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Phenotypic characterization of various Salmonella species isolated from food products

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ABSTRACT*: *Salmonella is recognized as the primary pathogenic bacterium responsible for foodborne diseases on a global scale, and the rise in salmonella infections is associated with the proliferation of antimicrobial-resistant strains. In this research, a total of 50 meat items were acquired from various supermarkets in Sharkia Province, Egypt, and subjected to microbiological analysis to assess the prevalence of salmonellosis infection. The findings revealed a prevalence rate of 26%, with the S. Typhimurium serotype being the most common, followed by S. Enteritidis and S. Virchow. All Salmonella strains demonstrated susceptibility to ceftriaxone (100%), while 61.5% of the isolates exhibited multidrug resistance (MDR). There was notable resistance observed against ceftazidime and ampicillin-sulbactam. In summary, this study provided a comprehensive overview of the incidence, drug resistance patterns, and phenotypic characteristics of various salmonella serovars found in food products.

KEYWORDS: ***Antimicrobial susceptibility, Meat products, Salmonella***.

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I. INTRODUCTION

Salmonella are Gram-negative bacteria with a rod-shaped (bacilli) and motile structure belonging to the Enterobacteriaceae family. The majority of non-typhoidal salmonella serovars are responsible for conditions like gastroenteritis, bacteremia, and localized infections. Human transmission is primarily associated with the consumption of contaminated animal products, including poultry, pork, and various types of meats. Additionally, direct contact with animals such as chicks, ducklings, and other potential carriers can serve as another means of transmission, with these animals potentially transferring the bacterium to humans (*Vora et al. 2012*).

Meat products play a crucial role in the transmission of salmonella. The primary sources of salmonellosis in humans often involve commonly contaminated foods like poultry, beef, chicken, turkey, pork, eggs, milk, and derived products (*Capita et al. 2003 and Ray 2004*). Among humans, the leading contributors to nontyphoidal Salmonella infections are typically poultry, eggs (which can become contaminated through infected ovaries), and various meat products (*Ray 2004 and Schlosser et al. 2000*). Salmonella contamination of food can occur at any stage along the entire journey from farm to table, encompassing production, processing, distribution, retail marketing, as well as handling or preparation (*Cui et al. 2006*).

Salmonella infections can be classified into two main categories: typhoidal salmonellosis (TS or enteric fever) and non-typhoidal Salmonella (NTS) related infections (*Akinyemi et al. 2021 and Ngogo et al. 2020*). Typhoid fever is caused by Salmonella enterica serovar Typhi, whereas S. Paratyphi A, B, and C are responsible for human host-restricted paratyphoid fever (Prasertsee et al. 2019). NTS infections, on the other hand, are attributed to various serovars of S. enterica (*Ngogo et al. 2020*). Examples of serovars that can lead to diseases in humans through contaminated food and food products include Salmonella Enteritidis, S. Cholerasuis, and S. Typhimurium (*Thung et al. 2018).*

Invasive typhoidal salmonellosis manifests as enteric fever, gastroenteritis, and bacteremia (*Lauteri et al. 2022*). In contrast, non-typhoidal salmonellosis primarily presents as gastroenteritis, a condition that affects the ileum and colon, leading to symptoms such as diarrhea, abdominal cramps, and vomiting (*Gong et al. 2022*).

The global rise in foodborne infections associated with antimicrobial-resistant pathogenic microorganisms and the dissemination of antimicrobial resistance (AR) is a major concern in countries worldwide *(Prasertsee et al. 2019*). Currently, the emergence and spread of antimicrobial resistance in zoonotic salmonella strains pose a significant public health threat (*Jajere 2019*). Of particular concern, salmonella strains with "clinically significant resistance" to certain agents like extended spectrum cephalosporins and fluoroquinolones have been found in livestock (*Li et al., 2013*). In many developing nations, the inappropriate use and excessive use of antibiotics have contributed to the growing prevalence of multidrug resistance in Salmonella (*Borah et al., 2021*).

Salmonella, along with their resistance genes, can easily be transmitted to humans through the food chain, leading to food poisoning. Therefore, the objective of the current study is to identify the phenotypic characteristics of salmonella isolates from various food products.

Materials and Methods

Sampling

Procuring a combined total of 50 meat products, equally distributed between sausages and minced meat (25 each), involved visiting various supermarkets within El-Sharika governorate's Zagazig city. The collection process adhered to strict hygiene standards, where samples were carefully gathered into sterile polyethylene bags, meticulously labeled, and promptly transported to the laboratory for thorough microbiological analysis.

Isolation and Identification

Following the recommended guidelines of the International Organization for Standardization (*ISO 6579 2002*), Salmonella isolation procedures were meticulously executed. Initially, a 25 g portion of the bacterial sample underwent pre-enrichment in buffered peptone water (BPW) at a temperature of 37°C for an overnight incubation. The enriched samples were subsequently inoculated onto modified semi-solid Rappaport–Vassiliadis (MSRV) and incubated at 42°C for 24 hours. A loopful of positive growth, extracted from the MSRV colony, was further transferred to both MacConkey's agar and xylose lysine deoxycholate (XLD) agar plates and left in an incubator overnight. These obtained colonies were then subjected to a series of biochemical tests, including triple sugar iron agar, Urea hydrolysis test, and Lysine decarboxylase test (*Quinn et al. 2002*).

For serotyping, Salmonella isolates were sent to the Serology Unit of the Animal Health Research Institute located in Doki, Giza, Egypt. Commercial antisera (Difco, Detroit, MI, USA) were employed in accordance with the manufacturer's instructions to determine the serotypes.

Antimicrobial susceptibility testing

To determine the antimicrobial susceptibility of the Salmonella isolates to a range of antibiotics, we employed the Kirby-Bauer disc diffusion method (2) on Trypticase soy agar (TSA) with commercially available discs. The panel of antimicrobial agents encompassed chloramphenicol (C, 30 µg), amikacin (AK, 30 µg), gentamicin (CN, 10 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), ofloxacin (OFX, 5 µg), amoxicillin (AX, 25 µg), and ampicillin/Sulbactam (SAM, 10/10 µg). Incubation of the plates occurred over a period of 16–24 hours at 37°C.

Subsequently, we measured the zones of growth inhibition surrounding each antibiotic disc, recording the values to the nearest millimeter. These zone diameters served as indicators of the isolate's susceptibility and the drug's diffusion rate within the agar medium. The interpretation of the zone diameters for each drug followed the criteria established by the Clinical and Laboratory Standards Institute in 2013.

Results

Isolation and identification of Salmonella isolates

1. **Colonial and biochemical appearance**

On MacConkey agar, salmonella colonies appear colorless and transparent (though they sometimes have dark centers) due to the lack of lactose fermentation which is of great importance in differentiating Salmonella from other bacteria present in the specimen. On XLD medium the majority of Salmonella serotypes produce hydrogen sulfide and have red colonies with a black (H2S) center (**Figure 1**). Positive triple sugar iron test, negative urease, and positive lysine decarboxylase.

2. **Prevalence and serotyping data**

Different Salmonella species were isolated with a total prevalence rate of 26% (13/50) with predominance for *the S.* Typhimurium serotype as illustrated in **Figure 2**.

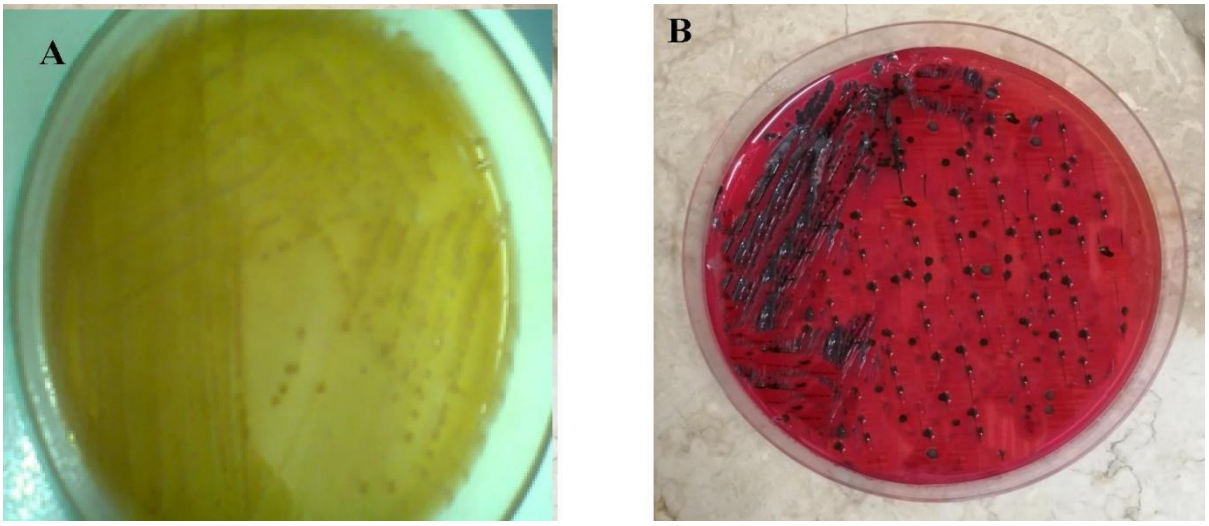

Figure 1. Cultural characters of Salmonella isolates on MacConkey (A) and XLD (B) media

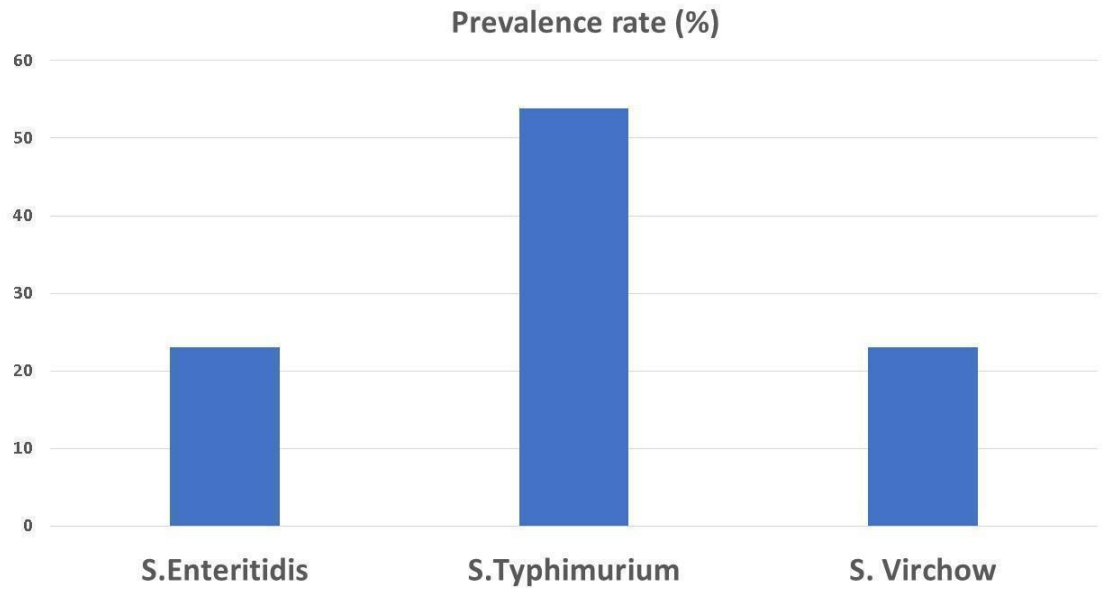

Figure 2. Prevalence rate of different salmonella serotypes in examined meat samples.

Antimicrobial Susceptibility pattern

Data of **Figure 3** show that all isolates were resistant to ceftazidime (100%) followed by ampicillin-sulbactam and chloramphenicol with percentages of 84.6 and 53.8%, respectively.

Moreover, ceftriaxone represented absolute susceptibility to all isolates and was regarded as a drug of choice for salmonellosis infection.

Interestingly, 61.5% of isolates displayed a multidrug-resistant pattern i.e., resistance to three or more drugs.

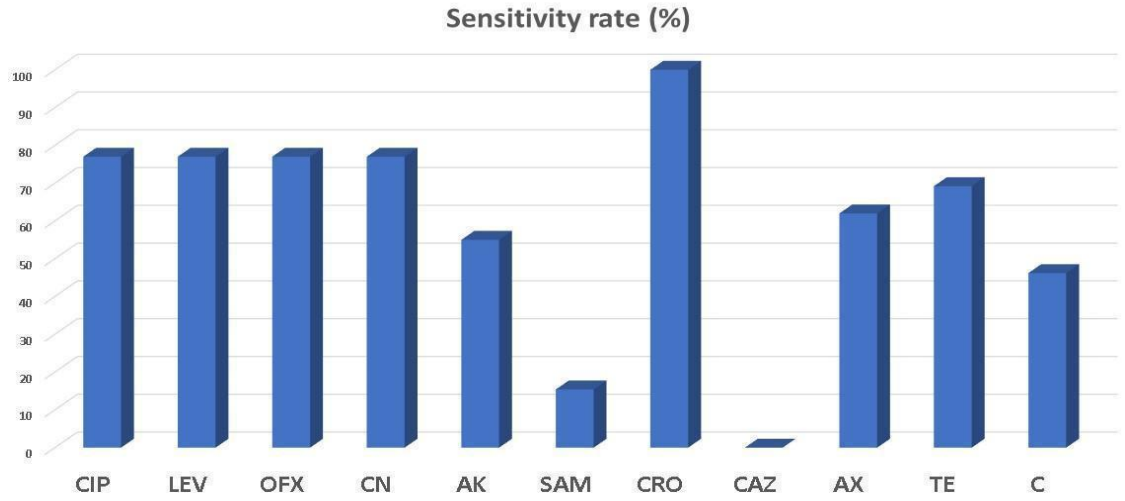

Figure 3. Frequency of antimicrobial susceptibility of Salmonella isolates from meat products. CIP: ciprofloxacin, LEV: levofloxacin, OFX: ofloxacin, CN: gentamicin, AK: amikacin, SAM: ampicillin/ sulbactam, CRO: ceftriaxone, CAZ: ceftazidime, AX: amoxicillin, TE: tetracycline, C: chloramphenicol.

Discussion

Contaminated food products are the main sources of transmission for salmonella species. It is the major cause of more disease in more countries and developing.

In this study, the prevalence rate of 26 % (13 / 50) in the studied food items. This can seriously affect the quality and safety of the processed meat and pose a potential risk to the consumer.

The recovery rate of Salmonella from meat products varied among several countries. It was less than 60.2 % of hind of sheep in Riyadh, Saudi Arabia (*Bosilevac et al.,2015*), in the range of 33.3% in China (*Yan et al. 2010*), 14.1% and 9.9% were from Addis Ababa, Ethiopia (*Ejeta et al., 2004 and Zewdu, E., & Cornelius, P. 2009)*. of interest, more than 10% were detected from the freshly dressed carcass in Spain (*Sierra et al.,1995*) and 4% from Hyderabad, India *(Kumar et al., 2014)*. Also, about 11.4% prevalence in meat products was recorded in Egypt (*El-Demerdash et al., 2021*). Therefore, this is considered a risk indicator for the increase in pathogenic bacteria, and care must be taken to limit its spread.

Regarding, the antimicrobial susceptibility profile, the isolates were resistant to ceftazidime (100%), followed by ampicillin-sulbactam and chloramphenicol with percentages of 84.6 and 53.8%, respectively.

ceftriaxone represented (100%) more sensitivity to all isolates. In another study, all isolates were susceptible to ciprofloxacin and meropenem and absolute resistance was obtained among the isolates against amoxicillin-clavulanic acid (100%) followed by novobiocin and sulfamethoxazole-trimethoprim (87.5%), ceftriaxone (75%), chloramphenicol (62.5%), gentamicin (50 %), ceftazidime and doxycycline (37.5%) and amikacin (12.5%) *(El-Demerdash et al., 2021).*

In the present investigation, 61.5% of isolates displayed multidrug resistance which is higher than the rates of reporting quinolone-resistant and multidrug resistance Salmonella spp. increased from 9.52% and 22.94% in 1990-999 (n = 283 and 329) to 84.40% and 29.08% in 2000-2009 (n= 2510 and 417), respectively (*Bokhary et al. 2021).* It is studied less than findings on the multidrug-resistant (MDR) profile showed that a total of 6/8 (75%) of Salmonella Enteritidis were resistant to 3 or more antibiotics *(El-Demerdash et al., 2021)*. These data represent a great hazard to public health. Finally, we strongly recommend the application of advanced biosafety manners during processing, transportation and storage to avoid this risk.

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