Aerobic Bacteria Associated with Omphalitis of Chicks

Khalil, S. A. and Einas, El-Shamy
Dept. of Microbiology Fac. of Vet. Med. Alex. Univ.

ABSTRACT

This study was aimed to determine bacterial agents which causing omphalitis in chicks. To achieve this purpose the following points were studied: Isolation and biochemical identification of *E. coli* and *Staphylococcus aureus* from yolk sac samples were collected from diseased chicks and detection virulence markers in isolated *E. coli* and *Staphylococcus aureus* by using phenotypic and genotypic markers of virulence. Phenotypic assay as the Congo red binding capacity of the isolated *E. coli* and the haemolytic activity of the isolated *E. coli* on blood agar medium were used. Genotypic assay as detection of Adhesin-encoding genes of *E. coli* by using multiplex PCR and detection of *Staphylococcus aureus* super antigen genes by using multiplex PCR. This study was carried-out on random samples (150 samples) of yolk sac that were collected from different private chick hatcheries from newly hatched chicks. The samples were collected, labeled and transported in ice box to the laboratory of Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University bacteriological identification was carried-out for the bacterial isolates of these samples. It could be concluded that, the main bacterial agents causing omphalitis include *Staphylococcus aureus*, *E. coli*, *Proteus vulgaris*, *Proteus mirabilis*, *klebsiella pneumonia* and *Enterobacter hafniae*.

INTRODUCTION

Omphalitis is infectious and non-contagious condition of yolk sac which accompanied by unhealed navels in young fowl. The affected chicks appear normal until a few hours before death (Kahn *et al.*, 2008). Bacterial infection of navel area is one of the most common causes of mortality in chicks during the first week after hatching (Pattison *et al.*, 2008). Several bacteria such as *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus* and *Enterococcus* have been isolated from yolk sac infection of birds (Cortes *et al.*, 2004).

*Escherichia coli* (*E. coli*) is the most common contaminant of yolk sacs in chickens and about 70% of chicks with omphalitis had this bacterium in their yolk sacs. On the other hand, it is common to recover low numbers of *E. coli* from normal yolk sacs (Saif *et al.*, 2008).

Omphalitis is caused by bacteria, which multiply under the same conditions as they do in yolk sac infection. If the navel becomes infected before drying out then it can become putrid and the chick dies. There are many causes for omphalitis in chicks that include Colibacillosis, Salmonellosis, clostredial diseases (Shivaprasad, 2003).

Brandly (2008) reported that, from the standpoint of avian pathology, some
recent observations indicate that the condition of infection and inflammation of the navel of baby chicks (omphalitis) has not been accorded the importance it warrants.

This condition is an infection of the navel. Just before a chick is hatched, it ‘absorbs' the yolk sac. This is done through the navel. Once complete, the navel will heal over and dry out. Before the navel completely dries out, it is at risk of becoming infected with bacteria. Omphalitis or yolk sac infection occurs frequently in commercial poultry. The most prevalent bacteria causing yolk sac infection is *Escherichia coli* (Deeming, 1995; Rehman et al., 1996; Anjum. 1997; Sharada a cil.. 1999). Besides E. coli, *Staphylococcus aureus* is next most important bacterium associated with yolk sac infection (Choudhury et al., 1993; Deeming. 1995; Rehman et al., 1996). The infected yolk sacs were large in size having yellowish brown and green to yellowish red appearances.


*Staphylococcus aureus* is a very ubiquitous microorganism associated with omphalitis, yolk sac and liver infections in first week dead chicks and in-shell dead embryos (*White et al., 2003*). The birds of group A infected with *Staphylococcus aureus* seemed weak, huddled together and had a watery diarrhea. The umbilicus was open. (*Rai et al., 2003*).

*E. coli* is one of the opportunistic pathogen responsible for number of disease conditions such as yolk sac infection, air sac disease, pericarditis, enteritis, omphalitis, coligranuloma, colibacillosis (*Ahmad et al., 2009*). Also, *Proteus Species* has been associated with in-shell embryos mortality (*Orajaka and Mohan, 1985*).

*Klebsiella pneumonia* also has been reported as one of the bacteria infecting the yolk sac and causing embryos and chicks mortalities during their first week of life (*Orajaka and Mohan, 1985*).

Laboratory isolation of *E. coli* from lesions, yolk, on MacConkey's or methylene blue agar (EMB). Colonies are pink on MacConkey's and dark with metallic sheen. It Simulates Salmonella, *Staphylococcus*, Fowl cholera. (*World Poultry Net, 2012*).

Bacterial colonization in the epithelial surfaces is considered a critical first step in the pathogenesis of avian pathogenic *E. coli* isolates (*Ramirez et al., 2009a*).

This study aimed to determine the main bacterial causes of omphalitis in chicks with determination of the virulence factors in recovered *E. coli* and *staphylococcus aureus* isolates.

**MATERIALS AND METHODS**

**1-Materials:**

**1.1. Samples**

Random samples (150 samples) of yolk sac were collected from different private chick hatcheries from newly hatched dead chicks. The samples were collected, labeled and transported in ice box to the laboratory of Department of Microbiology, Fac. of Vet. Med. Alex. Univ.
Bacteriological examination:
I-Cultivation of the samples for bacterial isolation:
The samples were cultivated into nutrient broth and tryptic Soya broth incubated aerobically at 37 °C for 18 – 24 hours. A loopful from the incubated nutrient broth and tryptic Soya broth was streaked onto MacConkey’s agar and salt mannitol agar media. The inoculated plates were incubated aerobically at 37 °C for 24 – 48 hours, and then examined for bacterial growth. The bacterial colonies were picked up and purified. The pure isolates were stored in soft agar till further identification.

I-Identification of the isolates:
The bacterial isolates were subjected to characterization by studying their morphological, cultural, and biochemical characteristics as well as their motility as follows:

1-Morphological characteristics:
Films were prepared from fresh cultures, stained with Gram’s stain and examined microscopically for the morphological characteristics of the isolate.

2-Cultural characteristics:
The colonial morphology on MacConkey’s agar and salt Mannitol agar media were studied.

3-Detection of motility:
The isolates were inoculated into tubes containing semi-solid nutrient agar medium and incubated for 24 hours at 37 °C. Inoculated tubes were examined for detecting motility of the inoculated isolates, then preserved in the refrigerator at 4 °C.

4-Biochemical characterization:
The isolated bacteria were identified according to (Quinn et al., 2002) as follows: catalase test, oxidase test, indole test, methyl red test, Vogas-Proskauer test, citrate utilization test, urease test, gelatin liquefaction, oxidation_ fermentation test, coagulase test and mannitol fermentation test.

5-Hemolytic activity and Congo red test were performed for differentiation of hemolytic and non-hemolytic isolates also for differentiation between pathogenic and non-pathogenic bacterial isolates.

6-Detection of the adhesin_encoding genes of E. coli isolates by multiplex polymerase chain reaction.

7-Detection of staphylococcus aureus super antigen genes by multiplex polymerase chain reaction.

RESULTS AND DISCUSSION
Omphalitis is infectious and non-contagious condition of yolk sac which accompanied by unhealed navels in young fowl. The affected chicks appear normal until a few hours before death (khan et al., 2008)
The results of typing bacteria isolated from examined samples cleared that, the most commonly isolated bacteria were Staphylococcus aureus, E.coli, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumonia and Enterobacter hafniae where they were isolated at a percentage of 20%, 19%, 6%, 5.3%, 4.6% and 4%,respectively (Table ,1).These results agreed with those of Cortes et al.,( 2004), where he reported that, several bacteria such as Proteus spp., Enterobacter spp., Pseudomonas spp., Klebsiella spp., Staphylococcus spp., Streptococcus spp., Clostridium spp., Bacillus cereus and Enterococcus have been isolated from yolk sac infection of birds. Also these results agreed with those of Saif et al.,( 2008),where he reported that, Escherichia coli (E. coli) is the most common contaminant of yolk sacs in chickens and about 70% of chicks with
Omphalitis had this bacterium in their yolk sacs. On the other hand, it is common to recover low numbers of E. coli from normal yolk sacs.

The results of pathogenicity determinants of the isolated E. coli isolates indicated that, most of E. coli isolates causing omphalitis are hemolytic causing alpha haemolysis (82%) and beta-haemolysis (18%) (Table, 2). These results agreed with those of Dho-Moulin and Fairbrother, (1999); El-Sukhon et al., (2002), where they reported that, the most important virulent isolates affecting broilers and chicks include E. coli and is the main cause of omphalitis and commonly causes alpha or beta haemolysis to RBCs.

The results of in vitro differentiation between pathogenic and non-pathogenic E. coli isolates by Congo red binding activity and haemolytic activity of E. coli isolates. The results indicated that, all isolates (100%) bound with Congo red dye giving red colonies (CR positive) and also gave hemolytic activity (Table, 3 and 4). These results agreed with those of Sharada, Wilfred Ruban and Thiaygeeswaran., (2010) where they reported that, The results of the Congo red (CR) binding assay indicated that majority (95.38%) were positive and only three isolates recovered from enteritis were negative which indicated that isolates of virulent avian E.coli can be identified by their ability to bind Congo red. These results also, agreed with those of Berkhoff and Vinal, (1986) who also reported a strong correlation between expression of CR phenotype and virulence in avian E.coli and suggested that it was associated with the presence of B-D-glucan in bacterial cell wall. Our results agreed with those of Rehab Saif et al., (2011) where she reported that, in vitro pathogenicity tests indicated that the E. coli isolates gave positive results with haemolysis tests and Congo red tests. The results also indicated that, the isolates that gave positive results with haemolytic activity gave also positive results with Congo red positive results. (Table, 5).

While, the results of amplification of adhesion encoding genes of E. coli by multiplex PCR (Curli gene and Fimbriae H gene) (Table, 6) (Fig., 1), indicated that all pathogenic E. coli isolates tested for PCR gave positive results for PCR. These results agreed with those of McPeake et al. (2005) and Delicato et al. (2003) where they reported that, 100% of avian pathogenic E.coli isolates were positive for curli encoding genes whereas Amabile de Campos et al. (2005) indicated that 16.7% of E. coli isolates from colisepticemic cases were positive for crl gene. These results also, agreed with those of Reza Ghanbarpour and Mahmood salehi, (2010) Out of 104 examined E. coli isolates 91 (87.50%) were positive for crl gene which was the most prevalent genetic marker.

The results of amplification of S. aureus super antigenic genes by multiplex PCR (SEB, SEC and SED) (Table, 7) cleared that, S. aureus isolates tested for PCR (SEB, SEC and SED) all of them gave negative results for PCR. These results attributed to the S. aureus yolk sac infection (omphalitis) with S. aureus led to decline in the immunity. (Rai et al., 2003).

It could be concluded that, the main bacteria causing omphalitis in chicks include staphylococcus aureus, E. coli, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumonia and Enterobacter hafniae.
**Table (1):** Results of typing bacteria isolated from different samples.

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>No. of the samples</th>
<th>Isolated bacteria</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>150</td>
<td>E. coli</td>
<td>28</td>
<td>19</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>Staphylococcus aureus</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td></td>
<td>Klebsiella pneumonia</td>
<td>7</td>
<td>4.6</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td></td>
<td>Proteus vulgaris</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td></td>
<td>Proteus mirabilis</td>
<td>8</td>
<td>5.3</td>
</tr>
<tr>
<td>Enterobacter hafniae</td>
<td></td>
<td>Enterobacter hafniae</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table (2):** Results of hemolytic activity of E. coli isolates on blood agar media.

<table>
<thead>
<tr>
<th>Total No. of examined E. coli isolates</th>
<th>Type of haemolysis</th>
<th>Alpha haemolysis</th>
<th>Beta haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>23</td>
<td>82</td>
</tr>
</tbody>
</table>

% according to number of E. coli isolates.

**Table (3):** Results of in vitro differentiation between pathogenic and non pathogenic E. coli isolates by Congo red binding activities.

<table>
<thead>
<tr>
<th>Total No. of examined E. coli isolates</th>
<th>Pathogenic E. coli</th>
<th>Non-pathogenic E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>100</td>
</tr>
</tbody>
</table>

Red colonies: Congo red positive = pathogenic
White colonies: Congo red negative = Non pathogen

**Table (4):** In vitro pathogenicity tests on E. coli isolates.

<table>
<thead>
<tr>
<th>Pathogenicity tests</th>
<th>Total No</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis</td>
<td>28</td>
<td>28 100</td>
</tr>
<tr>
<td>Congo red test</td>
<td>28</td>
<td>28 100</td>
</tr>
</tbody>
</table>
Table (5): Correlation between Congo red test and alpha haemolysis.

<table>
<thead>
<tr>
<th>Total No. of examined E. coli isolates</th>
<th>Agreement</th>
<th>Disagreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>28</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>18</td>
</tr>
</tbody>
</table>

Table (6): Results of amplification of adhesion encoding genes of E. coli by multiplex PCR (Curli gene and Fimbriae H gene).

<table>
<thead>
<tr>
<th>Total No. of examined E. coli isolates</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table (7): Results of amplification of S. aureus super antigen genes by multiplex PCR (SEB, SEC and SED).

<table>
<thead>
<tr>
<th>Total No. of examined S. aureus isolates</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
Aerobic Bacteria Associated With OMPHALITIS of Chicks

REFERENCES


Figure 1. Finger printing of E. coli isolates of four groups (RAPD D-PCR) amplified polymorphic DNA above lanes 1-4 corresponds to E. coli strain groups respectively, size strand 250 bp while amplified polymorphic DNA above lanes 1-4 corresponds to Staph. aureus strain give no size strand.


Aerobic Bacteria Associated With OMPHALITIS of Chicks


Shivaprasad, H. L. (2003): Pathology of Birds – An Overview. California Animal Health and Food Safety Laboratory System, Fresno Branch School of Veterinary Medicine, University of California, Davis 2789 South Orange Avenue. Fresno, CA 93725