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DETERMINATION OF ANTIBIOTIC SENSITIVITY OF BACTERIA ISOLATED FROM INFECTED YOLK SAC IN BROILER CHICKEN FARMS AROUND TABRIZ CITY, EAST AZERBAIJAN OF IRAN

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INTRODUCTION

Iran increasing modern poultry production, both for local use and for export. However, expansion was constrained by an unsteady supply of hatching eggs, day-old-chicks, premix, or veterinary drugs, diseases, a lack of support services, insufficient data with which to plan improved services and inadequate information on how to improve animal breeding, marketing and processing **[1]**. Infectious diseases are remaining among the major health constraints which hampering its intended potential **[2]**. Yolk sac infection is the main infectious cause of chick mortality during the first week of the post-hatching period **[3, 4]** and is the main cause of chick's mortality accounting for large economic losses to the poultry industry **[5]**. It can cause mortality rate of about 5-10%; however the condition has also been associated with much higher mortality especially in chicks during first week of age **[6]**. Contamination of unhealed navels has been suggested as a cause of yolk sac infection in newly hatched chicks **[7]**. Different types of bacterial agents are attributed for causation of yolk sac infection/omphalitis in chicks **[8]** *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus* and *Enterococcus* spp. were some bacteria that have been isolated from yolk sac infections in chicks in different locations all over the world. Nevertheless, *Escherichia coli* (*E. coli*) was frequently observed **[5, 9, and**

10]. In Iran, investigations on poultry diseases in general and yolk sac infections (omphalitis) in particular have received little attention. Till now no significant research has been reported in the country pertaining to yolk sac infections during their first week of life and continued to be the most neglected and devastating diseases of chicken. Therefore, the objective of this study was determination of antibiotic sensitivity of bacteria isolated from infected yolk sac in broiler chicken farms around Tabriz city, East Azerbaijan of Iran.

MATERIALS AND METHODS

The study was conducted during the period between December 2012 to June 2012 in East Azerbaijan of Iran. Yolk swabs were aseptically collected from broiler chicken in farms. 45 samples were collected from chicks 1-7 days old. Samples were enriched by overnight incubation in nutrient broth at 37ºC. Cultures were inoculated onto nutrient agar (NA), blood agar (BA), eosin methylene blue (EMB) agar, brilliant green agar (BGA), mannitol salt agar (MSA), salmonella-shigella (SS) agar and triple sugar iron (TSI) agar and incubated at 37°C. Discrete bacterial colonies were sub-cultured until pure cultures were obtained **[11]**. Bacteria were characterised by recording morphology of colonies (size, margin, elevation and colour), Gram stain **[12]** sugar fermentation, catalase, coagulase, M-R, V-P, indole, and triple sugar iron tests **[11]**.

Antimicrobial sensitivity was tested using 0.5 McFarland turbidity standard inoculums and freshly prepared, dried Mueller Hinton agar (Oxoid, UK) against 10 common antibiotics: nalidixic acid, ampicillin, amoxycillin, chloramphenicol, ciprofloxacin, tetracycline, kanamycin, gentamicin, sulphamethoxazole and erythromycin (Oxoid, UK). Five isolates of *E.coli, Salmonella* and *Staphylococci* were selected randomly for the test. Disc diffusion or Kirby-Bauer method **[13]** was used. The results were expressed as resistant, intermediate or sensitive according to the guidelines of National Committee for Clinical Laboratory Standards **[14]**.

RESULTS AND DISCUSSION

Three genera of bacteria were isolated from yolk swab samples of chicks, *E. coli, Salmonella* and *Staphylococci*. Bacterial genera recovered are in agreement with earlier studies **[15]**.

The prevalence of bacteria associated with omphalitis in chicks is presented in **Figure 1**.

In this study *Salmonella* showed the highest prevalence both in chicks aged 1-3 days and 4-7 days (68 and 54.3%, respectively). These findings contradict the observation of **Iqbal *et al.* (2006)** who recorded a prevalence of *E.*

coli 47.9% and only 0.5% prevalence of *Salmonella*. The prevalence of *Staphylococci* ranked third in this study (24% in 1-3 days old chicks and 28.6% in 4-7 days old chicks), but a previous study recorded 0.5% prevalence of Staphylococci **[16]**. The cultural characteristics of *E. coli, Salmonella and Staphylococci* **(Table 2)** were similar to the findings of other authors **[17, 18, 19 and 20]**.

E. coli fermented dextrose, lactose, sucrose and mannitol with the production of acid and gas. *E. coli* gave positive reaction to catalase and MR and indole tests and negative reaction in V-P test. *Salmonella* fermented dextrose, maltose and mannitol with acid and gas production. *Salmonella* were MR and catalase positive and negative to V-P and indole tests. *Staphylococci* fermented all five basic sugars with only acid production. Catalase, MR and V-P tests were positive but indole and coagulase tests were negative. These results are similar to those of **Sato *et al.* (1961); Zahdeh *et al.* (1984) and OIE (2004) [21, 22 and 23]**. Antibiotic sensitivity of *E. coli, Salmonella* and *Staphylococci* has shown in **Table 2**.

All *E. coli* isolates were resistant to eight antibiotics: ciprofloxacin, gentamicin, amoxycillin, ampicillin, tetracycline, erythromycin, nalidixic acid and sulphamethoxazole. All *Salmonella* isolates were resistant to tetracycline and erythromycin. All *Staphylococci* were resistant to nalidixic acid and tetracycline. The results are identical with those by **Klein *et al.* (1996); Khan *et al.* (2002); Lee *et al.* (2005); Nazir *et al.* (2005a, b); Akond *et al.* (2009)**.

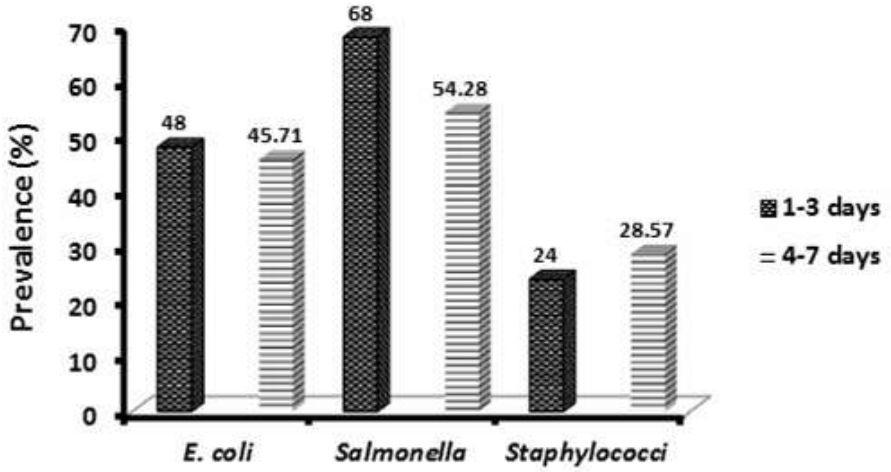

Figure 1: Prevalence of *E. coli, Salmonella and Staphylococci* in 1-3 days and 4-7 days old chicks with clinical signs of omphalitis

Table1: Bacteria isolated from yolk swabs of chicks suffering from omphalitis

Chicken age	No of samples	No of bacterial isolates		
		E. coli	*Salmonella*	*Staphylococci*
1-3 days	15	8	13	5
4-7 days	27	15	18	7

Table 2: Antibiotic sensitivity of *E. coli, Salmonella* and *Staphylococci*

Antimicrobial agents	Disc concentration (µg/ml)	*E. coli* (n = 5)			*Salmonella* (n = 5)			*Staphylococci* (n = 5)		
		R	I	S	R	I	S	R	I	S
Nalidixic Acid	25	3	0	0	4	1	0	5	0	0
Ampicillin	8	3	0	0	2	0	3	0	1	4
Amoxicillin	8	3	0	0	3	0	2	0	1	4
Chloramphenicol	25	0	0	3	2	1	2	0	1	4
Ciprofloxacin	3	3	0	0	0	0	5	0	0	5
Tetracycline	25	3	0	0	5	0	0	5	0	0
Kanamycin	25	1	2	1	1	2	2	2	0	3
Gentamicin	8	3	0	0	0	3	2	0	1	4
Sulphamethoxazole	20	3	0	0	3	0	2	2	0	3
Erythromycin	10	3	0	0	5	0	0	1	2	2

R = resistant; I = intermediate; S = sensitive

CONCLUSIONS

The occurrence of multi-drug resistance in bacteria in chicks suffering from omphalitis is alarming as this resistance may gain access to man and animals, which might result in difficulties in treatment of bacterial infection. Further studies are required to formulate guidelines for the prevention and control of bacterial omphalitis in chicks.

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