STUDIES ON SOME AEROBIC BACTERIA CAUSING DEATH OF BROILER CHICKENS

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(Manuscript received 3 Decempre 2003)

Abstract

One hundred and twenty chicks were collected from twenty-one broilers flocks representing 130000 birds from different localities in North Sinai Governorate. The chickens were either diseased or freshly dead. Some bacteria were isolated from the examined birds and identified as (72) *E.coli,* (34) *Kelbsiella,* spp., (31) *Proteus,* spp., (19) *S.aureus,*spp., (8) *Pseudomonas aeruginosa* and (11) *Salmonella,* spp., Some strains of *E.coli* and *Salmonella* species were identified serologically. Experimental infection of day – old broiler chickens, with representative isolate from each 4 genera was carried out.

The clinical signs and macro-pathological finding varied according to the type of microorganism. Microscopically, the liver of birds showed focal coagulative necrosis and congestion of hepatic blood vessel. The heart of infected birds had showed fibrinous pericarditis represented by congestion, fibrin threads, fibrinous tissues proliferation with leukocytic infiltration, and the spleen of infected birds showed focal coagultive necrosis of the splenic tissue. In addition, the intestines of the infected birds showed desqumation of the villus epithelium with leukocytic infiltration in the mucosa and sub-mucosa.

The antibiogram for *E.coli* isolated reveled that they were all sensitive to Gentamycin, Norfloxacin, Colistin sulphate and variably sensitive to antibiotics used in the test.

INTRODUCTION

Variable higher mortality rate and economic losses were recorded in broilers in many countries (Jordan,1990 and Padron, 1990). In Egypt, the mortality in broilers is mostly due to primary or secondary infection with bacterial agents. Mainly *Salmonella, E.coli, Proteus,* spp., Klebesiella, spp., *Enterobacter, Bacillus,* spp., *S. aureus* spp., and *Streptococcus* spp., (Abd-allah, 1981, Emad *et al.*,1996 and Osman,

1992). The bacterial infection in chicks can be transmitted by many ways including feed and water and direct and indirect contact (Andrews, 1980).

The aim of the present investigation was to isolate and identify the causative bacterial agents which cause mortality in broilers in North Sinai, recording the pathological alterations in different organs (Butura and cernea, 1969) and studying the effective antibiotics for *E.coli*.

MATERIALS AND METHODS

Specimens: One hundred and twenty broilers (7-45 days old) were collected from different localities at North Sinai Governorate. Ninety were in diseased condition, while, the remaining were freshly dead. The examined samples were collected from liver, kidney, spleen, lung and heart blood and subjected to bacteriological and histopathological examination.

Media: The media used were nutrient broth, nutrient agar, Eosin methylen blue media, XLD agar. Moreover, media for identification as T.S.I agar, urea agar base, Simmons citrate agar indol nitrite medium, sugar fermentation as adonitol, lactose, fructose, glucose, semisolid agar medium were used.

Antisera: Kovac, reagent *E.coli* antiserum polyvalent and monovalent O.K. antisera (Behering werke A.G., Marburg, Germany) was used. Salmonella antisera, Somatic agglutinating serum and salmonella flagelar agglutinating serum (Difico. Lab.) were kindly supplied by Dr. Adel F.Faried Prof. of Microbiology, Animal Health Research Institute Dokki, Egypt.

Antibiotic: susceptibility testing: Antibiotic sensitivity discs obtained from oxoid (National committee for clinical lab standard, 1994)

Culture: Under aseptic precaution, samples were directly taken from different organs and cultivated on media and incubated at 37°C for 24 h. Sub culturing was carried out onto selective media.

Identification: Selected colonies were picked up from selective media for biochemical and serological identification based on criteria adapted (Edward and Ewing1972). The antibiogram was tested by streaking the incubated broth culture on Mueller Hinton agar plate and using antibiotic discs for antibiotics (Gentamycin, Norfloxacin, Colistin sulphate, Chloramphenicol, Neomycin, Lincomycin, Kanamycin, Tarramycine and Streptomycine). Isolation and identification of the causative bacterial agents were done according to Cruickshank *et al.* (1975).

Histopathology: Samples from different organs were fixed in formalin 10% embedded in paraffin, sectioned at 5mn and stained with H&E. (Lillie and Fullmer, 1976).

Experimental infection: one hundred and sixity-one days old Hubbard chicks were obtained from Commercial Poultry Company.Ten chicks were taken randomly killed and examined bacteriologically to establish freedom from bacteria.

Birds were maintained in isolation units and fed unmedicated balanced ration, the remaining (150) birds were divided into five groups each contained 30 birds, group one was inoculated with o.1ml of *E.coli* isolated strain contained 10^7 C.F.U. /bird via air sac. Group two was inoculated with o.5ml of *Psedomonas aeruginosa* isolated which contained (10^6 C.F.U./bird subcutaneously). Group three was inoculated with o.2 ml of *S.aureus* isolate which contained (10^8 C.F.U. /bird subcutaneously). Group four was inoculated with o.1ml of *Salmonella pullorum Gallinarum* isolate which contained (10^7 C.F.U./bird intra muscular). Group five was left uninoculated negative as control. Birds survived after four weeks post- infection were sacrificed and subjected to post mortem examination and reisolation.

RESULTS

The clinical signs observed in the diseased broilers were depression, weakness, loss of appetite and diarrhoea. The gross lesions varied according to the type of infection as septicemic carcasses, congestion of lungs, swollen liver and pale kidney. The relative incidence of the recovered bacterial isolated was as shown in Tables 1,2,3. *E.coli* constitued (41.1%) of the total isolates, *Kelebsiella* spp. (19.4%), *Proteus* spp. (17.7%), *S.aureus* spp. (10.8%), *Salmonella* spp. (6.3%), and *Pseudomonas aeruginosa* spp. (4.6%).

The results of antibiogram on the *E.coli* isolates revealed that Gentamycin, Colisten sulphate, were the most effective against all isolates, whereas, Neomycn, Choloromphenicol, Lincomycin, Kanamycin, Terramycin and Streptomycine were only effective against (0-65%) (Table 4).

The clinical signs and macro pathological findings observed in infected group with *E.coli*, *Pseudomonas aeruginosa* spp., S.*aureus I8*spp and *Salmonella* spp. were congested lung, fibrenous pericaditis and fibrinous Perihepatitis. The spleen showed hyperplasia of the white pulp and thickening of the wall of splenic blood vessels.The intestine showed severe desquamation of villus epithelium and infiltration of the mucosa and sub- mucosa with leukocytes, mainly lymphocytes and heterophils.

The result of artificially inoculated tested birds and frequency of reisolation trials for inoculated bacterial agent from different internal organs of experimentally infected birds are shown in Table 5.

Histopathological examination of the liver of birds infected with salmonella showed congestion of the hepatic blood vessels and focal leukocytic aggregation (Fig.1), numerous scattered areas of coagulative necrosis infiltrated with leukocytes with dilation of hepatic sinusoids (Fig.2), hepatic cells suffered from pressure atrophy, degenerative changes and congestion with dilation of hepatic sinusoids (Fig.3). The kidney of birds infected with *E.coli*

showed focal coagulative necrosis of some renal tubules and cystic dilatation of others with leukocytic infiltration (Fig.4) , cystic dilation of some renal tubules and interstitial leukocytic infiltration (Fig.5), the heart showed fibrinous pericarditis represented by fibrin threads fibrous tissue poliferation with leukocytic infiltration (Fig.6). The spleen showed hyperplasia of the white pulp and thickening of the wall of splenic blood vessels (Fig. 7).The intestine showed severe desquamation of villus epithelium and infiltration of the mucosa and sub mucosa with leukocytes mainly lymphocytes and heterophils (Fig. 8).

Table 1. Incidence of bacterial infection in broiler chickens in North Sinai .

No. of Examined birds	E.coli		Kelebsiella		Proteus		Staphylo-coccus		Psedomonus		Salmonella	
175	No	%	No	%	No	%	No	%	No	%	No	%
	72	41.1	34	29.4	31	17.7	19	10.8	8	4.6	11	6.3

Table 2. Biochemical identification of the bacterial isolates.

Si	Biochemical tests								
	M	I	U	S	L	V	A	TSIA	Mo
E.coli	+	+	-	-	A G	-	-	-	+
Kelbsiella	-	+	-	-	A G	+	+	-	-
Shigella	+	-	-	-	-	-	-	+	-
Proteus	+	+	+	-	-	-	-	+	+
Salmonella	+	-	-	+	-	-	-	+	+
Pseudomonas Spp.	+	+	+	+	A G	-	-	-	+

M. =Methyl red reaction
V. = Voges proskauer reaction
T.S.I.A= triple sugar iron agar.
U. = Urea
Si. = Suspected isolate
L.=Lactose fermentation
A.=Adonitol
Mo.= Motility
S.= Simmons citrate
A. Acid G. Gas

Table 3. Serological identification of *E.coli* isolates.

Serial No.	E.coli serotype	No. of typed strain
1	O36	25
2	O2	28
3	O128	29

Table 4. Antibiotic susceptibility test for *E.coli* isolates.

Anti.	O36	O2	O128	No. of sensitive strain	Total no. of test strain
Ge	++	+++	++++	72	72
NF	+	++	+++	72	72
CL	+	+	++	72	72
N	-	++	+++	47	72
C	-	++	++	47	72
L	++	-	+++	43	72
K	+	-	++	43	72
TE	-	-	++	29	72
S	-	-	-	0	72

(9 Or less) - =Resistant (14-17) ++ =Low sensitive

(10-14)+ = Sensitive (18 or moor) +++ =Highly sensitive

Table 5. Result of mortality rate and reisolation in experimentally infected groups.

group	No of birds	Bacterial type	dose	titer	Rout of ioculation	No.of dead birds	Mortality rate	reisolation
1	30	E.coli	o.1	10.7	Air sac	15	50%	+
2	30	P. aeruginosa	o.5	10.6	S/c	18	60%	+
3	30	Staph aureus	o.2	10.8	S/c	15	50%	+
4	30	Salmonella	o.1	10.7	I/m	12	40%	+
5	30	Control	-	-	-	-	-	-

Titer= Colony forming unit + = positive - = negative ui = uninoculated

S/c subcutaneous I/m interamuscular

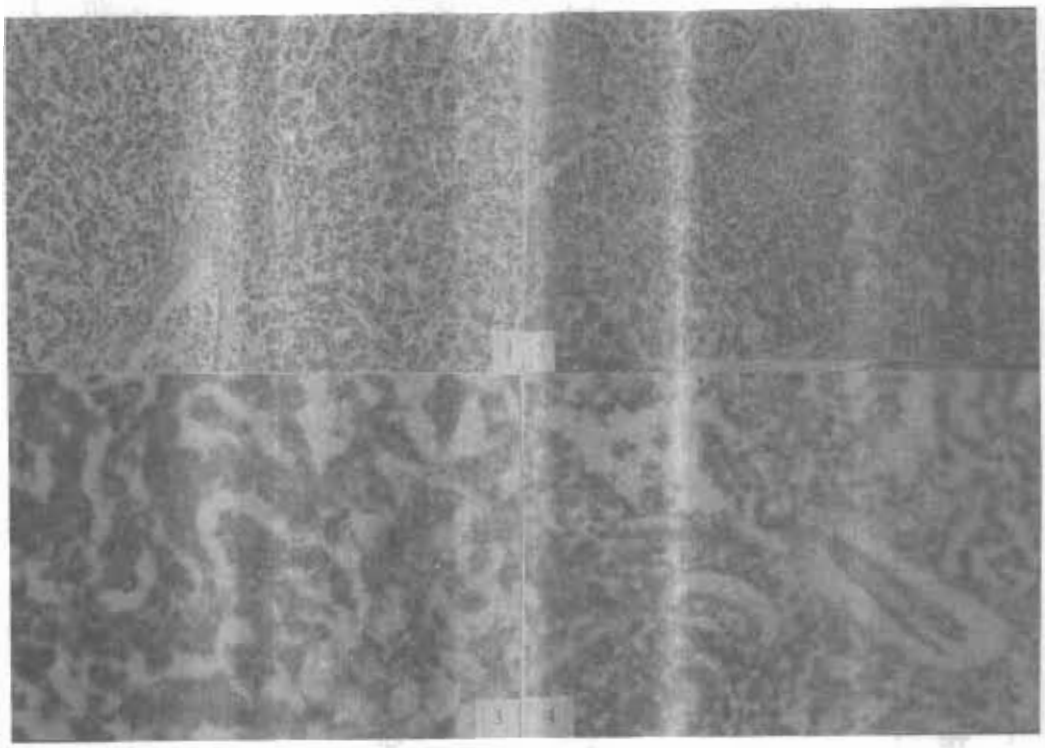

Fig. 1. Liver of birds experimentally infected with Salmonella isolates showing congestion of hepatic blood vessels and focal leukocytic aggregation in portal area. (H&E X200)

Fig. 2. Liver of birds experimentally infected with Salmonella isolates showing numerous scattered areas of coagulative necrosis infiltrated with leukocytes with dilatation of hepatic sinusoids. (H&E X200)

Fig. 3. Liver of birds experimentally infected with Salmonella isolates showing hepatic cells suffered from pressure atrophy, degenerative changes and dilatation with congestion of hepatic sinusoid. (H&E X300)

Fig. 4. Kidney of birds experimentally infected with E.coli showing focal coagulative necrosis of some renal tubules and cystic dilation of others with leukocytic infiltration H&EX300)

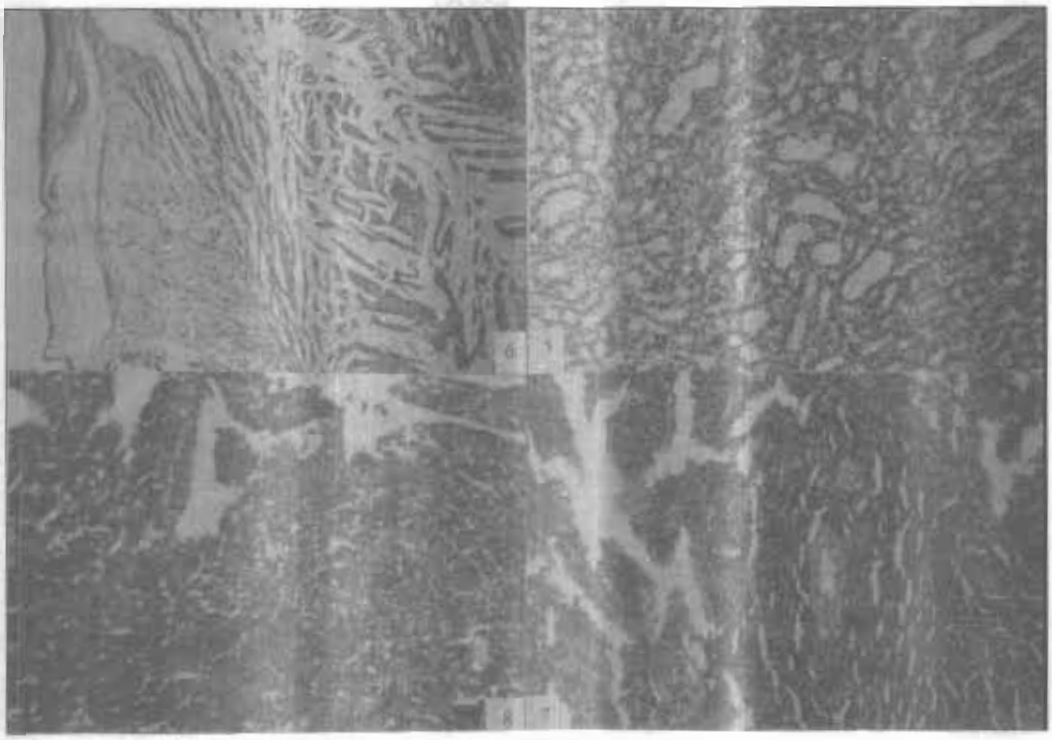

Fig. 5. Kidney of birds experimentally infected with *E.coli* showing cystic dilation of some renal tubules and intrstital leukocytic infiltration. (H&E X200)

Fig. 6. Heart of birds experimentally infected with *E.coli* showing fibrinous pericarditis represented by fibrin threads fibrous tissue proliferation with leukocytic infiltration (H&EX100)

Fig. 7. Spleen of birds experimentally infected with salmonella showing hyperplasia of thewhite pulp and thickening of the wall of spienic blood vessels (H&EX200)

Fig. 8. Intestine of birds experimentally infected with Salmonella showing desquamation of epithelium cells of the villi and leukocytic infiltration of the mucosa and submucosa (H&E X 200).

DISCUSSION

Intensive breeding of broilers in Egypt is facing different problems and severe losses in broilers industry. The bacterial agents are the major causes of these losses. The clinical symptoms observed on the naturally examined birds are depression, loss of appetite, weakness and diarrhoea. These results are in agreement with those described by Emad *et al.* (1996). The postmortem of examined birds varied according to severity and the types of infection, which are mainly congested lungs swollen livers, pale kidneys, air sacculitis, pericarditis, ureters filed with urates and septicemic carcasses. These lesions are in agreement with those described by Niazi *et al.* (1981), Kumar *et al.* (1988) and Abdel Gani *et al.* (1995,). The data in Table 1 revealed that *E.coli* were isolated with an incidence of 43.6% that is in agreement with that reported by Youseif (1995). On the other hand, Kamel *et al.* (1997) reported that *E.coli* were isolated from infected broilers with an incidence 28%. *Kelebsiela* species was isolated with an incidence of 19.4.% that nearly agree with the results obtained by (Seedy *et al.* 1994). *Pseudomonas aeruginosa* spp. was recorded with an incidence of 4.8% (Table 1), which nearly agreed with that recorded by Yousnes *et al.* (1990) and Osman who reported an incidence of 10%. The same incidence 5%was recorded by Emad *et al.* (1996). *S. aureus* spp. was isolated with an incidence of 11.5% (Table 1). The obtained results were in agreement with the results recorded by Youseif (1995). *Proteus* species was recorded with an incidence of 17.7% (Table 1) which nearly agreed with the result obtained by Abd-El Gawad (1989) who reported an incidence of 22%, and 29%, respectively, controversy disagreed with the result obtained by Osman (1992) who reported an incidence of 11%. *Salmonella* species was isolated with an incidence of 6.3% (Table 1) which agreed with the result obtained by Padron (1990), controversy with a higher incidence of 20% mentioned by Kamel *et al.* 1997), and a lower incidence of 4% (Youseif, 1995).

The histopathological changes of the liver, spleen and intestine for Salmonella experimental changes in the kidneyand heart for *E. coli* are similar to those reported by Abdel Gawad, (1989) and Kamel *et al.* (1997).

The histopathological alterations recorded in different internal organs could be attributed to the infection and its endotoxin production, severerity of infection route of type of microorganism. Thus, mortaiity varied from 40- 60% with different isolates

used in the experimental infection. The result of antibiotic susceptibility testing against *E. coli* isolate revealed that all strains were sensitive to Gentamycin, Norfloxacin, and Colisten sulphate, while, the percentage (0-65%) of the isolates were sensitive to the other antibiotics. The obtained results were in agreement with those obtained by Younes *et al.* (1990).

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دراســـــة على بعـــــض المـــسببات البكتيرية الهوائية للنفوق فى بداري دجاج التـــــــسمين

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تـسم جمـــع ١٢٠ عينة من بدارى دجاج التسمين المريضة والنافقة حديثا من اماكن مختلفة بمحافظـــة شمال سيناء وتم فحصها بكتريولوجيا وباثولوجيا وتم عزل بعض المسببات البكترية مثل ميكروب القولون العصوى المعدى بنسبة ٤١,١% و ميكروب الكليبسيلا بنسبة ١٩,٤% وميكروب البروتـــيس بنسبة ١٧,٧% وميكروب استافيلوكوكاس بنسبة ١٠,٨% وميكروب السيدوموناس بنسبة ٤,٦% وميكروب السالمونيلا بنسبة ٦,٣% وبعد عمل الصفة التشريحية ودراسة التغيرات الباثولجية فـــي الاعضـــاء الداخلـــية للعـــدوي التجريبية لبعض انواع المعزولات وجد أحتقان شديد فى معظم الاعضـــاء الداخلـــية ووجـــد تنكرز فى الكبد والكلى و وجدت الأمعاء بها أحتقان شديد وبالفحص الميكرســـوكوبى وجـــد تـــنكرز بالأمعاء ، وبعمل اختبار الحساسية ضد ميكروب القولون العصوى المعـــدى (عدد ٧٢) وجد ان جميع المعزولات (١٠٠%) حساسة للمضادات الحيوية الجنتاميسين ، النوروفلوكساســـين، ســـلفات الكولســـتين، ومتبايـــنة فـــي الحساسية للنيومايسين ، الكلورامفنكول، اللنكومايسين، الكاناميسين ، الترمايسين والاستربتومايسين وننصح بستعمالها فى برامج العلاج اثناء فترات التربية .