and gentamicin was 72% and 100% (Al-Arfaj *et al.,* 2015). Bhusan Das et al, found that 100%, more than 92%, and about 96% of the *E. coli* strains isolated from both broiler and layer farms were resistant to erythromycin, doxycycline, and sultrim, respectively. Additionally, only 3% of the isolates were resistant to colistin. These results were concomitant with the results of our study. They found that 95.4% of the isolates were resistant to enrofloxacin; whereas, in our study resistance to enrofloxacin was 30% (Bhusan Das, 2020). Although bacterial resistance to some antibiotics like colistin is still scarce, their resistance development is growing rapidly (El-Mokhtar *et al.,* 2019).

Conclusion

Due to the threats of drug and multi-drug-resistant species for global health and wealth, it is necessary to manage antibiotic prescription and consumption (Mancilla-Becerra *et al.,* 2019; Van Duin & Paterson, 2016). Probiotics and acidifiers are among the products that reduce the need for antibiotics (Wijayanti *et al.,* 2019). Another important way to slower antibacterial resistance is to prohibit the usage of human antibiotics in the poultry industry (Smoglica *et al.,* 2022).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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مطالعه‌ی گذشته نگر حساسیت آنتی بیوتیکی باکتری های جداشده از تلفات پرندگان گوشتی ارجاعی به آزمایشگاه دامپزشکی شهر کازرون

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چکیده

زمینه و هدف: عفونت های باکتریایی از علل مهم تلفات و خسارات مالی در صنایع پرورش طیور به شمار می روند. با وجود نقش کلیدی آنتی بیوتیک ها در درمان این بیماری ها، افزایش چشمگیر مقاومت نسبت به آنها تهدیدی برای جوامع به حساب می آید. مطالعات متعددی در زمینه ی بررسی میزان مقاومت آنتی بیوتیکی در گونه های مختلف باکتری های بیماری زای طیور انجام گرفته است. هدف ما از انجام این مطالعه بررسی علل باکتریایی تلفات حاصل از مرغداری های شهرستان کازرون و همچنین ارزیابی حساسیت دارویی این باکتری ها بود.

مواد و روش‌ها: نمونه ها از تلفات ۱۱۸ فارم مرغداری صنعتی در اطراف شهرستان کازرون در طی سال ۱۴۰۱–۱۴۰۰ بدست آمد. نمونه ها بر روی محیط کشت های مک کانکی آگار و ائوزین متیلن بلو آگار کشت داده شدند. سپس حساسیت نمونه هایی که برای اشریشیا کولی مثبت شدند توسط آنتی بیوتیک های مختلف (انروفلوکسازین، اریترومایسین، اکسی تتراسیکلین، تیلوزین، جنتامایسین، داکسی سیکلین، دانوفلوکسازین، دی فلوکسازین، سولتریم، فلورفنیکل، فلومکوئین، فوزبک، کلرتتراسیکلین، کلیستین، لینکوسپکتین و نئومایسین) مورد ارزیابی قرار گرفت.

یافته‌ها: از میان ۱۱۸ نمونه ی مورد بررسی ۱۰۰ نمونه آلوده به اشریشیا کولی بودند. ۱۰۰ نمونه از ۱۰۶ نمونه گرم منفی برای اشریشیا کلی مثبت بود. تمام نمونه ها به آنتی بیوتیک های نئومایسین، کلرتتراسیکلین، فلومکوئین، جنتامایسین و تیلوزین مقاوم بودند. همچنین ۹۹٪ و ۹۷٪ از نمونه های دارای اشریشیا کولی به ترتیب به لینکوسپکتین و دیفلوکسازین مقاوم بودند. بیشترین حساسیت آنتی بیوتیکی نمونه ها نسبت به آنتی بیوتیکهای فوزبک (۹۴٪) و کلیستین (۹۰٪) یافت شد.

نتیجه‌گیری: با توجه به افزایش مقاومت دارویی نسبت به آنتی بیوتیکهای متداول، روش های جایگزین جهت کاهش سرعت رشد مقاومت دارویی و همچنین کاهش مرگ و میر حاصل از آنها مورد نیاز است که از جمله ی آنها می توان به رعایت امنیت زیستی و استفاده از پروبیوتیکها و اسیدی فایرها جهت کاهش ابتلا به بیماری ونیاز به درمان آنتی بیوتیکی در طیور اشاره کرد.

واژه‌های کلیدی: *آنتی بیوتیک، مقاومت آنتی بیوتیکی، طیور، جوجه گوشتی، اشریشیا کولی*

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