Frequency, serotyping, antibiogram, and seasonality of *Salmonella* isolated from red meat markets

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Abstract

Bacterial pathogenic strains are as adaptable as *Salmonella* strains and cause diverse intestinal and extraintestinal diseases in humans and other mammals worldwide. Red meat and its products are important hosts for many zoonotic diseases. This work was designed to investigate the frequency, serotypes, and antimicrobial resistance profile of isolated *Salmonella* spp. in red meat (cattle, sheep, and goats) sold in Dhamar Governorate, Yemen. A total of 250 red meat samples were collected from the retail seller market between July and December 2022. All samples were transported immediately to the laboratory, subcultured on selective enrichment agar, and identified by serotyping and antimicrobial susceptibility tests *via* disk diffusion methods. The results indicated 26 positive samples of *Salmonella* out of the 250 samples (10.4%). Notably, isolates belong to ten various serotypes: *S.* Typhimurium 19.2%, *S.* Anatum 15.4%, *S.* Newport 11.5%, *S.* Enteritidis 11.5%, *S.* Muenchen 11.5%, *S.* Infantis 7.7%, *S.* Montevideo 7.7%, *S.* Dublin 7.7%, *S.* Senftenberg 3.9%, and *S.* Arizona 3.9%. The antibiotic resistance profile revealed that 57.5%, 53.9%, and 53.9% of isolates are resistant to erythromycin, tetracycline, and norfloxacin, respectively. This resistance among *Salmonella* spp. suggests a significant threat to health, which will in turn require an active safety measure and response. On the other hand, the seasonal variations "August and July" were found to be associated with an increased frequency of *Salmonella* isolation.

Introduction

The *Salmonella* genus is the leading zoonotic infection and one of the most essential foodborne diseases (FBDs) all over the world. The first salmonellosis infection was recorded in swine by two researchers, Salmon and Smith, in 1885 (Chlebicz and Śliżewska, 2018; Al-Gamal *et al.*, 2019; AAP, 2024; CDC, 2024). In addition, *Salmonella* spp. is a common occurrence in the enteric cannel of birds, animals, and a wide range of other mammals and humans. It is a universal bacterium observed in nature and is capable of surviving for various months in soil and water (Silva *et al.*, 2021). Among only two types, *S. enterica* and *S. bongori,* more than 2600 serotypes of *Salmonella* are prevalent in the environment (Almashhadany and Osman, 2019; Peruzy *et al.*, 2023).

In humans, pathogenic strains can cause certain life-threatening illnesses, most of which are foodborne infections (Mikoleit, 2010; Ammar *et al.*, 2019; Almashhadany *et al.*, 2022a). Nevertheless, the various strains of *Salmonella* are mainly categorized into two types: typhoidal and non-typhoidal (Woldemariam *et al.*, 2005). The first group includes various species of *Salmonella*-producing typhoid fever and paratyphoid fever (*S. Typhi, Paratyphi* A, B, and C) (Hugho *et al.*, 2024). However, unfortunately, these species are carried only by humans, as confirmed by previous studies reported in most countries in the Middle East, Africa, and the European Union (González-Santamarina *et al.*, 2021; Almashhadany *et al.*, 2022a). Furthermore, it is worth noting that the nontyphoidal *Salmonella* type plays a significant role in the transmission of various *Salmonella* strains across individuals, various animal species, humans, and different mammals (Tan *et al.*, 2022). Therefore, non-typhoidal *Salmonellae* are the predominant strains associated with foodborne illnesses (El-Prince *et al.*, 2019; Serter *et al.*, 2024).

Human salmonellosis is a serious FBD. According to estimates from the World Health Organization (WHO), around 1.3 billion occurrences of salmonellosis species are reported annually (Almashhadany, 2019). According to Smith (2017), the prevalence of foodborne illnesses is estimated to be 80.3 million incidents, accounting for 85.6% of the total. However, it has been estimated that the occurrence of non-typhoidal *Salmonella* leads to approximately 500,000 fatalities per year. The WHO stated an incidence of 94 million cases of non-typhoidal salmonellosis gastroenteritis in 2018, with foodborne cases accounting for around 85% of the total (Mikoleit, 2010). The WHO has indicated that FBDs have emerged as a significant public health concern, experiencing a notable rise over the past decade in numerous countries across Asia, Africa, and South America (Almashhadany *et al.*, 2022b; DOH, 2024).

Several studies have shown that in developing countries, salmonellosis contributes to diarrhea morbidity (Ngogo *et al.*, 2020) and mortality in childhood, as *Salmonella* is accountable for about

20% of childhood illness cases (Shmeleva, 2020). Correspondingly, some *Salmonella* spp. have been host-specific, while others have a more common host range. Nonetheless, the serovars that cause asymptomatic disease in animals may result in human illnesses (Hohmann, 2001; Shmeleva, 2020). Thus, severe individuals for salmonellosis are children (<5 years), the elderly (Van Duijkeren *et al*., 2002), and immunocompromised individuals (Heikinheimo *et al*., 2006; Coburn *et al*., 2007; Balasubramanian *et al*., 2019). The main causes of *Salmonella* spp. in individuals are various kinds of food, especially meat and its products. It is understood that *Salmonella* can survive for long periods in most domestic freezers (Ballout *et al*., 2023).

Conventional, meat-borne *Salmonella* pathogens are usually related to the feeding of raw or undercooked meat. Moreover, *Salmonella* spp. also contaminates meat products throughout and after handling, storage, and manufacturing (Almashhadany *et al*., 2016; El-Bagoury *et al*., 2019; Garba *et al*., 2020; CFIA, 2024).

In Yemen, data are deficient on detailed statistics on the occurrence and cases of *Salmonella* contamination caused by foodborne pathogens, as there are no surveillance systems implemented similar to those in other countries. Therefore, this particular study highlighted the existence of *Salmonella* spp. in meat and its products, particularly in street vendors and in huckster outlets, restaurants, and hotels. Consequently, this study aimed to monitor the frequency and antibiogram profiles of *Salmonella* among red meat sold at retail vending in Dhamar Governorate.

Materials and Methods

Study design
This study was conducted from July to December 2022. 250 samples were collected as follows 80 cattle, 95 sheep, and 75 goats from different places in Dhamar City. The aforementioned specimens were obtained in sterilized plastic bags under a controlled environment at the Laboratory of Veterinary Public Health, located within the Faculty of Veterinary Medicine at Thamar University in Dhamar, Yemen, as per prior study (Almashhadany and Osman, 2019).

Isolation, identification, and characterization of Salmonella
Salmonella isolated from red meat was used according to information provided by Mikoleit (2010). Initial detection was done on the Gram stain reaction of all suspected *Salmonella* colonies (Taha *et al*., 2013; Almashhadany and Osman, 2019), and followed by conventional biochemical tests were indicated according to standardized techniques (Mikoleit, 2010; Liu *et al*., 2018). A series of biochemical assays were conducted on all possible colonies using a Triple Sugar Iron agar slant to examine the triple sugar iron responses, along with other relevant biochemical investigations (Mir *et al*., 2015, Garba *et al*., 2019).

Salmonella serotyping test
All the isolates of *Salmonella* that exhibited distinguishing biochemical reactions have been subjected to serotyping by slide agglutination performing a Remel® kit and manufacturer information for *Salmonella* isolation according to guidelines (Remel Europe Ltd. Clipper Boulevard West, Crossways Dartford, Kent, UK).

Antimicrobial sensitivity testing profile
This study aims to evaluate the susceptibility of isolates to a screen of 8 commonly utilized antibiotics employing the Mueller-Hinton agar disc diffusion assessment, as outlined by HiMedia (Mumbai, India). The Modified Kirby-Bauer technique and analysis of inhibition zone diameters around antibiotic discs were conducted following the guidelines set out by the Clinical & Laboratory Standards Institute (Sahu *et al*., 2018). Table 1 presents the antimicrobial agents that were examined, along with their respective concentrations and inhibition zone diameters for the resistant, intermediate, and susceptible strains.

Statistical analysis

A comprehensive analysis of the data was conducted using version 21 of the SPSS software package (IBM, Armonk, NY, USA). In essence, the normal approximation process has transformed estimates into confidence intervals. The researchers employed the Chi-square test to examine potential differences among groups and the extent of contamination. A statistical significance criterion of $p<0.05$ was established.

Results

Frequency of Salmonella in raw red meat

Out of 250 various red meat samples, the species of *Salmonella* was detected in 26 samples, and the rate of occurrence was 10.4%. Of note, 8.7% (7/80) of samples from cattle meat, 11.6% (11/95) of sheep meat, and 10.7% (8/75) of goat meat contained *Salmonella* (Table 2). Based on statistical inference, it is estimated that contamination was slightly higher in sheep meat compared to cattle and goat meat. There was no significant difference ($p<0.05$) between types of meat in terms of *Salmonella* prevalence.

Identified Salmonella serotypes

Regarding serotyping, 10 distinct species of Salmonella have been identified in various types of red meat. *S. Typhimurium* (19.2%) and *S. Anatum* (15.4%) were the most often identified serotypes, whereas *S. Senftenberg* and *S. Arizona* were the least reported (3.9%) for each serotype. Additional serotypes and their respective proportions are visually depicted in Table 3.

Antimicrobial sensitivity pattern of the Salmonella isolates

All strains isolated (n=26) were examined toward 8 generally used antibiotics. Complete sensitivity has been found towards Colistin and Gentamicin, whereas higher resistance phenotypes to erythromycin (57.7%), as well as tetracycline and norfloxacin with a rate of (53.9%), were found. The complete antibiogram profile is shown in Table 4.

Salmonella spp. temporal distribution

The most significant incidence rate was observed in August (16.3%) and followed by July (14.0%), while the lowest rate was found in December (5.1%) and November (5.4%). Of note, the summer season was associated with an incremental increase in *Salmonella* incidence (Table 5).

Discussion

Salmonellosis has a worldwide occurrence and, according to the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (OIE), has been a predominant and important zoonosis since 1950. A few studies have indicated the significance of red meat and meat products in the spread of *Salmonella* to humans (Musawa *et al*., 2021; Hugho *et al*., 2024). It is the main cause of human bacterial intestinal inflammation worldwide. The prevalence levels differed from high to low among red meat, including 14/50 (28%) of beef, 10/40 (25%) of mutton, and 5/30 (16.67%) of meat contact surfaces (Mir *et al*., 2015).

In the current study, the frequency of *Salmonella* in beef, mutton, and chevon meat (goat meat) samples was 26/250 (10.4%) (Table 2). These results are in agreement with those of Taha *et al.* (2013) who carried out a study in Sana'a city from April 2009 to April 2010, which showed that the isolation percentage of *Salmonella* in red meat examples was 14.7%. However, the general spread followed the study executed in China by Yang *et al.* (2020), who illustrated that the beef, mutton, and dumplings showed an average level of contamination with a percentage of 16.1% (n=161) and 10.9% (n=92), respectively.

Recently, a study conducted in Yemen by Al-Khadher (2015) reported *Salmonella* spp. in native beef, imported beef, camel meat, minced meats, poultry meat, fish meat, table eggs, and raw milk, which had contamination percentages with *Salmonella* of 0.11%, 0.14%, 0.05%, 0.11%, 0.05%, 0.16%,

0.19%, 0.14%, and 0.05%, respectively. These studies confirm that *Salmonella* spp. is a frequent contaminant of red meat including meat products, which could pose an increased risk of infection to humans. The results of the current study also point to poor hygienic practices in the preparation and processing of the carcasses which serve as a source of contamination.

Nevertheless, the pooled estimates of Salmonella contaminated goat carcasses, beef carcasses, minced beef and milk were 3.86%, 4.53%, 8.34% and 10.76% respectively in Ethiopia (Tadesse and Gebremedhin, 2015).

Also, this study showed lower results than those found in Riyadh, Saudi Arabia, where the prevalence of Salmonella was 11.2, 13.5, 23.2, and 18.8% in feces and 80.2, 51.2 67.6, and 60.2% on hides of cattle, goats, camels, and sheep, respectively (Bosilevac *et al*., 2015).

Conversely, in Addis Ababa, Ethiopia, out of 384 samples, a total of 4 (1.04%) salmonella was isolated of which (1.5%) and (0.5%) were from sheep and goats respectively (Kassaye *et al*., 2015). Differences in lower prevalence can be attributable to various factors, including variations in geographical location (Almashhadany, 2021), sanitation practices, laboratory detection methodologies, the number of specimens involved in the investigation, and seasonal shifts (Nair *et al.*, 2015). The contamination observed in the current investigation may be attributed to the direct transmission of *Salmonella* within the animals, or it could be a result of unsanitary circumstances in the abattoirs throughout the processing and handling of carcasses, inadequate waste treatment facilities, unchlorinated water supplies, and substandard personal hygiene practices. These factors may contribute to cross-contamination of red meat samples at any point during the slaughtering process (Woldemariam *et al*., 2005).

In society-based works, FAO and OIE indicated a relation between the increase in the spread of *Salmonella* spp. in food from animal sources and an increase in human salmonellosis cases (Doyle *et al*., 2013; El-Bagoury *et al*., 2019). Thus, reducing ruminant animal contamination would reduce salmonellosis in humans. The sources of contamination exhibit significant variation, primarily encompassing fecal matter derived from livestock and other animal species (Doyle *et al*., 2013; Almashhadany and Osman, 2019; El-Bagoury *et al*., 2019). However, these variations have been well-known throughout the world and are impacted by a variety of variables, such as geographic location (Granato *et al.*, 2018), time, the type of food contaminated with salmonella, and the technique of detection (Bell *et al.*, 2016; Galié *et al*., 2018).

Concerning *Salmonella* strains isolated in the current study (Table 3), *S. Typhimurium* was the most predominant at 5/26 (19.2%), which is consistent with previous reports in Yemen (Al-Khadher, 2015) and Egypt (Reda and Mohamed, 2014). In addition, a recent study in Iraq showed that the isolated *Salmonella* from sheep meat (3.6%) was similar to that from goat (3.3%) (Hanoun and Al-Samrraae, 2019).

The serovar *S. Typhimurium* was found more frequently, as Fatima *et al*. (2023), in Pakistan,confirmed that the predominant Salmonella strains were Salmonella enterica serovars; Typhimurium (45.4%).Yang *et al.*, 2020), found that the predominant serovars included Salmonella enterica serovar Enteritidis (32.7%), Salmonella enterica serovar Indiana (14.2%) and Salmonella enterica serovar Typhimurium (11.9%).

Hawwas *et al*. (2022), in Egypt, reported that four serotypes; *S. Typhimurium*, *S*. Enteritidis, S. Dublin, S. Saintpaul, were shared between sheep, goats, and humans.

Additionally, this may cause antibiotic resistance in *Salmonella* spp. to increase continuously. Besides, observation logs indicated a two-fold increment in resistant phenotypes of *Salmonella* isolates (from 30% to more than 70%) from the early 1990s to the 2000s (Su *et al*., 2004). The current result concerning the sensitivity testing showed 57.7%, 53.9%, and 53.9% resistance to erythromycin, norfloxacin, and tetracycline, respectively (Table 4). Conversely, the use of antimicrobials in meat and meat products, particularly red meat, can result in antibiotic-resistant strains (Soufi *et al*., 2012). The antimicrobial susceptibility of *S. Typhimurium* species regained from the food chain and its products has been studied lately (Van Duijkeren *et al*., 2002; Xu *et al*., 2020).

This is thought to be because it is the most commonly used drug as a growth promoter, according to prior studies (Alfama *et al.*, 2019; Almashhadany, 2019). Changing resistance levels among isolates to the antibiotics used in this study were shown. Nevertheless, *Salmonella* isolates showed susceptibility to colistin, and this phenomenon could potentially be attributed to the limited application of these two antimicrobials within the domain of veterinary medicine in Yemen (Almashhadany, 2019; Elsayed *et al.*, 2024).

However, *Salmonella* strains discovered in raw red meats and chicken meat in the UK and Tunisia have shown complete susceptibility to levofloxacin, ceftriaxone, as reported by Little *et al.* (2008) and Soufi *et al.* (2012). According to Eng *et al.* (2015) and Soufi *et al.* (2012), the presence of antimicrobial-resistant Salmonella in meat and its derivatives poses challenges in maintaining food safety and may be associated with human infections.

In this regard, *Salmonella* occurrence had the highest frequency in August (16.3%) and July (14.0%) (Table 5), while the lowest frequencies were found in November (5.1%) and December (5.4%). In terms of time, a few studies have connected warm periods to a high occurrence of *Salmonella* (Alfama *et al.*, 2019).

Based on prior research conducted in the United States, it was observed that the incidence of salmonellosis exhibited a notable rise throughout the summer season (38.6%) and reached its lowest point in winter (14.5%) (Judd *et al.*, 2019). Furthermore, recent research has indicated that *Salmonella* spp. exhibits variability in their typical reservoirs, as well as in terms of environmental factors, seasonal dissemination, and their capacity to induce human infections (Judd *et al.*, 2019, Kadry *et al.*, 2019). However, a study conducted in Mexico revealed that there was a greater occurrence ($p<0.05$) of this disease in supermarkets throughout the year compared to wet markets (Regalado-Pineda *et al.*, 2020).

Anyhow, in the study conducted by Al-Khadher (2015) in Yemen, it was confirmed that food of animal origin in Dhamar Governorate is highly contaminated with certain *Salmonella* spp., with these species representing the main source of *Salmonella* infections in patients in the governorate. Al-Khadher (2015) stated that out of the 35 stool and 45 blood samples examined, 8 (22.8%) and 22 (48.9%) respectively tested positive for salmonellosis.

Conclusions

The objective of the present investigation was to assess the occurrence, serovars, antibiotic resistance, and seasonality of *Salmonella* spp. in red meat (namely beef, mutton, and chevon) obtained from specific retail establishments and butcheries located in Dhamar governorate. In general, there was a notable occurrence (10.4%) of *Salmonella* spp., with the identified serovars being *S. Typhimurium*, *S. Anatum*, *S. Newport*, *S. Enteritidis*, *S. Muenchen*, *S. Infantis*, *S. Montevideo*, *S. Dublin*, *S. Senftenberg*, and *S. Arizona*. This work should be followed by molecular and epidemiological investigations to evaluate the potential risks to consumer health and analyze the prevalence of antibiotic resistance. Nevertheless, it is recommended to conduct a comprehensive four-season study to assess the evolving epidemiology of *Salmonella* in red meat and its derivatives.

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Table 1. Concentrations, inhibition zone diameter for resistant, intermediate, and susceptible tested antibiotics.

Inhibition zone diameter (mm)				
Antibiotic	Antibiotic concentration	Resistant	Intermediate	Susceptible
Colistin	10 μg	≤8	11-9	≥11
Erythromycin	15 μg	≤13	18-14	≥18
Gentamicin	10 μg	≤12	-	≥13
Kanamycin	30 μg	≤13	17-14	≥18
Neomycin	30 μg	≤12	16-13	≥17
Norfloxacin	10 μg	≤10	15-11	≥16
Streptomycin	10 μg	≤11	14-12	≥15
Tetracycline	30 μg	≤14	18-15	≥19

Table 2. Frequency of *Salmonella* among red meat samples.

Red meat	No. samples	+ive samples, n (%)	-ive samples, n (%)
Cattle meat	80	7 (8.7)	73 (91.3)
Sheep meat	95	11 (11.6)	84 (88.4)
Goat meats	75	8 (10.7)	67(89.3)
Total	**250**	**26 (10.4)**	**224(89.6)**

+ive samples, number and percentages of *Salmonella* isolates; -ive samples, *Salmonella* not isolated.

Table 3. Serotypes of 26 isolated *Salmonellae* from red meat.

Serotypes	Strains	%
S. Typhimurium	5	19.2
S. Anatum	4	15.4
S. Newport	3	11.5
S. Enteritidis	3	11.5
S. Muenchen	3	11.5
S. Infantis	2	7.7
S. Montevideo	2	7.7
S. Dublin	2	7.7
S. Senftenberg	1	3.9
S. Arizona	1	3.9

Table 4. Antimicrobial of isolates to a panel of eight antibiotics.

Antimicrobial agents	No. Examined	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)
Colistin	26	26 (100)	0 (0)	0 (0)
Erythromycin	26	9 (34.6)	2 (7.7)	15 (57.7)
Gentamicin	26	26 (100.0)	0 (0)	0 (0)
Kanamycin	26	17 (65.4)	2 (7.7)	7 (26.9)
Neomycin	26	11 (42.3)	2 (7.7)	13 (50.0)
Norfloxacin	26	9 (34.6)	3 (11.5)	14 (53.9)
Streptomycin	26	14 (53.8)	2 (7.7)	10 (38.5)
Tetracycline	26	11 (42.3)	1 (3.8)	14 (53.9)

Table 5. *Salmonella* **spp temporal distribution in raw red meat samples during the period of study.**

Month	No. of examined Cattle meat (No. of positive)	No. of examined Sheep meat (No. of positive)	No. of examined goat meat (No. of positive)	Total examined	No. of positive	%
July	16	19	15	50	7	14.0
August	14	16	13	43	7	16.3
September	13	15	12	40	4	10.0
October	13	16	12	41	4	9.8
November	12	14	11	37	2	5.4
December	12	15	12	39	2	5.1
Total	**80**	**95**	**75**	**250**	**26**	**10.4**