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Research Article

Prevalence and antimicrobial resistance pattern of *Salmonella* spp. in raw chicken meat samples from local meat shops in Anand

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Abstract

***Salmonella*, a dangerous zoonotic food-borne bacterium, is widely acknowledged as the primary cause of diarrheal illness, which affects 10% of people annually. In the present study, a total of 150 raw chicken samples (75 each of muscle and liver) were collected aseptically from retail meat shops in Anand. On the basis of cultural isolation and biochemical characterization, 40 (26.60%) samples out of 150 were positive for *Salmonella* spp. On screening the isolates against different antibiotics, all isolates were sensitive to ciprofloxacin and ceftriaxone, followed by decreasing order of sensitivity to chloramphenicol, gentamicin, amikacin, enrofloxacin, streptomycin, amoxiclav, nalidixic acid, trimethoprim, tetracycline and ampicillin as 90%, 87.50%, 82.50%, 70%, 65%, 55%, 32.50%, 20%, 12.50%, and 10%, respectively. So, it can be concluded that meat can be a possible intermediary vehicle for the spread of multidrug-resistant *Salmonella* strains to humans.**

Keywords: Antimicrobial resistance, Prevalence, Raw chicken, *Salmonella*

Highlights

- 40/150 (26.67%) chicken samples were found positive for *Salmonella* species.
- All isolates were 100% sensitive to ciprofloxacin and ceftriaxone.
- 57% of isolates were multi-drug resistant.

INTRODUCTION

Food-borne microorganisms are major pathogens that cause human illness worldwide due to the consumption of foodstuff contaminated with vegetative pathogens or their toxins (Abebe *et al*., 2020). One such pathogen that increases morbidity and mortality is *Salmonella* spp. It is the third most common cause of death among food-transmitted infections and one of the 31 pathogens with the highest potential to cause intestinal or systemic disease in humans (World Health Organization, 2015). According to reports, Salmonella infections cause 3.4 million illnesses and 155,000 fatalities worldwide each year (Stanaway *et al*., 2019). The Centers for Disease Control and Prevention in the USA recorded more than 1 million instances of food-borne salmonellosis each year.

Salmonella spp. are gram-negative, facultative anaerobes with a rod shape that are members of the *Enterobacteriaceae* family (Thames and Sukumaran, 2020). More than 150 serotypes/serovars such as *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium can cause food-borne salmonellosis, and more than 2,600 serovars have been identified to date. A study of confirmed outbreaks of food-borne disease in India from 1980 to 2016 shows that *Salmonella* spp., along with a few other pathogens is responsible for most of the food-borne illnesses (Tegegne, 2019). The main reservoirs for its transmission are thought to be

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animal-derived foods, particularly chicken and pig. Salmonella symptoms can appear anywhere from a few hours to a few days after consuming contaminated food. Gastroenteritis, abdominal pain, bloody diarrhoea, fever, myalgia, headache, nausea, and vomiting are among the symptoms (Ehuwa *et al*., 2021).

To prevent and treat bacterial infections, antimicrobials are crucial. However, due to their overuse, drug resistance is rising, and multidrug-resistant strains have developed. Antimicrobial-resistant *Salmonella* has become a significant problem worldwide. This has a significant negative effects on public health and the economy, including failure to treat patients with gastroenteritis, extended hospital stays, and rising medical costs (Su *et al*., 2018). Therefore, it is preferable to evaluate meat samples due to the overuse and misuse of antibiotics in the chicken industry and the disease brought on by *Salmonella* spp. Many food safety and public health laboratories use traditional microbiological methods as the foundation for analysis because they are simple to use, consistently produce accurate results, have high sensitivity and specificity, and are less expensive than newly developed molecular-based technologies (Lee *et al*., 2015). Hence this study aimed to look at the prevalence and antibiotic sensitivity pattern of *salmonella* spp. isolated from raw chicken meat.

MATERIALS AND METHODS

Sample collection: A total of 150 raw chicken meat samples (75 livers and 75 muscles) were obtained from different retail meat shops in Anand in sterile sample collection vials under aseptic conditions. Following that, these samples were then immediately transferred to the laboratory and were preserved at 4°C.

Isolation and identification of *Salmonella* spp.: The pre-enrichment in Buffered Peptone Water (BPW) (HiMedia, India) of the meat samples was performed. Twenty-five gram sample was cut into small pieces, homogenized with 225 mL BPW, and then incubated for 24 hours at 37°C. After incubation, 0.1 mL of the pre-enrichment inoculum was added to 10 mL of the Rappaport-Vassiliadis (RV) medium (HiMedia, India) for enrichment, and the mixture was then incubated for further 24 hrs at 37°C±1°C. After enrichment, Xylose Lysine Deoxycholate (XLD) agar (Hi-Media, India) and Brilliant Green Agar (BGA) (HiMedia, India) were employed as the selective medium, and a loop-full of the inoculum from RV broth was streaked on it. The inoculated plates were then incubated at 37°C±1°C for 24 hrs for the growth of *Salmonella* spp. Cultures identified using XLD, and BGA agar were further confirmed by gram staining and biochemical tests (Ejo *et al*., 2016).

Biochemical characterization of *Salmonella* spp. isolates: The biochemical tests like Indole, Methyl red, Voges-Proskauer, Citrate, Urease, and Triple Sugar Iron Agar test were performed as per standard protocols for further confirmation of *Salmonella* spp. isolates (International Organization for Standardization, 2007).

- Indole test: Isolated colonies showing characteristic colony morphology were cultured in tryptone water for 18–24 hrs. After completion of incubation, the addition of Kovac's reagent to tryptone water enabled the detection of indole synthesis.
- Methyl Red test: Isolated colonies of *Salmonella* spp. were inoculated in 0.5 mL of peptone water and incubated overnight at 35-37°C. After overnight incubation, a drop of methyl red solution was added to check for red colour development.
- Voges-Proskauer (V-P) test: *Salmonella* spp. isolated colonies were inoculated into 2 mL of sterile peptone water, which was then incubated at 35–37°C for 24 hrs. After incubation, 6 drops of 5% alpha naphthol (solution A) and 2 drops of 40% potassium hydroxide (solution B) were added to check the development of the pink colour/ring.
- Citrate test: Using a sterile loop, 3-4 mL of broth culture of *Salmonella* spp. isolate was

inoculated on Simmon's Citrate agar slant (HiMedia, India) and was incubated at 37°C for 24 hrs. After the incubation, the slant was examined for colour change.

- Urease test: Inoculate a test tube of Urease agar slant with a colony of *Salmonella* sp. from XLD agar. It was incubated at 37°C for 48 hrs and then examined for colour change.
- Triple Sugar Iron (TSI) agar test: Well-isolated colony was selected and touched from XLD plate using a sterile straight nichrome wire loop. The TSI medium was inoculated first by stabbing the butt and then by streaking the slant. After incubation for 24 hrs at 37°C, the slant and the butt were examined for colour change due to the reaction.

Antibiogram pattern of the isolated *Salmonella* spp.: The *Salmonella* spp. isolates were subjected to *in-vitro* antibiotic sensitivity tests against 12 antibiotics as per the method described by Bauer *et al.* (1966). On the basis of the results, the isolates were classified as sensitive, intermediate, and resistant according to Clinical Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2020). The antibiotics used were amikacin (30 mcg), amoxyclav (30 mcg), ampicillin (10 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg), chloramphenicol (30 mcg), enrofloxacin (10 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg), streptomycin (10 mcg), tetracycline (30 mcg) and trimethoprim (5 mcg). The reason for choosing the above antimicrobials was that these are commonly used for treating people suffering from Salmonella infection.

RESULTS

Isolation of *Salmonella* spp.: The overall prevalence of *Salmonella* spp. was 26.67% (40/150) by cultural isolation. Out of these 40 positive samples, 28 (18.67%) were muscles, and 12 (8%) were liver samples. All the isolates showed pink to red coloured colonies with a black centre on XLD agar and pinkish white colonies on BGA, which are typical to *Salmonella* spp. (Fig. 1, 2). In gram staining, pink-coloured short rods were seen (Fig. 3). On biochemical characterization, all these isolates were indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, [IMViC (-/+/-/+)] urease negative and on TSI produced black butt (Fig. 4, 5, 6).

Antibiogram: All *Salmonella* spp. isolates were 100% sensitive to ciprofloxacin and ceftriaxone, followed by decreasing order of sensitivity of chloramphenicol, gentamicin, amikacin, enrofloxacin, streptomycin, amoxiclav, nalidixic acid, trimethoprim, tetracycline, and ampicillin as 90%, 87.50%, 82.50%, 70%, 65%, 55%, 32.50%, 20%, 12.50%, and 10%, respectively (Fig.7, 8). Fifty-seven percent (23/40) isolates also displayed resistance to two or more two antibiotics and thus were called multi-drug resistant (Nikaido, 2009). The details of multi-drug resistance are given in Table 1.

Table. 1. Multi-drug resistance patterns of *Salmonella* isolates resistant to two or more antibiotics

Number of isolates	Antimicrobial agents
7	Ampicillin, Amoxyclav, Streptomycin, Tetracycline
5	Ampicillin, Tetracycline, Trimethoprim
4	Ampicillin, Enrofloxacin, Tetracycline
3	Amoxyclav, Nalidixic acid, Tetracycline
2	Ampicillin, Tetracycline
1	Ampicillin, Amoxyclav, Enrofloxacin, Tetracycline
1	Nalidixic acid, Tetracycline

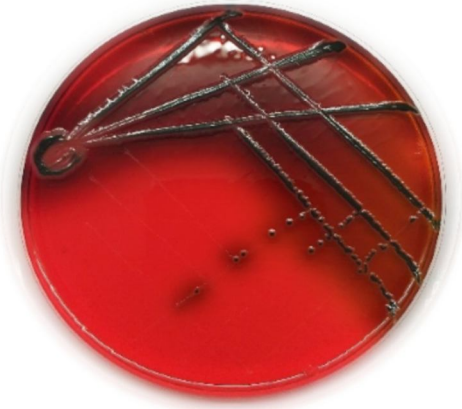


Fig. 1. Pink colonies with black centers of *Salmonella* spp. on Xylose Lysine Deoxycholate (XLD) agar

Fig. 2. Pinkish white colonies of *Salmonella* spp. on Brilliant Green Agar (BGA)

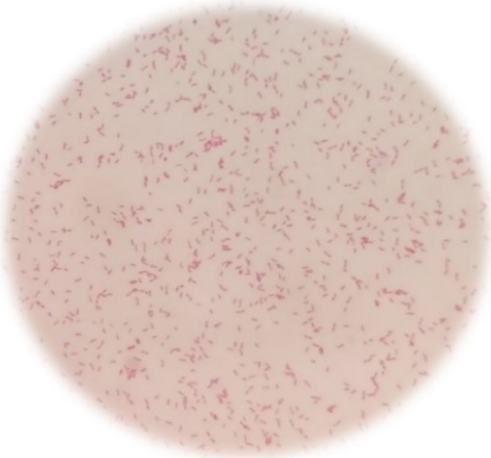
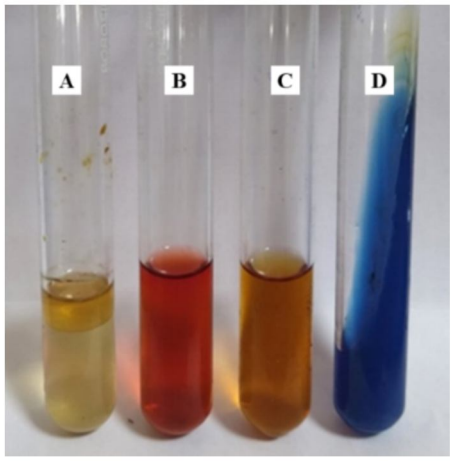
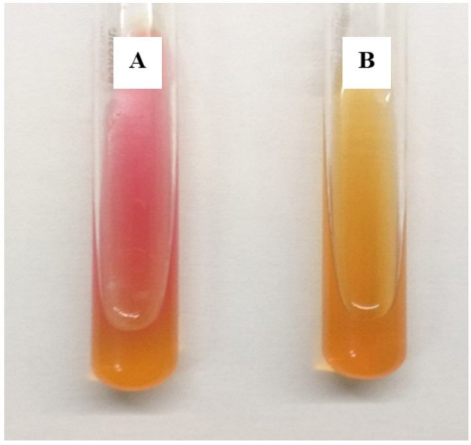


Fig. 3. Gram negative bacilli



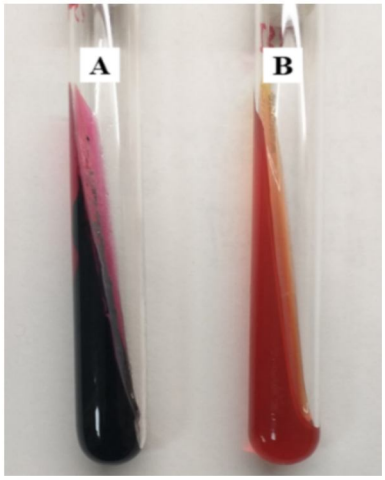
[A - Indole (-), B - Methyl Red (+), C - Voges-Proskauer (-), D - Citrate (+)]

Fig. 4. IMViC pattern of *Salmonella* spp.



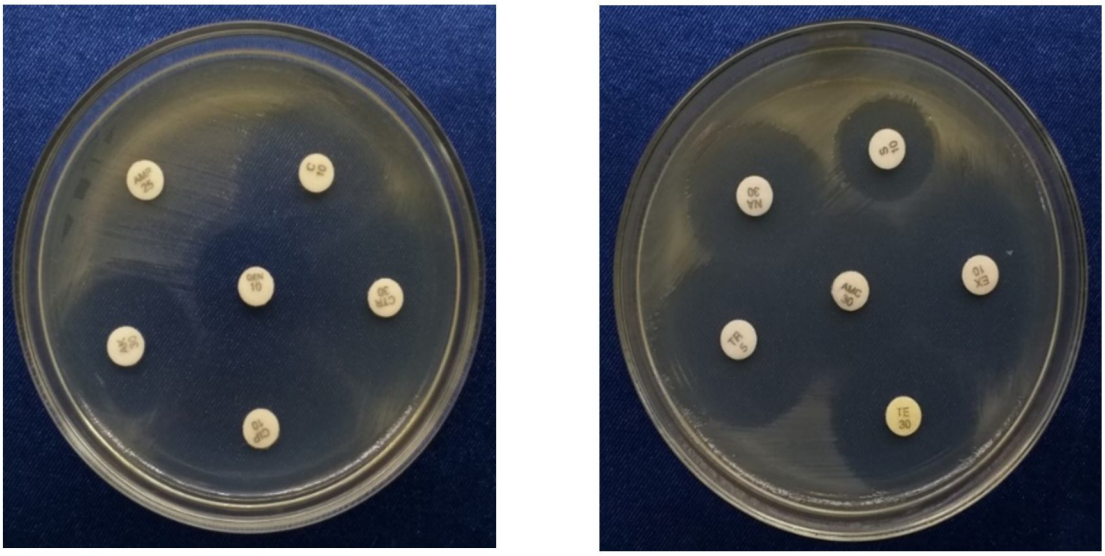
[A- Positive, B- Negative]

Fig. 5. Urease test



[A- Positive, B- Negative]

Fig. 6. Triple Sugar Iron test



[AK- Amikacin; AMP- Ampicillin; CTR- Ceftriaxone; C- Chloramphenicol; CIP- Ciprofloxacin; GEN- Gentamicin; AMC- Amoxycillin clavulanic acid; NA- Nalidixic acid; S- Streptomycin; EX- Enrofloxacin; TE- Tetracycline; TR- Trimethoprim]

Fig. 7. *In vitro* antimicrobial sensitivity test for *Salmonella* isolates

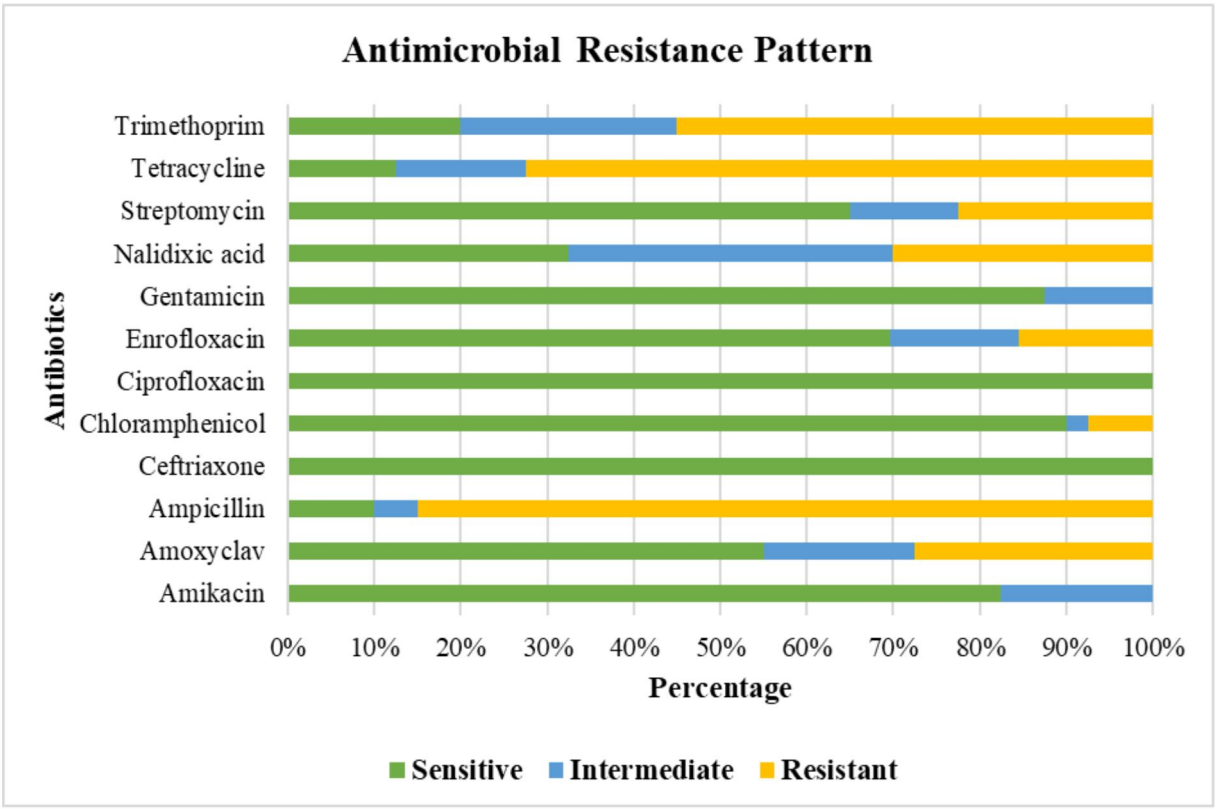


Fig. 8. Antimicrobial resistance pattern of *Salmonella* isolates

DISCUSSION

Isolation of *Salmonella* spp.: *Salmonella*, an anthroponotic pathogen, is one of the leading causes of food-borne illnesses all over the world. Food of animal origin such as chicken meat is also one of the vehicles of transmission to humans. The results of our study agree with some other studies. An almost similar finding of about 23.70% and 33.30% was observed by Kaushik *et al*. (2014) and Balakrishnan *et al*. (2018), respectively. However, the lower prevalence rate of 4.80% by Procura *et al*. (2017), 18% by Uddin *et al*. (2018), 14.64% by Bangera *et al*. (2019), and 14.89% by Sharma *et al*. (2019) was also reported which may be due to good hygienic practices followed during meat processing and low temperature to keep meat as bacteria grow rapidly at warm temperatures. In contrast, high prevalence rate of 55.90% and 63.60% was recorded by Mori *et al*. (2018) and Zhang *et al*. (2018), respectively, indicating unhygienic practices during meat processing.

Antibiogram: In contrast to our study's antibiotic sensitivity result, Perin *et al*. (2020) examined the antibiotic sensitivity profile of 95 *Salmonella* spp. isolates from chicken meat. Chloramphenicol and amikacin were sensitive to all isolates. Nalidixic acid (95%), tetracycline (94%), ampicillin (87%), amoxicillin with clavulanic acid (84%), ceftriaxone (79%), and ciprofloxacin (76%) had the highest rates of resistance. Zwe *et al*. (2018) studied the antimicrobial sensitivity pattern of fresh chicken meat sold in Singapore meat markets. More than 80% of *Salmonella* spp. isolates exhibited resistance to at least one of the eleven antibiotics tested. The most common phenotypic resistances exhibited were ampicillin (78.80%), tetracycline and chloramphenicol (61.50%), and nalidixic acid (30.8%). Other antibiotics such as gentamicin, amoxiclav, ceftriaxone, and ciprofloxacin showed 23.10%, 15.40%, 9.60%, and 3.80% resistance, respectively.

These results indicate that the resistance patterns of *Salmonella* spp. to all the examined antibiotics varied considerably. They are becoming more resistant to specific antibiotics, which suggests that these medications have been widely used in chickens for therapeutic purposes or to promote growth at doses below therapeutic levels. Mutational alterations may potentially cause the development of resistance. *Salmonella* sensitivity to particular antibiotics suggests that these antibiotics were used less frequently on the farm and may be suggested for the treatment of *Salmonella* infection.

Out of a total of 150 chicken samples (75 each of muscles and liver), 40 (26.67%) samples were found positive for *Salmonella* species by cultural isolation. All *Salmonella* spp. isolates were 100% sensitive to ciprofloxacin and ceftriaxone, followed by decreasing order of sensitivity of chloramphenicol, gentamicin, amikacin, enrofloxacin, streptomycin, amoxyclav, nalidixic acid, trimethoprim, and ampicillin as 90%, 87.50%, 82.50%, 70%, 65%, 55%, 32.50%, 20%, and 10%, respectively.

Conflict of interest: The authors of this research article declare no conflict of interest.

Author's contributions: ZBP: Collected the samples, carried out the antimicrobial sensitivity part and drafted the manuscript; JBN, RAM: Conceived the study and participated in its designing; JHC: Oversaw the research; BCP: Checked the manuscript; ST: Helped in sample collection and did the cultural isolation of the bacteria; KSM: Did the biochemical characterization of the isolates.

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