Preliminary Study for Detection of Salmonella Species Isolated from Luncheon
Abdelazeem M. Algammal1, Abdallah A. Shehata2,*, Ahmed A. Abdelrehim2, and Mohamed A. Salem3

1 Department of Bacteriology, Immunology, and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
2 Sokhna labs for imports and exports – Royal labs, Suez, Egypt.
3 Animal Medicine Department (infectious diseases), Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

Abstract
Salmonella, members of the family Enterobactreciae, are the most dangerous of the pathogenic microorganisms in the intestinal tracts and are responsible for the occurrence of a significant number of food-borne diseases throughout the world for a great many people. This study aimed to determine the prevalence of Salmonella spp. in retail luncheon meat, microscopically, bacteriologically, and biochemically examination, serotyping of Salmonella isolates for detection, and the identification of Salmonella spp. in luncheon samples. Out of 40 luncheon meat samples, three were positively identified as Salmonella, with a prevalence of 7.5 % (3/40). Salmonella isolates were serologically identified as S. Typhimurium. All confirmed Salmonella isolates exhibited red colonies with a black center on XLD agar. Catalase, methyl-red, citrate utilization, lysine, ornithine, and H2S generation tests were positive in all isolates and negative for indole, oxidase, Voges-Proskauer, and urease and Salmonella have the ability to ferment glucose, mannitol, and xylose.

Key word: Salmonella, Typhimurium, luncheon, identification

Introduction
Meat is high in nutrients necessary for microorganism growing that can become contaminated from various causes, including handling, the environment, human manipulation, and/or the animal itself (El-Gendy et al., 2014). Carcass contamination is affected by several factors, including transport stress, hygienic level, and time spent in lairages during slaughter (Marritto and Gravani, 2006; Algammal et al., 2020d).

Despite advances in technology and sanitary standards in advanced countries at all steps of beef and
poultry meat production, food-borne illnesses continue to pose a hazard to human and animal health. The most common Enterobacteriaceae members that cause food-borne infections are E. coli and S. enterica serovars (Moawad et al., 2017). According to food safety guidelines, contaminated with Salmonella are unsafe for consumption (Agunos, 2007). People still die from typhoid fever, the most dangerous type of human salmonellosis, in developing countries where sanitation and hygiene standards are lacking (Dougan et al., 2011) and (Algammal et al., 2020c). According to the World Health Organization, the illness affects more than 90 million individuals worldwide each year, with varying morbidity and mortality rates (Majowicz et al., 2010). S. Typhimurium is a gram-negative bacillus that is aerobic to facultatively anaerobic, motile, and non-sporulated (Delano et al., 2002).

Multidrug resistance has increased worldwide and is reflected a public health threat. Several latest investigations reported the occurrence of multidrug-resistant bacterial pathogens from different origins, increasing the necessity of new powerful and safe alternatives for antibiotics such as probiotics (Enany et al., 2018; Eid et al., 2019; Algammal et al., 2020a; Batia et al., 2021; Hetta et al., 2021; Algammal et al., 2021b; Algammal et al., 2022a). Besides, the routine application of antimicrobial susceptibility testing to detect the antibiotic of choice in addition to the screening of the emerging MDR strains. Therefore, the present study aimed to isolate Salmonella spp. from luncheon as well as biochemical and serological identification of recovered isolates (Eid et al., 2016; Algammal et al., 2019; Algammal et al., 2022; Makharita et al., 2020; Abolghait et al., 2020a; Algammal et al., 2020e; Algammal et al., 2021a; Kareem et al., 2021; Algammal et al., 2022c).

Materials and methods

Sampling

Forty samples of luncheon were randomly collected from different supermarkets in Suez Governorate. All samples were collected under aseptic conditions; each sample was packed in a frozen package from (400-500g) in an icebox and transferred immediately with a minimum delay to a microbiological lab where conventional bacteriological analyses were done.

Isolation of Salmonella

Each sample was placed into 45 ml of nutrient broth in a homogenizer flask (Oxoid, Manchester, UK). The mixture was preserved at room temperature for 15 minutes. Rappaport–Vassiliadis broth (Oxoid) was used for Salmonella. Loops of Rappaport–Vassiliadis broth streaked into xylose lysine deoxycholate (XLD), incubated at 37°C (Oxoid). Plates were incubated at 37 °C for 18–24 h after being
injected with bacteria (Moawad et al., 2017).

Biochemical identification
The suspected colonies were recognized based on colonial characteristics, Gram staining microscopical analysis, and biochemical reactions (catalase test, indole, methyl-red, oxidase test, citrate-utilization, H2S, urease, Voges-Proskauer and fermentation of glucose, mannitol, and xylose).

Serological identification
Slide agglutination tests utilizing marketable antisera (SIFIN, Berlin, Germany) following the Kauffman–White system were used for serotyping all biochemically verified Salmonella isolates. At the Animal Health Research Institute in Dokki, Egypt, and the Bacteriology Laboratory in Cairo, Egypt, the serotyping was performed as described by (Popoff et al., 2000).

Results
Phenotypic characters of recovered Salmonella species isolated from luncheon samples:
A. Colonial appearance:
Salmonella is grown on XLD agar with a slightly transparent zone of reddish color with a black center, as illustrated in figure (1).

B. Microscopical examination:
Salmonella isolates were Gram-negative, medium-sized bacilli, arranged singly, in pairs, and groups, and they were non-spore-forming.

C. Biochemical identification
All Salmonella isolates gave violet slant and butt color with H2S production (black color) on Lysine iron agar, were negative urea agar with acid butt (yellow color) and alkaline slant with H2S production (black coloration) with gas production on TSI agar, as shown in table (1).

Occurrence of Salmonella spp. in luncheon samples:
The Prevalence of Salmonella isolates based on conventional methods and biochemical tests, 7.5% (3/40) isolates were retrieved from the examined 40 luncheon samples.

Serotyping of Salmonella isolates
Salmonella isolates were serologically recognized as follows, Salmonella enterica serovar Typhimurium.

![Fig. (1): Suspected typical Salmonella colonies on XLD. -Slightly transparent zone of reddish color with black center.](image-url)
Table (1): Biochemical reactions of Salmonella isolates:

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Salmonella spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Positive</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Positive</td>
</tr>
<tr>
<td>H2S</td>
<td>Positive</td>
</tr>
<tr>
<td>Glucose</td>
<td>Positive</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Positive</td>
</tr>
<tr>
<td>Xylose</td>
<td>Positive</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative</td>
</tr>
<tr>
<td>Urease</td>
<td>Negative</td>
</tr>
<tr>
<td>Oxidase</td>
<td>Negative</td>
</tr>
<tr>
<td>Catalase</td>
<td>Positive</td>
</tr>
<tr>
<td>VP</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Discussion

*Salmonella* species are the most frequent cause of food-borne illnesses in various countries (Switt et al., 2009). Every year, *Salmonella* outbreaks cause great economic losses because of hospitalization, medical treatment, and reprocessing or recall of contaminated food (Germini et al., 2009). *S. Typhimurium* was involved several times in food poisoning outbreaks in Egypt due to consuming meat and meat products (Ramadan and Sadek, 1971). These results agreed with the (Garai et al., 2012) that reported *Salmonella Typhimurium* occurred more and was more widely distributed than any other serovars; this organism caused severe outbreaks of salmonellosis in all kinds of animals and was frequently the cause of both periodic cases and outbreaks of gastroenteritis in man all over the world. Also, these results were agreed with (Herikstad et al., 2002), who stated that *Salmonella Typhimurium* and *Salmonella Enteritidis* are the most commonly isolated serovar from food-borne outbreaks all over the world. The present study found a lower prevalence of *Salmonella* (7.5%) than (Ruban et al., 2010), who found 31.99% of *Salmonella*, and (Abo Hashem et al., 2022), who detected the prevalence rate was 8.3%. (Essa et al., 2009) isolated four strains of *S. Typhimurium* were detected in the tested beef samples and (Saad et al., 2018), who isolated *S. Typhimurium* (4%) in the Luncheon. According to (Fallah et al., 2013), a higher occurrence of *Salmonella* was found (44 %). It is obvious from the previous results that the *Salmonella* spp. appear to be high, which attracts our attention to the contamination from enteritis sources, and it can prove enteric contamination (El-Gendy et al., 2014).

In Egypt, the predominant serotype differs from one geographic area to another. This may be due to contamination during production, handling, packing, and storage (Rabei et al., 2012).

In conclusion, luncheon is one of processed meat products which concern favorable media for the growth of *Salmonella* spp. The most common *Salmonella* serovars which
contaminated processed meat products is S. Typhimurium.

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唾液中的研究关于从鸡只肉中分离的沙门氏菌

研究目的

沙门氏菌是导致胃肠道疾病的最危险的非致病性细菌，属于Enterobactrecia科，是造成全世界大量人群患病的主要原因。本研究旨在通过显微镜、细菌学和化学方法检测和鉴定从零售鸡只肉中分离的沙门氏菌。在从40个样本中筛选出的3个阳性样本中，7.5%(3/40)为沙门氏菌。通过显微镜观察，这些菌株具有红色中央黑色的特征。通过Catalase、methyl-red、citrate、lysine、ornithine、H2S、Voges-Proskauer和XLD等试验，所有阳性菌株均为阳性，且XLD试验呈阴性。值得注意的是，所有阳性菌株均能利用葡萄糖、麦芽糖和木糖，但不能利用乳糖、甘露糖、山梨糖醇、L-鸟氨酸、乳酸和尿酸。沙门氏菌

阿拉伯文翻译

دراسة مبدئية للكشف عن عزلات السالمونيلا المعزولة من اللانشون

تعتبر السالمونيلا أخطر الكائنات الحية الدقيقة المسببة للأمراض في الأمعاء، التي تنتمي إلى Enterobactrecia عائلة Enterobacteriales. السالمونيلا هي مسببة لفيروسات كهرباء، كما أنها مسؤولة عن حدوث عدد كبير من الأمراض التي تنتقلها الغذاء في جميع أنحاء العالم لعدد كبير من الناس. هدفت الدراسة إلى تحديد وجود بكتيريا في لحوم اللانشون بالتجزئة، من خلال الفحص المجهري والبكتريولوجي والكيميائي الحيوي، التمديد المصلي لعزلات السالمونيلا للكشف، والتعرف على السالمونيلا. في عينات الغداء، من بين 40 عينة من لحوم اللانشون، تم تحديد ثلاث عينات بشكل إيجابي على أنها السالمونيلا، مع انتشار 7.5% (3/40). تم التعرف على عزلات السالمونيلا مصليا كما يلي: Salmonella enterica serovar Typhimurium. أظهرت جميع عزلات السالمونيلا المؤكدة مستعمرة حمراء مع مركز أسود على XLD. كانت اختبارات Catalase وmethyl-red وcitrate وlysine وأي indeole وVoges-Proskauer إيجابية في جميع العزلات، ولكن L-ornithine وH2S كانت سلبية. لأغزب على Salmonella.