JOURNAL OF VETERINARY MEDICAL RESEARCH 2018, 25 (1): 68 - 79

Journal homepage:
http://www.bsu.edu.eg/bsujournals/JVMR.aspx

Online ISSN: 2357-0520 Print ISSN: 2357-0512

Original Research Article

Bacterial pathogens associated with cellulitis in chickens

Radwan, I.A.[a], Abed, A.H.[a,*], Abd Allah, M.M.[2] and Abd El-Latif, M.A.A.[2]

[a]*Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.*

[b] *Animal Health Research Institute Dokki, El Fayoum branch, Egypt.*

ABSTRACT

Cellulitis is a serious problem for the poultry industry because of increased condemnations, carcass downgrading at processing, and higher labor costs to process affected flocks. In the present study, the prevalence of cellulitis was studied in 240 broiler chickens. The correlation between cellulitis and other systemic lesions of the same bird was investigated also. Moreover, identification of the causative bacterial agents was conducted focusing on *E. coli* and *Salmonella* isolates. The prevalence rate of cellulitis in examined broiler chickens was 38.3%. Cellulitis without systemic lesion was observed in 14.2% of birds while 24.2% of birds had cellulitis associated with other systemic lesions in the internal organs while hepatitis was the most frequent. The bacteriological examination revealed that of 253 samples collected, a total of 157 bacterial isolates were recovered (62.1%). Among the recovered isolates, *E. coli* was the most prevalent (126 isolates; 80.3%) as well as 4 *Salmonella* species (2.5%), 9 *Proteus* species (5.7%), 7 *Pseudomonas aeruginosa* (4.5%), 3 *Enterobacter* species (1.9%) and 8 *Staphylococcus aureus* (5.1%). Serogrouping of *E. coli* isolates revealed that O_{125} was the most prevalent; 32%, followed by serogroups O_{158}, O_{55}, O_{78} as 24%, 12%, 10%, respectively, then both O_1 and O_8; 6% for each, and finally O_{15}; 4%. Antibiogram of *E. coli* isolates showed a high sensitivity against enrofloxacin only (81%) while they were moderately sensitive to apramycin (65.9%) and colistin sulphate (61.9%) as well as ciprofloxacin and cefotaxime sodium (56.3% and 55.6%, respectively). On the other hand, high moderate degrees of resistances were observed against the other antimicrobials. *Salmonella* isolates showed complete sensitivities to ciprofloxacin and enrofloxacin while they were completely resistant to most of antimicrbials.

ARTICLE INFO

Article history:

Received 5/2018

Accepted 6 /2018

Online 6/2018

Keywords:

Bacterial pathogens, cellulitis, chickens

*Corresponding author. *Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.* Email: aboabedelmasry@yahoo.com, ahmed.moawad@vet.bsu.edu.eg

Introduction

Avian cellulitis is a serious problem for the commercial broiler industry because of increased condemnations, carcass downgrading at processing, and higher labor costs to process affected flocks. Between 1986 and 1996, condemnations for coliform cellulitis increased almost 12-fold in Canada. In 1996, more than 2.6 million broilers were condemned for the disease, which represented 0.568% of all birds processed and approximately 30% of total condemnations **(Kumor *et al*., 1998)**. Estimated annual losses to the U.S. broiler industry due to cellulitis have increased from $20 million in 1991 to more than $80 million in 1998 **(Singer *et al*., 1999)**. Condemnation rates due to cellulitis increased; especially coliform cellulitis, during the past 15 years **(Umar *et al*., 2015)** till cellulitis becomes now one of the major causes of condemnation in broiler chickens in slaughterhouses all around the world, which makes it a source of major financial losses **(Fard *et al*., 2007)**.

Cellulitis; also known as necrotic dermatitis, was firstly reported by **Randall *et al*. (1984)** in England. Cellulitis refers to inflammation of the subcutaneous tissue and is typically seen adjacent to the lower abdomen and thigh of broiler chickens **(Barnes and Gross, 1997)**. The affected animals usually do not show any clinical signs, and the lesions are sometimes only detected at the slaughter plant **(Gomis *et al*., 2001)**. The animals are infected through skin lesions **(Elfadil *et al*., 1996b)**, but symptoms are probably only seen if a minimum infection pressure of avian pathogenic *E. coli* (APEC) and possibly also other predisposing factors are present in the house **(Onderka *et al*., 1997)**. Cellulitis was referred as a consequence of overpopulation and poor house hygiene rather than a specific disease **(Glünder, 1990)**.

E. coli has been reported as the predominant microorganism isolated from cellulitis lesions in previous studies**(Randall *et al*., 1984, Eterradossi *et al*., 1989 and Messier *et al*., 1993)**. Other agents such as *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterobacter agglomerans*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Aeromonas*, *Citrobacter ferundi* and *Aerobacter* have been isolated but are not believed to be significant **(Glünder, 1990; Messier *et al*., 1993; Norton, 1998; Singer *et al*., 2000 and Fard *et al*., 2007)**. Moreover, *Actinomyces pyogenes* **(Derakhshanfar and Ghanbarpour, 2002)** and *Erysipelothrix rhusiopathiae* **(Derakhshanfar *et al*., 2004)** have been considered as a causative agent of avian cellulitis, with public health hazards. The more recent field situation in broilers is specifically associated with anaerobes such as *Clostridium* species. *C. colinum C. septicum*, *C. perfringens* and *C. sordelli* can cause cellulitis **(Fard *et al*., 2007 and Umar *et al*., 2015)**.

E. coli is the principal infectious agent and it is considered the cause of coliform cellulitis in chickens **(Saif *et al*., 2003)**. *E. coli* of many different serogroups may be isolated from cases of cellulitis, but serogroups O_{78}, O_1, and O_2 were the most predominated isolates **(Glünder, 1990; Allan *et al*., 1993; Messier *et al*., 1993; Peighambari *et al*., 1995b; Gomis *et al*., 1997 and Fard *et al*., 2007)**, which are serogroups typically associated with pathogenicity in poultry. Large groups of non-serotyped *E. coli* isolates, which collectively outnumber those which can be serotyped, have also been isolated from cellulitis lesions **(Macklin *et al*., 1999)**.

Although antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive birds, antimicrobial drug use in livestock production has been implicated as a risk factor in the development and dissemination of drug resistance from livestock production farms **(Gosh and LaPara, 2007)**. Food animals and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact between animals and humans or indirectly via the food production chain **(WHO, 2011)**; or as a result of

the spread of animal waste on land **(Heuer and Smalla, 2007)**. Therefore, the appropriate antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examination.

The present study aimed to investigate the prevalence of cellulitis in chickens with detection of the causative bacterial pathogens.

Material and Methods

2.1. Chickens. A total of 240 broiler chickens of different ages (3-5weeks) from different farms in Beni-Suef and El-Fayoum Governorates were subjected to the present study during the period from January 2014 up to December 2014. These chickens were subjected to clinical and postmortem examinations to detect cellulitis.

2.2. Samples. Samples were collected from 92 broiler chickens suffered from cellulitis with or without septicaemia signs. A total of 253 samples were collected from the affected tissues. Samples from the muscles of the lower abdomen and thigh; in case of cellulitis, as well as the other internal lesions; airsacculitis, pericarditis and hepatitis, were collected. Heart blood samples were collected from all cases either cellulitis associated with septicaemic lesions or not.

2.3. Bacteriological examination. The collected samples were cultivated under aseptic condition into Tryptone Soya broth and MacConkey broth then inoculated aerobically at 37ºC for 24 hrs. Then loopfulls from the inoculated broth were streaked onto Tryptone Soya agar (TSA) and MacConkey's agar then, incubated aerobically at 37ºC for 24-72hr. All the recovered isolates were identified morphologically and biochemically according to schemes described by **Kreig and Holt (1984)**, **Collee *et al.* (1996)** and **Quinn *et al*. (2002)**.

2.4. Serological identification. Randomly selected 50 *E. coli* isolates as well as 4 *Salmonella* isolates that were preliminarily identified morphologically and biochemically were subjected to serological identification.

2.4.1. Serogrouping of *E. coli* isolates. *E. coli* serogroups were identified serologically by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera according to **Quinn *et al*. (2002)**.

2.4.2. Serotyping of Salmonella. Four *Salmonella* isolates; 2 from muscles and 2 from heart blood of two birds, were identified serologically by slide agglutination test using diagnostic polyvalent and monovalent O and H *Salmonella* antisera according to Kauffman-white scheme **(Kauffmann, 1974)**.

2.5. Antibiotic susceptibility testing. All *E. coli* (126 isolates) and Salmonellae (4 isolates) recovered from cellulitis chickens were tested for their antimicrobial susceptibility to 14 different antimicrobial discs including; apramycin (15µg), ciprofloxacin (15µg), cefotaxime sodium (30µg), colistin sulphate (10µg), sulphamethoxazol-trimethoprim (1.25+23.75µg), doxycycline HCl (30µg), enrofloxacin (5µg), lincomycin (10µg), spectinomycin (100µg), fosfomycin (300µg), gentamycin (10µg), florophenicol (30µg), streptomycin (10µg) and spiramycin (100µg) (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to **CLSI (2012)**. The antibiotic susceptibility was based on the induced inhibition zones according to the guidelines of the **CLSI (2012)**.

Resistance to more than four antibiotics was taken as multidrug resistance (MDR). MDR index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed **(Chandran *et al*., 2008)**.Isolates with MDRI values of more than 0.2 or 20% were considered highly resistant.

$$\text{MDR index} = \frac{\text{Number of antibiotics resisted x 100}}{\text{Total number of antibiotics used}}$$

Results

3.1. Prevalence of cellulitis in the examined broiler chickens. Out of 240 broiler chicken, 92 birds (38.3%) showed cellulitis (inflammation

of the muscles of the lower abdomen and thigh) in PM examination **(Table 1)**. Of them, 34 birds (14.2%) had cellulitis only while 58 birds (24.2%) had cellulitis associated with septicaemic lesions in the internal organs (at least one organ was affected). The affected organs included liver (n=51), air sacs (n=11) and pericardium (n=7). On the other hand, 148 (61.7%) had no cellulitis symptoms.

Table (1): Prevalence of cellulitis in the examined broiler chickens.

No. of birds	Positive Cellulitis						Negative Cellulitis	
	Cellulitis only		Cellulitis + Septicaemia		Total			
	No.	%	No.	%	No.	%	No.	%
240	34	14.2	58	24.2	92	38.3	148	61.7

%: was calculated according to the number (No.) of birds.

3.2. Bacteriological examination. Out of 253 samples collected from different lesions of broiler chickens with cellulitis; with and without septicaemia, a total of 157 bacterial isolates were recovered with a rate of 62.1%. Bacterial isolation was distributed as follows; 73 bacterial isolates (79.3%) from muscle samples, 30 (32.6%) from heart blood samples, 41 (80.4%) from liver samples, 7 (63.6%) from air sacs and 6 (85.7%) from pericardium **(Table 2)**.

Table (2): Results of bacteriological examination of different samples collected from broiler chickens with cellulitis/septicaemic lesions.

Samples (lesion)	No. of samples	Bacterial isolation	
		No.	%
Cellulitis	92	73	79.3
Heart blood	92	30	32.6
Hepatitis	51	41	80.4
Airsacculitis	11	7	63.6
Pericarditis	7	6	85.7
Total	253	157	62.1

%: was calculated according to the number (No.) of the samples.

were identified as follow; 126 *E. coli* isolates with a prevalence rate of 80.3%, 4 *Salmonella* species (2.5%), 9 *Proteus* species (5.7%), 7 *Pseudomonas aeruginosa* (4.5%), 3 *Enterobacter* species (1.9%) and 8 *Staphylococcus aureus* (5.1%) **(Table 3)**. Concerning muscles` bacterial isolates (n=73), 59 isolates (80.8%) were *E. coli*; of which 55 (93.2%) were single and 4 (6.8%) were mixed with other bacteria (2 isolates were mixed with *S. aureus*, one isolate was mixed with *Proteus* and another isolate was mixed with *Enterobacter*). Moreover, 2 *Salmonella* (2.7%), 3 *Proteus* species (4.1%), 3 *P. aeruginosa* (4.1%), 2 *Enterobacter* species (2.7%) and 4 *S. aureus* (5.5%) were identified. Out of 30 bacterial isolates recovered from heart blood, 24 (80%) were *E. coli*, 2 *Salmonella* (6.7%), 2 *Proteus* species (6.7%), one isolate (1.33%) for both *Enterobacter* species and *S. aureus*. Concerning the liver isolates (n=41), they were represented as 34 *E. coli* (82.9%), 2 *Proteus* species (4.9%), 2 *P. aeruginosa* (4.9%) and 3 *S. aureus* (7.3%). Belonging the air sac (n=7) and pericardium (n=6) isolates, *E. coli* represented 5 (71.4%) and 4 (66.7), respectively while *Proteus* species represented one isolate for both with prevalence rate of 14.3% and 16.7%, respectively. Also, one isolate of *P. aeruginosa* was recovered from both sites with a rate of 14.3% and 16.7%, respectively.

3.3. Prevalences of different bacterial pathogens recovered from cellulitis/septicaemic lesions in broiler chickens. The recovered bacterial isolates (157)

Table (3): Prevalences of bacterial pathogens causing cellulitis and different septicaemic lesions in broiler chickens.

Site of Samples	No. of isolates	*E. coli*		*Salmonella* species		*Proteus* spp.		*P. aeruginosa*		*Enterobacter* spp.		*S. aureus*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Muscles	**73**	59	80.8	2	2.7	3	4.1	3	4.1	2	2.7	4	5.5
Heart blood	**30**	24	80	2	6.7	2	6.7	0	0	1	3.3	1	3.3
Liver	**41**	34*	82.9	0	0	2	4.9	2	4.9	0	0	3	7.3
Air sac	**7**	5*	71.4	0	0	1	14.3	1	14.3	0	0	0	0
Pericardium	**6**	4*	66.7	0	0	1	16.7	1	16.7	0	0	0	0
Total	**157**	**126**	**80.3**	**4**	**2.5**	**9**	**5.7**	**7**	**4.5**	**3**	**1.9**	**8**	**5.1**

%: was calculated according to the number (No.) of isolates.

***: *E. coli* isolates from the colisepticaemic lesions of the internal organs (Total No. = 43 isolates)**

3.4. Serological identification

3.4.1Serogrouping of *E. coli* isolates. Out of 50 *E. coli* isolates, 7 O-serogroups were obtained. The serogroups O_{125} was the most prevalent represented 16 isolates (32%) followed by serogroups O_{158}; 12 (24%) and O_{55}; 6 (12%). Then, the serogroup O_{78} as 5 isolates (10%). After that, both O_1 and O_8 serogroups represented 3 isolates (6%) for each and finally serogroup O_{15}; 2 isolates (4%). Moreover, there were 3 isolates (6%) were untyped with the available antisera.

3.4.2. Serotyping of *Salmonella* isolates. All the recovered *Salmonella* isolates were serotyped as *Salmonella* Kossen.

3.5. Antibiotic susceptibility testing. Results of *in-vitro* sensitivity tests showed that *E. coli* isolates were highly sensitive to enrofloxacin only (81%) while they were moderately sensitive to apramycin (65.9%) and colistin sulphate (61.9%) as well as ciprofloxacin and cefotaxime sodium (56.3% and 55.6%, respectively). On the other hand, they were highly resistant to lincomycin (87.3%), streptomycin (78.6%), spiramycin (75.4%) and trimethoprim-sulphamethoxazol (73%). Moderate resistances against fosfomycin, spectinomycin and florophenicol were also recorded; 58.7%, 54.8% and 54%, respectively. Multi drug resistance (MDR) was detected amongst 116 *E. coli* isolates (MDRI= 92.1%) which observed as resistance for four or more antimicrobials of different categories. On the other hand, *Salmonella* isolates were completely sensitive to ciprofloxacin and enrofloxacin. On the other hand, they were completely resistant to colistin sulphate, trimethoprim-sulphamethoxazol, lincomycin, spectinomycin, florophenicol, streptomycin and spiramycin. MDR was detected among All *Salmonella* isolates (MDRI= 100%) which observed as resistance for four or more antimicrobials of different categories.

Discussion

Avian cellulitis, sometimes referred to as Inflammatory Process (IP), is a serious problem for the commercial broiler industry. It is a diffuse spreading, edematous, infective inflammation of the deep subcutaneous tissues, occasionally extending into the muscle, which is characterized by sheets of caseated and fibrinoheterophilic exudates in subcutaneous tissues located in the skin between the thigh and midline **(Peighambari *et al.*, 1995b; Ghanbarpour *et al.*, 2003 and Saif *et al.*, 2003)**. The condition occurs primarily in broilers and less in turkeys **(Ghanbarpour *et al.*, 2003)**. Cellulitis is caused by damages to the skin in 2-3 week-old chickens, by introduction of bacteria and yielding plaque-like lesions under the skin. The mortality in mild and sporadic cases, without septicemia is very low **(Elfadil *et al.*, 1996a** and **Ghanbarpour *et al.*, 2003)**. It is difficult to detect birds with cellulitis in the live flock, because the lesions are not readily apparent, as the affected sites of the skin are covered with feathers and the infected birds show no clinical signs of disease **(Gomis *et al.*, 1997)**.

There are some different related factors such as farm management, breed and sex, chick quality, heat stress, nervousness of flocks, environmental and temperature conditions, density, lighting programs, wet and unsuitable litters, ventilation, nutritional entities, calorie/protein ratio, feed additives, amino acids and vitamin deficiency could be associated with an increase in skin scratches that could lead to cellulitis **(Elfadil *et al.*, 1996a)**. The severity of the disease is related to genetic status and immunosuppressive diseases **(Peighambari *et al.*,**

1995a; Jeffrey *et al.*, 1999; Derakhshanfar and Ghanbarpour, 2002 and Fard *et al.*, 2007). Other observations have suggested that any insult to the integrity of the skin, regardless of when it occurs, should be considered a significant route of cellulitis pathogenesis **(Peighambari *et al.*, 1995b and Norton *et al*., 1999)**.

Condemnation rates due to cellulitis increased; especially coliform cellulitis, during the past 15 years **(Umar *et al.*, 2015)** till cellulitis becomes now one of the major causes of condemnation in broiler chickens in slaughterhouses all around the world, which makes it a source of major financial losses **(Fard *et al.*, 2007)**. The increased incidence of cellulitis over the past several years is probably related to various factors. Considering different results, it seems decreasing the age of slaughtering, upgrading immunity status and improvement the welfare and management policies of broiler flocks lead to less carcass condemnations **(Fard *et al.*, 2007)**.

Many bacterial agents; either Gram positive or Gram negative, were isolated from cellulitis lesions and considered as a cause for cellulitis but still *E. coli* is the principal infectious agent and it is considered the cause of coliform cellulitis in chickens **(Saif *et al.*, 2003)**.

In the present study, the prevalence of cellulitis was studied in 240 broiler chickens. The correlation between cellulitis and other systemic lesions of the same bird was studied also. Moreover, identification of the causative bacterial agents was conducted focusing on *E. coli* and *Salmonella* isolates.

The data illustrated in **table (1)** revealed that the prevalence rate of cellulitis in examined broiler chickens was 38.3%. Cellulitis without systemic lesion was observed in 14.2% of birds while 24.2% of birds had cellulitis associated with other systemic lesions in the internal organs (at least one organ). The affected organs included liver (n=51), air sacs (n=11) and pericardium (n=7). These results were nearly similar to that obtained by **Gomis *et al.* (1997)** and **Gomis *et al.* (2001)** who remarked that prevalence rate of cellulitis with other systemic manifestations in broilers were 30.5% and 34.6%, respectively. This observation was also in agreement with that previously described (**Eterradossi *et al.*, 1989) and Morris (1991)** and suggested that the occurrence of cellulitis and other diseases especially caused by *E. coli* may be related. The relationship between cellulitis and other lesions; especially colibacillosis lesions, of the different organs in broilers appears to be complex and varies from flock to flock. The present results suggested that common predisposing factors may exist for both types of disease. The occurrence of multiple lesions may be underestimated, because other types of lesions in conjunction with cellulitis may not be detected at the time of inspection, as birds condemned for cellulitis are not examined further.

Usually, an affected bird has only skin lesions, but concurrent lesions of systemic colibacillosis occasionally can be found, suggesting that cellulitis may result from systemic spread or, conversely, that localized lesions in the skin can be a source for systemic disease **(Saif *et al.*, 2003)**. Other previous findings have proved that the bacteria entering through the skin sometimes find their way into the blood circulation and caused septicaemia **(Peighambari *et al.*, 1995a)**. The latter is inversely correlated with age (i.e., the younger the bird, the more likely it is to develop systemic disease) **(Johnson *et al.*, 2001)**.

From other point of view, the present results showed that hepatitis was the most frequently associated with cellulitis while airsacculitis and pericarditis were less frequent. These findings were opposed to those of **Gomis *et al.* (1997)** and **Gomis *et al.* (2001)** who found that airsacculitis and pericarditis were more frequent than hepatitis.

The previously obtained and discussed results in **table (1)** were reinforced by the data illustrated in **table (2)** which studied the bacteriological examination of samples collected from different lesions of broiler chickens with cellulitis either associated with septicaemic lesions or not. Samples were collected from the muscles of the lower abdomen and thigh; in case of cellulitis, as well as the other internal lesions; airsacculitis, pericarditis and hepatitis. Heart blood samples were collected from all cases either cellulitis associated with septicaemic lesions or not. The results revealed that out of 253 samples collected, a total of 157 bacterial isolates were recovered; with a rate of 62.1%. The isolates were distributed in samples from 92 muscle, 92 heart blood, 51 liver, 11 air sacs and 7 pericardium as follow; 73 isolates (79.3%), 30 isolates (32.6%), 41 isolates (81.4%), 7 isolates (63.6%) and 6 isolates (85.7%), respectively. The reasons for the negative isolation of bacteria from birds with gross lesions are not known, but it is

possible that birds were able to clear the infection completely, while fibrin deposits of inflammation remain **(Gomis *et al.*, 2001)**.

Detailed data of the previous results were illustrated in **table (3)** which showed the results of identification and the prevalences of different bacterial pathogens recovered from broiler chickens with cellulitis. Among the recovered isolates (n=157), *E. coli* was the most prevalent as 126 isolates were identified with a prevalence rate of 80.3%. This result coincided with the previous studies reported*E. coli* has been as the predominant microorganism isolated from cellulitis lesions **(Randall *et al.*, 1984; Eterradossi*et al.*, 1989**; **Messier*et al.*, 1993; Derakhshanfar and Ghanbarpour, 2002; Saif *et al.*, 2003; Abd El-Latif, 2004 and Fard *et al.*, 2007)**. Most of cellulitis cases resulting from an infection by *E. coli* associated with litter **(Schrader *etal.*, 2004)**. Mannan Oligosaccharides, and to a less extent lignin, can be used to reduce *E. coli*proliferation in poultry litter and this would offer an opportunity for dietary control of the cellulitis problem **(Baurhoo *et al.*, 2007)**.Moreover, other bacteria have been identified including 9 *Proteus* species (5.7%), 7 *P. aeruginosa* (4.5%), 3 *Enterobacter* species (1.9%) and 8 *S. aureus* (5.1%).These results run were similar to other authors who recovered the same agents **(Glünder, 1990; Messier *et al.*, 1993; Norton, 1998; Singer *etal.*, 2000 and Fard *et al.*, 2007)**. Additionally, in the current study there was an unprecedented result where 4*Salmonella* species (2.5%) were recovered from cellulitis lesions and that is considered; as we believe, the first record for *Salmonella* as a cause of cellulitis.

Belonging the bacterial isolates from muscles, *E. coli* was the most frequent representing 59 isolates (80.8%); of them 55 (93.2%) were single and 4 (6.8%) were mixed with other bacteria (2 isolates were mixed with *S. aureus*, one isolate was mixed with *Proteus* and another isolate was mixed with *Enterobacter*). These results were coincided with those of **Derakhshanfar and Ghanbarpour (2002)** who reported that 91.1% of *E. coli* isolates were the single isolate recovered while the remainders were mixed with *S. aureus* and other bacteria. Their study confirmed the frequent association of *E. coli* with cellulitis lesions in broiler chickens, along with isolation of *S. aureus*. Moreover, in the present study, 2 *Salmonella* (2.7%), 3 *Proteus* species (4.1%), 3 *P. aeruginosa* (4.1%), 2 *Enterobacter* species (2.7%) and 4 *S. aureus* (5.5%) have been identified.

Moreover, out 30 bacterial isolates recovered from heart blood, *E. coli* was the most prevalent; 24 isolates (80%), followed by both *Salmonella* and *Proteus* species; 2 isolates (6.7%) for both, then both of *Enterobacter* species and *S. aureus*; one isolate (1.33%) for both. On the other hand the liver isolates (n=41) were represented as 34 *E. coli* (82.9%), 2 *Proteus* species (4.9%), 2 *P. aeruginosa* (4.9%) and 3 *S. aureus* (7.3%). Belonging the air sac (n=7) and pericardium (n=6) isolates, *E. coli* represented 5 isolates (71.4%) and 4 isolates (66.7), respectively while *Proteus* species represented one isolate for both with prevalence rate of 14.3% and 16.7%, respectively. Also, one isolate of *P. aeruginosa* was recovered from both sites with a rate of 14.3% and 16.7%, respectively.

Serogrouping of *E. coli* isolates was conducted on randomly selected 50 isolates representing the entire affected organs.Results of serogrouping of *E. coli* isolates showed that 7 O-serogroups were obtained. Serogroups O_{125} was the most prevalent represented 16 isolates (32%) followed by serogroups O_{158}, O_{55}, O_{78} as 12 (24%), 6 (12%), 5 (10%), respectively, then both O_1 and O_8; 3 (6%) for each, and finally O_{15}; 2 (4%). Moreover, there were 3 isolates (6%) were untyped with the available antisera. Although only 40% of the isolates were serogrouped, the distribution of O antigens was nearly similar to that reported in previous studies (**Valvano *et al*, 1992; Messier *et al.*, 1993; Gomis *et al.*, 2001 and Schouler*et al.*, 2012)** who recovered nearly the same serogroups; beside other serogroups, from cellulitis lesions. On the contrary they differed from those obtained by **Tana *et al.* (2013)** how recovered 8 different serogroups *E. coli* including O_2, O_8, O_{15}, O_{73}, O_{86}, O_{102}, O_{115} and O_{139}, and **Wang *et al.* (2010)** who recovered 8 serogroups; O_{65}, O_{78}, O_8, O_{120}, O_2, O_{92}, O_{108}, and O_{26}.On the other hand, Serogroups O_{125} was the most prevalent followed by serogroups O_{158}, O_{55}. These results were completely different from several authors who reported that O_{78}, O_2 and O_1 were the main serotypes in different disease types; cellulitis, septicaemia, and airsacculitis, **(Cloud *et al.*, 1985; Glünder, 1990; Allan *et al.*,1993; Dozois *et al.*, 1992; Peighambari *et al.*, 1995b; Gomis *et al.*, 1997; Fard *et al.*, 2007; Abd El-Hamid and Hebib, 2008)**. Others reported that $O_{78}$was the most frequently isolated **(Messier *et al.*, 1993; Derakhshanfar and Ghanbarpour,**

2002; Zhao *et al*., 2005 and Ammar *et al.*, 2011). Additionally, **Ngeleka *et al.* (1996)** found that O_{25} and O_{78} were the most frequently observed while **Wang *et al.* (2010)** and **Tana *et al.* (2013)** found that O_{65} and O_{86} were the most prevalent serogroups, respectively. Moreover, **Onderka *et al.* (1997)** isolated 85 *E. coli* strains from cases of cellulitis, which belonged to 19 different O-types. Nevertheless, there is frequently also a great diversity of O-types in the isolates.

Serotyping of *Salmonella* isolates recovered from cellulitis in broiler chickens was also applied on 4 isolates from 2 birds; 2 from cellulitis lesions and 2 from heart blood of the same birds. These 4 isolates were preliminarily identified morphologically and biochemically as *Salmonella* and subjected to serological identification to detect their serotypes.The results of serotyping revealed that, all the isolates were serotyped as *Salmonella* Kossen. This result; as we believe, was an unprecedented and was considered the first record for *Salmonella* as a cause of cellulitis.

Antimicrobial therapy is one of the primary control for reducing both the incidence and mortality associated with avian colibacillosis therefore reducing their enormous losses in the poultry industry **(Blanco *et al.*, 1997)**. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians **(Allan *et al.*, 1993 and Peighambari *et al.*, 1995b)**. In vitro antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy **(Blanco *et al.*, 1997)**. Moreover, it is very useful to detect the multidrug resistant isolates.

In the present work, all the recovered *E. coli* (n=126) and *Salmonella* (n=4) isolates were subjected to *in-vitro* antibiotic sensitivity tests against 14 different antimicrobial drugs to detect the drug of choice for treatment as well as to detect MDR isolates for further analyses of the isolates. The results of antibiogram of *E. coli* isolates showed that a high sensitivity was observed against enrofloxacin only (81%) while they were moderately sensitive to apramycin (65.9%) and colistinsulphate (61.9%) as well as ciprofloxacin and cefotaxime sodium (56.3% and 55.6%, respectively). On the other hand, high resistances were observed against lincomycin (87.3%), streptomycin (78.6%), spiramycin (75.4%) and trimethoprim-sulphamethoxazol (73%) while moderate resistances against fosfomycin, spectinomycin and florophenicol were also recorded; 58.7%, 54.8% and 54%, respectively. These results agreed with several previous reports**(Allan *et al.*, 1993; Peighambari *et al.*, 1995b; Negeleka *et al.*, 1996; Blanco *et al.*, 1997; Gomis *et al.*, 2001; Fard *et al.*, 2007; Hammoudi and Agaad, 2008 and Radwan *et al.*, 2014)** which have indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens with cellulitis and other lesions to several of the antibiotics frequently used in the poultry industry. Also, **Sharada*et al.* (2001)** found that no single antimicrobial drug was effective by 100% against *E. coli* isolates, which might be due to development of resistance due to indiscriminate use of antibiotics.Moreover, in the present study, multidrug resistance (MDR) was detected among 116 *E. coli* isolates (MDR index was equal to 92.1%) which observed a resistance to 4 or more antimicrobials of different categories. This result was similar to that of **Radwan *et al.* (2014)** who reported that MDRI was 90.4% for *E. coli* isolates. Moreover, about half (54.6%) exhibited resistance to four or more antibiotics, as observed in previous work of **Yang *et al*. (2004); Zhao *et al*., (2005) and Ozawa *et al*., (2008)**. Moreover, **Blanco *et al.* (1997); Chen and Wang (1997) and Hammoudi and Aggad (2008)** found high levels of resistance to antibacterial drugs in pathogenic strains of *E. coli* isolated from chickens ensuring that multiple drug resistance was common.

On the other hand, the results of antibiogram of *Salmonella* isolates showed complete sensitivities to ciprofloxacin and enrofloxacin. On the other hand, they were completely resistant to colistinsulphate, trimethoprim-sulphamethoxazol, lincomycin, spectinomycin, florophenicol, streptomycin and spiramycin. These results coincided with those reported by **Yoshida *et al.* (1993); Yah and Eghafona (2007); Khan *et al.* (2010) and Fallah *et al*. (2013)**who reported the high resistance of *Salmonella* isolates chicken against most of these antimicrobials.Moreover, in the present study, multidrug resistance (MDR) was detected among All *Salmonella* isolates (MDR index was equal to 100%) which observed a resistance to 4 or more antimicrobials of different categories. **Yah and Eghafona (2007) and Fallah *et al*. (2013)** reported lower values of MDRI for *Salmonella* recovered from chickens; 42.6% and 34.1%, respectively. Antimicrobial-resistant *Salmonella* is a public

healthconcern since resistance in *Salmonella* limits the therapeutic options available to veterinarians and physicians in the treatment of human salmonellosis **(Witte, 1998)**.

Our field observations indicated that the abusive and anarchic use of antibiotics is probably the cause of the high percentages of resistance detected and these finding agreed with those report by **Blanco *et al.*, (1997)** who attributed the development of drug resistance to frequent usage of drugs in veterinary practices at sub-optimal concentrations. Since the use of these antimicrobial agents may cause cross-resistance with human enteric pathogens, prudent use of them in veterinary medicine is highly recommended.

Concerning selection of the drug of choice for treatment either in both of *E. coli* and *Salmonella*, the obtained results revealed that fluoroquinolones were recommended. These results supported by what recorded by **García-Rodríguez *et al.* (1995)** and **Raemdonck *et al.* (1992)** who reported that fluoroquinolones were class of antimicrobials that exhibit excellent activity against Gram negative bacilli, although their use in poultry may be inappropriate because of cross-resistance with fluoroquinolones used for treatment of important human enteric infections **(García-Rodríguez *et al.*,1995 and Piddock *et al.*, 1990)**.

Conclusion

Avian cellulitis is a serious problem for the commercial broiler industry causing great economic losses.The prevalence rate of cellulitis in examined broiler chickens was 38.3%.Cellulitis may be associated with other systemic lesions in the internal organs. *E. coli* is the most prevalent bacterial agent causing cellulitis.

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