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
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 37°C. 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 Staphylococi 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 Staphylococi [16]. The cultural characteristics of E. coli, Salmonella and Staphylococi (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. Staphylococi 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 Staphylococi 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 Staphylococi 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 Staphylococi in 1-3 days and 4-7 days old chicks with clinical signs of omphalitis
Table 1: Bacteria isolated from yolk swabs of chicks suffering from omphalitis

<table>
<thead>
<tr>
<th>Chicken age</th>
<th>No of samples</th>
<th>No of bacterial isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>1-3 days</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>4-7 days</td>
<td>27</td>
<td>15</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Disc concentration (μg/ml)</th>
<th>E. coli (n = 5)</th>
<th>Salmonella (n = 5)</th>
<th>Staphylococci (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>25</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>25</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>25</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>20</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
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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.

REFERENCES


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