Epidemiological studies on salmonellosis in broiler chicken farms in Alexandria governorate
(With 3 Tables)

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A survey on Salmonella infection was carried out on 50 broiler chicken farms located at Alexandria Governorate. A total of 5 Salmonella isolates were isolated and serotyped using different selective enriched media for isolation and a variety of biochemical and serological tests for identification. The in-vetro sensitivity test revealed that Neomycin and Ceftiofur gave the highest activity against Salmonella isolates. On the other hand, Salmonella typhimurium isolates showed resistance against Erythromycin, Amoxycillin and Ampicillin. Moreover, Salmonella isolates were tested for their virulence using Congo red binding test and ability to produce hemolysin.

Key words: Broilers, salmonellosis, S.typhimurium.
INTRODUCTION

Salmonella is one of the most important microorganisms that cause diseases in man and animals and among common causes implicated in outbreaks of food borne infectious disease around the world, (Abou-Zeed et al., 2000). Salmonellae have a wide host range, including human, animals and birds (Duce et al., 1991). Poultry have been implicated as a major source of Salmonella contaminated food products as poultry eggs or meat that cause human salmonellosis (Tauxe, 1991 and Humaphrey, 1999). Salmonella pullorum has a long history of causing economically significant illness and mortality in young commercial chickens (Snoeyenbos, 1991). Salmonella typhimurium found to be the main cause of Salmonella in human and animals (Groisman et al., 1989). Much more, Salmonella enteritidis, only arose to its predominant position in man and poultry in the mid-1980s as a result of vertical and horizontal transmission within and between large poultry organization in many parts of the world (Hartung, 1992). Sensitivity of isolation of these organisms by culture depends on various factors including the method used for collecting the sample, the amount of sample analyzed and seasonal variation in shedding the organism (Cohen et al., 1994). The growth inhibition by other bacteria can be a particular problem, especially with faecal samples which are often heavily contaminated with other microorganisms, moreover, in chronic Salmonella infection in fowl, Salmonella organisms are excreted only intermittently in faeces. Standard procedures for isolation and identification of Salmonella using growth media are slow and often these tests fail to detect bacteria that may be present for that, numerous schemes for pre enrichment, direct enrichment and secondary enrichment have been examined for recovering and isolating Salmonella from meat, poultry and enviroment (Tate et al., 1990). There is a need for Salmonella testing methods that provide more rapidly and sensitivity results. These rapid methods should be robust and reliable and have a specificity that minimize false positive results. (Blackburn, 1993).

Plasmid analysis has a great effect as an epidemiological tool in an outbreak of salmonellosis (Kapperud et al., 1989). More, plasmid analysis is relatively simple to perform, does not require aspecialized reference laboratory and can be accomplished in a short period of time and not expensive (Mayer, 1988)

The purpose of this work was designed to study the incidence of Salmonella infections in broiler chicken farms by using different
methods of enrichment for isolation and applying biochemical and serological methods for identification, Moreover study some virulence factors for Salmonella microorganisms and finally isolated Salmonella were tested for antimicrobial drug susceptibility.

**MATERIALS and METHODS**

1 - **Bacteriological isolation:**
A total of 50 broiler chicken farms of different ages at different localities in Alexandria Governorate were subjected for bacteriological studies, from each flock 5-7 live and freshly dead chicken aged from 1-5 week were taken for isolation and identification of Salmonella using enriched media as “Selenite F broth and Tetrathionate brilliant green broth” incubated at 37 – 42°C for 24-48 h. (Oxoid 1987) and delayed secondary enrichment for 5 days (Waltman et al., 1991), then cultured on MacConkey’s agar, Salmonella-Shigella (SS) agar and Brilliant green agar incubated at 37°C for 24 h. (Oxoid, 1987).

The suspected colonies were examined morphologically and biochemically.

2 - **Morphological identification:**
Purified suspected Salmonella isolates were examined morphologically as described by Cruickshank et al. (1975).

3 - **Biochemical identification:**
Suspected Salmonella isolates were identified biochemically using criteria of Quinn et al. (1994).

4 - **Serological identification:**
Cultures biochemically identified as Salmonella were typed serologically according to Kauffmann White Scheme (Kauffmann, 1972) by slide agglutination test using polyvalent “O” Salmonella antiserum. Colonies which gave positive results were similarly tested using monovalent “O” and “H” Salmonella antisera.

5 - **In vitro antibiotic sensitivity test:**
It was done according to Finegold and Martin (1982) using Muller Hinton agar (Oxoid) and commercial antibiotic discs (Oxoid and BBL) including Neomycin (30µg), Gentamycin (10 µg), Amoxycillin (25 µg ug), Norfloxacin (10 µg), Stryptomycin (10 µg), Ampicillin (40 µg), Oxytetracyclin (30ug µg), Chloramphenicol (30 µg), Ceftiofur (30 µg), Doxycycline (30 µg), and Erythromycin (15 µg).
6 - Detection of virulence factors:
1 – Congo red binding activity (Agenta et al., 1977). *Salmonella* strains were cultured on Congo medium. Congo red positive salmonella isolates were identified by appearance of red colonies. The reaction was best seen after 24 hours incubation at 37°C, followed by an addition of dys at room temperature. Congo red negative *Salmonella* colonies did not bind the dye (white colonies).
2 – Detection of haemolysin (Tange et al., 1993). *Salmonella* isolates were inoculated into blood agar plates containing 5% sheep blood, after 24 hours of incubation at 37°C positive B- haemolysis production was indicated by clear zone of haemolysis.

RESULTS

Incidence of Salmonella among broiler chicken flocks:
Out of 50 examined broiler chicken flocks, a total of 5 suspected *Salmonella* isolates were recovered. These isolates were subjected to further examination by polyvalent and monovalent *Salmonella* antisera. Five *Salmonella* isolates were obtained and identified as *Salmonella typhimurum*. (Table 1), and the biochemical characteristics of *Salmonella* isolates are illustrated in Table (2). The results of drug sensitivity was studied and summarized in Table (3). which indicated that *Salmonella typhimurum* was found sensitive to Neomycin, Ceftiofur and Gentamycin, while it was resistant to Ampicillin, Erythromycin, Oxytetracyclin and Amoxycillin, and showed variable degrees of sensitivity against other antimicrobial agents. Regarding to virulence activity of *Salmonella* isolates it was found that the examined isolates were bounded with Congo red dye giving red colonies and produce B- haemolycin on blood agar.

Table 1: Incidence of *Salmonella* among broiler chicken flocks.

<table>
<thead>
<tr>
<th>Age of broiler chickens</th>
<th>No. of examined flocks</th>
<th>Positive flocks</th>
<th>No. of isolates</th>
<th>Serotype of salmonella</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td><em>S.typhimurum</em></td>
<td>10</td>
</tr>
<tr>
<td>2nd week</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td><em>S.typhimurum</em></td>
<td>20</td>
</tr>
<tr>
<td>3rd week</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td><em>S.typhimurum</em></td>
<td>10</td>
</tr>
<tr>
<td>4th week</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td><em>S.typhimurum</em></td>
<td>10</td>
</tr>
<tr>
<td>5th week</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td><em>S.typhimurum</em></td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>5</td>
<td>5</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2: Biochemical reactions of suspected *Salmonella* isolates

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+ ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>- ve</td>
</tr>
<tr>
<td>Indol</td>
<td>- ve</td>
</tr>
<tr>
<td>Methyl red</td>
<td>+ ve</td>
</tr>
<tr>
<td>Voges proskauer</td>
<td>- ve</td>
</tr>
<tr>
<td>Citrate</td>
<td>+ ve</td>
</tr>
<tr>
<td>Urease</td>
<td>- ve</td>
</tr>
<tr>
<td>TSI</td>
<td></td>
</tr>
<tr>
<td>Slant</td>
<td>Red</td>
</tr>
<tr>
<td>Butt</td>
<td>Yellow</td>
</tr>
<tr>
<td>H₂S</td>
<td>+ ve</td>
</tr>
<tr>
<td>Sugar fermentation</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>+ positive</td>
<td>- negative</td>
</tr>
</tbody>
</table>

Table 3: Antibiotic sensitivity of isolated *Salmonella*

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neomycin (N 30 µg)</td>
<td>++++</td>
</tr>
<tr>
<td>Gentamycin (GM 10 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Amoxycillin (AML 25 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Norfloxacin (NOR 10 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Stryptomycin (S 10 µg)</td>
<td>++</td>
</tr>
<tr>
<td>Ampicillin (AMB 40 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Oxytetracyclin (T 30 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Chloramphenicol (C 30 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Ceftiofur (CEF 30 µg)</td>
<td>+++</td>
</tr>
<tr>
<td>Doxycilline (DO 30 µg)</td>
<td>++</td>
</tr>
<tr>
<td>Erythromycin (E 15 µg)</td>
<td>+++</td>
</tr>
</tbody>
</table>

DISCUSSION

The incidence of salmonellosis has been increased as a major public health hazard (Rose *et al.*, 1991). Contaminated poultry meat and eggs have been frequently implicated as sources for human *Salmonella* outbreaks in many countries (Humphrey 1999, Gast 1993, and Gast 2001). In the present work 5 - 7 freshly dead or alive bird were collected from each one of 50 broiler chicken flocks to represent as a sample of
the flock and were examined bacteriologically and serologically for isolation and identification of Salmonella. Results were tabulated (Table 1, 2) indicating recognition of 5 Salmonella isolates and serotyped as Salmonella typhimurum. The relation between Salmonella infection and age of broiler flocks (50 flocks) was also investigated. The result revealed that 2 isolates of Salmonella typhimurum were recovered at the 2nd week of age at a percent of 20 %, while 1 isolate as a percent of 10 % was recovered at the 1st, 3rd, and 4th week of age. In Egypt, different Salmonella serotypes were isolated from broiler chicken as Salmonella typhimurum and Salmonella enteritidis (Shahata, 1980 and Abd-Alla, 1991). Salmonella typhimurum was isolated by (Abd El-Hamid et al., 1984) who examined 555 autopsied chicks and isolated Salmonella typhimurum in 26.1%, Ibrahim and Abd El-Lateif (1997) recovered 7 Salmonella isolates which were identified as Salmonella typhimurum from diseased and dead baby chicks (5.6%) while Hegazy (2002) examined 138 broiler flocks and found Salmonella typhimurum at a rate of 5.41%. At the age of 5 weeks in 50 broiler flocks all samples were negative and non of the salmonellae were isolated which could be attributed to either large doses of antibiotics which were used till the 5th week, or to the fact that Salmonella colonization of birds decreased with increasing broiler age. This idea was supported by Barnes et al., (1980), Williams (1984) and Rose et al. (1999). Since, one of the main purpose of such investigation is to find out the most suitable method for control, the antibiogram was carried out against Salmonella typhimurum isolate. The results showed that Salmonella was sensitive to Neomycin, Cetiofur and Gentamycin. However, they were moderately sensitive to Norfloxacin, Chloramphenicol and Doxycylin. Meanwhile, it was resistant to Amoxycillin, Ampicillin, Oxytetracyclin and Erythromycin. The high incidence of resistance could be attributed to the routien usage of subtherapeutic doses of antibiotics as feed additives to raise the feeding efficiency and the rate of weight gain. These findings were supported by Tourfrade (1984) and Coghlan (1991). Regardless of the different sensitivity patterns of Salmonella, the antibiogram is still the valied method for the drug of choice to control Salmonella infection. Abd El-Hamid et al., 1984, Heffernan, 1991 and Hegazy, 2002).

Identification of virulence of Salmonella isolates had led to better understanding of pathogenesis of diarrheal disease caused by them and providing a new dimension to their diagnosis. Our results showed that Salmonella isolate recovered from broiler chicken bind Congo red and pathogenic Salmonella have envolved some unique cellular products
associated with virulence of organism. Also, the result indicated that
Salmonella isolate was hemolysin producer and this could be used as a
phenotypic maker or virulence factor.

These results were supported and in agreement with Gorman and

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