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Antibiotic Profile of Bacterial Species Isolated from Broiler Chickens with Cellulitis

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ABSTRACT

The present study was carried out to isolate and identify the bacterial agents involved in field cases of avian cellulitis in broiler chickens and also to examine isolated bacteria for antibiotic susceptibility. The study was applied on 290 broiler chickens, aged 30-35 days, suffered from cellulitis (65 with head and 225 body lesions) to isolate bacterial agents. All obtained isolates were identified and tested for the pathogenicity based on Congo red assay. Disc diffusion test was used to study the sensitivity pattern of bacterial isolates with determination of multiple antibiotic resistance index. Results revealed that all head and 91.5% of body samples were positive on bacteriological examination. *E. coli* was the most prevalent isolate (45.2%), followed by staphylococci (33.2%), Clostridia (5.4%), streptococci (5.1%), *Proteus mirabilis* (4.4%), *Enterobacter* spp. (3.2%), *Pseudomonas aeruginosa* (2.2%), and *Aeromonas* spp. (1.2%). Congo red binding test was positive for *P. aeruginosa* (100%), Clostridia (72.7%), *E. coli* (65.8%), staphylococci (62.2%), *Aeromonas* spp. (60%), *P. mirabilis* (38.9%), *Enterobacter* spp. (38.5%) and streptococci (33.3%). Serological typing of *E. coli* identified nine O serotypes, with high predominance of O78 (19%). On antibiotic susceptibility profiling, *E. coli* isolates demonstrated 83.1-92.9% resistance to chloramphenicol, tetracycline, and enrofloxacin. Staphylococci isolates showed high resistance to ampicillin (97.0%) and clindamycin (82.9%). Clostridial and *Aeromonas* spp. isolates showed 100% resistant to tetracycline, enrofloxacin, and cefotaxime. *Enterobacter* spp. showed 100% resistance to chloramphenicol and cefotaxime. *P. aeruginosa* had 100% resistance to tetracycline and enrofloxacin. Also, streptococci isolates showed 100% resistance to erythromycin. Totally, 56.3% bacterial isolates were multidrug-resistant, 23.8% extensively drug-resistant and 1.5% pan drug-resistant. The present study concluded that *E. coli* is the most predominant pathogen involved in cellulitis, particularly O78 serotype. In addition, this study demonstrated high prevalence of multidrug-resistant bacteria among isolates, particularly against commonly used antibiotics. Therefore, it is recommended to use antibiotic sensitivity tests and accurate therapeutic doses to efficiently treat and control bacterial infections in poultry.

Key words: Antibacterial susceptibility, Bacterial isolates, Broiler, Cellulitis, Sensitivity classes.

INTRODUCTION

Avian cellulitis, known as necrotic dermatitis, is one of the most prevalent infections in broiler chickens and has been reported from many countries around the world with a developed poultry industry. Birds with cellulitis often do not show clinical symptoms (Norton 1997). Macroscopically, cellulitis lesions are characterized by deposition of yellowish fibrin under discolored or thickened skin. In cases of involvement of the skin surface, there may be an oozing exudate over the skin "waffle skin", and infections are most common over the thigh muscle, breast, legs, abdomen, head and neck (Randall et al., 1984; Fallavena et al., 2000; Gomis et al., 2000). Clinically, cellulitis can be seen in affected chicken if infection occurs in the head region, and it looks as swollen head syndrome in 5-6 weeks old chickens (Morley and Thomson 1984**)**, while affection of other body sites can be only detected accidentally in post mortem examination or during inspection in slaughterhouse (Bianco et al., 2016). At processing, the presence of subcutaneous fibrinonecrotic plaques accompanied by inflammation of overlying skin lead to total or partial carcass rejection (de Brito et al., 2003). Hence, economic losses are mainly due to increased condemnation rate and downgrading of affected carcasses (Bianco et al., 2016). In the USA, losses due to cellulitis are estimated to be 40-50 million dollars annually and cause up to 30% of total carcass condemnation in broilers (Norton, 1997). In Canada, 0.8% of slaughtered broilers were condemned for cellulitis in 2004 (Paniago, 2009). In Brazil, cellulitis lesions are estimated to cause at least 18 thousand tons meat losses in 2011 (Barbieri et al., 2013). Unfortunately, in Egypt, there is no accurate data about losses attributed to this problem.

Avian cellulitis is mainly caused by bacterial agents such as *Actinomyces pyogenes, Pasteurella multocida*, *Pseudomonas aeruginosa, Streptococcus dysagalactae, Staphylococcus* spp., *Aeromonas* spp., *Proteus vulgaris,* and Enterobacteriaceae through the invasion of subcutaneous tissue. *Escherichia coli* is the predominant organism (Barros et al., 2013) colonized in the subcutaneous tissues in avian cellulitis (Peighambari et al., 1995; Gomis et al., 2000; Derakhshanfar and Ghanbarpour 2002). Cellulitis lesions caused by loss of skin integrity (as a predisposing factor) in a

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susceptible host infected with bacterial agents. Trauma to the skin is essential for the development of avian cellulitis, as there are no records for lesions in chickens with non-traumatized skin (Peighambari et al.1995). Injurious behavior, cannibalism, biting insects, poor litter conditions, foot problems that lead to long sitting of birds, immunodeficiency and systemic infections are considered as predisposing factors in avian cellulitis (Rosenberger et al., 1975; Peighambari et al. 1995; Norton 1997; Wang et al., 2005; Bianco et al., 2016).

The development of antimicrobial resistance among bacteria makes antimicrobial susceptibility tests essential to detect drug resistance and to identify susceptibility to drugs for proper treatment of particular infections (Jorgensen and Ferraro, 2009). For interpretation of antimicrobial susceptibility, isolates have Multiple Antibiotic Resistance (MAR) index ≥ 0.3 are either multidrug-resistant (MDR) (resistant to 3 or 4 class of antibiotics) or expanded drug-resistant (resistant to more than five antibiotics classes). The most resistance patterns observed among the MDR isolates indicate that these isolates originate from a high-risk source of contamination where antibiotics are often used (Christopher et al., 2013).

The present study aimed to isolate and identify the bacterial agents involved in field cases of avian cellulitis in broiler chicken as well as examine the isolated bacteria for antibiotic susceptibility.

MATERIAL AND METHODS

Ethical approval

This study was approved by the Ethical Committee for Medical Research at the National Research Centre, Egypt and in accordance with local laws and regulations.

Chicken flocks

This study was conducted on 290 broiler chickens, ranged from 30 to 35 days old, suffered from cellulitis (65 with head and 225 body lesions). The chickens were collected from 45 flocks, with an average stock density of 21,000 chicken/house, located in full integrated poultry farms in Giza, Behera, and Sharkia governorates, Egypt from March 2017 to March 2019. Birds with head cellulitis were diagnosed at farms while body cellulitis was identified in carcasses at slaughterhouses.

Flocks with head cellulitis (13 flocks) were sampled directly at the farm (5 birds/flock) and resampled again at slaughterhouse after de-feathering (5 carcasses with body lesions/flock). The rest of the chicken flocks (32 flocks) were sampled at slaughterhouse after de-feathering (5 carcasses with body lesions/flock). All birds were subjected to postmortem examination and samples collected from cellulitis lesion for bacteriological examination.

Bacteriological examination

Sampling

For bacteriological examination, sterile swabs were individually collected from subcutaneous exudates of cellulitis lesions (3 swabs/bird). The collected samples were labeled and transported in cool boxes to the laboratory.

Culture media

Bacterial enrichment was done using nutrient broth, tryptone soya broth, and LB broth as liquid media at 37 ˚C for 18 hours. Further bacterial isolation and identification were carried out using selective and differential media, including Salmonella-Shigella agar and MacConkey agar (for Enterobacteriaceae), nutrient agar and blood agar (for Gram-positive bacteria) and Pseudomonas isolation agar (for *P. aeruginosa*), which were prepared and used according to Collee et al., (1996); Forbes et al., (2002) and Greenwood et al., (2005).

Bacterial isolation and identification

After initial enrichment, a loopful of the enriched broth was streaked on solid media and incubated at the recommended temperature and time, then examined for bacterial growth according to Quinn et al., (1994) and Collee et al., (1996). The suspected colonies were picked up, purified and kept in semi-solid agar for further morphological and biochemical analysis (Konemann et al., 1992; Quinn et al., 2002). Identification and characterization of the obtained isolates were done according to colony morphology and Gram staining (Forbes et al., 2002; Greenwood et al., 2005). Proper biochemical characterization was done using API identification kits (API System, France) and was analyzed using Bergey's manual of systematic bacteriology (Sneath et al., 1986).

Serological typing of *E. coli*

The obtained *E. coli* isolates were subjected to serological identification according to Edward and Ewing (1972) using polyvalent and monovalent diagnostic *E. coli* antisera (Deben Diagnostics Ltd., UK) through the application of slide agglutination test.

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***In vitro* pathogenicity test**

The purified isolates of all bacterial spp. were tested based on Congo red (CR) dye-binding assay in order to differentiate between pathogenic and non-pathogenic microorganisms according to Berkhoff and Vinal (1986). Each isolate was cultured on a separate plate of Trypticase soy agar supplemented with 0.003% CR dye (Sigma, UK) and 0.15% bile salts. Plates were incubated aerobically at 37°C for 24 hours. Then, the cultures were left at room temperature for an additional 48 hours to obtain clear results. The appearance of deep brick-red colonies between 24 and 72 hours of incubation was recorded as positive (CR+).

Antibiogram

Antibiotic discs

The following 10 antibiotic discs were used: gentamycin 10 µg/ml (CN), oxacillin 30 µg/ml (OX), erythromycin 15 µg/ml (ERI), chloramphenicol 30 µg/ml (C30), tetracycline 30 µg/ml (T30), clindamycin 2 µg/ml (DA), enrofloxacin 5 µg/ml (ENR), ampicillin 10 µg/ml (AMP), cefotaxime 30 µg/ml (CTX) and vancomycin 30 µg/ml (VA), representing antibacterial categories of aminoglycosides, *b*-lactams, macrolides, phenicols, tetracycline, lincosamides, fluoroquinolones, penicillin, cephalosporin and glycopeptides, respectively. The selection of disk concentrations and interpretations of zone diameters were done as recommended by the manufacturers (Difco Laboratories, Detroit, MI, USA) and CLSI (2016).

Antibiotic susceptibility test

Antibiotic susceptibility of the identified isolates was determined using the disc agar diffusion test according to Watts (2008) and CLSI (2016). Separate and similar colonies on solid media plate were emulsified in 3 ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard. Using sterile swab sticks, the Muller Hinton agar plates, 9 cm-diameter, were inoculated with the bacterial suspension by streaking the surface of the agar and rotating the plate to ensure even distribution. The inoculated plates were allowed to dry at room temperature for 10 minutes and then antibiotic discs were placed on the surface of the agar. The plates were left at room temperature for the pre-diffusion time before aerobic incubation at 37°C for 16-18 hours. Growth inhibition zones were measured to the nearest millimeter and isolates classified as sensitive, intermediate and resistant based on CLSI (2016).

Determination of multiple antibiotic resistances index

The MAR index was determined by the following formula: MAR= a/b

where a is the number of antibiotics to which the test isolate was resistant; and b is the total number of antibiotics that the test isolate has been evaluated for susceptibility (Krumperman, 1983; Paul et al., 1997). According to standardized international terminology created by European Centre for Disease Control and Centre for Disease Control and Prevention, Atlanta, the MDR bacteria were defined as non-susceptible to at least one agent in three or more antimicrobial categories, Extensively Drug-Resistant (XDR) bacteria were defined as non-susceptible to at least one agent in all but two or fewer antimicrobial categories, and Pan Drug-Resistant (PDR) bacteria were defined as non-susceptible to all agents in all antimicrobial categories (Magiorakos et al., 2012).

RESULT AND DISCUSSION

In the present study, the mortality rate in sampled flocks ranged from 5.6 to 10.5%. The number of transported chickens to slaughterhouse ranged from 18795 to 19845 per house. The incidence rate of head cellulitis in 13 flocks was 0.2-1.26 percent while the rate of rejected carcasses due to cellulitis after de-feathering was 0.9-1.7 percent. These findings are consistent with the data recorded by Amini et al. (2015) who reported the average overall condemnation rate for 16 broiler farms processed by two processing plants was 1.4%; while the average total condemnation rate due to cellulitis was 0.83% over the 12-months period. Also, cellulitis was recorded in 126 condemned carcasses and 272 broilers dead on their own and reported as the main cause of condemnation in 13 broiler flocks between 2014 and 2016 (Poulsen, 2018).

In the present study, 290 cellulitis samples were represented by 65 head cellulitis and 225 body cellulitis. Concerning body cellulitis samples, the most common lesions were located as diffuse lesions in general body region, followed by the abdomen and the thigh. The lowest incidence was in the back region (Table 1). These findings are similar to those described in previous studies (Messier et al., 1993; Fallavena et al., 2000; Alves et al., 2007). One study recorded that well-characterized lesions were generally located in the thigh, back and cloacal area (Alves et al., 2007).

Cellulitis lesions appeared as irregular and thick skin with dark to brown discoloration, either circumscribed localized or generalized throughout the body (Figure 1). Skinning of these lesions revealed the existence of the characteristic yellowish to green subcutaneous exudates which were fibrinous, serosanguineous, or suppurative (Figure

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2). The detected exudate was found to extend to the subcutaneous layers in some cases. Fibrinous to caseous pericarditis, airsacculitis, bursal and kidney lesions were recorded in some cases.

In the present study, all head cellulitis samples and 91.5% of body cellulitis samples had positive results for bacterial examination where 407 bacterial isolates were recovered (Table 1). The most prevalent isolated bacteria were *E. coli* (45.2%), followed by *Staphylococcus* spp. (33.2%), Clostridia (5.4%), *Streptococcus* spp. (5.1%), *Proteus mirabilis* (4.4%), *Enterobacter* spp. (3.2%), *P. aeruginosa* (2.2%) and *Aeromonas* spp. (1.2%) (Table 2). Similar results were obtained by Santos et al. (2014) who isolated 25 avian cellulitis lesion samples, of which 11 isolates were *E. coli* strains, 9 were *S. epidermidis* strains, 7 were *Proteus mirabilis*, and 3 were *Manheimia haemolytica*. In another study, bacteriological and mycological examination for 40 cellulitis lesions from 28 poultry farms indicated that the most prevalent bacteria were *E. coli* (96.4%), followed by *Citrobacter* spp. (10.7%), *Proteus vulgaris* (7.1%), *Staphylococcus* spp., *Streptococcus* spp., *Candida albicans*, *P. aeruginosa*, *Klebsiella* spp., *Serratia* spp., *Penicillium* spp. and *Aspergillus* spp. (Brito et al., 2011). Also, in previous studies, a number of bacteria such as *Aeromonas*, *Enterobacter*, *Pasteurella*, *Proteus*, *Pseudomonas*, *Streptococcus* and *E. coli*, as the most predominant bacteria, were recovered from cellulitis lesions (Norton et al., 1997; Gomis et al., 2002; Shawki et al., 2017). However, in some studies, only *E. coli* was isolated from all (100%) broiler carcasses-affected cellulitis (Andrade, 2005; Vieira et al., 2006).

Results from CR binding test indicated that *E. coli* (65.8%), *Staphylococcus* (62.2%), Clostridia (72.7%), *Aeromonas* spp. (60%), *Enterobacter* spp. (38.5%), *Proteus mirabilis* (38.9%), *P. aeruginosa* (100%), and *Streptococcus* spp. (33.3%) were positive (Table 2). CR binding activity test was applied in order to distinguish pathogenic from nonpathogenic strains of bacteria *in vitro* (Berkhoff and Vinal, 1986; Parul et al., 2014). A strong correlation between virulence of clinical isolates of *E. coli* and their expression on the CR agar medium was discovered by Berhkoff and Vinal (1986). So, the CR dye-binding could be applied as a phenotypic marker or virulence factor for pathogenic bacteria. A similar relationship between virulence and ability to bind to the CR dye was observed for other bacteria (Surgalla and Beesley, 1969; Payne and Finklestein, 1977; Prpic et al., 1983; Yoder, 1989). However, the exact mechanism of action of this test is unknown, but it is proposed that the presence of B-D-glycan in the bacterial cell wall may be involved (Vinal, 1988).

Table 1. Results of the bacteriological examination in head and body cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Site of lesion	Number of samples	Number of samples positive for bacterial culture (%)	Number of bacterial isolates
Head	65	65 (100%)	129
Thigh	44	38 (86.4%)	41
Abdomen	71	67 (94.4%)	86
Back	27	25 (92.6%)	37
Generalized	83	76 (91.6%)	114
Total Body	225	206 (91.5%)	278
Total	290	271 (93.4%)	407

Table 2. Bacterial species isolated from head and body cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Bacterial spp.	Sample site		Total Number (%)	Congo red positive	
	Head	Body		Number	%
E. coli	47 (36.4%)	137 (49.3%)	184 (45.2%)	121	65.8%
Staphylococcus	43 (33.3%)	92 (33.1%)	135 (33.2%)	84	62.2%
Clostridia	7 (5.4%)	15 (5.4%)	22 (5.4%)	16	72.7%
Aeromonas spp.	3 (2.3%)	2 (0.7%)	5 (1.2%)	3	60%
Enterobacter spp.	5 (3.9%)	8 (2.9%)	13 (3.2%)	5	38.5%
Proteus mirabilis	5 (3.9%)	13 (4.7%)	18 (4.4%)	7	38.9%
P. aeruginosa	6 (4.6%)	3 (1.1%)	9 (2.2%)	9	100%
Streptococcus spp.	13 (10.1%)	8 (2.9%)	21 (5.1%)	7	33.3%
Total	129	278	407	252	61.9%

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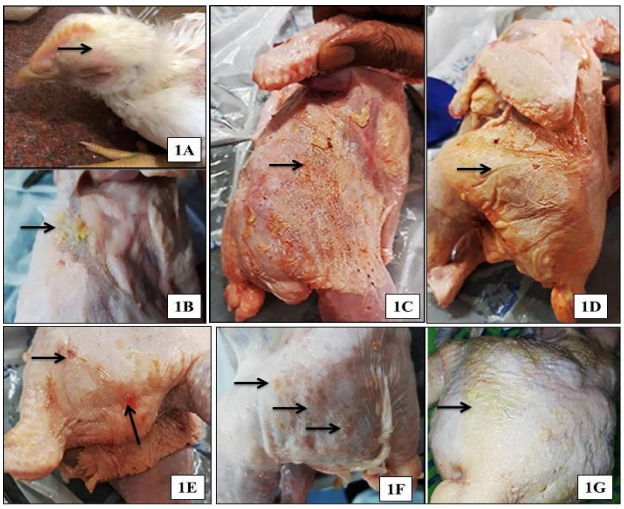

Figure 1. Macroscopic appearance of avian cellulitis at various body regions of broiler chickens. **1A:** Swollen head (arrow); **1B:** Irregular yellowish discoloration (arrow); **1C:** Generalized brownish discoloration (arrow); **1D:** Generalized dark yellow discoloration (arrow); **1E & 1F:** Localized circumscribed focal brownish discoloration (arrows), **1G:** Focal asymmetrical yellow discoloration (arrow).

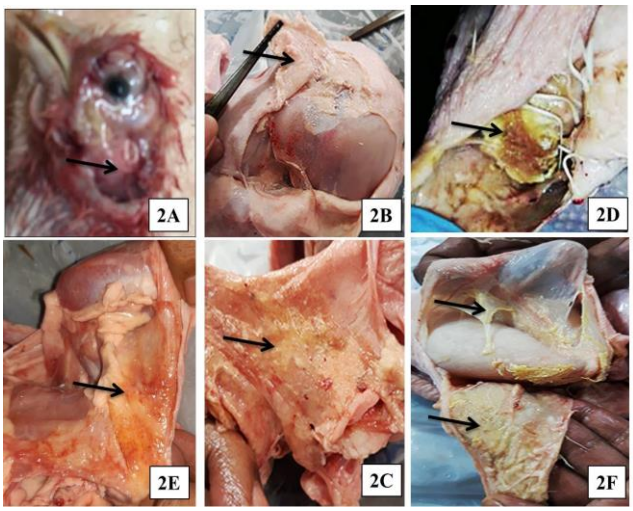

Figure 2. Macroscopic appearance of avian cellulitis after skinning in broiler chickens. **2A:** Serosanguineous (arrow), **2B:** Fibrinous subcutaneous exudate (arrow); **2E and 2C:** Yellowish subcutaneous exudate (arrow); **2D:** Greenish subcutaneous exudates (arrow); **2F:** Suppurative subcutaneous exudate (arrow).

Table 3. Determination of serotypes of 184 *E. coli* isolates recovered from broiler chickens-affected cellulitis in Egypt (March 2017 to March 2019)

E. coli serotype (O group)	Number of isolates (%)
O11	7 (3.8%)
O55	28 (15.2%)
O78	35 (19%)
O114	15 (8.1%)
O125	9 (4.9%)
O128	17 (9.2%)
0146	13 (7.1%)
O157	9 (4.9%)
O158	33 (17.9%)
un-typed	18 (9.8%)

Table 4. The species of coagulase-positive and coagulase-negative staphylococci isolated from cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Staphylococci isolates	*Staphylococcus* spp.	Number of isolates (%)
Coagulase-positive staphylococci isolates (78 isolates)	***S. aureus***	43 (55.1%)
	S. intermedius	15 (19.2%)
	S. hyicus	9 (11.5%)
	non-identified isolates	11 (14.1%)
Coagulase-negative staphylococci isolates (57 isolates)	***S. gallinarum***	8 (14%)
	S. sciuri	14 (24.6%)
	S. epidermidis	6 (10.5%)
	S. lentus	7 (12.3%)
	S. xylosus	11 (19.3%)
	S. haemolyticus	9 (15.8%)
	S. saprophyticus	2 (3.5%)

In the present study, serotyping of isolated *E. coli* from cellulitis lesions revealed the presence of nine different O serotypes of *E. coli*, which O78 was the most predominant serotype (19%) (Table 3). Similar results were obtained previously, where *E. coli* were isolated from cellulitis lesions and belonged to six O-groups, with O78 (52.2%) being the most prevailing serotype (Derakhshanfar and Ghanbarpour, 2002). Noteworthy, O78 serotype of *E. coli* contains virulent strains related to severe infections in poultry. Also, serotype O78 has public health implications because it is considered one of the serotypes accompanied by enterotoxigenic *E. coli* strains that can infect humans directly by contact with infected birds (Messier et al., 1993).

The isolated staphylococci in this study were identified into three coagulase-positive staphylococci with *S. aureus* as the most prevalent strain (55.1%), and seven coagulase-negative staphylococci in which *S. sciuri* was the most predominant strain (24.6%) (Table 4). Similarly, 17 *Staphylococcus* spp. (three coagulase-positive spp. and 14 coagulase-negative spp.) were isolated and identified from 100 cellulitis lesion samples in broiler chickens (Hilmy, 2002). Also, *S. aureus* was isolated from 12 out of 98 broiler carcasses with cellulitis (Derakhshanfar and Ghanbarpour, 2002).

Antibiotics are used in poultry farms as therapeutic agents and growth promoters, which have favorable and economic benefits for farmers. However, its excessive use is a big threat and results in emerging and dissemination of antibiotic-resistant strains of pathogenic and non-pathogenic organisms that could be transferred to humans via the food chain (Kariuki, et al., 1999; Apata, 2009; Suleiman et al., 2013). The rapid surge in the development and spread of antibiotic resistance is the main cause of concern (Aarestrup et al., 2008). Thus, testing isolated pathogens for antibiotic resistance has become a global interest in efficient preventive treatment and control measures.

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Table 5. The results of antibiotic susceptibility profile of bacterial pathogens isolated from cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

Bacterial species	State	Antimicrobial agents									
		CN	OX	ERI	C30	T30	DA	ENR	AMP	CTX	VA
E. coli **(n=184)**	**S**	43 (23.4%)	16 (8.7%)	58 (31.5%)	22 (11.9%)	15 (8.2%)	181 (98.4%)	12 (6.5%)	29 (15.8%)	69 (37.5%)	23 (12.5%)
	R	86 (46.7%)	137 (74.5%)	79 (42.9%)	153 (83.1%)	164 (89.1%)	2 (1.1%)	171 (92.9%)	124 (67.4%)	98 (53.3%)	138 (75%)
	I	55 (29.9%)	31 (16.8%)	47 (25.5%)	9 (4.9%)	5 (2.7%)	1 (0.5%)	1 (0.5%)	31 (16.8%)	17 (9.2%)	23 (12.5%)
Staphylococcus **spp. (n=135)**	**S**	132 (97.8%)	28 (20.7%)	89 (65.9%)	105 (77.8%)	78 (57.8%)	9 (6.7%)	38 (28.1%)	4 (2.9%)	97 (71.8%)	47 (34.8%)
	R	3 (2.2%)	103 (76.3%)	42 (31.1%)	29 (21.5%)	55 (40.7%)	112 (82.9%)	88 (65.2%)	131 (97.0%)	21 (15.5%)	84 (62.2%)
	I	0 (0%)	4 (2.97%)	4 (2.97%)	1 (0.7%)	2 (1.5%)	14 (10.4%)	9 (6.7%)	0 (0%)	17 (12.59%)	4 (2.9%)
Clostridia spp. (n=22)	**S**	3 (13.6%)	14 (63.6%)	20 (90.9%)	22 (100%)	0 (0%)	4 (18.2%)	0 (0%)	16 (72.7%)	0 (0%)	3 (13.6%)
	R	18 (81.8%)	1 (4.5%)	2 (9.1%)	0 (0%)	22 (100%)	14 (63.6%)	22 (100%)	4 (18.2%)	22 (100%)	18 (81.8%)
	I	1 (4.5%)	7 (31.8%)	0 (0%)	0 (0%)	0 (0%)	4 (18.2%)	0 (0%)	2 (9.1%)	0 (0%)	1 (4.5%)
Aeromonas **spp. (n=5)**	**S**	0 (0%)	2 (40%)	2 (40%)	5 (100%)	0 (0%)	1 (20%)	0 (0%)	4 (80%)	0 (0%)	1 (20%)
	R	3 (60%)	0 (0%)	3 (60%)	0 (0%)	5 (100%)	3 (60%)	5 (100%)	1 (20%)	5 (100%)	4 (80%)
	I	2 (40%)	3 (60%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter **spp. (n=13)**	**S**	11 (84.6%)	2 (15.4%)	2 (15.4%)	0 (0%)	8 (61.5%)	1 (7.7%)	3 (23.1%)	1 (7.7%)	0 (0%)	2 (15.4%)
	R	2 (15.4%)	10 (76.9%)	8 (61.5%)	13 (100%)	3 (23.1%)	12 (92.3%)	8 (61.5%)	10 (76.9%)	13 (100%)	11 (84.6%)
	I	0 (0%)	1 (7.7%)	3 (23.1%)	0 (0%)	2 (15.4%)	0 (0%)	2 (15.4%)	2 (15.4%)	0 (0%)	0 (0%)
Proteus mirabilis **(n=18)**	**S**	7 (38.8%)	2 (11.1%)	4 (22.2%)	14 (77.7%)	6 (33.3%)	8 (44.4%)	5 (27.7%)	10 (55.5%)	13 (72.2%)	16 (88.9%)
	R	10 (55.5%)	14 (77.7%)	14 (77.7%)	2 (11.1%)	12 (66.7%)	9 (50%)	9 (50%)	5 (27.7%)	5 (27.7%)	2 (11.1%)
	I	1 (5.6%)	2 (11.1%)	0 (0%)	2 (11.1%)	0 (0%)	1 (5.6%)	4 (22.2%)	3 (16.6%)	0 (0%)	0 (0%)
P. aeruginosa **(n=9)**	**S**	4 (44.4%)	2 (22.2%)	0 (0%)	1 (11.1%)	0 (0%)	2 (22.2%)	0 (0%)	2 (22.2%)	3 (33.3%)	2 (22.2%)
	R	5 (55.5%)	7 (77.7%)	8 (88.8%)	8 (88.8%)	9 (100%)	7 (77.7%)	9 (100%)	7 (77.7%)	5 (55.5%)	7 (77.7%)
	I	0 (0%)	0 (0%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (11.1%)	0 (0%)
Streptococcus **spp. (n=21)**	**S**	3 (14.3%)	2 (9.5%)	0 (0%)	7 (33.3%)	3 (14.3%)	18 (85.7%)	5 (23.8%)	7 (33.3%)	9 (42.8%)	6 (28.6%)
	R	18 (85.7%)	16 (76.2%)	21 (100%)	13 (61.9%)	15 (71.4%)	2 (9.5%)	14 (66.7%)	10 (47.6%)	7 (33.3%)	13 (61.9%)
	I	0 (0%)	3 (14.3%)	0 (0%)	1 (4.7%)	3 (14.3%)	1 (4.7%)	2 (9.5%)	4 (19.0%)	5 (23.8%)	2 (9.5%)

S: Sensitive; R: Resistant; I: Intermediate; CN: Gentamycin 10 µg/ml; OX: Oxacillin 30 µg/ml; ERI: Erythromycin 15 µg/ml; C30: Chloramphenicol 30 µg/ml; T30: Tetracycline 30 µg/ml; DA: Clindamycin 2 µg/ml; ENR: Enrofloxacin 5 µg/ml; AMP: Ampicillin 10 µg/ml; CTX: Cefatoxaime 30 µg/ml; VA: Vancomycin 30 µg/ml n: number

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Table 6. Antibiotic sensitivity patterns of bacterial spp. isolated from cellulitis lesions of broiler chickens in Egypt (March 2017 to March 2019)

No. of antibiotics to which the organism is resistant	Resistance index	Resistance class	Distribution of bacterial spp. according to drug resistance index; n (%)								
			E. coli **n=184**	***Staphylococcus*** **spp. n=135**	**Clostridia spp. n=22**	***Aeromonas*** **spp. n=5**	***Enterobacter*** **spp. n=13**	***Proteus mirabilis*** **n=18**	***P. aeruginosa*** **n=9**	***Streptococcus*** **spp. n=21**	**Total isolates n=407**
0	0	S	12 (6.5%)	4 (2.9%)	0 (0%)	0 (0%)	2 (15.4%)	0 (0%)	0 (0%)	0 (0%)	18 (4.4%)
1-2	0.1-0.2	NDR	20 (10.9%)	9 (6.6%)	1 (4.5%)	0 (0%)	2 (15.4%)	2 (11.1%)	1 (11.1%)	3 (14.2%)	38 (9.3%)
3-7	0.3-0. 7	MDR	99 (53.8%)	82(60.7%)	9 (40.9%)	3 (60%)	9 (69.2%)	10 (55.5%)	1 (11.1%)	16 (76.1%)	229 (56.3%)
8-9	0. 8-0.9	XDR	44 (23.9%)	27 (20%)	10 (45.5%)	2 (40%)	0 (0%)	5 (27.8%)	7 (77.8%)	2 (9.5%)	97 (23.8%)
10	1	PDR	3 (1.6%)	3 (2.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (1.5%)

S: Sensitive; NDR: Narrow drug-resistant; MDR: Multidrug-resistant; XDR: Extensively drug-resistant; PDR: Pandrug-resistant

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The results of *in vitro* sensitivity testing of the isolated bacterial strains are presented in table 5. It was found that the antibiogram profile of *E. coli* isolates showed highest resistance rate to enrofloxacin (92.9%), followed by tetracycline (89.1%), chloramphenicol (83.1%), vancomycin (75%), oxacillin (74.5%), ampicillin (67.4%), cefotaxime (53.3%), gentamycin (46.7%) and erythromycin (42.9%). High susceptibility and lowest resistance to clindamycin (98.4%) were recorded. These findings are in line with a previous study that indicated the high prevalence of resistance in *E. coli* isolated from broiler chickens against 10 different antimicrobial agents including ampicillin (100%), tetracycline (93.1%), nalidixic acid (84.5%), chloramphenicol (84.5%), kanamycin (69.0%), sulfamethoxazole-trimethoprim (58.6%), cefotaxime (58.6%), streptomycin (50.0%), gentamicin (48.3%), and ciprofloxacin (41.4%) (Awad et al., 2016). In Egypt, another study reported that *E. coli* has variable sensitivity ranged from 14.3% to trimethoprim + sulfamethoxazole to 64.3% to clindamycin (Amer et al., 2017). Also, the resistance rate to oxacillin among the *E. coli* recovered from broilers ranged from 78.1% to 100% (Ahmed et al., 2013; Ibrahim et al., 2019).

The antibiogram profile of *Staphylococcus* spp. indicated high resistance rate to ampicillin (97.0%) and clindamycin (82.9%), followed by oxacillin (76.3%), enrofloxacin (65.2%), vancomycin (62.2%) and tetracycline (40.7%). On the other hand, the lower rates of resistance to erythromycin (31.1%), chloramphenicol (21.5%) and cefotaxime (15.5%) were recorded. High susceptibility and lowest resistance to gentamycin were detected (97.8%). Similarly, Bala et al. (2016) concluded that antibiotics such as oxytetracycline, oxacillin, and ampicillin have low activity against the tested isolates of *Staphylococcus* spp. Similar patterns of antimicrobial susceptibility have been previously reported (Pesavento et al., 2007; Otalu et al., 2011; Leonard and Markey, 2008; Waters et al., 2011). These findings may be a result of the extensive usage of these antimicrobial agents in animal husbandry over time, which has contributed to the development of drug-resistant strains (Nemati et al., 2008). On the other hand, Suleiman et al. (2013) reported 100% susceptibility to gentamicin and 66.7% to Augmentin.

The antibiogram profile of Clostridia isolates showed 100% resistance to tetracycline, enrofloxacin, and cefotaxime, as well as a high resistance rate of 81.8%, 81.8% and 63.6% to gentamycin, vancomycin, and clindamycin, respectively. However, these isolates showed low rates of resistance and high susceptibility to oxacillin (4.5%), erythromycin (9.1%), ampicillin (18.2%) and complete sensitivity to chloramphenicol.

In Egypt, Osman and Elhariri (2013) recorded that clostridial isolates obtained from broiler flocks exhibited resistance toward gentamicin, streptomycin, oxolinic acid, lincomycin, erythromycin, and spiramycin. The prevalence of resistance to other antibiotics was also high, as doxycycline (98%), trimethoprim-sulfamethoxazole (98%), colistin (94%), pefloxacin (94%), neomycin (93%), enrofloxacin (82%), flumequine (78%), oxytetracycline (71%), norfloxacin (67%), tylosin-fosfomycin (52%), ciprofloxacin (58%), spectinomycin (50%), chloramphenicol (46%), and rifampicin (34%). The aforementioned study recommended drugs such as amoxicillin, ampicillin, cephradine, fosfomycin, and florfenicol for *C. perfringens* infection treatment in Egypt. Another study conducted in Korea recorded that *C. perfringens* isolated from chickens were susceptible to ampicillin, amoxicillin/clavulanic acid, cephalothin, cefepime, chloramphenicol, cefoxitin, ceftiofur, florfenicol, and penicillin but resistant to gentamycin, neomycin, streptomycin, apramycin and colistin (Park et al., 2015). This trend of resistance was similar to that detected in Taiwan by Fan et al. (2016) who found that most *C. perfringens* isolates from broiler chickens showed resistance against erythromycin, lincomycin, and chlortetracycline but susceptibility to amoxicillin, bacitracin, and enrofloxacin.

The result from the antibiogram profile of *Aeromonas* spp. revealed complete resistance to tetracycline, enrofloxacin, and cefotaxime, followed by vancomycin (80%), gentamycin (60%), erythromycin (60%) and clindamycin (60%). while low rates of resistance to ampicillin (20%) and complete sensitivity to chloramphenicol were detected. These results were in accordance with that obtained by Ghenghesh et al. (2013) who reported *Aeromonas* isolated from chicken carcasses were susceptible to ciprofloxacin, ceftriaxone, and gentamicin. The isolates showed a significantly higher resistance rate to tetracycline. On the contrary, some studies reported complete resistance of *Aeromonas* to ampicillin and other penicillins (Ghenghesh et al., 2001; Hammad et al., 2018).

Antibiogram profile of *Enterobacter* spp. showed complete resistance to both chloramphenicol and cefotaxime, followed by clindamycin (92.3%), vancomycin (84.6%), oxacillin (76.9%) and ampicillin (76.9%), erythromycin (61.5%), enrofloxacin (61.5%), tetracycline (23.1%) and gentamycin (15.4%). Previous studies reported that *Enterobacter* spp. isolated from chickens were resistant to multiple antibiotics including ampicillin, cefotaxime, and gentamicin (Dennison and Morris, 2002; Kilonzo-Nthenge et al., 2008).

Antibiogram profile of *Proteus mirabilis* demonstrated 77.7% resistance to both oxacillin and erythromycin, followed by tetracycline (66.7%), gentamycin (55.5%), clindamycin (50%), enrofloxacin (50%), ampicillin (27.7%), cefotaxime (27.7%), chloramphenicol (11.1%) and vancomycin (11.1%). In a previous study by Nemati, (2013), *Proteus* isolates recovered from poultry were found to be highly resistant to nalidixic acid (93%), doxycycline (91%) and oxytetracycline (89%). Moreover, low resistance to norfloxacin (24%), ampicillin (22%), ceftriaxone (22.4%) and amikacin (24%) and high susceptibility to gentamycin were reported **(**Nemati, 2013). In Bangladesh, a similar trend of antibiotic resistance was noticed in 36 *Proteus* isolates from chicken and 95% of the isolates showed resistance against

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tetracycline, 89% against nalidixic acid and 20% were resistant against ciprofloxacin. Totally, 84% of the isolates exhibited MDR (Nahar et al., 2014).

The antibiogram profile of *P. aeruginosa* showed complete resistance to tetracycline and enrofloxacin, followed by erythromycin and chloramphenicol with the same resistance rate of 88.8%. In addition, a resistance rate of 77.7% to oxacillin, clindamycin, ampicillin, and vancomycin was found. Also, gentamycin and cefotaxime had the same resistance rate of 55.5%. In Pakistan, Sharma et al. (2017) investigated that *P. aeruginosa* isolates from chicken exhibited 100% resistance toward ceftriaxone, meropenem, ciprofloxacin, erythromycin, and colistin, while 60% sensitivity was noticed to ampicillin-sulbactam, ceftazidime, cefoperazone, and rifampicin. Isolates exhibited variable multidrug resistance patterns to other antibiotics. Another study carried out in Nigeria demonstrated that the *P. aeruginosa* isolates showed high resistance to β-lactams, tetracycline, tobramycin, nitrofurantoin, and sulfamethoxazole-trimethoprim, while ofloxacin, imipenem, and ertapenem appeared highly effective against the bacterial pathogens (Aniokette et al., 2016).

The antibiogram profile of *Streptococcus* spp. revealed complete resistance to erythromycin, followed by gentamycin (85.7%), oxacillin (76.2%), tetracycline (71.4%), chloramphenicol (61.9%), vancomycin (61.9%), enrofloxacin (66.7%), ampicillin (47.6%), cefotaxime (33.3%) and clindamycin (9.5%). A study carried out in Japan reported that most of the examined *Streptococcus* isolates appeared susceptible to vancomycin, penicillin G and ampicillin, while some showed resistance to tetracycline, doxycycline, and lincomycin (Nomoto et al., 2013).

The misuse of antimicrobial at sub-therapeutic doses or unneeded doses contributes to the emergence of MDR bacteria (Yang et al., 2004). Concerning the result of MDR, it was demonstrated that only three bacterial spp., including *Staphylococcus* spp. (2.9%), *E. coli* (6.5%) and *Enterobacter* spp. (15.4%), were sensitive to all tested antibiotics. All tested bacteria spp. had narrow drug-resistant isolates ranged from 4.5% to 15.4%. *E. coli* (1.6%) and *Staphylococcus* spp. (2.2%) had isolates related to the PDR group. The prevalence of MDR, XDR, and PDR isolates was 56.3%, 23.8%, and 1.5%, respectively (Table 6). Among the total of 407 bacterial isolates, 332 isolates (81.6%) were found to be MDR with MDR Index ≥ 0.3. A previous study by Xia et al. (2011) reported that over 58% of *E. coli* isolates showed resistance to four or more antimicrobial agents. The growing incidence of MDR is a public health issue due to the danger of entering the human food chain (Angulo et al., 2005).

In Egypt, 42%-83.3% of examined *E. coli* isolates were MDR to 5-10 out of 12 tested antibiotics (Amer et al., 2018). Another study reported a high prevalence of MDR as all examined *E. coli* isolates showed resistance to at least five anti-microbial agents (Ibrahim et al., 2019). The study of resistance patterns of the *C. perfringens* isolates indicated that all examined isolates exhibited resistance to 8-11 types of antibiotics and all were MDR (Osman and Elhariri, 2013). In Poland, over half of 302 *Staphylococcus* strains isolated from poultry were resistant to five of the used antibiotics, with the highest percentage recorded for enrofloxacin (Marek et al., 2016).

CONCLUSION

In conclusion, avian cellulitis had economic damage due to the high rate of carcass condemnation at slaughterhouses. *E. coli* serotype O78 as a zoonotic pathogen was the most predominant pathogen involved in cellulitis. A high prevalence of MDR among bacterial isolates was found, particularly against commonly used antibiotics. Therefore, it is recommended that the use of antimicrobial agents should follow prudent guidelines to minimize the development and spread of resistant bacteria. Also, the utilization of some antibiotics such as tetracycline, oxytetracycline, and erythromycin in poultry farms should be revised. Moreover, susceptibility testing should be performed to assure drugs of choice.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Mohamed M. Amer and Hoda M. Mekky designed the study, drafted and revised the manuscript. Hanaa S. Fedawy, Kh. M. Elbayoumi and Dalia M. Sedeek shared in samples collection, performing the tests, manuscript writing and data analysis. All authors read and approved the final manuscript.

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