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ANTIBIOTIC RESISTANCE PATTERN OF AVIAN PATHOGENIC *ESCHERICHIA COLI* IN BROILERS BEL0NGING TO SOME EGYPTIAN FARMS

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) are causative agents of extraintestinal infections, collectively known as colibacillosis, which results in significant economic losses in poultry industries. Colibacillosis is the most frequent bacterial disease in avian species and antimicrobials are the main weapon to reduce incidence and mortality associated with it. However, inappropriate use of antibiotics may lead to therapy failure. This study was conducted to isolate APEC and to investigate the antibiotic resistance profiles of the isolates for further development of different control strategies. A total of 138 samples (liver, heart blood and lung) were collected from different broiler chicken farms in Giza, Sharqia and Qalyubiyya Governorates. All samples were subjected to bacteriological examination for isolation and identification of *E. coli*. Confirmed that *E. coli* strains were subjected to antimicrobial susceptibility test. The results revealed that 114 out of 138 samples (82.6%) were positive for *E. coli* isolation. Concerning antibiotic sensitivity test, high resistance was detected against ampicillin, tetracycline and nalidixic acid. Meanwhile *E. coli* isolates showed less resistance to ciprofloxacin and erythromycin. Overall, all - isolates showed resistance to at least three or more antimicrobials. These findings suggest the need for surveillance and intervention system to control misuse of antibiotics and APEC outbreak in- poultry farms.

Keywords:

Antimicrobial resistance; Avian pathogenic *Escherichia coli* (APEC); Broiler chickens.

INTRODUCTION

Avian colibacillosis is one of the most common bacterial diseases leading to significant economic losses to the poultry industry in the world **(Schouler *et al.*, 2012; Paixao *et al.*, 2016).** APEC is the causative agent of colibacillosis. It is a Gram-negative bacterium that belongs to the Enterobacteriaceae family and considered to be a member of the extraintestinal pathogenic *Escherichia coli* (ExPEC) group **(De Oliveira *et al.*, 2015; Wang *et al.*, 2015).**

The most common infections caused by APEC in chickens are perihepatitis, swollen head syndrome, airsacculitis, pericarditis, egg peritonitis, salpingitis, coli granuloma, omphalitis, cellulitis, and osteomyelitis/arthritis; those are commonly referred as avian colibacillosis **(Moulin and Fairbrother, 1999; Mellata *et al.*, 2013).** Colibacillosis is one of the leading causes of mortality (up to 20%) and morbidity in poultry and also results in decreased meat gain (2% decline in live weight, as result of 2.7% deterioration in feed conversion ratio) and egg production (up to 20%), decreased hatching rates, and increased condemnation of carcasses (up to 43%) at slaughter **(Moulin and Fairbrother,1999;Guabiraba and Schuler, 2015; Mellata, 2013).** By entering through the oral and respiratory routes, APEC induces systemic infections in chickens as a primary pathogen or as a secondary pathogen to infectious bronchitis virus (IBV), Newcastle disease virus (NDV), avian influenza virus (AIV) infections, immunosuppressive disease (Infectious bursal disease (IBD), and environmental stresses as result of overcrowding and high ammonia level **(Moulin and Fairbrother, 1999; Guabiraba and Schouler, 2015).** APEC can affect all species of poultry in all types of production systems **(Guabiraba and Schouler 2015).** APEC is also prevalent (9.52% to 36.73%) in all age groups of chickens **(Kabir *et al.*, 2015).** Taken together, along with the treatment expenses, APEC costs the poultry industry hundreds of millions of dollars as economic losses worldwide **(Ghunaim *et al*., 2014).**

Antibiotic resistance represents a serious problem to global public health, resulting in a significant impact on animal health and food safety **(Aarestrup, 2004).** The misuse of antimicrobial agents could lead to selection and diffusion of resistant microorganisms with related increase of antibiotic resistance rate **(Spellberg, 2014).** Furthermore, the problem of multidrug resistance (MDR) can be transmitted and disseminated between animal and human pathogens, leading to treatment problems both in animal and human diseases. Poultry industries consume wide range of antibiotics, because only few regulations are controlling their use **(Hvistendahl, 2012).**

In poultry industries, antibiotics have been used as growth promoter and disease preventive measures **(Bhandari and Singh, 2004; Osti *et al.*, 2017; Shrestha *et al.*, 2017).** Management treatments, infection control, and vaccination techniques are all crucial elements of avian colibacillosis control **(Manyi-Loh *et al.*, 2018).** There are a wide variety of antimicrobial agents used in poultry colibacillosis treatment, including: aminoglycosides, tetracyclines, sulphonamides, fluoroquinolones and β-lactams(Penicillin and cephalosporin)**(Zhuge *et al.*,**

2017). Due to the frequent use of antimicrobial agents, antimicrobial resistance against APEC can develop **(Agyare *et al*., 2019).**

The aim of this study was to determine the antibiotic susceptibility phenotypes of APEC isolated from different broiler farms in Egypt for further development of appropriate control measures.

MATERIAL AND METHODS

Sample Collection:

From November 2020 to June 2021, 138 samples were obtained from various broiler frams in Giza, Sharqia and Qalyubiyya Governorates. Samples were taken from the visceral organs (liver, lung, and heart blood) of broilers of various breeds died from colibacillosis with typical symptoms such as septicemia, respiratory infections, and pathologic findings such as pneumonia, tracheid, air saculitis, pericarditis, peritonitis, and hepatitis. The samples were collected aseptically in separate zipper lock bags, maintained in an ice box, and quickly transferred to the laboratory for detailed bacteriological investigation.

Isolation and identification of *E. coli*:

Under aseptic conditions, a portion from each sample was inoculated into nutrient broth and incubated aerobically at 37°C for 12h. Loopfuls from incubated nutrient culture were streaked onto MacConkey agar (HiMedia, M081) and incubated aerobically at 37 °C for 24 h.The pure colonies were further streaked onto eosin methylene blue (EMB) agar (HiMedia, M317) and incubated overnight at 37°C. Colonies with the green metallic sheen on EMB agar were suspected as *E. coli* isolates and further confirmation was done by following standard microbiological techniques including colonial morphology, Gram staining and biochemical tests (indole, methyl red, Voges-Proskauer, citrate, catalase, oxidase, and motility indole ornithine test) **(Konemann *et al*., 1997; Quinn *et al*., 2002)**

Antimicrobial Susceptibility Testing:

Confirmed *E. coli* isolates were further tested for AMR. The susceptibility of identified *E. coli* isolates to a panel of twelve commonly used antimicrobial agents was performed by the standard Kirby-Bauer disc diffusion method. The susceptibility of isolates to 12 different antimicrobial agents was determined as described by **Clinical and Laboratory Standards Institute (CLSI, 2021).** According to the measurement of inhibition zones, tested strains were considered as susceptible or resistant. The following antimicrobial discs (Oxoid,

Basingstoke, UK) were used: gentamicin (GN, 10 µg), kanamycin (KAN, 30 µg), ampicillin (AMP, 10 µg), cefotaxime (CTX, 30 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg),tetracycline(TET,30 µg),sulfamethoxazole-trimethoprim(STX, 25 µg), chloramphenicol (C,30 µg), erythromycin (E,5µg) ,enrofloxacin (ENR, 5 µg) and streptomycin (S, 10 µg).

RESULTS

Incidence of *E. coli* isolation:

The cultural characterization on MacConkey and Eosin Methylene Blue (EMB) agar plates, as well as biochemical tests, revealed that the overall percentage of *E. coli* isolated from diseased and freshly dead broiler chickens is 82.6% (114/138 samples).As shown in(Table 1), the highest prevalence was met with liver samples (89.13%) followed by the heart (84.7%) while the lowest prevalence was found in lung samples (73.9%).

Table (1): Prevalence of *E.coli* in different organs of diseased and freshly dead broiler chickens

Source of samples	Number of samples	Positive samples		Negative samples	
		N	%*	N	%
Liver	**46**	**41**	**89.13**	**5**	**10.8**
Heart (blood)	**46**	**39**	**84.7**	**7**	**15.2**
lung	**46**	**34**	**73.9**	**12**	**26**
Total	**138**	**114**	**82.6**	**24**	**17.4**

***The percentage was calculated according to the number of examined samples.**

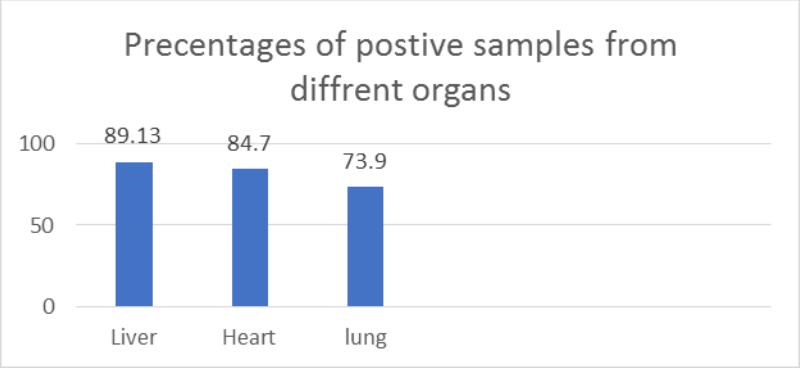

Fig. (1): prevalence of positive *E. coli* in different organs of diseased and freshly dead broiler chickens.

Results of Antibiotic Sensitivity Testing:

Antibiotic susceptibility of 114 APEC isolates against 12 different antimicrobial drugs result is shown in Fig. (2). The highest frequencies of resistance detected were against ampicillin (100%), tetracycline 105/114 (92.1%), chloramphenicol 95/114 (85%), nalidixic acid 92/114 (80.7%), kanamycin 81/114 (71%), enrofloxacin 75/114 (65.7%) while moderate resistance was detected against sulfamethoxazole-trimethoprim 65/114 (57%), cefotaxime 59/114 (51.7%), streptomycin 49/114 (42.9%), gentamicin 46/114 (40.3%), ciprofloxacin 42/114 (36.8%). and erythromycin 33/114 (28.9%).

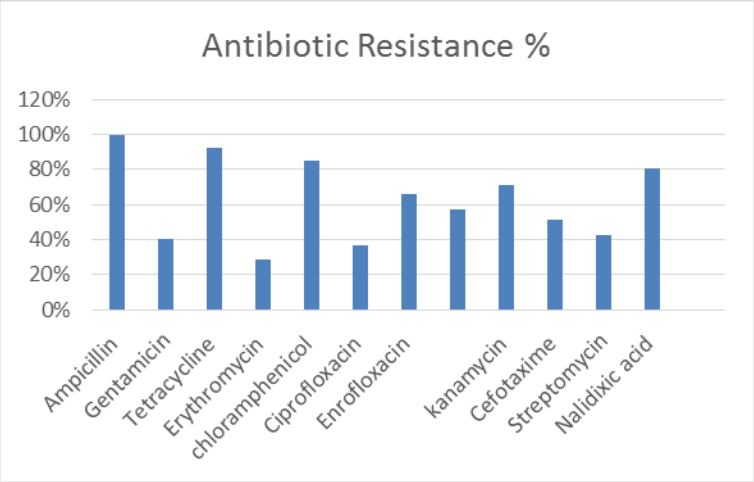

Fig. (2): Antibiotic resistance percentages of APEC against 12 antimicrobial agents

DISCUSSION

Poultry infection with *E. coli* causes high economic losses due to high mortality and morbidity rates, loss of body weight, decreased egg production, and condemnation of whole carcasses or organs **(Salama *et al.*, 2007).** The emerging antimicrobial resistance among different APEC isolates has stimulated researcher's interest in exploring the developing resistance for further adaptation of effective preventive measures as vaccine development. In this study, the overall percentage of recovered *E. coli* isolates was 82.6 % (114/138) from ill and freshly dead broiler chickens. This finding is almost similar to the findings of previous studies **(Eid and Erfan, 2013; Peer *et al.*, 2013**; **Mahmud *et al.*, 2018)** where they reported elevated levels of *E. coli* isolation (80 %, 84 %, and 83.08%, respectively). In contrast significant lower rates (38.7 %, 44.61 %, 36.20 %, and 35.74 %, respectively) were recorded by other authors **(Zhao *et al.*, 2001; Sharada *et al.*, 2008; Hasan *et al.*, 2011; and Literak**

***et al.*, 2013).** Based on the recovery rates of APEC from liver, heart blood, and lung samples, liver has the highest rate of isolation (89.13 %), followed by the heart blood (84.7 %), and the lung (73.9 %). Studies conducted by **Roshdy *et al.* (2012) and Abd El -Tawab *et al.* (2016)** reported similar results, with the highest isolation rate found in liver, followed by heart blood, and lung. Similarly, **Sarah *et al.* (2013)** found that 88 of 95 liver samples tested positive for *E. coli* (92.6 %), however lower rates (14%) were recorded by **(Ola *et al., 2017*).**

The indiscriminate use of the antibiotics exerts a selection pressure leading to the development of drugs resistant strains of bacteria. The antibiotic resistant patterns found in this study suggest a threatening situation of prevalence of the antibiotic resistant *E. coli* strains among broiler chickens farms. Out of twelve antibiotics tested, none of the antibiotic showed 100% effectiveness against the recovered *E coli* isolates. The highest resistance (100%) was found against ampicillin and the lowest resistance (28.9%) was exerted towards erythromycin.This was in agreement with the findings of previous studies **(Ahmed *et al.*, 2013)** as they reported that 84 to100% of APEC isolates were highly resistant to ampicillin, tetracycline, nalidixic acid and chloramphenicol. Also, **(Qabajah and Ashhab,2012)** reported a higher percentage of resistance against enrofloxacin, spectinomycin and gentamicin. Considering the increasing incidence of antibiotic-resistant *E.coli* isolates in the current study; these results were similar to those reported in other Egyptian publications **(Abd El-Tawab *et al.*, 2014 and 2015; Awad *et al.*, 2016**; **El-Shazly *et al.*, 2017**;**Amer *et al.*, 2018; El-Seedy *et al.*, 2019**; **Qurani 2019).** There have been a number of reports confirming this finding worldwide, **(Saidi *et al.* 2012 in Zimbabwe) (Momtaz and Jamshidi, 2013 and Rahimi, 2013 in Iran) (Solà-Ginés *et al.*, 2015 in Spain) (Dou *et al.*, 2016** and **Li *et al.*, 2016 in China), (Rahman *et al*., 2017 in Bangladesh); (Dandachi *et al.*, 2018 in Lebanon) and (Subedi *et al.*, 2018 in Nepal).**

Such results suggest a strong indication about an indiscriminative and abusive use of multiple antibiotics for prophylaxis or control of infections. Such multidrug resistant ultimately replace the drug sensitive microorganisms from the antibiotic saturated environment **(Van den Bogaard *et al*., 2001)**

CONCLUSION

Colibacillosis is one of the most important diseases of poultry, resulting in high economic losses as well as high morbidity and mortality. Presence of multidrug resistant pathogens are reported because of the abuse of the antimicrobials. The in-vitro antimicrobial susceptibility

testing revealed high prevalence of antibiotic resistance among APEC isolates obtained from different poultry farms in Egypt against different antibiotic groups namely, broad-spectrum types such as β-lactams, third generation cephalosporins, and quinolones. Attention should be paid to those antimicrobials used in broilers feed, drinking water, and as growth promoter in suboptimal doses such as erythromycin, enrofloxacin, oxytetracycline and sulfonamides. The high resistance levels observed for these antibiotic classes reflect the widespread use of them in poultry. In Egypt, high frequencies of antimicrobial resistance found in chicken isolates can be attributed to the large-scale use of antimicrobials for disease treatment and prevention without veterinary consultation.

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