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Identification, Prevalence, and Antibiotic Resistance Profiles of *Salmonella* Isolates from Food Samples

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Astract: This study investigated the prevalence and antimicrobial resistance of *Salmonella enterica* subspecies *enterica* in food samples. Using standard methods (ISO 6579/2017) and VITEK MS (MALDI-TOF), all 30 examined samples yielded isolates confirmed as *Salmonella enterica* subspecies *enterica*. PCR analysis confirmed the presence of the *inv*A gene in 100% (30/30) of isolates, indicating their pathogenic potential. . Growth kinetics analysis revealed the dynamic growth of *Salmonella*, reaching 10 log10 CFU/ml within 6 hours. Antibiotic susceptibility testing revealed a concerning trend. Nearly half (46.67%) of the isolates exhibited multidrug resistance (MDR), defined as resistance to three antibiotic classes or more. Nalidixic acid resistance was most prevalent (66.67%), followed by Ciprofloxacin (50%) and Tetracycline (46.67%). The Multiple Antibiotic Resistance index (MAR) ranged from 0.1 to 0.5, with 15 isolates exceeding 0.2, suggesting a high-risk contamination source for these isolates. These findings highlight the significant presence of MDR *Salmonella enterica* in the food chain. The observed resistance patterns pose a public health threat. Implementing stricter regulations on antibiotic use in food production and improving sanitation practices are crucial to mitigate the spread of antibiotic-resistant *Salmonella*.This study indicates the urgent need for stringent antimicrobial stewardship measures to mitigate the dissemination of resistant strains. Strategies such as restricting antibiotic usage in agriculture and implementing robust sanitation practices in food production are imperative to safeguard public health against the escalating threat of *Salmonella* antimicrobial resistance.

Key words: *Salmonella* · Antibiotic Resistance · Salmonellosis · Foodborne Pathogens

INTRODUCTION

Salmonella remains a major public health concern globally, consistently ranking among the most frequently reported foodborne illnesses [1]. The World Health Organization (WHO) estimates a significant disease burden, with *Salmonella* causing an estimated 7.6 million cases worldwide in 2010 [1, 2]. Furthermore, WHO highlights the severity of *Salmonella* infections, with S. enterica and S. Typhi responsible for an estimated 59,000 and 52,000 deaths, respectively, in 2010 [1, 2].

Salmonella poses a significant global public health burden. Data suggests annual cases reach over 90,000 in the European Union and exceed 45,000 in the United States alone [3, 4]. The economic impact is significant, with the US Department of Agriculture estimating the total cost of human Salmonellosis in the US to be over $3.6 billion annually [5]. Furthermore, *Salmonella* infections are a leading cause of foodborne illness worldwide, with estimates suggesting nearly 80 million infections and over 155,000 deaths annually [6, 7]. The severity of this burden is further highlighted by the fact that hospitalizations for *Salmonella* in the US were nearly double those for Campylobacter and comparable to the combined total of hospitalizations from several other foodborne pathogens combined [8].

Salmonella enterica, a bacterium subdivided into six subspecies and over 2600 serovars, represents a major

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public health concern due to its ability to cause salmonellosis [9, 10]. This foodborne illness commonly results from consuming contaminated food or water, or through direct contact with infected animals [11]. Symptoms typically develop within 12-72 hours and include fever, abdominal cramps, nausea, and vomiting [11].

Antimicrobial resistance (AMR) in foodborne pathogens like *Salmonella* poses a significant danger to worldwide public health. Currently, the yearly death toll from AMR is estimated at 700,000; by 2050, the number is anticipated to increase to 10 million, resulting in a $100 trillion economic loss [12]. Antimicrobial resistance (AMR) can emerge naturally in bacteria through spontaneous mutations within their genomes, leading to the inheritance of resistance-mediating genes. However, the widespread misuse and abuse of antimicrobial agents in both human and animal settings has significantly accelerated the emergence and spread of AMR [13, 14].The increase of multidrug resistance (MDR) in *Salmonella* to important antimicrobial drugs like fluoroquinolones and α-lactams is a major concern for human Salmonellosis treatment [15, 16]. The presence of multidrug-resistant (MDR) *Salmonella* in food poses a significant public health concern. Food serves as a potential reservoir and vector for these resistant strains, facilitating their dissemination to humans through direct consumption of contaminated food or indirectly through the food chain [17]. This highlights the critical need for effective strategies to minimize foodborne *Salmonella* contamination and curb the emergence and spread of MDR strains..

Due to its persistence in animal production systems and the surrounding environment, *Salmonella* can act as a constant reservoir for reintroduction into the food chain, inhibiting control measures [18]. It is able to form biofilms on food [19], this biofilm can act as a bacterial reservoir for recurring bacterial contamination in the food processing facility, resulting in several food-borne outbreaks [20]. Microbial biofilms are a severe hazard to the food industry because they are resistant to antimicrobial and physical treatments, making them difficult to inactivate or remove [21]. Consuming food contaminated with antimicrobial-resistant (AMR) bacteria poses a serious health risk beyond direct infection. This practice can facilitate the horizontal transfer of AMR genes between ingested bacteria and the human gut microbiome. The accumulation of these genes in human gut bacteria can significantly decrease the effectiveness of antibiotic drugs in treating future infections [22].

MATERIALS AND METHODS

Bacterial Strains Isolation: Different food samples were collected from the local markets and provided to Zewail City of Science, Technology, and Innovation's, center of Scientific Excellence for Food Research and Analysis (cSEFRA). According to ISO 6579:2017 [23], 25 grams of food sample were added to 225 ml buffer peptone water (BD Difco, USA) and incubated at 37°C for 18 hours ± 2 hrs. One ml and 0.1 ml were transferred to 10 ml of MKTTn (Merck, Germany) and RVS (BD, USA), incubated for 24 hours ± 3 hrs at 37°C and 41.5°C respectively. A plating-out was made on XLD agar (BD Difco, USA) and Hektoen Enteric agar, HEA, plates (BD Difco, USA) from the MKTTn and RVS selective enrichments, the plates of XLD and HEA were incubated for 24 hours at 37°C. Thirty presumptive *Salmonella* colonies were taken from different sample source for biochemical confirmation, in Triple Sugar Iron agar (OXOID, UK) slant tubes, the slant surface was streaked and the butt was stabbed, in Urea Agar (OXOID, UK) slant tubes, the surface was streaked, after that the slants tubes were incubated at 37°C for 24 hrs.

Bacterial Isolates Confirmation by VITEK MS and Stocking: Thirty presumptive *Salmonella* isolates were confirmed by the VITEK MS system (BioMérieux, France) according to the manufacturer method, a single colony from fresh culture plate was picked, placed, on the target slide (BioMérieux, France), *E. coli* ATCC 8739 was placed on the calibration spots as a calibration control, and a 1 µl of CHCA matrix (BioMérieux, France) was added to the colony on the target slide and left to dry, the target slide with the sample were loaded into the instrument and the instrument was set to run.

Bacterial Strain Growth and Stock Preparation: Thirty *Salmonella* confirmed isolates were cultured overnight in Tryptone Soya Broth (TSB) (Merck, Germany) at 37°C and were preserved at -80°C freezer (Thermofisher scientific, USA), and added to TSB that includes 20% glycerol w/v (Sigma Aldrich, USA) (Bacteriology Culture Guide, ATCC).

Molecular Detection of *Salmonella* isolates by PCR:

DNA Extraction: A single colony of each thirty *Salmonella* isolates was picked and cultured into a 1.5 ml micro-centrifuge tube containing 1 ml TSB and incubated for 24 hrs. at 37°C. Tryptone Soya Agar plates are streaked from the overnight culture tubes and incubated

overnight at 37°C, the growing bacterial colonies were harvested and the DNA extraction was performed using the boiling method [25, 26]. We collected the supernatant and stored it at -20°C to be used for PCR.

PCR: Isolated *Salmonella* species were confirmed by Polymerase chain reaction, PCR, *inv*A gene was assessed for its presence, with a set of primers as follow: *F:* 5' *GTGAAATTATCGCCACGTTCGGGCAA* 3' and *R:* 5' *TCATCG CACCGTCAAAGGAACC 3'* [27]. *Salmonella entrica* ATCC 14028 was used as a positive control.

The PCR reactions were conducted in a 25 µl reaction that contain 12.5 µl OnePCR master mix (GeneDirex, Taiwan), 1 µl of each primer of 10 pmol concentrations, 4.5 µl of DNase/RNase-free water, and 6µl of isolated DNA of Salmonella. The T100 thermal cycler (Biorad, USA) was set at different profiles as follow: Initial denaturation at 94°C, for 5 min, denaturation at 94°C, for 30 sec, annealing at 55°C, for 30 sec, extension at 72°C, for 30 sec, final extension at 72°C, for 10 min, and the number of cycles was 35.

Gel Electrophoresis and Imaging of PCR Products: The PCR products were electrophoresed on (*w/v*) 1.5 % agarose (Sigma Aldrich, USA) embrace 0.5 µg/mL ethidium bromide (Fisher Scientific, USA) using TBE buffer 1X at 100 V for 1 hour, A DNA ladder of 100 bp size (Bio-Helix, Taiwan) was used. The bands were visualized and detected under a UV transilluminator with expected amplified fragment size of 284 bp.

Bacterial Growth Curve: One *Salmonella* isolate was selected to do the growth curve to study its growth kinetics as described before [28] with minor modifications, overnight culture of the isolate was performed in TSB (Merck, Germany) in 1.5 ml tube. A new flask containing 50 ml of TSB was inoculated from the overnight culture to obtain ~10^6 CFU/ml, and immediately a 100 µl of the flask was withdrawn. A serial dilution was made and a spott assay was performed by spotting 10 µl of each serial dilution in triplicates on the surface of Tryptone Soya agar (TSA) and this step was repeated at each time point for 6 hours.

Antibiotic Sensitivity Testing by Disk Diffusion: All *Salmonella* isolates included in this study were tested against fifteen antibiotics on Mueller-Hinton agar (Sigma Aldrich, USA) using Kirby-Bauer disc diffusion method [29]. The antibiotics used were: Nalidixic acid (30 µg), Gentamycin (10 µg), Chloramphenicol (30 µg), Cefixime (5 µg), Ceftazidime (30 µg), Sulphamethoxazole trimethoprim (25 µg), Tetracyclin (30 µg), Ciprofloxacin (5 µg), Kanamycin (30 µg), Amoxicillin Clavulanic Acid (30 µg), Ampicillin (10 µg), Imipenem (10 µg), Ertapenem (10 µg), ceftriaxone (30 µg), and Norfloxacin (10 µg), all obtained from (OXOID, UK). The Clinical and Laboratory Standards Institute's (CLSI, 2018) criteria were used to determine sensitivity and resistance.

RESULTS AND DISSCUSSION

Bacterial Isolation and Confirmation: Salmonellosis is a zoonotic disease, which can be transmitted from animals to human, caused by *Salmonella* [31, 32] and many of foodborne illness are frequently caused by *Salmonella* [33]. *Salmonella* species were isolated from examined food samples using the standard method ISO 6579/2017. Thirty *Salmonella* spp. isolates were further identified and confirmed by VITEK MS (MALDI-TOF) which allows identifying the bacteria to genus, species, and subspecies levels and the results demonstrated that all thirty bacterial isolates were *Salmonella enterica* subspecies *enterica* as previously reported [34]. All confirmed 30 *Salmonella* spp. were subjected to PCR confirmation to detect specific chromosomally encoded determinants using specific oligonucleotide primer pairs. Rahn *et al.* [35] demonstrated that the *inv*A gene contains *Salmonella*-specific regions and might be exploited as a PCR target for diagnostic purposes. The results showed that the *inv*A gene was detected among all the tested strains 100% (30/30) as indicated in Fig. 1, and this gene plays an essential role in the invasion of host epithelial cells, induces the infection, and enhances the pathogenicity of the bacteria [36, 37].

The growth kinetics of *Salmonella* isolate no. 30 showed that the bacteria stayed in the lag phase for about 1 hour at 6 Log_{10} CFU/ml, and started the log phase after this period till 6 hours, the duration of the experiment, and reached 10 log_{10} CFU/ml at the end point as described in Fig. 2.

Antibiotic Sensitivity Profile: Antibiotic resistance is rapidly growing in bacteria isolated from animals and humans, and it has emerged as a major global issue. In particular, antimicrobial-resistant strains is reported to be a major concern among *Salmonella enterica* [38, 39]. *Salmonella* isolates that showed resistance to at least three classes of antimicrobials were classified as multidrug-resistant (MDR) [40]. In our study, 14 out of 30 (46.67%) *Salmonella* isolates showed multidrug resistance to a minimum three classes of antibiotics, as

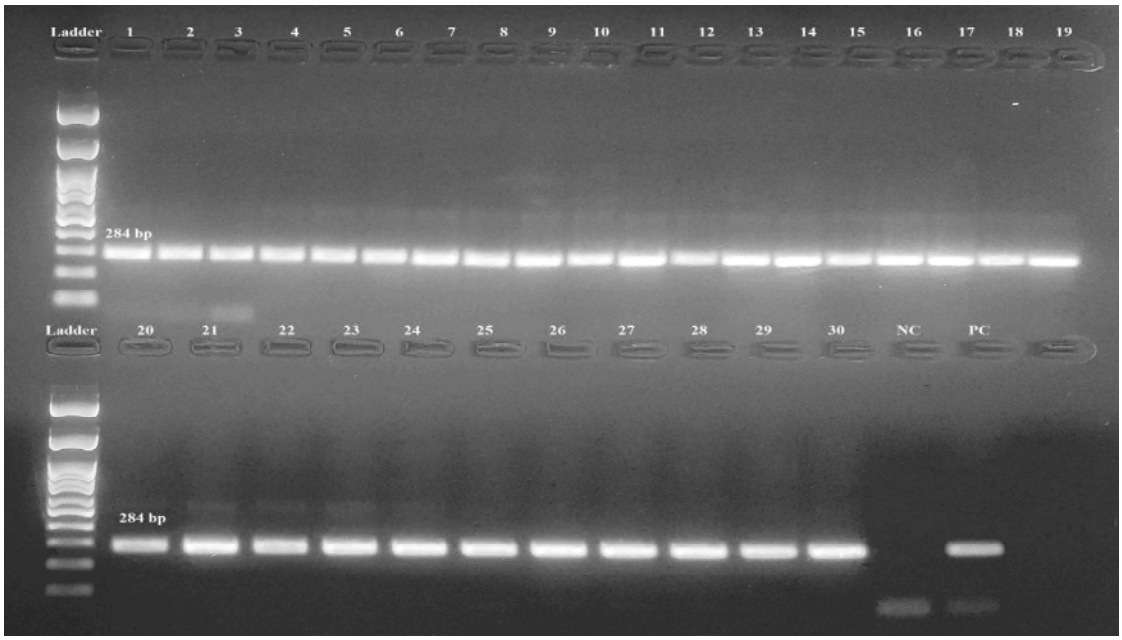

Fig. 1: *inv*A gene (284 bp) of *Salmonella* isolates displayed on 1.5% agarose gel. Lane (ladder) represents the NDA marker (100 bp), lanes from 1 to 30 represents the isolates samples, lane (NC) represents the negative control, lane (PC) represents the positive control.

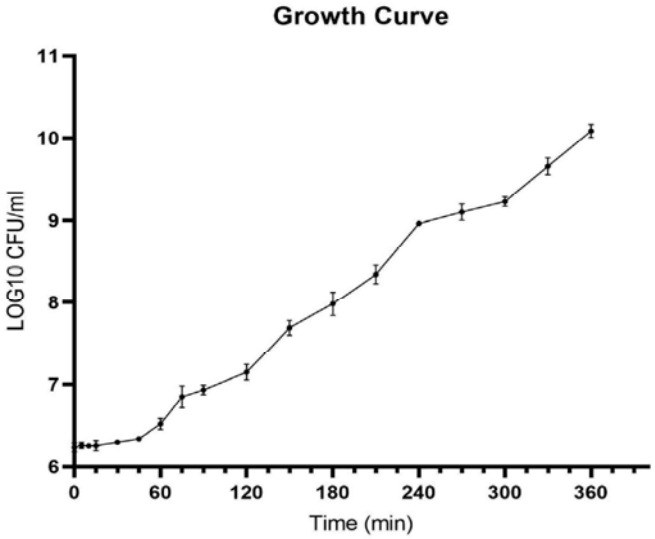

Fig. 2: *Salmonella* growth curve.

shown in Table (1) and these finidings are greater than the previously stated finidings [41, 42]. However, it is less than other reported results [43]. MDR strains within foods directly impact human health, as they can cause diseases that are difficult to treat. Antibiotic sensitivity testing of all 30 *Salmonella* isolates indicated that, resistance to Nalidixic acid (NA30) was the most prevalent with 20 (66.67%) resistant *Salmonella* strains, followed by Ciprofloxacin (CIP5) with 15 (50%), Tetracyclin (TE30) with 14 (46.67%), Kanamycin (K30) with 11 (36.67%), Gentamycin (CN10) and Ampicillin (AM10) with 7 (23.33%), Chloramphenicol (C30) with 5 (16.67%), Amoxicillin Clavulanic Acid (AMC30) with 4 (13.33%), Sulphamethoxazole trimethoprim (SXT25) with 3 (10%), and Norfloxacin (NOR10) with 1 (3.33%) resistant strains. However, previous results [44, 45] found that Ampicillin resistance was the most common. The MAR index, which is the number of non-effective antibiotics/total number of

Table 1: Antimicrobial susceptibility pattern of Salmonella isolates to tested antimicrobials.

	No. of isolates (%)n=30		
Antimicrobial agents	Susceptible (S)	Intermediate (I)	Resistant (R)
Nalidixic acid (NA30)	10 (33.33)	0 (0)	20 (66.67)
Gentamycin (CN10)	14 (46.67)	9 (30)	7 (23.33)
Chloramphenicol (C30)	20 (66.67)	5 (16.67)	5 (16.67)
Cefixime (CFM5)	26 (86.67)	4 (13.33)	0 (0)
Ceftazidime (CAZ30)	18 (60)	12 (40)	0 (0)
Sulphamethoxazole trimethoprim (SXT25)	27 (90)	0 (0)	3 (10)
Tetracyclin (TE30)	15 (50)	1 (3.33)	14 (46.67)
Ciprofloxacin (CIP5)	0 (0)	15 (50)	15 (50)
Kanamycin (K30)	0 (0)	19 (63.33)	11 (36.67)
Amoxicillin Clavulanic Acid (AMC30)	12 (40)	14 (46.67)	4 (13.33)
Ampicillin (AM10)	17 (56.67)	6 (20)	7 (23.33)
Imipenem (IPM10)	29 (76.67)	1 (3.33)	0 (0)
Ertapenem (ETP10)	23 (76.67)	7 (23.33)	0 (0)
Ceftriaxone (30)	23 (76.67)	7 (23.33)	0 (0)
Norfloxacin (NOR10)	25 (83.33)	4 (13.33)	1 (3.33)

tested antibiotics, of the thirty isolates ranges from 0.1 to 0.5 which is similar to the previously reported results [41]. The MAR index of fifteen out of the thirty *Salmonella* isolates was greater than 0.2, which indicates that the tested isolates came from a high-risk contamination sources where antibiotics were commonly used [46]. *Salmonella* resistance to these antimicrobials poses a public health risk. Over time, the abuse and misuse of these affordable and easily accessible over-the-counter medications made them ineffective [47]. Addressing the growing threat of antimicrobial resistance (AMR) necessitates a multifaceted approach. Restricting antibiotic use in agricultural settings can significantly reduce the selective pressure that favors the emergence and persistence of resistant bacterial populations within animal hosts. Additionally, implementing and enforcing stricter sanitation practices throughout the food supply chain can effectively minimize cross-contamination events, thereby impeding the dissemination of AMR bacteria along the food chain [46].

CONCLUSION AND RECOMMENDATIONS

This study highlights the critical need to address the rising prevalence of *Salmonella* contamination and antibiotic resistance (AMR) in foodborne pathogens. The high proportion of multidrug-resistant (MDR) *Salmonella* isolates emphasizes the urgency for proactive treatments to inhibit AMR spread in clinical, agricultural, and food settings. The observed predominance of resistance to nalidixic acid, ciprofloxacin, and tetracycline highlights the importance of ongoing surveillance and responsible antibiotic use to preserve their efficacy in treating infections.

The study recommends implementing stricter regulations on antibiotic usage in food production to minimize the selective pressure promoting the emergence of resistant strains. Additionally, enforcing improved sanitation practices throughout the food supply chain is crucial to reduce the risk of *Salmonella* contamination. Finally, ongoing surveillance of antibiotic resistance patterns in *Salmonella* is essential to inform future public health interventions and treatment strategies.

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