



**Research Paper**

**ANTIBIOGRAM FOR MAJOR PATHOGENS RECOVERED FROM BROILERS  
SWOLLEN HEAD SYNDROME**

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**Abstract**

This study was carried out on 250 chickens head were collected from broilers farms in Egypt, the samples were examined on different microbiological media in which, the result revealed that the major pathogens associated with swollen head syndrome in broilers were *E.coli*, *K. pneumoniae*, *P. aeruginosa*, *S.aureus*, *S.xylous*, *S.intermedius*, *E.faecalis*, *S.epidermidis*, and *E.faecium* in percentage of 28% (70\250), 2.8% (7\250), 2.4%(6\250), 1.6%(4\250), 1.2%(3\250), 1.2%(3\250), 0.8%(2\250), 0.8%(2\250) respectively. Serotyping of recovered *E.coli* revealed that the main serotypes recovered from swollen head syndrome were O78:K80 followed by O8: K60 in percentage 20% (14\70) and 17.2% (12\70) respectively. Antibioqram for the recovered pathogens showed that low incidence of antibiotic resistance among recovered *Staphylococcus* and *Streptococcus* spp. while *E.coli* isolates showed 43%(30\70) and 29%(20\70) resistance to Colistin and Erythromycin respectively, in another hand *K.pneumoniae* isolates were resistant to tetracycline and Ciprofloxacin, Cefotaxime, Neomycin, Erythromycin, Sulfa-trimethoprim in percentage of 57% (4\7) and 43(3\7)% respectively and also *P. aeruginosa* were 80%(4\6) and 60 (3\6)% resist to Cefotaxime and Colistin, Tetracycline respectively.

Key words: Swollen head, Broilers, Antibioqram.

**INTRODUCTION**

Swollen-head syndrome (SHS) is a recently described, acute respiratory disease that is observed in a variety of domestic poultry and is characterized by swelling of the periand infraorbital sinuses, torticollis, opisthotonos, and incoordination. Since the syndrome was described in South Africa, SHS or SHS-like disease has been reported in The Netherlands, United Kingdom, France, Spain, Israel, Germany, and Canada. Typically, disease episodes last for 2 to 3 weeks, during which time there is a many cases, diseased birds become reluctant to move and eventually die as a result of their inability to feed. Although the etiology of SHS is uncertain and its clinical appearance is quite variable, the disease is thought to result from a mixed infection with *Escherichia coli* and paramyxovirus, coronavirus, or pneumovirus in which the initial viral infection causes acute rhinitis that permits *E. coli* to invade the subcutaneous facial tissues (Zellen, G. et al., 1988). In broilers and hatchers chickens, APEC strains also cause a syndrome named "swollen head syndrome". Lesions observed in this syndrome include

gelatinous edema on the skin head and peri orbital tissues, and, in some cases, fibrous exudates in the subcutaneous head tissues and in the lachrymal glands (Nunoya *et al.* 1991). Swollen head syndrome considered to be an important avian disease in various countries including Brazil (Arns & Hafez 1992). This syndrome has caused considerable losses in the avian industry because it is responsible for mortality of 3 to 4% of the birds and for reduction of 2 to 3 % at the egg production (Morley & Thomson 1984). Swollen head syndrome usually begins after an acute rhinitis caused by pneumovirus being followed by the invasion of the subcutaneous skin tissues by *E.coli* what causes the characteristic edema (Hafez & Loehren 1990). Swollen head syndrome (SHS) in avian species was described by Morley and Thomson, 1984, who attributed the disease to a mixed infection with *Escherichia coli* and *Coronavirus*, *Paramyxovirus*, or *Pneumovirus* in which the initial viral infection caused acute rhinitis that permitted *E. coli* to invade the subcutaneous facial tissues (Pages Mante, *et al* 1987 ). This acute respiratory disease observed in domestic poultry is characterized by swelling of the peri- and infra-orbital sinuses, torticollis, opisthotonus and lack of coordination (Pattison M, *et al.*, 1989 ). There are many pathogens had far relation with swollen head syndrome as *Pseudomonas* spp., *Klebsiella* spp. and *Enterococcus* spp. Enterococci are gram positive cocci that often occur in pairs (diplococci) or short chains. They are non-motile, nonspore forming, facultative anaerobic bacteria. The genus *Enterococcus* includes more than 17 species, but only few cause clinical infections. Although a dozen *Enterococcus* species have been identified, only two are responsible for the majority of human infections. Until recently, *Enterococcus faecalis* had been the predominant enterococcal species, accounting for 80 to 90% of all clinical isolates, and *Enterococcus faecium* had accounted for 5 to 15%. Other *Enterococcus* species (*E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. avium*, and *E. raffinosi*) are isolated frequently (Gilmore *et al.*, 2002). With increasing antibiotic resistance, enterococci are recognized as feared nosocomial pathogens that can be challenging to treat (Franz *et al.*, 1999). On the other hand, recently, many other pathogens found to be a community acquired pathogens and become a public health hazard such as enterotoxigenic *S. aureus* (Abdel Moein and Samir, 2011) These bacteria are usually found in large numbers in food of animal origin, such as farm animals, pig, and poultry carcasses, and their presence is an indication of fecal contamination, which commonly occurs during slaughter of the animals. They have the potential to easily spread through the food chain and contaminate water and the environment (Bates *et al.*, 1994). Enterococcosis has been reported in a variety of avian species worldwide.

## MATERIAL AND METHODS

### Samples

A total of 250 chickens head were collected from broilers farms, then the samples were brought to the Microbiology department, under cooling condition .

### Isolation and Identification

Under complete aseptic condition, the collected broilers head were incised and swabs were collected followed by inoculation of these swabs on tryptone soya broth all of the inoculated tubes were incubated aerobically at 37°C for 24 hr, then plated on MacConkey's agar, Cetramide agar and Mannitol salt agar "all media obtained from Oxoid", incubate the plates aerobically at 37°C for 24 hr., the recovered isolates primary identified by using Gram's stain followed by biochemical identification by using API 20E, API 20NE, API Staph, and API Strept (Quinn *et al.*, 2002).

### Serotyping of *E.coli* isolates:

Somatic (O), and capsular (K) antigens are used for serotyping of *E. coli*. The suspected *E. coli* isolates were subjected to serological identification by slide agglutination test according to Edwards and Ewing, (1972) using standard polyvalent and monovalent *E. coli* antisera in the Central Laboratories of Ministry of Public Health.

### Antibiogram study for pathogens recovered from swollen head syndrome in broilers .

Using the Disc diffusion method which applied following the guidelines of the National Committee on Clinical Laboratory (NCCLS 2014)

## RESULTS

Survey on major pathogens associated with swollen head syndrome in broilers illustrated in table (1) which revealed that there were 82/250 (32.8%) gram -ve bacilli which divided biochemically to 70/250 (28%) *E.coli* isolates, 7/250 (2.8%) *K. pneumoniae* isolates and 5/250 (2%) *P. aeruginosa*, while there were 15/250 (6%) Staphylococci which divided in base of Staph API system into 6/250 (2.4%) *S.aureus*, 2/250 (0.8%) *S.epidermidis*, 3/250 (1.2%) *S.intermedius* and 4/250 (0.8%) *S.xylous*, also there were 5/250 (2%) Streptococci which confirmed by using strept API system as 3/250 (1.2%) *E.faecalis* and 2/250 (0.8%) *E.faecium*. Serotyping of recovered *E.coli* isolates illustrated in table (2) as the most common serotypes associated with swollen head syndrome were O78:K80 followed by O8:K60 in percentage 20% (14/70) and 17.2% (12/70) respectively. AntibioGram for the recovered pathogens in (table 3 and 4) showed that low incidence of antibiotic resistance among recovered *Staphylococcus* and *Streptococcus* spp. while *E.coli* isolates showed 43% (30/70) and 29% (20/70) resistance to Colistin and Erythromycin respectively, in another hand *K.pneumoniae* isolates were resistant to tetracycline and Ciprofloxacin, Cefotaxime, Neomycin, Erythromycin, Sulfa-trimethoprim in percentage of 57% (4/7) and 43% (3/7) respectively and also *P. aeruginosa* were 80% (4/6) and 60% (3/6) resist to Cefotaxime and Colistin, Tetracycline respectively.

## DISCUSSION

In poultry, the gram-negative bacterium *Escherichia coli* is an important cause of diseases resulting in serious economic losses to the poultry industry. These strains are designated avian pathogenic *E. coli* (APEC) and the diseases they cause are mainly septicemia, swollen head syndrome, omphalitis, cellulitis, yolk-sac infection and respiratory tract infections. It has been proposed that some of the above mentioned diseases such as septicemia and swollen head syndrome start as secondary infections triggered by an initial *Mycoplasma* or viral infection followed by an invasive phase (Silveira et al. 2002a). Poultry industry is still in the grip of various diseases of bacterial, viral, fungal and parasitic origin. Among the bacterial diseases, colibacillosis is one of the most frequently encountered problems. It is an acute septicemic disease in intensively raised birds, caused by *Escherichia coli* (*E. coli*) and characterized by pansystemic involvement and great economic losses. The disease causes high morbidity and mortality throughout the life span of poultry from an egg to an adult bird and constantly results in huge economic losses (Ewers et al., 2003). The risk for swollen head syndrome increases with increasing infection pressure in the environment. A good housing hygiene and avoiding overcrowding are very important. Other principal risk factors are the duration of exposure, virulence of the strain, breed, and immune status of the bird. Every damage to the respiratory system favours infection with APEC. Several pathogens, like NDV, IBV and MG, both wild type and vaccine strains, may play a part in this process. An unfavourable housing climate, like an excess of ammonia or dust, renders the respiratory system more susceptible to APEC infections through deciliation of the upper respiratory tract (Barnes and Gross, 1997). *E. coli* infections are of significant concern to the poultry industry. It is one of the most important and frequently encountered bacterial avian pathogens causing a wide variety of disease syndrome in birds causing up to 30% of poultry mortality (Geornaras et al., 2001). Colisepticaemia in poultry is an important disease of chicks. It is also associated with other disease conditions like acute enteritis, air sacculitis, pericarditis, peritonitis, coligranuloma of liver, synovitis, swollen-head syndrome and osteomyelitis (Amara et al., 1995). The species of *E. coli* are serologically divided in serogroups and serotypes on basis of their antigenic composition. More than 1000 *E. coli* serotypes have been reported but only small percentages have been implicated in poultry diseases (Cloud et al., 1985). Our Survey on major pathogens associated with swollen head syndrome in broilers illustrated in table (1) which revealed that there were 82/250 (32.8%) gram -ve bacilli which divided biochemically to 70/250 (28%) *E.coli* isolates, 7/250 (2.8%) *K. pneumoniae* isolates and 5/250 (2%) *P. aeruginosa*, while there were 15/250 (6%) Staphylococci which divided in base of Staph API system into 6/250 (2.4%) *S.aureus*, 2/250 (0.8%) *S.epidermidis*, 3/250 (1.2%) *S.intermedius* and 4/250 (0.8%) *S.xylous*, also there were 5/250 (2%) Streptococci which confirmed by using

strept API system as 3\250(1.2 %) *E.faecalis* and 2\250(0.8%) *E.faecium* . Serotyping of recovered *E.coli* isolates illustrated in table (2) as the most common serotypes associated with swollen head syndrome were O78:K80 followed by O8: K60 in percentage 20% (14\70) and 17.2%. (12\70) respectively .Epidemiological tracing of *E. coli* strains is of considerable importance in veterinary microbiology. The data can be used to monitor trends in the occurrence of pathogenic strains or to identify possible source of infection. Autologous bacterins provide limited serotypes specific protection, because multiple serogroups are associated with disease, especially O1, O2 and O78 among many others (**Dziva and Stevens, 2008**).Avian pathogenic *E. coli* strains are often resistant to antimicrobials approved for poultry including cephradine, tetracyclines, chloramphenicol, sulfonamides, amino-glycosides and  $\beta$ -lactam antibiotics(**Li, X.S et al 2007** ). Resistance to fluoroquinolones was reported within several years of the approval of this class of drugs for use in poultry(**Van den Bogaard et al 2001**). There is reason for concern that genes conferring resistance to extended-spectrum beta-lactams will emerge in avian pathogenic *E. coli* strains (**Zhao, S et al 2001** ) and reduce the efficacy of ceftiofur, which is currently used on a limited basis in poultry breeding flocks and hatcheries. In one study, conducted at the University of Georgia, 97 of 100 avian pathogenic *E. coli* isolates were resistant to streptomycin and sulfonamide and 87% of these multiple antimicrobial resistant strains contained a class 1 integron, *intI1*, which carried multiple antibiotic resistance genes. Multiple antimicrobial resistance traits of avian pathogenic *E. coli* have also been associated with transmissible R-plasmids (**Wooley, R.E et al 1991**). The present study antibiogram for the recovered pathogens showed that low incidence of antibiotic resistance among recovered *Staphylococcus* and *Streptococcus* spp. while *E.coli* isolates showed 43%(30\70) and 29%(20\70) resistance to Colistin and Erythromycin respectively ,in another hand *K.pneumoniae* isolates were resistant to tetracycline and Ciprofloxacin, Cefotaxime ,Neomycin, Erythromycin ,Sulfa-trimethoprim in percentage of 57% (4\7)and 43(3\7)% respectively and also *P. aeruginosa* were 80%(4\6) and 60 (3\6)% resist to Cefotaxime and Colistin ,Tetracycline respectively .The ubiquitous nature of the enterococci and resistance to adverse environmental conditions takes account for their ability to colonize different habitats, and for their potential for easy spreading through the food chain (**Giraffa 2002**). The present study reports the occurrence of the *Enterococcus* species in the broilers internal organs and swollen head , this is considered a public health hazard with may be a potential zoonotic pathway that infects human contacts.

## CONCLUSION

Swollen head syndrome is one of the most clinical case in broilers chickens which caused due to mixed infection viral and bacterial and bad hygienic measures is predisposing for its occurrence. There are event for recovering of antibiotic resistance pathogens from this case which make treatment decision is difficult and increase public health hazard

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**Table (1) Collective table for major pathogens recovered from broilers swollen head syndrome**

Samples	Recovered pathogens					
250 swollen broilers head	Gram's - ve			Gram's + ve		
	Species	n=isolates	%	Species	n=isolates	%
	<i>E.coli</i>	70	28	<i>S.aureus</i>	6	2.4
				<i>S.epidermidius</i>	2	0.8
	<i>K.pneumoniae</i>	7	2.8	<i>S.intermedius</i>	3	1.2
				<i>S.xylous</i>	4	1.6
	<i>P.aeruginosa</i>	5	2	<i>E.faecalis</i>	3	1.2
Total		82	32.8	<i>E.faecium</i>	2	0.8
				Total	20\250	8

**Table (2) Results of serological identification of the examined *E. coli***

serogroups	Isolates	
	Number	%
<i>E. coli</i> 086:K61	7	10
<i>E. coli</i> 025:K-	1	1.4
<i>E. coli</i> 0158:K-	4	5.7
<i>E. coli</i> 0119:K69	1	1.4
<i>E. coli</i> 078:K80	14	20
<i>E. coli</i> 026:K60	3	4.3
<i>E. coli</i> 0126:K71	3	4.3
<i>E. coli</i> 0127:K-	1	1.4
<i>E. coli</i> 0111:K58	1	1.4
<i>E. coli</i> 08: K60	12	17.2
<i>E. coli</i> 02: K40	10	10
<i>E. coli</i> 01: K24	6	8.6
Untypable	7	10
Total	70	100

Table (3): Antibigram for gram -ve microorganisms recovered from swollen head syndrome in broilers .

Chemotherapeutic discs	Conc (µg)	<i>E. coli</i>						<i>K.pneumoniae</i>						<i>P.aeruginosa</i>					
		Resistant		Intermediate		Sensitive		Resistant		Intermediate		Sensitive		Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ciprofloxacin	5	12	17	8	11	50	71	3	43	2	29	2	29	1	20	1	20	3	60
Cefotaxime	30	22	31	18	26	30	43	3	43	3	43	1	14	4	80	0	0	1	20
Colistin sulphate	10	30	43	5	7	35	50	4	57	0	0	3	43	3	60	1	20	1	20
Streptomycin	10	10	14	22	31	38	54	0	0	2	29	5	71	2	40	2	40	1	20
Tetracycline	30	14	20	16	23	40	57	4	57	2	29	1	14	3	60	0	0	2	40
Kanamycin	10	12	17	8	11	50	71	2	29	3	43	2	29	2	40	1	20	2	40
Neomycin	30	18	26	14	20	38	54	3	43	2	29	2	29	0	0	3	60	2	40
Erythromycin	15	20	29	23	33	27	39	3	43	2	29	2	29	4	80	1	20	0	0
Amoxicillin	10	19	27	10	14	41	59	2	29	4	57	1	14	1	20	3	60	1	20
Gentamycin	10	5	7	10	14	55	79	1	14	2	29	4	57	0	0	1	20	4	80
Sulpha\trimethoprim	1.25	15	21	12	17	43	61	3	43	2	29	2	29	2	40	2	40	1	20
Total No. of the tested strains		70						7						5					

No.: Number of positive cases.

%: Calculated according to the number of tested isolates.

Table ( 4 ): Antibigram for gram +ve microorganisms recovered from swollen head syndrome in broilers .

Chemotherapeutic discs	Conc ( µg)	<i>S. aureus</i>						<i>S.epidermidis</i>						<i>S.intermedius</i>					
		Resistant		Intermedia te		Sensitive		Resistant		Intermedia te		Sensitive		Resistant		Intermediat e		Sensitive	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Penicillin	10	0	0	0	0	6	100	0	0	0	0	2	100	0	0	0	0	3	100
Ampicillin	10	0	0	0	0	6	100	0	0	0	0	2	100	0	0	0	0	3	100
Ciprofloxacin	5	0	0	0	0	6	100	0	0	0	0	2	100	0	0	0	0	3	100
Cefotaxime	30	2	33.3	0	0	4	66.7	0	0	0	0	2	100	0	0	0	0	3	100
Vancomycin	10	0	0	0	0	6	100	0	0	0	0	2	100	0	0	0	0	3	100
Streptomycin	10	1	16.7	0	0	5	83.3	0	0	0	0	2	100	0	0	0	0	3	100
oxacillin	30	1	16.7	0	0	5	83.3	0	0	0	0	2	100	0	0	0	0	3	100
Amoxicillin	10	0	0	0	0	6	100	0	0	0	0	2	100	0	0	0	0	3	100
Gentamycin	10	0	0	0	0	6	100	0	0	0	0	2	100	0	0	0	0	3	100
Total No. of the tested strains		6						2						3					

No.: Number of positive cases.

%: Calculated according to the number of tested isolates.



Continued ,Table ( 4 ): Antibigram for gram +ve microorganisms recovered from swollen head syndrome in broilers .

Chemotherapeutic discs	Conc (µg)	<i>S. xylosus</i>						<i>E.faecalis</i>						<i>E.faecium</i>					
		Resistant		Intermedia		Sensitive		Resistant		Intermedia		Sensitive		Resistant		Intermediat		Sensitive	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Penicillin	10	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
Ampicillin	10	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
Ciprofloxacin	5	0	0	0	0	4	100	0	0	0	0	3	100	1	50	0	0	1	50
Cefotaxime	30	1	25	1	25	2	50	0	0	0	0	3	100	0	0	0	0	2	100
Vancomycin	30	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
Streptomycin	10	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
oxacillin	1	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
Amoxicillin	10	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
Gentamycin	10	0	0	0	0	4	100	0	0	0	0	3	100	0	0	0	0	2	100
Total No. of the tested strains		4						3						2					

No.: Number of positive cases.

%: Calculated according to the number of tested isolates.