



Preliminary studies on *E. Coli* implicated in avian Colibacillosis with reference to their antibiotic resistance profiles

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ABSTRACT

Colibacillosis constituted as one of the most important disease condition affecting poultry with significant implications on production. Herein, examination of 451 organ samples including trachea, lung, heart blood, liver and spleen collected from freshly slaughtered chickens using standard methods for isolation and identification of *E. coli* showed that 236 samples were positive for *E. coli* with an incidence rate (52%). Twenty-five *E. coli* strains were serogrouped and 6 different serogroups were successfully identified, the most prevalent serogroup was O78 (20%), followed by O157 and O27 with incidence rate (16%) for each, O168 and O125 (12%) each. and finally O115 (8%), in addition to 4 untypable strains. The antibiotic susceptibility of 60 isolates against ten antimicrobials was performed by disc diffusion method and the results revealed that gentamicin and colistin were the most effective antibiotics on the isolates. On the other hand, all isolates exhibited absolute resistance to erythromycin and rifampicin, followed by high level of resistance ranging from (75-95%) for ciprofloxacin, sulfamethoxazole–trimethoprim, ceftiofur, chloramphenicol, doxycycline and amoxicillin- clavulanic acid. All isolates exhibited multidrug resistance phenotypes.

Key words: *Escherichia coli*, Biochemical identification, Serogrouping, Antibiotic resistance.

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(BVMJ-30(1): 68-77, 2016)

1. INTRODUCTION

Escherichia coli is the most common etiological agent involved in many disease conditions in poultry (Barnes et al., 2008). Although *E. coli* presents as normal microflora in the intestinal tract of chickens, extra intestinal pathogenic *E. coli* (ExPEC) infections generates a negative economic impact (Barnes et al., 2003 and Mellata, 2013). The subpathotype of ExPEC that infects poultry causing colibacillosis, known as avian pathogenic *E. coli* (APEC) and is considered a heterogeneous group of pathogens present in all stages of the poultry production chain, causing several types of lesions and diseases (Cunha et al., 2014). This infectious disease is considered to be initiated in the avian upper respiratory

tract; air sacs being the first organs infected, followed by septicemia and organ colonization (Germon et al., 2005 and Saberfar et al., 2008). APECs have diverse serotypes, but certain ones (O78, O2, and O1) are more frequently observed than others (Jeong et al., 2012). The frequencies of O78 among APEC isolates vary according to location and host (Ewers et al. 2004). Antimicrobial therapy is one of the primary control measures for reducing the morbidity and mortality caused by APEC infections. In the latest decades, an increasing resistance against various classes of antibiotics has become exaggerated due to the excessive and misuse of antibiotics either as treatment or as growth promoters (Teuber, 2001). That represents a serious

danger to poultry industry and consequently to human health (Pidcock, 1996) so they need to be used prudently in order to preserve their therapeutic usefulness in both animals and humans (Gyles, 2008). The development and dissemination of antibacterial resistance had become a topic of concern due to its direct influence on public health, through elevating the morbidity, mortality, and treatment costs of infectious diseases. In view of these considerations, the present study was undertaken for isolation and identification of *E. coli* to record the prevalence of *E. coli* infection in chickens and serotyping of the obtained isolates then performing the antibiogram susceptibility test on the isolates.

2. MATERIAL AND METHODS

2.1. Samples collection:

Four hundred and fifty-one tissue specimens including tracheas, lungs, heart blood, livers and spleens of freshly slaughtered broilers showing different disease manifestations clinically diagnosed as colibacillosis and obtained from farms of different localities in Sharkia (103) and Dakahlia (56) Governorates, were submitted to the bacteriological examination for *E. coli* isolation.

2.2. Isolation and identification of *E. coli* (Cruickshank et al. 1975):

The surface of organs was seared by hot spatula, and then a sterilized loopfuls were inoculated onto MacConkey's broth and incubated aerobically at 37°C for 12 hours. Loopfuls from incubated MacConkey's broth were streaked onto MacConkey's agar plates and incubated for 24 hours at 37°C. Suspected lactose fermented colonies (pink colonies) were picked up and streaked on the eosin methylene blue (EMB) then incubated for another 24-48 hours at 37°C. The suspected purified colonies were picked up for examination by IMVC and TSI biochemical tests, followed by typing of the isolates by RapID™ ONE system

(Remel), which is a qualitative micro method employing conventional and chromogenic substrate for the identification of medically important Enterobacteriaceae and other selected oxidase-negative, G-negative bacilli by following the kit manual instructions.

2.3. Serological typing of *E. coli*

Twenty-five isolates that were preliminary identified as *E. coli*, were taken randomly and subjected to serological identification (Edward and Ewing, 1972) using slide agglutination test.

2.4. Antimicrobial susceptibility testing

Antibiotic susceptibilities of *E. coli* isolates were determined by the standard disk diffusion method (Finegold and Martin, 1982), *E. coli* isolates were tested against the following antimicrobial agents: gentamicin, rifampicin, ciprofloxacin, colistin, amoxicillin-clavulanic acid, ceftiofur, doxycycline, chloramphenicol, erythromycin and sulfamethoxazole-trimethoprim.

3. RESULTS

3.1. The total Recovery rate of *E. coli* isolated from different organs in broilers:

The obtained results revealed that 236 isolates were identified as *E. coli* with percentage of 52.3% distributed as (64%, 51%, 48%, 52% and 41% out of the examined lung, liver, heart blood and trachea and spleen, respectively. with a total number of 145 (56.6%) and 101 (51.8%) *E. coli* isolates out of 256 and 195 examined specimens from Sharkia and Dakahlia Governorates, respectively as shown in Table (1).

3.2. Serological typing of *E. coli* isolates:

The results of serological identification of 25 *E. coli* Isolates revealed that O78 was the most predominant serotype with a percentage of (20%) followed by O157 and O27 (16%) each, other serotypes as O168,

Table (1): The total recovery rate of *E. coli* isolated from different organs in broilers.

Organ Specimen	Examined No.	Positive No.	Percent
Lung	100	64	64%
Liver	100	51	51%
Heart blood	100	48	48%
Trachea	100	52	52%
Spleen	51	21	41%
Total	451	236	52%

Table (2): The incidence and frequency distribution of *E. coli* serogroups

<i>E. coli</i> Serogroup	No. of isolates	Percent
O78	5	20%
O27	4	16%
O157	4	16%
O168	3	12%
O125	3	12%
O115	2	8%
Untypable	4	16%

Table (3): Resistance Profile of the *E. coli* isolates against ten antimicrobial agents

Antimicrobial Agents	<i>E. coli</i> Isolates (60)	
	No.	(%)
C	50	83%
CT	27	45%
AMC	45	75%
E	60	100%
FOX	51	85%
CN	24	40%
CIP	57	95%
RF	60	100%
SXT	56	93%
DO	48	80%

(AMA): Antimicrobial agents; (C): chloramphenicol, (cT): colistin, (AMC): amoxicillin- clavulanic acid; (E): erythromycin; (FOX): ceftiofur; (CN): gentamicin; (CIP): ciprofloxacin; (RF): rifampicin; (SXT): sulfamethoxazole-trimethoprim and (DO): doxycyclin.

Table (4): Prevalence of the multidrug resistant *E. coli* isolates

No. of antimicrobial agents	No. of <i>E. coli</i> isolates
Three drugs	1
Four drugs	0
Five drugs	4
Six drugs	3
Seven drugs	10
Eight drugs	12
Nine drugs	21
Ten drugs	9

O125 were also recorded with percentage of (12%) each, and O115 with percentage of (8%) (while, 4 isolates were untypable. As shown in Table (2).

3.3. Antimicrobial susceptibility testing:

The results revealed that all isolates showed absolute resistance to rifampicin and erythromycin (100%) followed by high frequencies of resistance observed to ciprofloxacin, sulfamethoxazole/trimethoprim (95% and 93%, respectively). The resistance to ceftiofur was (85%), chloramphenicol (83%), doxycycline (80%) and amoxicillin/ clavulanic acid (75%). Meanwhile, least level of resistance in *E. coli* isolates was reported to colistin and gentamycin (45% and 40%), respectively as shown in Table (3). Additionally, no isolate was completely sensitive to all antibiotics while high level of multidrug resistance was reported, 9 isolates were resistant to ten antimicrobial agents tested, 21 were resistant to 9 antimicrobial agents and 12 isolates were resistant to 8 antimicrobial agents. None of the tested antibiotics showed 100% efficacy on all isolates, as shown in Table (4).

4. DISCUSSION

APEC causes avian colibacillosis, a disease of poultry that occurs worldwide and associated with heavy economic losses (Barnes et al., 2008). APEC identification has passed through a number of developments, beginning with conventional

methods of identification based on chemical characterization and the nature of selective media. A more specific identification approach is the serotyping analysis that aims at classifying pathogenic strains based on their surface antigens. Based on O, H, and K antigens. O Serogrouping is one of the basic diagnostic methods for the classification of pathogenic strains of *E. coli*. It is suggested that some strains (O1, O2 and O78) could be classified as avian pathogenic strains (Giovanardi et al., 2005, Kawano et al., 2006, Yaguchi et al., 2007, Ozawa et al., 2008). In the present investigation, out of 451 examined tissue specimens representing different organs collected from freshly slaughtered diseased broilers, results revealed that *E. coli* were recovered from 236 samples with an overall prevalence rate (52%). Nearly similar results were recorded in other studies in Egypt as those of Sharada et al., (2010); Norhan (2011); Wafaa (2012) and Ammar et al., (2015) who isolated *E. coli* from diseased broilers with total recovery rates (44.6%, 63.6%, 46.6% and 63.0%), respectively. Higher recovery rates were recorded in a previous study carried out by Abd El Aziz et al. (2007) who isolated *E. coli* from different poultry farms showing signs of CRD in Dakahlia governorate with percentage of (90%). In addition to Nashwa et al., (2010) and Shimaa (2013) who isolated *E. coli* with percentage of (75%) each and Samanta et al. (2014) (75.5%) from backyard chicken in India. However,

lower isolation rate was reported by Momtaz *et al.* (2012) (15.8%). Moreover, the highest isolation rate of *E. coli* was from lung (64%) followed by trachea (52%); liver (51%); heart blood (48%) and finally spleen with incidence rate (41%). The high isolation rate from lung and trachea may be attributed to the fact that the infection usually begins at the respiratory tract and then spread to other internal organs resulting in systemic infection. (Barnes *et al.*, 1999). The biochemical profile of *E. coli* isolates was similar to those previously reported such as fermentation of certain sugars or enzymatic reaction (Gomis *et al.*, 2001; Quinn *et al.*, 2002; Bopp *et al.*, 2005 and Raji *et al.*, 2007). It has been suggested that high metabolic activity might be characteristic of highly virulent strains associated with colibacillosis (LeBouguenec and Schouler, 2011).

Concerning identification of isolates depending on RapID one identification system (Remel). *E. coli* strains belonged to four profiles (4361011, 4161001, 0261001 and 4341011) this is in accordance with previous studies which proved accuracy rate for this test exceeding 91% in identification of *Enterobacteriaceae* (Kitch *et al.*, 1994 and Lee *et al.*, 1994).

In the present study, 25 of the obtained *E. coli* isolates recovered from broilers with different disease manifestations were serotyped by slide agglutination test. The results showed that O78 was the most predominant serogroup with a percentage 20%, followed by O157 and O27 with percentage 16%; O168, respectively and O125 (12%); finally, O115 (8%). While 4 other strains were untypable. These results are in line with those recorded by Zhao *et al.*, (2005) and Jin *et al.*, (2008) who stated that the most common *E. coli* serotype in avian colibacillosis cases was O78 and McPeake *et al.*, (2005) who recovered *E. coli* O78 serogroup from cases of colisepticaemia in broilers with a percentage of 55%. In addition to Kumar *et al.*, (2003) who found that the most predominant APEC serogroups were O78

(23.5%). Also, these results go hand in hand with those recorded by Wen-Jie *et al.*, (2008) who reported that the most prevalent APEC serotype isolated from poultry with colibacillosis in different areas of China was O78 (17.6%). In addition, other serotypes as, O115 was also recorded, Rezk *et al.*, (2010) detected *E. coli* O78 and O125 serogroups in diseased chickens collected from different localities in Ismailia Governorate and Al-Ajimi (2011) who isolated APEC O125 serotype from lung of broilers. Shimaa and Mosalem (2013) obtained similar results and isolated *E. coli* O78, O157, O125 and other serogroups from sever outbreaks of *E. coli*. These variations indicated that *E. coli* serogroups are country specific, which is important in bacterines preparation that must be specific to the predominant serogroup. Antimicrobial agents have been widely used in poultry to treat infections caused by a variety of bacterial pathogens. However, this wide spread use of large quantities of antimicrobials in poultry without professional supervision causes many problems. Furthermore, the use of antibiotics as growth promoters in poultry feed for the last 60 years (Aarestrup, 2005) led to development of antimicrobial resistance and zoonotic transfer of antibiotic resistance genes resulted in prevention of antibiotic usage as growth promoters in Europe (Castanon, 2007). Antibiotic susceptibility test was performed on 60 *E. coli* isolates using disc diffusion method to determine the differentiation between the isolates according to variation in their inhibition to different antibiotics. The results revealed that all the isolates were resistant to rifampicin and erythromycin (100%) followed by high frequencies of resistance to ciprofloxacin and sulfamethoxazole/ trimethoprim (95% and 93%, respectively). Resistance to ceftioxin, chloramphenicol, doxycycline and amoxicillin-clavulanic acid were (85%, 83%, 80% and 75%), respectively. Meanwhile, the least level of resistance was reported to colistin (45%) and gentamicin

with (40%). Additionally, no isolate was completely sensitive to all the antibiotics. Gentamycin and colistin were the most effective chemotherapeutic agents on the tested isolates, and similar finding was reported by Blanco et al. (1996) who found that colistin was one of the antibiotics that showed low level of resistance against *E. coli* isolates. High level of erythromycin resistance was also reported in many previous studies as Nazir, 2004; Afshin and Kashefi, 2012; Ezzeldeen et al., 2013 and Rahimi, 2013) who reported resistance rate to erythromycin with percentage of 92.3%, 100%, 100% and 96.1%, respectively. As well as a very high level of resistance to chloramphenicol was detected by other investigators (Islam et al., 2004 and Rahman et al., 2008). In the existing study, a very high level of multidrug resistant *E. coli* isolates was recorded, similar findings had been reported before in different parts of the world (Amara et al., 1995; Guerra et al., 2003; Saenz et al., 2003; Rahman et al., 2008 and Rahimi, 2013). Yet it was higher than those reported by other researchers in European countries (Blanco et al., 1996 and David and Burch, 2000). According to Pessanha et al., (2001) broilers can act as reservoirs of important antibiotic resistance genes in veterinary and human medicine and the use of growth promoters in animal feed may contribute to the occurrence of multidrug resistant isolates as well.

5. CONCLUSION

In conclusion, the current study showed that multidrug resistant *E. coli* isolates are prevalent among broilers. The increase and spread of multidrug resistance is alarming so it is important to understand the mechanism of resistance of bacteria to antibiotic to be able to overcome it. Besides there should be a renewed interest for the development of new derivatives and generations of antibiotics. safety measures as strict biosecurity, correct management and effective vaccination programs should

be employed and limiting the random use of antibiotics in the veterinary field.

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