Studies on bacterial causes of joint infection in chickens

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A B S T R A C T

Pathogenic bacteria play a serious role in arthritis in chickens which decrease chickens productivity and increase in economic losses so this study was conducted to assess the most pathogenic bacteria causing arthritis in chickens. In the present study 100 chickens showing arthritis were collected from different localities in Sharkia governorates. Positive bacterial isolates (123) were isolated from hock joint and foot pad with their percentage classified as follow, 44 *S.aureus* (35.77%), 37 *E.coli*(30.08%), 5 *Salmonella*(4.06%), 21 *proteus* (17.07%), 12 *pseudomonas*(9.75%), 1 *Enterobacter* (0.81%) and 3 *Shigella* (2.43%). The prevalence of bacterial isolates from different joints of the examined chickens revealed that the percentage of *Staph aureus* in hock joint, foot pad was (65.90%) and (34.09%) respectively but in case of *E.coli* the percentage was (89.18%) and (10.81%) respectively and *S.Typhimurium* percentage was (100%), (0%)Serologically *E.coli* serotypes were O55,O78,O158,O128,O111and untyped with a percentage (9.09, 27.2, 18.1, 9.09, 9.09 and 27.2% respectively while salmonella serotypes into *S. Typhimurium* with a percentage 100%.Experimentally 130 day old Hubbard chichens were infected with the isolated bacteria showed lameness, reluctant to move and swelling of hock joint & foot pad leading to high economic losses. Also the sensitivity test of the isolatedM.O showed *S. aureus* was highly sensitive to enerofloxacin, ciprofloxacin and norofloxacin. While *E. coli* was highly sensitive to enrofloxacin, chlormphenicol and norofloxacin On the other hand*S. Typhimurium* was highly sensitive to enrofloxacin, ciprofloxacin and doxycycline.

Keywords: joint infection, enrofloxacin, norofloxacin, *S. Typhimurium*

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1. INTRODUCTION

Lameness may result from tenosynovitis, arthritis, and/or osteomyelitis in chickens. Many bacteria associated with synovitis and arthritis including: *Staphylococcus* species, *E. coli*, *P. multocida, Mycoplasma species and Salmonella*, (Katherine, 1997). *Staphylococcus* species are ubiquitous organisms in the breeder house environments; they are normal inhabitants of skin, feathers, mucous membranes, respiratory and intestinal tract also itis the most important cause of arthritis with synovitis and osteomyelitis in chickens and it causes a great economic loss in poultry industry, Staphylococcal arthritis infects joints and cause irreversible joint destruction and significant mortality (. Kohler et al., 1980, Jensen and Miller, 2001, Huang et al., 2002 and White et al., 2003). Infection by *S. aureus* may occur in the joints or it may be generalized septicemia infection. The most common problem in broiler breeders is foot infection (bumble foot) or the hock (swelling of the joint). (Bowles, 2003). *In Egypt):* S. aureus was isolated from joint lesions in chickens at different ages (18 to 140 day of age,4-18-month age,16-72 weeks old) as well as *E. coli, salmonella, P. multocida, proteus, Enterococcus species, Ps. aeruginosa and M. synoviae (Omayma2005, Hebat-Allah et al., 2006, Mohamed and Mona 2008*). *Clifford and Robert (2001)* described joint and bone infection after *E. coli* septicemia in young birds of all species. They observed that tibiotarsal-tarsometatarsal region (hock joint) was the most commonly involved area with the thickening and swelling of the associated tendon causing sever lameness as the disease progressed. *Omayma (2005)* showed that the bacterial isolation from affected joints yielded single bacterial agents in 32.1% of cases while mixed bacterial agents were recorded in 48.8% of cases. The major agent isolated from affected joints were staphylococcus species (36.6%) followed by *Enterococcus spp (27.6%), E. coli (17.1%), erysipelas spp (5.7%), salmonella spp (4.1%) and proteus spp (4.9%), pseudomonas spp*

(1.6%), Pasteurella spp (1.6%) and mycoplasma spp (0.8%) respectively.

The aim of this study was the isolation and identification of bacterial causes of the affected joints and induce arthritis experimentally with the isolated bacteria as well as its sensitivity for antibiotics

2. Material &Methods:

2.1. Specimens:

A total of 100 diseased or freshly dead broiler chickens from flocks of different breeds (Saso, Balady and Hubbard) with different ages ranged from 3 to 6 weeks suffering from arthritis were collected from different localities at Sharkia province. These birds were subjected to clinical, post-mortem and bacteriological examination, isolation and identification.

2.2. 2-Media:

Different bacteriological media for isolation and identification were used such as Nutrient agar, MacConkey, s agar, Blood agar, Slope agar, Semi solid nutrient agar, Simmons citrate agar, Triple sugar iron agar media, Urea agar base, Baird parker agar and Eosin Methylene blue agar

2.3. 3-Diagnostic antisera:

2.3.1. E.coli diagnostic antisera:

Polyvalent and monovalent *E.coli* antisera obtained from Mast, Denka and Remef companies were used for serological identification of pathogenic E.coli

2.3.2. Salmonella typhimurium antisera:

Were kindly supplied by central lab of ministry of medicine, Egypt.

2.4. Experimental chicks:

One hundred and thirty, day old Hubbard chicks were obtained from AL-Kahera Poultry company to be used for experimental study

2.5. Antibiotic discs used for sensitivity test were obtained from (Oxoid).

2.6. Clinical and postmortem examination for diseased birds were recorded.

2.7. Bacteriological and serological identification of the isolates were carried out according to Edwards and Ewing, 1972

2.8. Bacterial isolation of collected samples was carried out according to Siam,1998

2.9. *Biochemical identification* according to Cruickshank et al., (1975)

2.10. Preparation of resistant strains (Konemann et al.,1997)

2.11. bacterial titeration according to Sambrook et al., 1989

2.12. Antibiotic sensitivity test according to (Blair et al., 1970) and (Finegold and Baron, 1986).

2.13. Experimental infection:

One hundred and thirty, day- old Hubbard chicks were used to study the pathogenicity of the isolated strains of M.Os. The experimental chicks were kept under complete isolation for 4 weeks received balanced ration before grouping. Five chicks were sacrificed and samples were collected from joints and cultured for trials of isolation to prove these chicks free from any bacterial infection. They were divided into five groups which subsequently divided into 11 subgroups each subgroup includes 10 chicks in number except control one was 20 bird. Reisolation trials were carried out as shown in Table (1).

3. RESULTS

3.1. Result of clinical & post mortem lesions:

Group (I) infected with *E. coli.* Subgroup (1), (3): infected with *E. coli* (serogroupsO158, *O78*) by I/V route: Showed depression, growth retardation, weakness and ruffling feather and lameness with swollen hock joint. the mortality rate was 30% and 20% respectively and the main lesions were septicemia, congestion, enlargement of all internal organs When we open the hock joint there was whitish to orange caseues exudates (table 3). In Subgroup (2), (4): infected with *E. coli* (serogroupsO158, *O78*) via foot/pad (F/P) route showed lower signs and lesions. Group (II) infected with *S. aureus.* Subgroup (1): chickens infected intravenously with isolated S. *aureus* showed lameness, reluctant to move. (one leg or bilaterally affected) with swollen hock joint, the mortality was 60%, the lesions were septicemia, congestion in the lung and enlargement of all internal organs. When opened the hock joint white to yellow purulent exudates was observed.

Subgroup (2)): chickens in this subgroup were infected with isolated *S. aureus* by F/P route: Showed a lower sign with 10%mortality and in necropsy yellow whitish exudate was observed in foot pad table (3).

Table (1): Experimental design:

Groups	Group I				Group II		Group III		Group IV			Group V
Sub Groups	1	2	3	4	1	2	1	2	1	2	3	
No. of bird	10	10	10	10	10	10	10	10	10	10	10	20
Inoculated M.O	*E. coli O78*	*E. coli O78*	*E. coli* O 158	*E. coli* O 158	*S. aureus*	*S. aureus*	*S. typhimurium*	*S. typhimurium*	*E. coli* (O78, O158) + *S. aureus* +*S.typhimurium*	*E. coli* (O78, O158) + *S. aureus* +*S.typhimurium*	*E. coli* (O78, O158) + *S. aureus s*+ *S.typhimurium*	Control negative non infected non treated
Route of infection	IV	FP	IV	FP	IV	FP	IV	FP	IV	FP	Oral	
Dose of infection	$1x10^4$ CFU	$1x10^6$ CFU	$1x10^4$ CFU	$1x10^6$ CFU	$1x10^4$ CFU	$1x10^6$ CFU	$1x10^4$ CFU	$1x10^6$ CFU	$1x10^4$ CFU	$1x10^6$ CFU	$1x10^8$ CFU	
Reisolation	From hock joint & foot pad at one week old PI				From hock joint &foot pad at one week old PI		-VE		From hock joint & foot pad at one week old PI		-VE	

-ve: Not reisolated. FP: Footpad inoculation, IV: Intravenous inoculation. PI: post infection

Table (2) Results of bacteriological examination:

Breed of chickens	Numbers of samples	S.aureus		E.coli		Salmonella		Ps.aeruginoisa		Pr.vulgaris		shigella		Enterobacter	
		+ve No	%	+ve No	%	+ve No	%	+ve No	%	+ve No	%	+ve No	%	+ve No	%
Hubbard 26-45d	67	25	37	28	42	3	4.48	7	10.4	13	19.4	3	4.48	1	1.5
Balady 24-36d	19	12	63	7	58	-	0	2	10.5	5	26	-	0	-	0
Saso 30-46d	14	7	50	2	21.4	2	14.3	2	14.3	3	21.4	-	0	-	0
Total		44		37		5		11		21		3		1	
Hock joint		29	65.9	33	89.13	5	100	11	19.6	19	90.4	2	66.66	1	100
Foot pad		15	34.09	4	10.81	0	0	1	8.3	2	9.5	1	33.33	0	0
Total		44		37				12		21		3		1	

Table (3) Results of the experimentally infected chickens with different infective strains:

Group	sub group	No. of chickens	Inoculated M.O.	Route of Infection	Dose of Infection (CFU)	Age	Reisolation		No. of dead /Total	Percent of mortality %
							hock joint	foot pad		
E.coli	1	10	*E.coli O78*	I/V	$1x10^4$	4week	+ve	-ve	3/10	30%
(I)	2	10	E.coli O158	F/P	$1x10^6$	4week	-ve	+ve	0/10	0%
	3	10	E.coli O78	I/V	$1x10^4$	4week	+ve	-ve	2/10	20%
	4	10	E.coli O158	F/P	$1x10^6$	4week	-ve	+ve	0/10	0%
S. aureus	1	10	*S. aureus*	*I/V	$1x10^4$	4week	+ve	-ve	6/10	60%
(II)	2	10	*S. aureus*	F/P	$1x10^6$	4week	-ve	+ve	1/10	10%
S. typhimurium	1	10	*S. typhimurium*	I/V	$1x10^4$	4week	-ve	-ve	0/10	0%
(III)	2	10	*S. typhimurium*	F/P	$1x10^6$	4week	-ve	-ve	0/10	0%
Mixed INFECTION (IV)	1	10	*E.coli O78*+ E.coli O158+ *S. aureus* + *S. typhimurium*	**I/V	$1x10^4$	4week	+ve	-ve	8/10	80%
	2	10	*E.coli O78*+ E.coli O158 *S. aureus* + *S. typhimurium*	F/P	$1x10^6$	4week	-ve	+ve	3/10	30%
	3	10	*E.coli O78*+ E.coli O158+ *S. aureus* + *S. typhimurium*	ORAL	$1x10^8$	1 day	-ve	-ve	0/10	0%
Control (V)		20			Control -ve non infected non treated					

*S.aureus I/V showed high mortality 60%**mixed infection with *E.coli* serogroups (O78, O158), *S. aureus* and *S. typhimurium* I/V showed very high mortality 80%

Table (4) Results of Serological identification of isolated *E.coli* and salmonellatyphimurium

E.coli			Salmonella					
			No of typed strains	Antigenic structure				Resulted serotypes
				O groups	O antigen	H antigen		
Serogroups	No	Percentage %	5 isolates	B	4.5	Specific phase	Group phase	Salmonella typhimurium
O55	1	9.09				1	1.2	
O78	3	27.2						
O158	2	18.1						
O128	1	9.09						
O111	1	9.09						
Untyped pathogenic strain	3	27.2						
Total	11							

Group (III)which infected with *S. Typhimurium*: In Subgroup (1): chickens infected with *S. Typhimurium by IV* route: infected chickens were Showed anorexia, depression growth retardation and poor feathering, whitish diarrhea decrease feed intake, decrease body weight with no lameness and no mortality. (table (3) P.M lesions showed pericarditis, greyish white nodule in lung and heart. In Subgroup (2): chickens which infected with *S. Typhimurium* by FP route: showed general signs of illness but without lameness and no P.M lesions were observed. Group (IV) mixed infection: In Subgroup (1): mixed infection with *E.coli* serogroups (O78, O158), *S. aureus* and *S. Typhimurium* by (I/V) route: 80% of chickens died at 5th day post infection while the other live birds showed lameness and reluctant to move the hock joints were hot, inflamed, swollen and chickens sit on their hocks. The main gross lesions were fibrinous pericarditis, perihepatitis and enlarged liver with bronzy discoloration (table (3). In Subgroup (2): mixed infection with *E.coli* serogroups (O78, O158), *S. aureus* and *S. typhimurium* by FP route: showed lower signs and lesions with 30% mortality only. In Subgroup (3): mixed infection with *E. coli* serogroups (O78, O158), *S. aureus* and *S. typhimurium* orally: No specific signs &lesions were observed.

3.2. Results of antibiotic sensitivity test:

Antibiotic sensitivity of *S. aureus* isolates showed that they were highly sensitive to enerofloxacin, ciprofloxacin, norofloxacin while *E. coli* serotypes were highly sensitive to enerofloxacin, chlormphenicol, colistinsulphate, and norofloxacin. While *S. typhimurium*highly sensitive to enerofloxacin, ciprofloxacin, sulfa-trimethoprime, and doxycycline

4. DISCUSSION

Poultry industry play major role as a source of animal protein for human consumption, but pathogenic bacteria play serious role in arthritis which decrease chickens productivity leading to increase in economic losses. In the present study bacterial isolation from chickens suffering from arthritis were 123 isolates. These isolates were at different ages and localities in Sharkia province from hock joints and foot pad. The isolates were biochemically identified into *S. aureus*44 isolates, *E. coli* 37 isolates, *Salmonella* 5 isolates, *Proteus* 21 isolates, *Pseudomonas* 12 isolates, *Shigella* 3 isolates, *Enterobacter* 1 isolate with a percentage 35.77, 30.08, 4.06, 17.07, 9.75, 0.81and 2.43% respectively (Table 1), it is clear that *Staphylococcus* isolates considered the most isolated bacteria from examined chickens (44 isolates) followed by *E. coli* isolates (37 isolates). The number of *Staphylococcus*, *E. coli*, *Salmonella*, *proteus, Pseudomonas, Shigella* and *Enterobacter* from hock joint were 29,33, 5, 19, 11, 2, and 1 isolates with a percentage 65.90%, 89.18%, 100%, 90.4%, 19.6%, 66.66% and 100% respectively while from foot pad were,15, 4,0, 2, 1, 1, 0 with a percentage 10.81%, 0%, 9.5%, 8.3%, 33.33% and 0% respectively (Table 2). These results near to that obtained by Omayma (2005*)*, who isolated *Staphylococcus*, *E.Coli, Salmonella*, *proteus*, *Pseudomonas* and *Enterobacter* from broilers with isolation percentage (36.6%, 17.1%, 4.1%, 4.9%, 1.6% and 2.6% respectively.

Staphylococcus aureus considered the main cause of arthritis in chickens with percentage 34.10% while Tran- Thi-Bich – Lien *et al.,* 2003*)* isolated *S. aureus*, S. *epidermidis* and *E . coli* from hock joint with a percentage of 100%, 0.5% & 0.7% respectively. Serological identification of eleven *E .coli* isolates showed the following serotypes O55 (1), O78 (3), O158 (2), O128 (1), O111 (1), and untyped pathogenic strains (3) with a percentage (3.09%, 27.2%, 18.1%, 9.09%, 9.09%, 27.2%) respectively (Table 3).Some of these serotypes were isolated by another authors Gomis*et al* (1997) and El-Sayed *et al., (*2001*).* While Serological identification of *4 Salmonella* isolates showed 100% *Salmonella Typhimurium*.Table (4), the same *Salmonella* was isolated by Pardon (1990) from chickens at different ages. Antibiotic sensitivity test for *E.coli* serotypes (*O78,O158*) showed that all strains were highly sensitive to enerofloxacin, chlormphenicol, colistinsulphate ,these results agree with Cloudss*et al* .,1985, Osman 1992, Bader 2003 and Mourad, 2008 they found *E.coli* isolates were highly sensitive to chlormphenicol, enerofloxacin,florofenicol and marbofloxaccin.

On the other hand, *S. aureus* isolates were highly sensitive to enerofloxacin, ciprofloxacin, norofloxaccin, streptomycin, gentamycin, these results agree with White *et al*., (2003), Elghaffar*et al*., (2004) Mohamed *et al*., (2006),

While *S. Typhimurium* was highly sensitive to ciprofloxacin, doxycycline, these results agree with Sunita-Schivhoer *et al*., (2001) and Bader (2003), found that *Salmonella spp*was sensitive to marbofloxacin, norofloxacin, florfenicol, amoxicillin, ampicillin, chlormphenicol, oxytetracyclin and lincospectin .

The clinical signs of experimentally infected broiler chickens (4weeks) with *E.coli*serotypes (O78, O158) I/V were depression growth

retardation, weakness and ruffling feather .the birds showed lameness, reluctant to move and recumbent with swollen hock joint .These signs were more severe in group infected with *E.coli*O78 than group infected with *E.coli* O158 and the mortality rate was 30% and 20% respectively.The main gross lesion was septicemia, congestion and enlargement of all internal organs. On the other hand birds those injected with *E.coli* serotypes (O78, O158) at 4w old via foot pad route showed swollen foot pad with lower signs and The main gross lesion was caseous whitish exudate within the foot pad, these results agree with Gardon and jorden 1982,gross 1991 .

Intravenous infection with *S. aureus*in Hubbard chickens (4 weeks age) showed 60% mortality within 72 hours post inoculation the birds showed lameness, reluctant to move, recumbent with swollen hock joint. the main gross lesion was septicemia, congestion and enlargement of all internal organs. At the end of the experiment sacrificed chickens showed white to yellow purulent exudates in the joint beside congestion with focal necrosis and bronzy discoloration of the liver. these finding agree withSkeeles (1991) Mutalibet *al*., (1982), Hebat-Allah et *al*., (2006) and Mohamed and Mona, (2008). Broiler chickens infected with *S. aureus*via IFP route showed swelling in foot pad, unable to stand with depression, inappetance and unable to move for eating and drinking and lose their body weight these signs less severe than injected via I/V route, the results agree with Kibenge*et al*., (1982) and Bowles (2003).

The isolated *S. Typhimurium* from field cases of arthritis couldn't produce arthritic lesion after I/V or IFP inoculation but it showed decrease in body weight this result agree with Samia*et al.,* (2000). While In mixed infection with *S. aureus, E.coli* (O78, O158) and *S. Typhimurium* intravenously at 4ws of age birds showed depression and lameness ,inflamed andswollen hock joint ; the main gross lesions were fibrinous Perihepatitis, enlarged liver with bronzy discoloration. Within the joint capsule yellow whitish exudate was observed. On the other hand, the clinical signs in broilers with the same bacteria via foot pad were swollen foot pad, unable to stand and to reach food and water. The main gross lesion was yellow whitish caseous exudate in the foot pad.

5. CONCLUSION:

It is concluded that the most bacterial causes of arthritis in chickens was *S. aureus* (35.77%), *E.coli* (30.08%) then *S. typhimurium*(4.06%) but other bacteria ranged from (0.81% to 17.07%) .Experimentally infected chickens with the isolated bacteria showed lameness, reluctant to move and swelling of hock joint & foot pad leading to high economic losses. Also the sensitivity test of the isolated M.O showed*S. Aureus* was highly sensitive to enerofloxaccin, ciprofloxacin and norofloxacin while *E.coli* was highly sensitive to enrofloxacin, chlormphenicol and norofloxacin .On the other hand *S. typhimurium* was highly sensitive to enrofloxacin, ciprofloxacin and doxycycline .

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