
***Salmonella enterica* Isolated from Diseased Broiler Chickens**

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Abstract

Salmonella infection in poultry is a problem for producers because it reduces flock performance. At the same time, *salmonella*-contaminated animal products endanger human health. *Salmonellae* is a genus in the Enterobacteriaceae family of gram-negative, rod-shaped, glucose-fermenting facultative anaerobes with motile peritrichous flagella. **In this study**, 100 cloacal swabs were collected from diseased broiler farms showing diarrhoea and depression at various intervals. Morphologically identified *Salmonella* appeared as smooth red colonies with a black centre on (XLD) media, while on McConkey agar appeared as pale, colourless smooth transparent colonies. Citrate Utilisation, Methylene Red, and Lysine Iron Agar showed positive results, whereas Oxidase, Urea, Indole, and Voges-Proskauer showed negative results.

Keywords: *Salmonella*, broiler, cloacal swabs

Introduction

Salmonella spp. is a bacterium that can cause Salmonellosis in humans and animals. Contact with the environment or infected things near poultry farms may result in indirect transmission. (Effendi et al., 2020). *Salmonella* is a Gram-negative, facultative anaerobic bacteria that is phylogenetically clustered in the Enterobacteriaceae family. Most *Salmonella* is motile,

with the exception of the poultry-specific serovars *Salmonella Gallinarium* and *Salmonella Pullorum* (Shafiullah et al., 2016). Also, *Salmonella* contamination has been reported in Spanish poultry products (Capita et al., 2003). Chicken carcasses and chicken parts (wings, legs, and giblets—liver and heart) were detected at a rate of 49% on average. *Salmonella Enteritidis*, *Salmonella Poona*,

Salmonella Paratyphi B, and *Salmonella Worthington* were isolated from 34.3%, 11.4%, 2.8%, and 1.4% of the samples, respectively. **Caffrey et al. (2021)** stated that the prevalence of antimicrobial resistance to antimicrobials deemed relevant to human health, as well as the incidence of *Salmonella* serovars in grill chicken and turkey flocks across Canada Between 2013 and 2018, 1,596 *Salmonella* isolates from 514 grill chicken flocks and 659 isolates from 217 turkey flocks were collected. **Cocciolo et al. (2020)** stated that *Salmonella* infection causes diarrhoea, anorexia, ruffled feathers, and pale combs, and patients can die within four days. Aside from death, decreased feed consumption and egg production are common. At necropsy, the liver is enlarged, bronze greenish, and friable, with scattered necrotic foci. **Logue et al. (2002)** reported that Rappaport-Vassiliadis broth is used for *Salmonella* enrichment, which is incubated for 24 hours at 42°C. After incubation, a loopful of the enrichment culture was streaked out on Brilliant Green agar to isolate *Salmonella* spp. and look for *Salmonella* with characteristic colonial morphology (purple colonies with a black centre) (**Goud and Pandey, 2021**). To confirm the diagnosis, biochemical and serological assays are used. Among the biochemical assays are sugar fermentation, decarboxylation and

dehydrogenation processes, and hydrogen sulphide formation, **Nghiem et al. (2019)** reported that A biochemical test (lactose, glucose, hydrogen sulphide formation, urease, lysine decarboxylase, and Indole) confirmed the presence of *Salmonella* colonies.

Materials and methods

Sampling

100 cloacal swabs were obtained from broiler farms of different ages (4-6 weeks) from diseased chickens at Giza governorates, Egypt.

Isolation of *Salmonella*

For *Salmonella* isolates, one millilitre of each sample was inoculated into 9 millilitres of Rappaport Vassiliadis broth. After 24-48 hours of aerobic incubation at 37°C, a loopful of the cultivated Rappaport Vassiliadis broth was streaked onto the surface of MacConkeys agar and XLD. For 24-48 hours, the inoculated plate was incubated aerobically at 37°C..

Biochemical identification

The obtained bacterial isolates were identified using the following tests: (Sugar fermentation test, Oxidase test, indol test, methyl red test, citrate test, vogues Proskauer, urea hydrolysis, triple sugar iron).

Serological identification

Salmonella isolates that were preliminary identified biochemically, were subjected to serological identification according to **Kauffmann and Das Kauffmann (2001)**.

Results

A.Colonial appearance

- *Salmonella* colonies with diameters of 2-4 mm appeared on (XLD) media as smooth red colonies with black centres due to H₂S production, whereas on Macconkey agar as pale, colourless smooth transparent and raised colonies (non-lactose fermenter)., **as shown in figure (1, A and B)**

B. Microscopical examination

The staining characters take the form of Gram-negative, short rods.

C. Biochemical identification

- Table(1) shows the biochemical identification of isolated *Salmonella*

spp. Lysine iron agar, citrate utilisation, and methyl red tests were all positive for all suspected *S.enterica* isolates. The oxidase, urea, indole, and voges-proskauer tests all came back negative for suspected isolates.

Prevalence of *S.enterica* serovars from poultry meat

A total of 8 *S.enterica* isolates were recovered from 100 bacteriologically examined cloacal swabs samples of broiler chickens. The percentage of *S.enterica* was 8% (8/100) in the examined chickens.

Table (1): Results of biochemical identification of isolated Salmonella spp.

Test	Result of reaction
Oxidase	-ve (colourless).
Urea	-ve (yellow color).
Triple Sugar Iron Agar	+ve (red slant, yellow Butt with H ₂ S&gas production).
Lysine Iron Agar	+ve (deep purple slant, and alkaline butt, no gas production& H ₂ Sproduction)
Indole	-ve (no change in broth)
Citrate Utilization	+ve (the medium converted from green neutral to blue)
Methyl Red	+ve (red color at the surface)
Voges-Proskauer	-ve (no bright red color)



Figure 1-(A): Typical colonies of *S. enterica* on XLD agar: Smooth red colonies with black centers due to H₂S production

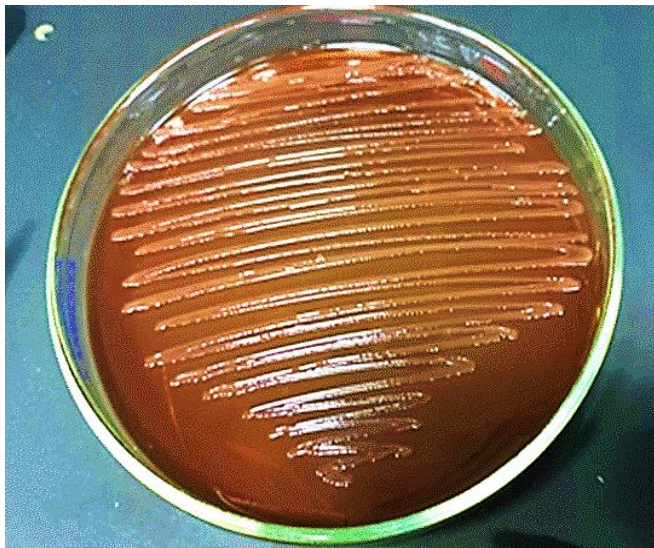


Figure (1),(B): Typical colonies of *S. enterica* on MacConkey agar: colorless smooth transparent colonies (non-lactose fermenter)

Discussion

Salmonella has been identified as a leading cause of foodborne illness worldwide, with an annual economic loss of \$3.7 billion

(Nidaullah et al., 2017). Since 1950, the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) have declared *Salmonella* to

be the most common and important zoonosis. This has resulted in its inclusion in the World Animal Health Organization's terrestrial animal health code (*Moultotou et al., 2015*). This study supports the findings of (*Attia et al., 2020*), who isolated *Salmonella* from 180 chicken meat and organ samples tested for *Salmonella*, with 15 (8.3%) positive and 165 (91.7%) negative for *Salmonella* isolation. These findings differed from those of *Mir et al. (2015)*, who discovered that 24 of the 32 isolates.

Conclusion

1- *Salmonella* spp are important pathogens of broiler chickens in Egypt.

2- *Salmonella enterica* is the most predominant spp isolated from broiler chickens.

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السالمونيلا المعوية المعزولة من دجاج التسمين المريض
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الملخص العربي

عدوى السالمونيلا في الدواجن مشكلة للمنتجين بسبب فقد أداء القطيع. في الوقت نفسه ، تشكل المنتجات الحيوانية الملوثة بالسالمونيلا تهديدًا خطيرًا على صحة الإنسان. السالمونيلا هو جنس في عائلة Enterobacteriaceae. وهي عبارة عن كائنات لاهوائية اختيارية سالبة الجرام ، على شكل قضيب ، تخمر الجلوكوز مع سوط صخري يسمح لها بالتحرك. في هذه الدراسة تم أخذ عينة من مزارع تسمين تظهر عليها حالات الإسهال على فترات مختلفة. ظهرت السالمونيلا على شكل مستعمرات حمراء ناعمة مع مركز أسود على وسط (XLD) ، بينما تظهر على أجار macconkey شاحب ، عديم اللون ناعم وشفاف ، تظهر أحرف التلوين على أنها سالبة الجرام. تم التعرف على عزلات السالمونيلا مصليا ، و باستخدام Citrate Utilization ، Methyle Red ، Lysine Iron Agar ، و Triple Sugar Iron agar كانت النتائج موجبة بينما كانت سلبية في كل من Oxidase و Urea و Indole و Voges-Proskauer.