Chromogenic Detection of *Salmonella* from Diarrheic Chickens

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**Abstract**

The present study was conducted to detect *Salmonella* species in fecal samples of chickens suffering from diarrhea in Khartoum State, using enrichment medium and *Salmonella* Chromogenic Medium. A total of 100 cloacal swabs were taken from five open system poultry farms of three layers farms and two broilers farms, 20 samples from each farm. Three farms were located in Khartoum North (Shambat and Alhalfaya localities) and the other two farms were located in Southern Khartoum (Soba and Jabal-Awlia localities). Using the Selenite-f-broth as an enrichment medium for *Salmonella* and a Chromogenic agar designed especially for *Salmonella* detection, a total of 15 *Salmonella* isolates (15%) were recovered. All of the 15 isolates were obtained from one layer chicken farm from Shambat area. The isolates were identified to the species level according to their cultural and biochemical properties to *Salmonella enteritidis*. The high isolation rate (75%) of *Salmonella* from one of Shambat farms was considered as an outbreak of salmonellosis, which was controlled, through this study, by rapid detection of the causative agent.

**Keywords:** Chromogenic detection, *Salmonella* Chromogenic, Diarrheic chickens.

**Introduction**

The members of *Salmonella* genus occupy a position of outstanding importance. Salmonellosis is one of the most common bacterial diseases in the globe and the excessive *Salmonella* infections in humans are food-borne with *Salmonella enteritidis*. *Salmonella pullorum, S. gallinarum* and *S. enteritidis* can infect the ovaries of hens and be transmitted through eggs (Cooper, 1994). Increase in *S. enteritidis* isolation rate in
the north-eastern region of the United States of America identified grade A shell eggs as the dominant vehicle for outbreaks of this infection (Anonymous, 1990). Zhao et al. (2001) reported the occurrence of 1.4 million cases of human salmonellosis in the United States of America.

Orhan and Guler (1993) isolated *Salmonella* from internal organs, cloacal swabs, feed samples and eggs. These strains were identified as *S. gallinarum* (25), *S. enteritidis* (13). While, Pan et al. (1993) reported the isolation of 63 strains of *S. pullorum*. In Sudan, Ezdihar (1996) examined 610 samples from infected chickens and reported the isolation of 14 bacterial genera which included *Salmonella*.

Chromogenic media are a somewhat recent development in the life sciences, having first been used in the late 1980s when the possibilities of selective detection of microorganisms through chromogenic reactions came to be recognized (Poupart et al., 1991). Several studies showed that newly chromogenic media such as Oxoid Salmonella Precis, Merck Salmonella Chromogenic Media and Rambach’s Agar, which have been developed for the detection of *Salmonella* species, have a higher specificity than conventional media. Some are also reported to have a higher sensitivity (Manafi, 2000).

*Salmonella* chromogenic agar is based on the combination of two chromogenic substrates that ease quick identification. These two chromogenes are X-gal and Magenta-caprylate. Xgal is a substrate incorporated to visualize the enzyme β-D-galactosidase producing organisms as blue colonies. Magenta colonies are a result of the hydrolysis of Magenta-caprylate by the *Salmonella* species. Thus, non-*Salmonella* organisms appear blue or are not stained by any of the chromogenes of the medium (Odonoughe, 1993).

Due to at most importance of urgent detection of *Salmonella* in poultry flocks to avoid large losses and spread of the bacteria to man and animals, accurate and rapid technique must be adopted.

The aim of this study was to isolate *Salmonella* from diarrheic chickens in Khartoum State using Salmonella Chromogenic Medium as specific and rapid tool of detection.
Materials and Methods

Sample size:
A total of 100 cloacal swabs were collected, from five poultry farms which located in Khartoum State, 20 samples from each farm.

Salmonella chromogenic medium (Conda Ltd. Spain):
Thirty-seven point one grams of the dehydrated chromogenic medium were suspended in one liter of distilled water and then boiled for one minute. The medium was poured onto Petri-dishes as 20 ml amounts. The plates were kept refrigerated and protected from light.

Results

Table (1): Biochemical testing of *Salmonella* isolates

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th><em>Salmonella enteritidis</em></th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
</tr>
<tr>
<td>Dolcitol</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
</tr>
<tr>
<td>Rhmanose</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
</tr>
<tr>
<td>Malonate</td>
<td>-</td>
</tr>
<tr>
<td>Malonate</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
</tr>
<tr>
<td>Salicin</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>-</td>
</tr>
<tr>
<td>H2S</td>
<td>+</td>
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</tbody>
</table>
Figure (1) Growth of *Salmonella enteritidis* on Salmonella Chromogenic Medium

Discussion

Out of five chicken farms in remote areas of the State, *Salmonella* was found in only one farm with detection rate of 75% (15 samples out of 20). This finding may indicate that *Salmonella* infections in chicken farms of Khartoum State are sporadic in nature and the infection in the positive farm is an outbreak. This high detection rate of the positive farm was not found reported before in this country. However, many outbreaks in the world were reported before in chickens due to this organism (Anonymous, 1990). Many studies were conducted before in Khartoum State and some other parts of the Sudan, e.g., Yagoab and Mohammed (1987), Ezdiher (1996), Hiba (2007) and Hisham, (2010) and their *Salmonella* detection rate varied between 2.9% and 7.4%, which is considered higher compared to the four negative farms and much lesser than the detection rate of the positive farm. The 15 isolates of *Salmonella* were identified as *Salmonella enteritidis*. This species was isolated before from chickens in Khartoum North (Hisham, 2010). So, its isolation is not unexpected. This species was also world-wide reported to be isolated
from chickens (Anonymous, 1990; Orhan and Guler, 1993; and Barrow et al., 2003). Chromogenic media allowed the differentiation of microorganisms, as different colonies appear in different colors. This is based on the presence of various chromogenic substrates in the media. When these substrates are cleaved by specific enzymes that are produced by specific organisms, chromogenic substrates (which are colorless) produce a characteristic color. The detection of a specific enzyme(s) can be used to identify genus, species, or groups of microorganisms. Colonies of microorganisms appear colored, providing simple recognition (Ronald, 2006). The utility of the Salmonella Chromogenic Medium as specific and time-saving method for the detection and presumptive identification of *Salmonella* species was demonstrated. It was used after enrichment in selenite-f-broth, but it can directly be used if number of cells in the clinical sample is expected to be high. Because the medium is designed to isolate *Salmonella* with high specificity and sensitivity, the result obtained is considered true. The *Salmonella* detection potential of this medium is based on the presence of the Chromogenic substrate, Magenta-caprylate which is hydrolyzed by *Salmonella* species to produce magenta colonies. This is beside X-gal which was incorporated to visualize β-glactosidase-producing organisms as blue colonies. The Chromogenic mixture plus sodium citrate inhibit Gram-positive bacteria, *Proteus* and *Coliforms* (Miles et al., 1992). This type of medium is highly recommended to be used by non-expert hands as it depends on observation of specific color of colonies. It can be said that one day is enough to start treatment, either after direct culture on this medium or after enrichment and sub-culture on the solid Chromogenic medium. This medium is more selective and the colonies are specially colored compared to conventional media. The owner of the positive farm was extremely happy after informing him, two days post sampling that indicted the causative agent is *Salmonella enteritidis*. Odonoughe (1993) found more specific and sensitive detection of *Salmonella* from foods for Salmonella Chromogenic media compared to conventional ones. Determination of *Salmonella* to the species level took one day using few sugar fermentation tests. The biochemical results showed that the *Salmonella* isolates belong to one strain.
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References


