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Bacterial Leg Infections in Broiler Chickens

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Abstract

In a trial to investigate the bacterial causes of leg infection in broiler chickens, a total of 308 samples (foot bad and hock joint) of broiler chickens suffering from lameness were collected randomly from different broiler farms at Ismailia, Sharkia and North Sinai Governorates. Clinical and post-mortem examination, besides bacteriological analysis of the samples were carried out. The bacteriological investigation revealed the isolation of *Staphylococcus aureus* (83.8%, 258/308), *Staphylococcus lentus* (16.2%, 50/308), *Escherichia coli* (28.6%, 88/308); *Salmonella* spp. (1.3%, 4/308), *Pseudomonas aeruginosa* (1.9%, 6/308) and *Pasteurella multocida* (1.9%, 6/308). Antimicrobial sensitivity tests of the isolated bacteria revealed that *S. aureus* isolates were sensitive to Cefotaxim, Ciprofloxacin, Enrofloxacin and sulpha Trimethoprim. Virulence associated factors (coagulase gene and clumping factor) were determined in *S. aureus* isolates by conventional PCR. The results showed that the coagulase gene was identified in 10 *S. aureus* isolates, while, the clumping factor was detected in only two isolates. It could be concluded that the staphylococcal infection is the most important cause of arthritis in broiler chickens.

Keywords: Leg infection, Bacteria, Antimicrobial sensitivity, *S. aureus*

Introduction

Bacterial leg infection is a case describing joint infection by several causes resulting in purulent arthritis and lameness [1]. This disease is more prominent at age 14-70 days with average 35 days - old [2]. Bacterial leg affection in chickens is common and is caused by *Staphylococcus aureus, Staphylococcus pyogens, Streptococcus fecalis, Salmonella* Enteritidies, *Pasteurella multocida, E. coli, Pseudomonas aureginosa, Campylobacter, Ornithobacterium rhinotrachealis* and the most common cause is *Staphylococcus aureus* with an estimated percentage of 50.9% [3].

S. aureus infection in chicken causes swollen hock joint and lameness, this condition is usually observed in broiler birds [4]. Also, it is the most important cause of skin infection and toxin production (toxic shock and staphylococcal scalded-skin syndromes) [5,6]. Although it is found in water, dust, and air, the bacterium is normal habitant and can be isolated from the skin and feathers as well as in the respiratory and intestinal tracts [7,8]. The common forms of *S. aureus* associated poultry infections include tenosynovitis [9], omphalitis [10], infected hock and stifle joints [2] and "bumblefoot" [11]. *S. aureus* expresses several different proteins including clumping factors A and B (ClfA and ClfB) that play an important role in the ability of *S. aureus* to cause disease [12,13]. Clumping factor A (ClfA) is a microbial surface protein that promotes *S. aureus* binding to fibrinogen, and is associated with septic arthritis and infective endocarditis [14].

This study was planned to investigate the bacterial infection of leg in broiler chickens at ages 22-25 days. This was achieved by isolation and identification of the causative bacteria, as well as, assessment of the antibiogram of the isolated bacteria. In addition, identification of *Coa* and clumping factor virulence associated genes in *S. aureus* isolates using PCR was carried out.

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Material and Methods

Chicken samples

A total of 308 samples (food bad and hock joint) of broiler chickens suffering from bumble foot were collected randomly from different broiler farms at different ages (22-35 days old). The chickens were examined for clinical symptoms and then post-mortem examination was carried out.

Bacteriological examination

Bacterial isolation

The surface of foot pad, hock joint and organs was seared by hot spatula, and then a sterilized loopful was introduced through the seared portion. The loopful was then inoculated into nutrient broth and Rappaport vassiliadies (RV) broth and was incubated aerobically at 37ºC for 12 hours. A loopful from the incubated nutrient broth and RV broth were streaked onto blood agar and MacConkey's agar plates and were incubated for 24 hours at 37ºC. Hemolysis producing colonies and non-hemolysis colony from blood agar and lactose and non-lactose fermenter were picked up and streaked onto Eosin methylene blue media (EMB) for another 24 hours at 37ºC.

Biochemical and microscopical examination

Suspected colonies were subjected to biochemical identification according to Quinn *et al.* [15]. Films were prepared from the suspected pure isolates and stained with Gram's and Giemsa stains then they were examined microscopically.

Serological identification

Biochemically suspected *E. coli* isolates were serotyped using somatic antisera (O) of 51 vials (polyvalent 8 vials and 43 monovalent vials) (DENKA SEIKEN CO., LTD.TOKYO, Japan) according to Ewing [16]. Serotyping was carried out at the serology Unit, Animal Health Research Institute, Dokki, Giza.

Antimicrobial sensitivity test

Different chemotherapeutic sensitivity discs (Oxoid) namely Cefotaxim (CTX-30 µg), Ciprofloxacin (Cip-5µg), Enrofloxacin (Enr-10 µg), Gentamycin (Cn-10 µg), Doxycycline (Do-100 µg), Cholormphenicol (C–10 µg), Streptomycin (S-10 µg), Erythromycin (E-15 µg), and Trimethoprim/ Sulphamethoxazol (SXT-30 µg) were used. Antimicrobial resistance pattern was determined by the Kirby-Bauer method according to National Committee for Clinical Laboratory Standards (NCCLS) and the zones of inhibition were measured according to CLSI standards [17].

PCR assay for the presence of Coa and clumping factor associated genes

A single colony from biochemically identified *S. aureus* isolates was picked and suspended in 100 µl of MilliQ water. The suspension was then heated at 95°C for 15 minutes. After centrifugation for one minute at 20,800 g, the clear supernatant was used as a template for PCR [18]. Synthesized primers for the amplification of *Coa* and *clf*A were used (Table 1).

Table 1: Oligonucleotide primers used for the amplification of virulence associated genes in *S. aureus* isolates

Gene	Primer sequence 5\- 3\	Product size bp	Reference
Coa	ATA GAG ATG CTG GTA CAG G	Four different types of bands may be detected 350 bp- 430 bp-570 bp- 630 bp	[19]
***clf*A**	forward: GGC TTC AGT GCT TGT AGG reverse: TTT TCA GGG TCA ATA TAA GC	1042	[20]

Results

Examined broilers showed swollen joints, sitting on their hocks and keel bone and were unable to stand, recumbent, with swelling of foot pad, sever inflammation in hock joint, gradual emaciation and finally died.

Postmortem examination of naturally infected chickens showed swelling of joints filled with inflammatory exudates.

The bacteriological examination revealed that 258 isolates from 308 samples (83.8%) were coagulase positive *Staphylococcus* and they were identified as *Staphylococcus aurues*, while, 50 isolates were coagulase negative *Staphylococcus* which were identified as *Staphylococcus lentus* (16.2%) (Table 2).

Table 2: Occurrence of the isolated bacterial agents from broiler chickens with leg affections

Age /day	Number of samples from chickens			No. of isolates					
	Foot pad	Hock joint	Total	*S. aureus*	*S. lentus*	*E. coli*	*Pseudomonas aerogenosa*	*Salmonella* spp.	*Pasteurella multocida*
24	12	12	24	20	2	12	-	-	-
26	12	12	24	20	4	-	-	-	2
27	12	12	24	20	4	-	-	-	2
25	12	12	24	24	--	-	-	-	--
30	6	6	12	10	2	6	-	-	-
28	8	8	16	16	-	-	-	-	-
24	12	12	24	20	4	10	-	2	-
35	6	6	12	10	2	-	-	-	-
28	12	12	24	20	4	4	-	-	-
30	12	12	24	18	6	10	6	--	--
30	8	8	16	16	-	8	-	-	-
28	8	8	16	14	2	6	-	-	-
30	12	12	24	18	6	12	-	-	-
22	6	6	12	10	2	6	-	2	2
30	8	8	16	10	6	8	-	-	1
23	8	8	16	10	6	6	-	-	-
Total	154	154	308	258	50	88	6	4	6

Eighty eight *E. coli* isolates were identified in the infected joints from broiler chicken (28.6%). Out of 88 *E. coli* isolates, 30 were serotyped and they belonged to 6 different sero-groups, namely O78, O125, O55, O166, O146 and untypable isolates with the percentages of 26.7%, 13.3%, 23.3%, 16.7%, 13.3% and 6.7%, respectively (Table 3). Four salmonella isolates (1.3%) from joints with percentage and 6 *pseudomonas aeruginosa* and *Pasteurella multocida*, each (1.9%, each) were isolated from foot pad and joints.

Table 3: Serogroups of *E. coli* (N=30) isolated from broiler chickens with leg affections

Serogroup	Total no. of isolates	
	No.	%
O78	8	26.7
O125	4	13.3
O55	7	23.3
O166	5	16.7
O146	4	13.3
Untypable	2	6.7
Total	30	100

The results of the antimicrobial sensitivity tests (Table 4) showed that *S. aureus* isolates were sensitive to Cefotaxim, Ciprofloxacin, Enrofloxacin, Erythromycin and sulpha Trimethoprim, while they were resistant to Doxycycline. Other bacterial isolates (*E. coli, Salmonella, pseudomans aerogenosa* and *Pasteurella multocida*) were sensitive to Ciprofloxacin, Enrofloxacin and Gentamycin but resistant to Doxycycline, Ampicillin and Erythromycin.

Ten representative *S. aureus* isolates were randomly chosen to investigate the presence of *Coa* and *clf*A associated genes. The results revealed that all the examined *S. aureus* isolates were positive for *Coa* associated gene at 570bp (Figure 1A). Only two isolates were positive for *clf*A associated gene at 1042 bp (Figure 1B).

Discussion

Leg arthritis and lameness is caused by variety of etiological agents elevating morbidity and mortality in broilers flocks causing economic losses. Leg arthritis known as bacterial chondronecrosis (BCN) are femoral hip or proximal femoral degeneration [21,22]. BCN with osteomyelitis is considered the most common cause of lameness and arthritis in commercial broilers flocks in Australia, Canada, Europe, and the US.

In the present study, the clinical signs of affected birds were swollen joints on the hocks keel bone and they were unable to stand with foot bad dermatitis, reluctance to move, gradual emaciation and finally they died. These results agreed with Bakheet [23] who reported that the clinical signs of affected birds by bacterial organisms were lameness and swollen joints. Also, these results coincided with Youssef and Hamed [4] who reported that the affected breeders had the same clinical signs reported in the present study. *Staphylococcus* infections in poultry cause synovitis, with lameness being the most common clinical presentation [24]. Wideman *et al.* [25] showed that lameness typically began after the age of 22 days and progresses rapidly within 24-48 hours.

Table 4: Sensitivity of isolated bacterial agents against different antimicrobial discs

Antibiotic disc / Microorganisms	CTX	CIP	Enr	Cn	DO	C	S	E	SXT	Am	Amx.
Staphylococcus aureus	S	S	S	I	R	I	I	S	S	I	I
E. coli	S	S	S	S	R	R	I	R	S	R	R
Ps. aeruginosa	I	S	S	S	R	S	I	R	R	R	R
***Salmonella* Typhimiurum**	R	S	S	S	I	S	I	I	-	I	S
Pasteurella multocida	S	S	S	S	S	S	S	I	S	R	S

S= sensitive R= resist I= intermediate CTX: Cefotaxim; CIP=ciptofloxacin; Enr= enrofloxacin; Cn= gentamycin; Do= doxycycline; c= choloramphinicol; S= stryptomycin; E= erythromycin; SXT= Sulpha-trimethoprim; Am= ampicillin and AMX= amoxicillin.

Postmortem examination of naturally infected chickens showed swelling of joints filled with inflammatory exudates and arthritis of the hock. These results agreed with Stalker *et al.* [26] who reported arthritis of the hock. Moreover, Youssef and Hamed [4] reported that postmortem examination of naturally infected chickens showed swelling joints filled

with inflammatory exudates. reported that Lesions with BCN can occur in all bones but they are most commonly found in regions of the leg bones that have the widest growth plates and are subjected to torque and stress, such as the proximal tibiotarsus (tibia) and proximal head of the femur [27,28]. In case of the lesions associated with the musculoskeletal system, the affected bones often have focal yellowish areas of necrosis, while lesions in the joints contain purulent exudate [24]. Adayel [29] showed that the most frequent sites of *Staphylococcus aureus* infections in poultry were bones, tendons, sheaths and joints, especially tibiotarsal.

In the present study, the most identified bacterial isolates were *S. aureus* (83.8) followed by *S. lentus*, *E. coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Pasturella multocida* with the respective percentages of 16.2%, 28.6%, 1.3%, 1.9% and 1.9%. McNamee *et al.* [30] reported that *S. aureus* was recovered from 62.5% of the examined samples. Bacterial chondronecrosis associated with *S. aureus* has thus been identified as an important cause of leg weakness in these commercial broilers. These results agreed with Bakheet [23] who reported the isolation of *S. aurues* (62%), *E. coli* (60%), *Enterococcus* (8.8%) and mycoplasma (11.1%) from arthritis cases in chickens. Also, these results agreed with Rasheed [3] who reported the isolation of *S. aureus* (50.98%), *Pseudomonas aerogenosa* (27%), *E. coli* (7.8%) and *Proteus* spp. (3.9%) from chicken with arthritis. Abo-Elil [31] reported also that the percentage of coagulase positive *S. aureus* from diseased chickens was 12.2% from joint samples.

The present data highlights the importance of joints as the site for isolation in screening live stock to identify immerging staphylococcal infection. In agreement, 20.4% of lame chicken in two commercial flocks had typical bacterial skeletal lesions that were primary associated with *S. aureus* and the most frequent local infection in poultry were bones, tendons, sheath and joints, especially tibiotarsal [32].

Thirty from the 88 *E. coli* isolates were identified and belonged to 6 different sero-groups, namely O78,O125, O55,O166 , O146 and untypale with the percentage of 26.7%, 13.3%, 23.3%, 16.7%, 13.3% and 6.7%, respectively. The most predominant *E.coli* sero-group from the examined broilers was O78 followed by O55. These results agreed with Yousseff *et al.* [33] reported that sero-group O78 was the most common (28.2%) from diseased broiler chicken (aged from 17 to 35 days). Also, 20 *E. coli* isolates were sero-grouped as O6 (6), O111 (3), O55 (2), O114 (2), O15 (3), O125 (2), and untyped (2) [34].

The results of the antimicrobial sensitivity test showed that *S. aureus* isolates were sensitive to Cefotaxim,: Ciprofloxacin, Enrofloxacin, Erythromycin, sulpha Trimethoprim, Gentamycin, Ampicillin, amoxacilline and streptomycine, while, they were resistant to doxycycline. These results nearly agreed with Rasheed [3] who reported *S. aureus* isolates were sensitive to amoxicillin and resistant to gentamycin and novobiocin. Also, these results nearly agreed with Youssef and Hamed [4] who reported that the *in-vitro* antibiotic sensitivity test of 10 isolates of *S. aureus* to 17 antibiotic discs revealed that 35.3% of the isolates were highly sensitive to enrofloxacin followed by cefotaxim and amoxicillin-claveulinic and ciprofloxacin. Moreover, isolates of *Pasteurella multocida, E. coli, Pseudomonas aeruginosa, Streptococcus pneumoniae and S. aureus* were highly sensitive to Cefotaxim, Ciprofloxacin and Enrofloxacin [35,36]. In addition, 100% of *E. coli* isolates were resistant to Amoxicillin, Tetracycline, Oxytetracycline, Dan-ofloxacin and Ampicillin [33]. Also, the obtained results agreed with other reported studies [37,38]. Enrofloxacin is frequently used in the treatment of *E. coli* infection in poultry [39]. The great variation in the results of the antibiotic sensitivity could be attributed to the uncontrolled use of antibiotics as a field practice and to the physiochemical properties of the cell wall rather the antibiotic inhibiting enzymes [40]. *Salmonella* isolates showed the highest sensitivity to Ciprofloxacin and Enrofloxacin which was in correlation to the reports of [41,42].

The coagulase protein is an important virulence factor for *S. aureus*. The coagulase gene amplification has been considered a simple and accurate method for typing. This method is found to be technically simple with a good reproducibility and discriminatory power [43]. The *coa* gene has polymorphic repeat regions that can be used for differentiating *S. aureus* isolates [44]. Ten identified field isolates by biochemical tests were tested for the presence of *coa* gene. All the isolates were positive for *coa* gene producing bands at 570 bp. Other studies reported multiple bands of the amplified *coa* gene ranging from 420-1060 bp [45-48].

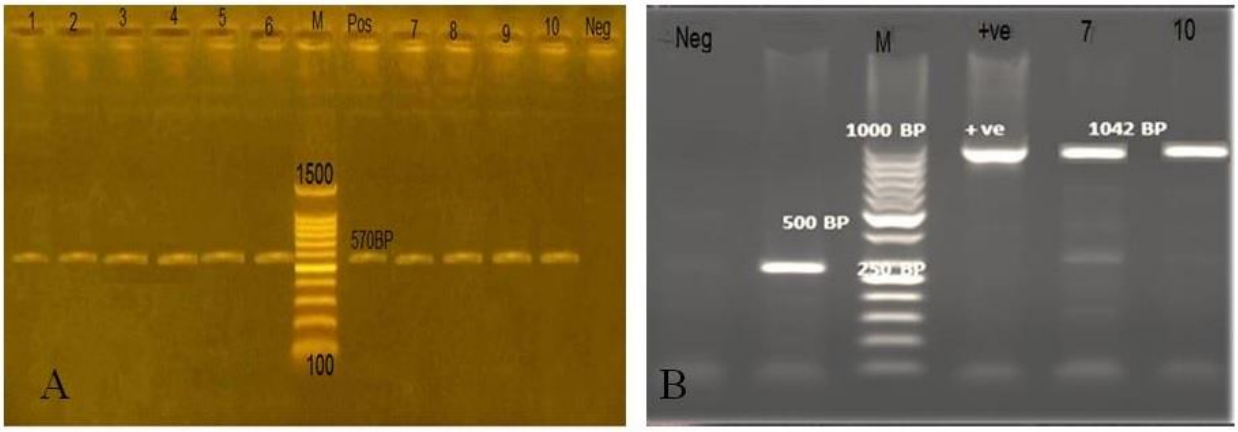

Figure 1: A: (Electrophoretic pattern of coagulase (*coa*) gene in different isolates: Lane: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 *S. aureus* field isolate; M- Marker GeneRuler (Fermentas) ; Pos: positive control at 570 bp Neg: negative control), B: (Electrophoretic pattern of clumping factor A (*clf*A) gene in different isolates: M-Marker GeneRuler (Fermentas). Neg: negative control, Lane 7 and 10 +ve *clf*A at 1042 bp).

Clumping factors are produced by *S. aureus* and two, namely, ClfA [clumping factor A] and ClfB [clumping factor B] have been identified [49,50]. In contrast to ClfA, which is present on cells at all stages of the growth cycle, ClfB is present only at a detectable and functional level on the surface of cells [50]. The ability of *S. aureus* to adhere to extracellular matrix proteins is thought to be essential for the colonization and the establishment of infections [51]. *S. aureus* possesses various adhesion genes, including clfA, fnbA, and cna [52]. Expression of this gene is thought to enhance bacterial growth and promote infection in the face of host defence mechanisms, such as phagocytosis [53].

Investigating the presence of clumping factor revealed only two positive isolates out of the tested ten isolates with a characteristic band at 1042 bp. In agreement, other studies reported the same findings [48,55].

Conclusion

It could be concluded that the most common cause of bacterial leg infection is *S. aureus*, but other bacteria may also cause the disease such as *E. coli*, *Salmonella*, *Pseudomonas and Pasteurella* which were isolated from leg infection of broilers. Staphylococcal infection is the leading cause of arthritis causing high morbidity with subsequent economic losses, therefore, care should be given to control the staphylococcal infection in poultry through means of good management practices especially biosecurity, and hopefully by developing an effective vaccine.

Conflict of Interest

The authors declare no conflict of interest.

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الملخص العربى

عدوى الأرجل البكتيرية في بدارى التسمين

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في محاولة لمعرفة الأسباب البكتيرية لعدوى الارجل في بدارى لتسمين. تم تجميع ٣٠٨ عينة من مختلف مزارع التسمين بمحافظات الاسماعيلية والشرقية وشمال سيناء التى كانت تعانى من عرج وتورمات في المفاصل. وتم فحصهم إكلينيكيا وكذلك اجراء الصفة التشريحية اضافة الى عزل البكتريا المصاحبة لتلك الاعراض. وقد اظهرت نتائج العزل البكتيري عن وجود ميكروب العنقودى الذهبى بنسبة ٨٣.٨% والعنقودى لنتس بنسبة ١٦.٢% و القولونى بنسبة ٢٨.٦% والسالمونيلا بنسبة ١.٣% والسودوموناس بنسبة ١.٩٥% والباستيريلا مالتوسيدا بنسبة ١.٩٥%. كما اظهرت نتائج اختبار الحساسية للميكروبات المعزولة الاستجابة العالية لميكروب العنقودى الذهبى لسيفوتاكسيم والسبروفلوكساسين والانروفلوكساسين والسلفاترايميثوبريم. كما اظهرت نتائج تفاعل البلمرة المتسلسل عن وجود جينات الضراو ة (جين التخثر) فى ١٠ معزولات و(جين والتلازن) فى معزولتين من ميكروب العنقودى الذهبى. وعليه نستنتج أن الميكروب العنقودى الذهبى هو من اهم اسباب عدوى الارجل البكتيرية والتهابات المفاصل في بدارى التسمين.