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Research Article

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Isolation of Escherichia Coli from the Yolk Sac of One-Day Old Chicks with Their Antibiogram in Mashhad-Iran

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Abstract

Background and aim: Yolk sac infection (YSI) and colibacillosis are the most common infectious diseases that lead to high rates of early chick mortalities (ECMs) in young chicks. In this study, by referring to the Mashhad Veterinary Laboratory, one hundred chicks from different poultry farms sent to the Veterinary Laboratory, were examined and sampling was performed. All specimens without a history of antibiotic use were gradually transferred to a laboratory to be cultured from their yolk sac.
Materials and Methods: For this purpose, MacConkey culture medium and eosin methylene blue were used to grow microbes. The morphological status of the microbe was examined by slide preparation and Gram staining. Also, using biochemical culture medium, indole and methyl red index were used to identify Escherichia coli bacteria. Then, antibiogram of the isolated strains was performed to determine the susceptibility and resistance of the microbes. For this purpose, the disk diffusion method was used by Kirby Bauer method and compared with standard drug disks. Finally, based on the results, the extent of microbial contamination and their drug sensitivity were determined.
Results: The findings showed that 40% of the samples contained Escherichia coli and the highest microbial susceptibility to Ceftiofur and gentamicin drugs was observed, such that their sensitivity was 57.5% and 60%, respectively. The three drugs of Colistine, phosphomycin and lincomycin were 30% sensitive. Oxytetracycline, Sultrim, Erythromycin, Fluorophenicol, Tiamulin and Tylosin showed more than 90% drug resistance. Also, other antibiotics had different degrees of drug resistance and in addition, multidrug resistance has been observed in abundance.
Conclusion: Since the indiscriminate use of antibiotics has caused high drug resistance in chickens, it is necessary to minimize the occurrence of microbial contamination by observing hygienic conditions and appropriate disinfection. Also, the prescription of drugs should only be done by a veterinarian and based on the antibiogram.

Keywords: *Escherichia coli, Yolk sac infection, Antibiogram, Drug resistance*

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Introduction

Avian Colibacillosis is one of the most infectious diseases of all ages of poultry which results a variety of disease conditions including yolk sac infection, omphalitis, swollen head syndrome, septicemia, enteritis, salpingitis and respiratory tract infection (Calnek, 1997). During the first week of post-hatching of broilers, yolk sac infection (YSI) may cause a high mortality. Yolk sac is the main source of infection for day old chicks. YSI causes deterioration and decomposition of yolk, leading to deficiency of necessary nutrients and maternal antibodies, retarded growth, poor carcass quality, and increased susceptibility to other infections (Allan *et al.,* 2018). Various risk factors may have synergistic effect to the susceptibility to colibacillosis in poultry. In addition, the concentration of E. coli in the broiler house environment acts as an important factor (Harry & Hemsley, 1965).

Nowadays, microbiological food safety has become an important public health issue worldwide. In the gastrointestinal tract of poultry and human being, E. coli is one of the most common microfloras (Jawetz *et al.,* 1984). Though most of E. coli is not pathogenic but they indicate contamination in food by fecal materials. At present, for different purposes such as prophylaxis, growth promotion or therapeutics, antibiotics have been used successfully in poultry industries (Barton, 2000). Moreover, in both veterinary and human medicine, reckless use of antibiotic is considered as the major issue for developing resistant microorganisms (Neu, 1992; Vounba *et al.,* 2019). Many well-established evidence proved that treatment by antibiotics can lead to the development and dissemination of resistant E. coli which can then be transferred to people through food or direct close contact with sick animals (Furtula *et al.,* 2010; Han *et al.,* 2020; De Mesquita Souza Saraiva *et al.,* 2022).

These resistant microbial organisms may act as a potential source in the transmission of antimicrobial resistance to human pathogenic organisms (Van de Bogaard & Stobberingh,2000; Solà-Ginés *et al.,* 2015). Due to reckless use of antibiotics in veterinary medicine sector, an increased number of multidrug resistant bacterial serotypes were developed over the last few years. The transmission of resistance among different bacterial species and genera has now widely happened by plasmid (Davies, 1994, Niero *et al.,* 2018; Koyama *et al.,* 2020). Multidrug resistant strains of E. coli are developing in both human and chicken isolates in different parts of the world, (Amara *et al.,* 1995; Solà-Ginés *et al.,* 2015). Having resistance to different antimicrobial agents is an extensive trouble in the case of management and treatment of intra and extra intestinal infections caused by E. coli, which is responsible for illness, increased healthcare costs and death (Gupta *et al.,* 2001). The present study was designed to isolate E. coli strain from day old chicks for assessing their susceptibility and resistance patterns to some selected antimicrobials.

Materials and Methods

The study was conducted on Day Old Chicks (DOC) from Mashhad city. A total number of 100 DOC were brought from broiler farm mortality and tested at Mashhad veterinary laboratory. Postmortem examination of the DOCs was done by maintaining standard procedures. The sample was collected from yolk sac by using sterile cotton swab and yolk sac was spread on the solid surface of Mac Conkey agar. All samples were incubated for 24 hours at 37C. Suspected colony was spread it over the solid surface of the eosin methylene blue (EMB) agar, and it also incubated for 24 hours at 37C. Bacterial colony identification was done according to following a series of Gram staining and biochemical tests including indole and methyl red (Melese *et al.,* 2017; Das *et al.,* 2017).

Results

Among 100 samples collected from yolk sac of DOC, a total of 40% individual colonies of E. coli were isolated through different tests. Results of cultural and biochemical examination of 40% samples were positive in MacConkey which was also positive in EMB agar.

In experiments, *Escherichia coli* produces light pink colonies in McConkey agar medium and metallic green shine colonies in eosin methylene blue. In the microscopic examination of Gram's staining, all the positive samples were found as Gram-negative, pink colored, rod-shaped bacteria which were arranged in single or in pairs. Red colored ring was also observed in indole agar positive.

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The final results obtained in our study indicate that the highest sensitivity to the gentamicin and Ceftifur drugs is 60% and 57.5%, respectively. Also, the three drugs of phosphomycin, lincomycin and colistine each had only 30% sensitivity. Erythromycin and fluorophenicol were also the least sensitive. Multidrug resistance is abundant in the samples (Table 1 and 2).

Positive result of antimicrobial sensitivity test of all 40 isolates were subjected to antimicrobial sensitivity test to different antimicrobial agents. For this purpose, the disk diffusion method was used by Kirby Bauer method and compared with standard drug disks (Bauer *et al.,* 1966). The diameter of the measured halos was reported by comparison with the standard tables of the sample under the headings of sensitive, semi-sensitive and resistant. In this research, the disks of Padten Teb Iran Company have been used.

Drugs	Fluorfenicol	Erythromycin	Enrofloxacin	Doxycycline	Danofloxacin	Chlortetracycline	Colistine	Ceftiofur
Sensitive %	0	0	10	0	0	0	30	57.5
Semi. Sensitive %	10	5	30	30	37.5	50	20	30
Resistance %	90	95	60	70	62.5	50	50	12.5

Table 1. Results of antibiograms.

Drugs	Tylosin	Tiamulin	Sultrim	Oxytetracycline	Neomycin	Lincomycin	Gentamicin	Phosphomycin
Sensitive %	0	0	10	0	0	30	60	30
Semi. Sensitive %	2.5	2.5	0	5	60	30	30	10
Resistance %	97.5	97.5	90	95	40	40	10	60

Table 2. Results of antibiograms.

Discussion

Colibacillosis is considered as one of the major bacterial infections in avian disease. The excessive use of antibiotics reduced their effectiveness, which eventually led to the risk of emergence of antibiotic resistance. The aim of this study was to isolate, and determine antibiotic susceptibility of yolk sac to E. coli infection. The isolation of *E. coli* was carried out using conventional techniques. Then, these strains were antibiogram and tested over 16 antibiotics. From the study, it can be concluded that the entire 100 sample collected from yolk sac of DOC investigated for the identification of E. coli, 40% were positive. Morphology, staining and cultural characteristics of the bacteria in different cultural media as recorded in the study were almost similar as reported by Choudhury *et al*. They reported that staining and morphology of isolated E. coli exhibited Gram negative, small-rod, arranged in single or pairs, non-spore former (Choudhury *et al.,* 1985).

In this study, the average finding of E. coli was 40% from DOC of commercial broiler which is similar to the earlier reports of Bhattacharjee *et al.,* who reported 40.82% of E. coli in chickens from Bangladesh (Bhattacharjee *et al.* 1996). Suha *et al.,* reported 43.50%, Das et al. (2017) reported 47%, Rahman & Kabir (2004) reported 67.73% and Ahmed *et al.,* found 52.26% colibacillosis which is higher than this study (Suha *et al.,* 2008; Das *et al.,* 2017; Rahman & Kabir, 2004; Ahmed *et al.,* 2009). Antibiogram was also done to determine the sensitivity and resistance pattern against different antibiotics.

The final results obtained in our study indicate that the highest sensitivity to the two drugs of gentamicin and Ceftiofur is 60% and 57.5%, respectively, which may be due to the type of use of these drugs, which is injectable. From the antimicrobial sensitivity test, it can be said that Gentamycin and Ceftiofur should be the best drugs of choice in case of this DOC of commercial broiler.

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Gentamicin has the lowest resistance rates because this molecule is not used in avian medicine. In other studies, most strains were highly sensitive to gentamicin and ceftriaxone (Melese *et al.,* 2017). Also, the three drugs phosphomycin, lincomycin and colistine each had only 30% sensitivity. Most of the isolates showed resistance against Neomycin, Lincomycin, phosphomycin, Colistin, Chlortetracycline, Danofloxacin and Enrofloxacin (40-60 %), Doxycyclin (70 %) and Erythromycin, Fluorophenicol, Oxytetracyclin, Sultrim, Tiamulin and Tylosin (90-100%).

The present study was consistent with the previous study, where the poultry E. coli isolates were found resistant to tetracycline (Biswas *et al.,* 2001; Melese *et al.,* 2017). High level of resistance was reported against Tetracycline because most of the farmers had regularly used commercially available tetracycline in the poultry feed. Most of isolates of this study exhibited multiple resistances to more antibiotics which are similar with few reports from Bangladesh and other parts of the world (Guerra *et al.,* 2003). Due to the misuse of antibiotics, such high incidence of multi drug resistance may apparently be occurred (Van de Boogard & Stobberingh, 2000). Multidrug resistance of E. coli has become a widespread pathogen. Therefore, it is important to monitor the occurrence of resistance among bacteria from animals and food, as these bacteria are able to spread through food product to human (Vounba *et al.,* 2019; Han *et al.,* 2020; De Mesquita Souza Saraiva *et al.,* 2022).

The commercial poultry industry depends on raising birds in large quantities at high stocking densities, especially in broiler production systems. Avian pathogenic *Escherichia coli* is the main cause of colibacillosis affecting poultry. The main challenge to the poultry industry is antimicrobial resistance and the emergence of multidrug resistant bacteria that threaten the safety of the food chain. Veterinary pharmaceuticals are commonly used in poultry farming to prevent and treat microbial infections as well as to increase feed efficiency, but their use has created public and environmental health concerns. Increasing antibiotic resistance is a matter of concern for consumers, public health authorities, farmers, and researchers.

Increased antibiotic resistance associated with the use or misuse of antibiotics in the animal industry has raised serious concerns. These concerns urgently require a focus on host-driven nonantibiotic approaches for the stimulation of protective antimicrobial immunity (Ageitos *et al.,* 2017).

Antimicrobial peptides (AMPs) are emerging as novel and effective non-antibiotic tools to combat infectious diseases in poultry. Most AMPs have the ability to kill microbial pathogens directly, whereas others act indirectly by modulating the host defense systems. Against a background of rapidly increasing resistance development to conventional antibiotics all over the world, efforts to bring AMPs into clinical use are accelerating. Several AMPs are currently being evaluated in clinical trials as novel anti-infectives. Thus, AMPS can be a significant addition to potential alternatives to antibiotics for the control of bacterial infections in young chicks. In ovo administration of these AMPs can offer significant protection from early chick mortality (Cuperus et al., 2016; Gunawardana *et al.,* 2019; Mahlapuu *et al.,* 2016; Nguyen *et al.,* 2021).

AMPs are ubiquitous, gene-encoded natural antibiotics that have gained recent attention in the search for new antimicrobials to combat infectious diseases. In multicellular organisms, AMPs, such as defensins and cathelicidins, provide a coordinated protective response against infection and are a principal component of innate immunity in vertebrates. In unicellular organisms, AMPs, such as bacteriocins, function to suppress competitor species. Because many AMPs kill bacteria by the disruption of membrane integrity and are thus thought to be less likely to induce resistance, AMPs are being extensively evaluated as novel antimicrobial drugs (Sang & Blecha, 2008).

Conclusion

Since the indiscriminate use of antibiotics has caused high drug resistance in chickens, it is necessary to minimize the occurrence of microbial contamination by observing hygienic conditions and appropriate disinfection. Also, the prescription of drugs should only be done by a veterinarian and based on the antibiogram. The use of new peptide drugs that do not cause drug resistance is recommended as an alternative to the currently used antibiotics.

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Conflict of interest
None.

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جداسازی اشریشیا کلی از کیسه زرده جوجه های یک روزه با آنتی بیوگرام آنها در مشهد، ایران

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چکیده

زمینه و هدف: عفونت کیسه زرده (YSI) و کلی باسیلوز شایع ترین بیماری های عفونی هستند که منجر به مرگ و میر زودرس جوجه ها (ECM) در جوجه های جوان می شوند. در این تحقیق با مراجعه به آزمایشگاه دامپزشکی مشهد، ۱۰۰ قطعه جوجه از مرغداری های مختلف ارسالی به آزمایشگاه دامپزشکی بررسی و نمونه برداری انجام شد. تمام نمونه های بدون سابقه مصرف آنتی بیوتیک به تدریج به آزمایشگاه منتقل شدند تا از کیسه زرده آن ها کشت داده شوند.

مواد و روش‌ها: برای این منظور از محیط کشت مک کانکی و ائوزین متیلن بلو برای رشد میکروب ها استفاده شد. وضعیت مورفولوژیکی میکروب با تهیه لام و رنگ‌آمیزی گرم مورد بررسی قرار گرفت. همچنین با استفاده از محیط کشت بیوشیمیایی، از اندول و شاخص متیل رد برای شناسایی باکتری اشریشیا کلی استفاده شد. سپس آنتی بیوگرام سویه های جدا شده برای تعیین حساسیت و مقاومت میکروب ها انجام شد. بدین منظور از روش دیسک دیفیوژن به روش کربی بائر و مقایسه آن با دیسک های دارویی استاندارد استفاده شد. در نهایت بر اساس نتایج، میزان آلودگی میکروبی و حساسیت دارویی آنها مشخص شد.

یافته‌ها: یافته ها نشان داد که ۴۰٪ نمونه ها حاوی اشریشیا کلی بودند و بیشترین حساسیت میکروبی به داروهای سفتیوفور و جنتامایسین مشاهده شد، به طوری که حساسیت آنها به ترتیب ۵۷/۵ و ۶۰ درصد بود. سه داروی کلستین، فسفومایسین و لینکومایسین ۳۰ درصد حساس بودند. دارو های اکسی تتراسایکلین، سولترم، اریترومایسین، فلوروفنیکول، تیامولین و تایلوزین بیش از ۹۰ درصد مقاومت دارویی نشان دادند. همچنین سایر آنتی بیوتیک ها دارای درجات مختلف مقاومت دارویی بودند و علاوه بر آن مقاومت چند دارویی به وفور مشاهده شد.

نتیجه‌گیری: از آنجا که مصرف بی رویه آنتی بیوتیک ها موجب بروز مقاومت دارویی بالایی در ماکیان شده است لذا بایستی با رعایت شرایط بهداشتی و ضدعفونی مناسب بروز آلودگی های میکروبی را به حداقل رساند. همچنین تجویز داروها صرفا بایستی توسط دامپزشک و بر اساس آنتی بیوگرام صورت گیرد.

واژه‌های کلیدی: *اشریشیا کلی، عفونت کیسه زرده، آنتی بیوگرام، مقاومت دارویی*

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