Preliminary Study for Detection of *Salmonella* Species Isolated from Luncheon

Abdelazeem M. Algammal[1], Abdallah A. Shehata[2*], Ahmed A. Abdelrehim[2], and Mohamed A. Salem [3]

[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 *Enterobactreciea*, 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; Algammal et al., 2022b)***. The most common sources of environmental contamination are air and water, but it also includes vehicles, insects, dust, rodents, dirty floors, tables, holding pens, equipment, and knives ***(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; Batiha 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., 2022b; 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 H_2S 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.

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

Biochemical tests	*Salmonella* spp.
Lysine	Positive
Ornithine	Positive
H2S	Positive
Glucose	Positive
Mannitol	Positive
Xylose	Positive
Indole	Negative
Urease	Negative
Oxidase	Negative
Catalase	Positive
VP	Negative
Citrate	Positive

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*.

References

Abo hashem, M.E., Enany, M.E., Ahmed, A.M, Huda, E.I. and Elsharawy, N.T. (2022): Estimation of potential bacteriological hazards and their genetic virulence determinants in beef meals provided to intensive care patients. Food Research Journal. 6 (2): 286 – 293.

Abolghait, S. K., Fathi, A. G., Youssef, F. M., & Algammal, A. M. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. International journal of food microbiology, 328, 108669.

Agunos A (2007) Effect of dietary beta1-4 mannobiose in the prevention of *Salmonella enteritidis* infection in broilers. Br Poult Sci 48: 331-341.

Algammal, A. M., Alfifi, K. J., Mabrok, M., Alatawy, M., Abdel-Moneam, D. A., Alghamdi, S., ... & El-Tarabili, R. M. (2022c). Newly Emerging MDR *B. cereus* in Mugil seheli as the First Report Commonly Harbor *nhe*, *hbl*, *cyt*K, and pc-plc Virulence Genes and *bla*1, *bla*2, *tet*A, and *erm*A Resistance Genes. Infection and Drug Resistance, 15, 2167-2185.

Algammal, A. M., El-Sayed, M. E., Youssef, F. M., Saad, S. A., Elhaig, M. M., Batiha, G. E., ... & Ghobashy, M. O. (2020a). Prevalence, the antibiogram and the frequency of virulence genes of the most predominant bacterial pathogens incriminated in calf pneumonia. AMB Express, 10(1), 1-8.

Algammal, A. M., El-Tarabili, R. M., Alfifi, K. J., Al-Otaibi, A. S., Hashem, M. E. A., El-Maghraby, M. M., & Mahmoud, A. E. (2022b). Virulence determinant and antimicrobial resistance traits of Emerging MDR Shiga toxigenic E. coli in diarrheic dogs. AMB Express, 12(1), 1-12.

Algammal, A. M., Hashem, H. R., Alfifi, K. J., Hetta, H. F., Sheraba, N. S., Ramadan, H., & El-Tarabili, R. M. (2021a). *atp*D gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-*Proteus mirabilis*. Scientific reports, 11(1), 1-15. https://doi.org/10.1038/s41598-021-88861-w

Algammal, A. M., Hetta, H. F., Batiha, G. E., Hozzein, W. N., El Kazzaz, W. M., Hashem, H. R., ... & El-Tarabili, R. M. (2020d). Virulence-determinants and antibiotic-resistance genes of MDR-*E. coli* isolated from secondary infections following FMD-outbreak in cattle. Scientific reports, 10(1), 1-13.

Algammal, A. M., Hetta, H. F., Elkelish, A., Alkhalifah, D. H. H., Hozzein, W. N., Batiha, G. E. S., ... & Mabrok, M. A. (2020b). Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infection and Drug Resistance, 13, 3255.

Algammal, A. M., Mabrok, M., Ezzat, M., Alfifi, K. J., Esawy, A. M.,

Elmasry, N., & El-Tarabili, R. M. (2022a). Prevalence, antimicrobial resistance (AMR) pattern, virulence determinant and AMR genes of emerging multi-drug resistant *Edwardsiella tarda* in Nile tilapia and African catfish. Aquaculture, 548, 737643. https://doi.org/10.1016/j.aquaculture.2021.737643

Algammal, A. M., Mabrok, M., Sivaramasamy, E., Youssef, F. M., Atwa, M. H., El-Kholy, A. W., ... & Hozzein, W. N. (2020a). Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor *opr*L and *tox*A virulence genes and *bla*TEM, *bla*CTX-M, and *tet*A antibiotic-resistance genes. Scientific reports, 10(1), 1-12.

Algammal, A. M., Mohamed, M. F., Tawfiek, B. A., Hozzein, W. N., El Kazzaz, W. M., & Mabrok, M. (2020e). Molecular typing, antibiogram and PCR-RFLP based detection of *Aeromonas hydrophila* complex isolated from *Oreochromis niloticus*. Pathogens, 9(3), 238.

Algammal, A. M., Wahdan, A., & Elhaig, M. M. (2019). Potential efficiency of conventional and advanced approaches used to detect Mycobacterium bovis in cattle. Microbial pathogenesis, 134, 103574.

Algammal, A.M., Hashem, H.R., Al-otaibi, A.S. et al (2021b)**.** Emerging MDR-Mycobacterium avium subsp. avium in house-reared domestic birds as the first report in Egypt. BMC Microbiology 21, 237. https://doi.org/10.1186/s12866-021-02287-y

Batiha, G. E. S., Hussein, D. E., Algammal, A. M., George, T. T., Jeandet, P., Al-Snafi, A. E., ... & Cruz-Martins, N. (2021). Application of natural antimicrobials in food preservation: Recent views. Food Control, 126, 108066.

Delano M.L., Mischler A.S. and Underwood J.W. (2002): Biology and Diseases of Ruminants: Sheep, Goats, and Cattle. Underwood, in Laboratory Animal Medicine (Second Edition). Pages 519-614

Dougan G, John V, Palmer S, Mastroeni P (2011) Immunity to salmonellosis. Immunol Rev 240: 196-210.

Eid HM, Algammal AM, Elfeil WK, Youssef FM, Harb SM, Abd-Allah EM (2019): Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks, Veterinary World, 12(5): 677-683.

Eid, H. I., Algammal, A. M., Nasef, S. A., Elfeil, W. K., & Mansour, G. H. (2016). Genetic variation among avian pathogenic E. coli strains isolated from broiler chickens. Asian J. Anim. Vet. Adv, 11(6), 350-356.

El-Gendy et. al. 2014.*Enterobacteriaceae* in Beef Products from Retail Outlets in Alexandria/ Alexandria Journal of Veterinary Sciences, 41: 80-86

Enany, M. E., Algammal, A. M., Shagar, G. I., Hanora, A. M., Elfeil, W. K., & Elshaffy, N. M. (2018). Molecular typing and evaluation of Sidr honey inhibitory effect on virulence genes of MRSA strains isolated from catfish in Egypt. Pakistan journal of pharmaceutical sciences, 31(5).

Essa, H. H., Manaa, A. M., Makar, N. H., & Sayed, S. M. (2009). Studies on *Salmonella* and *E. coli* in some meat products (beef burgers and luncheon) sold in Assiut city. Assiut Veterinary Medical Journal, 55(121), 126-135.

Fallah S.H, Asgharpour F, Naderian Z, Moulana Z(2013). Isolation and determination of antibiotic resistance patterns in nontyphoid *Salmonella* spp isolated from chicken. Int. J. Entric Pathog. 1(1):17–21.

Garai, P., Gnanadhas, D. P., & Chakravortty, D. (2012). *Salmonella enterica serovars Typhimurium* and *Typhi* as model organisms: revealing paradigm of host-pathogen interactions. Virulence, 3(4), 377-388.

Germini, A., Masola, A., Carnevali, P. & Marchelli, R. (2009). Simultaneous detection of *Escherichia coli* O175:H7, Salmonella spp., and *Listeria monocytogenes* by multiplex PCR. Food Control, 20, 733–738

Herikstad, H., Motarjemi, Y., & Tauxe, R. (2002). *Salmonella* surveillance: a global survey of public health serotyping. Epidemiology & Infection, 129(1), 1-8.

Hetta, H.F., Al-Kadmy, I.M.S., Khazaal, S.S. et al (2021). Antibiofilm and antivirulence potential of silver nanoparticles against multidrug-resistant *Acinetobacter baumannii*. Sci Rep 11, 10751. https://doi.org/10.1038/s41598-021-90208-4

Kareem, S. M., Al-Kadmy, I. M., Kazaal, S. S., Ali, A. N. M., Aziz, S. N., Makharita, R. R., ... & Hetta, H. F. (2021). Detection of *gyr*A and *par*C mutations and prevalence of plasmid-mediated quinolone resistance genes in *Klebsiella pneumoniae*. Infection and Drug Resistance, 14, 555.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM (2010) The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50: 882-889

Makharita, R. R., El-Kholy, I., Hetta, H. F., Abdelaziz, M. H., Hagagy, F. I., Ahmed, A. A., & Algammal, A. M. (2020). Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incriminated in healthcare-associated infections. Infection and drug resistance, 13, 3991.

Marritto, N.G., Gravani, R.B. (2006). Principles of food sanitation 5th edition, 2006. Springer Science Business Media. Inc

Moawad AA, Hotzel H, Awad O, Tomaso H, Neubauer H, Hafez HM, El-Adawy H (2017). Occurrence of *Salmonella enterica and Escherichia coli* in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. Gut Pathog. 18;9:57. doi: 10.1186/s13099-017-0206-9. PMID: 29075329; PMCID: PMC5648511.

Popoff MY, Bockemuhl J, Gheesling LL. Supplement (2002) (no. 46) to the Kauffmann–White scheme. Res Microbiol. 2004;155:568–570. doi: 10.1016/j.resmic.2004.04.005.

Rabie, N.S., Khalifa, N.O., Radwan, M.E.I. and Afify, J.S.A. (2012). Epidemiological and Molecular Studies of *Salmonella* Isolates from Chicken, Chicken Meat and Human in Toukh,

Egypt. Global Veterinarian 8(2):128-132.

Ramadan, K., & Sadek, L. (1971). Parameters of salmonellosis in Egypt. Vet. Med. Ass, 31, 193-218.

Ruban S.W, Thiyageeswaran M, Sharadha R. Isolation and identification of *Salmonella spp*. from retail chicken meat by polymerase chain reaction. Int. J. Microbiol. Res. 2010;1(3):106–109.

Saad, M.S, S., Hassan, M. A., Abou El Ros, N., & Abou Arayes, W. A. (2018). Prevalence of *Salmonella* and *Escherichia Coli* Organisms as Bacteriological Hazards in some Meat Products. Benha Veterinary Medical Journal, 34(3), 150-157.

Switt, A.I.M., Soyer, Y., Warnick, L.D. & Wiedmann, M. (2009). Emergence, distribution and molecular and phenotypic characteristics of *Salmonella enterica serotype* 4,5,12: i: -. Food-borne Pathogens and Disease, 6, 407–415.

دراسة مبدئية للكشف عن عترات السالمونيلا المعزوله من اللانشون

الملخص العربي

تعتبر السالمونيلا أخطر الكائنات الحية الدقيقة المسببة للأمراض في الأمعاء، التي تنتمي إلى *Enterobactreciea* وهي مسؤولة عن حدوث عدد كبير من الأمراض التي تنقلها الأغذية في جميع أنحاء العالم لعدد كبير من الناس. هدفت الدراسة إلى تحديد وجود بكتيريا *Salmonella spp.* في لحوم اللانشون بالتجزئة ، الفحص المجهري والبكتريولوجي والكيميائي الحيوي ، التنميط المصلي لعزلات السالمونيلا للكشف ، والتعرف على السالمونيلا. في عينات الغذاء. من بين 40 عينة من لحوم اللانشون ، تم تحديد ثلاث عينات بشكل إيجابي على أنها السالمونيلا ، مع انتشار 7.5 ٪ (40/3). تم التعرف على عزلات السالمونيلا مصليا كما يلي *Salmonella enterica serovar Typhimurium*: أظهرت جميع عزلات السالمونيلا المؤكدة مستعمرات حمراء مع مركز أسود على XLD. كانت اختبارات Catalase و methyl-red و citrate و lysine و ornithine و H2S إيجابية في جميع العزلات وسلبية لأوكسيديز و indole و Voges-Proskauer و urease و *Salmonella* لديها القدرة على تخمير الجلوكوز والمانيتول والزيلوز.