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# Prevalence, genetic characterization, and antibiogram of Salmonella enterica recovered from buffalo meat

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**ABSTRACT**

***Objective:*** This study aimed to determine the prevalence, virulence-associated genes and antimicrobial resistance of *Salmonella* species recovered from buffalo meat at Mansoura city in Egypt. *Salmonella* virulence genes were detected using polymerase chain reaction targeting *invA*, *stn*, and *hilA* genes.
***Design:*** Observational study.
***Samples:*** 120 samples.
***Procedures:*** A total of 120 buffalo meat samples were bacteriologically analyzed to isolate and characterize the *Salmonella* spp. and its virulence genes, in addition to their antimicrobial resistance.
***Results:*** Thirty (25%) out of 120-samples from buffalo meat were positive *Salmonella* spp. Out of the 191 phenotypically identified *Salmonella* isolates, only 58 strains were molecularly confirmed as *Salmonella* spp. based on *invA* gene detection. The *hilA* and *stn* genes were detected in 79.3% (46/58) and 72.4% (42/58) of the tested isolates, respectively. *S.* Enteritidis and *S.* Typhimurium were the predominant serovars of the tested isolates. All recovered isolates (n=58) were found to be resistant to erythromycin. A high percentage of isolates recovered from buffalo meat were resistant to at least one antibiotic with a MAR average of 0.459.
***Conclusion and clinical relevance:*** The high level of *Salmonella* contamination reported in Egyptian buffalo meat can constitute a potential risk for public health. Consequently, special programs are urgently needed to control *Salmonella* contamination in Egypt.
Key words: Buffalo meat, Salmonella, Virulence genes, Antibiogram

## 1. Introduction

Buffalo is considered an important source of economy in many African countries. Buffalo population is about 185.29 million all over the world and lives in different environments due to its genetic characteristics and strong musculature **[1]**. Buffaloes are more disease resistant than other animals and can tolerate a wide range of nutritional and environmental changes such as high temperatures, water scarcity, poor vegetation, and rough topography. They have a lot of capacity for development genetically in meat production**.**

Buffalo meat is a good source of healthiest red meat, because of its nutritive value, as it is rich in protein of high biological value, iron content, and low cholesterol. The major attractive features of buffalo meat are its good marbling, dark red color, firm consistency, low connective tissue, water holding capacity desirable texture and high protein, so it enters in the manufacturing of many meat products **[2]**.

Carcass is exposed during its slaughtering and preparation to microbial contaminants from different sources, either external or internal sources such as polluted air and water, dirty skin, hooves and hair, knives, cutting tools, infected personnel, intestinal contents, handling during processing and storage. These microbial contaminants are categories of spoilage bacteria causing food spoilage or pathogenic bacteria causing foodborne illnesses like *Salmonella* spp., *Listeria* spp., *Escherichia coli* O157:H7, and *Staphylococcus. aureus* **[3]**.

Non typhoidal *Salmonella* causes approximately 155,000 human deaths and 93.8 million cases of acute gastroenteritis worldwide. Most human *Salmonella* outbreaks are associated with different food, including meat and meat products. Human gastroenteritis symptoms caused by *Salmonella* include fever, abdominal cramps and diarrhea with an outbreak usually lasts 3 to 7 days. Immunosuppressed persons, young children and older people are more susceptible to human *Salmonella* infections **[4]**.

*S.* Typhimurium and *S.* Enteritidis are the most prominent serotypes responsible for *Salmonella* infections with percentage 46% and 24%, respectively **[5].** Additionally, these serovars are the most frequent strains recovered from humans worldwide. All *Salmonella* serovars are potentially pathogenic, there are significant variances in their virulence to humans, which has been related to the presence or absence of virulence-associated genes **[6]**.

The *invA* gene is essential for pathogen invasion in host cells, it has also been used as a PCR target for *Salmonella* strain detection, *while* the enterotoxin (*stn*) gene encoding a protein that causes severe diarrhea. The *hilA* gene is involved in adhesion and invasion of *Salmonella* to host cells **[7]**.

Misuse and overuse of antimicrobial agents in veterinary medicine as growth promoter or therapeutics leads to the emergence of multidrug-resistant bacteria (MDR bacteria), such as *Salmonella* that has evolved as a major health hazard all over the world, and makes it difficult to utilize traditional antibiotics **[4]**.

Because of the increased consumption and marketing of buffalo meat in Egypt, and the increased incidence of resistant or MDR *Salmonella* isolates worldwide against antimicrobials, this study was designed to determine the prevalence and the detection of virulence genes of *Salmonella* spp. recovered from buffalo meat distributed at Mansoura city in Egypt. Moreover, the present work highlighted the antimicrobial resistance profiles of *Salmonella* strains using 14 antimicrobial agents: amikacin (AK), ampicillin (AM), cefepime (FEP), cefotaxim (CF), ciprofloxacin (CP), clindamycin (CL), enrofloxacin (EN), erythromycin (E), gentamicin (G), ipipenem (IPM), , nalidixic acid (NA), streptomycin (S), sulphamethoxazol (SXT) and tetracycline (T) commonly used in human and veterinary medicines.

## 2. MATERIALS AND METHODS

### *2.1. Collection of samples*

A total of one hundred and twenty buffalo meat samples (250 grams each) were randomly purchased during the period from October 2020 to July 2021, from different retail butcher shopsdistributed at Mansoura city in Egypt. Each Sample kept separately in a sterile polyethylene bag, labeled and delivered in an ice container with a minimum delay to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, Egypt, wherein the preliminary microbial analyses were done rapidly.

### *2.2. Preparation of samples*

Preparation of meat samples was done according to techniques recommended by International Standards Organization **[8]**. Briefly, twenty-five grams from each whole individual collected meat sample were cut into small pieces using a sterile scalpel blade then homogenized with 225 ml of sterile buffered peptone water (Oxoid CM0509) in a sterile stomacher (Moulinex, made in France, speed: 2000 rpm) for 1 min The homogenate of each sample was poured aseptically into a sterile screw capped wide mouth jar and incubated at 37 ˚C for 24 h.

### *2.3. Isolation and identification of Salmonella spp.*

Isolation and identification of Salmonella spp. Was carried out according to International Organization for Standardization "ISO" **[9]**. From each pre-enriched culture, 0.1 ml was inoculated into 10 ml of Rappaport Vassilliadis enrichment broth (RV; Oxoid CM0669), followed by incubation at 42 °C for 24 h. A loopful from each enriched broth was streaked onto Xylose-Lysine-Desoxycholate (XLD) agar (CM0469; Oxoid Ltd., Basingstoke, UK) and incubated at 37 °C for 24h. Typical presumptive *Salmonella* colonies were purified onto nutrient agar plates, followed by incubation at 37 °C for 18-24 h. The purified colonies were subcultured onto nutrient agar slops then incubated at 37 °C for 18-24 h for further biochemical identification.

The biochemical identification of the recovered isolates was based on the triple sugar iron (TSI) test, production of indole from tryptophan, urease, citrate utilization, methyl red, Voges-Proskauer testes, in addition to lysine decarboxylation and carbohydrate, lactose and glucose fermentation. All biochemically identified isolates in the present work were further confirmed using PCR assay.

### *2.4. Molecular analyses*

All identified *Salmonella* isolates were molecularly confirmed according to the method reported by **[10]**. Genomic DNA was extracted by using the Gene JET genomic DNA Purification Kit (K0721, Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. Genomic DNA of *Escherichia coli* K12DH5α, and *S.* Typhimurium (RIMD 1985009) obtained from National Research Centre (NRC), Cairo, Egypt was used as negative and positive control reference strains, respectively, for the determination of *invA*, *stn*, and *hilA* genes.

The detection of *Salmonella* virulence genes was performed using the multiplex polymerase chain reaction, using specific oligonucleotide primers sequences constructed to yield DNA fragments of 275 bp, 617 bp and 854 bp for *invA, stn* and *hilA* genes, respectively. The molecularly confirmed *Salmonella* strains recovered from buffalo meat samples examined were subjected to slide agglutination technique for *Salmonella* serotyping based on detection of flagellar (H) and somatic (O) antigens by using of separated H and O *Salmonella* antisera (Denka Seiken Co., Tokyo, Japan).

### *2.5. Antimicrobial susceptibility tests*

Antibiograms of the identified *Salmonella* isolates were determined using the agar diffusion method according to the guideline of Clinical and Laboratory Standards Institute **[11]**, using Mueller-Hinton agar (Oxoid, Basingstoke, Ltd, UK). Antimicrobial agents (Difco Laboratories, and BioMerieux, France) were tested as follows: ampicillin (10 µg), amikacin (30 µg), cefotaxime (30 µg), cefepime (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), gentamicin (10 µg), erythromycin (15 µg), imipenem (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30 µg) and trimethoprim / sulphamethoxazole (25 µg). *Escherichia coli*

ATCC 25922 was tested as a reference strain for antimicrobial disc control. Multiple Antibiotic Resistance (MAR) index for each *Salmonella* strain was calculated by the formula: MAR index = No. of resistance antimicrobials / Total No. of tested antimicrobials.

## 3. RESULTS AND DISCUSSIONS

### *3.1. Prevalence of Salmonella enterica isolated from buffalo meat*

In the present study, the conventional cultural method based on the colonial appearance could detect presumptive *Salmonella* in 65 (54.2%) out of the 120 buffalo meat samples examined, while only 42.5% (51/120) of the tested samples were biochemically identified as *Salmonella* (Figure, 1A).

*Salmonella* was detected in 25% (30/120) of the buffalo meat samples examined based on molecular confirmation by PCR technique (Figure, 1A). Similar incidences of *Salmonella* spp., of 23.61%, 23.3% and 23% were recorded in red meat in Algeria **[12],** Egypt **[4]** and Turkey **[13]**, respectively. Conversely, our findings were higher than those detected in Iran at 7% ***[14],*** Laos at 7.11% **[15]**, Nepal at 7.4% **[16]**, India at 10.66% **[17]** and Egypt, at 18% **[18]**. Higher *Salmonella* prevalence was reported by other authors in different countries. In Laos, **[19]** who recovered *Salmonella* from 80% of buffalo meat and also in Bangladesh, **[20],** who found *Salmonella* in 46.67% of buffalo meat.

Out of 191 selected presumptive *Salmonella* colonies of all buffalo meat samples examined in our study, 96 (50.26%) and 58 (30.37%) were biochemically and molecularly confirmed as *Salmonella* spp., respectively (Figure, 1B). This result indicated that the molecular technique is more accurate and specific for detection of *Salmonella* isolates when compared to conventional culture method and biochemical identification, which is consistent with previous studies **[21, 22]**.

These wide variations between the results of *Salmonella* prevalence in buffalo meat examined with previous different studies could be attributed to differences in geographic regions and hygienic conditions during slaughtering and processing as well as sampling season and isolation method.

### *3.2. Distribution of the identified Salmonella serovars in buffalo meat examined.*

Out of the 58 serologically confirmed *Salmonella* strains recovered from buffalo meat samples examined, 12 different serotypes were identified. The most dominant serotypes were *S*. Enteritidis, *S*. Typhimurium, and *S*. Montevideo with an incidence of 20.7%, 17.2% and 12.1%, respectively. On the other hand, *S*. Derby, *S*. Saintpaul, and *S*. Chester were the least commonly identified serotypes (1.7% for each) (Table, 1). *S*. Enteritidis and *S*. Typhymiurium were the most prevalent serotypes isolated in the current study. our results are in close agreement with the results reported by **[4]** in Egypt, who revealed that the most prevalent serotype was *S*. Typhimurium (46/99) originated from fresh beef followed by *S*. Enteritidis (32/99). On the contrary, *S*. Typhi and *S*. Paratyphi were the most frequent serotypes isolated from buffalo meat in Egypt **[18],** whereas **[19]** reported that *S.* Stanley was the most common among isolates from buffalo meat in Laos. In India, *S*. Weltevreden was reported to be the most frequent isolate in buffalo meat **[17].** The variations seen in the list of prevalent *Salmonella* serotypes could attribute to differences in climate and geographical locations which may give certain serotypes priority over others.

### *3.3. Prevalence of virulence genes among identified Salmonella* serovars *isolated from buffalo meat.*

All serologically identified *Salmonella* isolates (n = 58) in this study were tested for the existence of virulence genes using a multiplex PCR (Figure, 2), with the findings indicating 100% of the isolates were harbored *invA* gene (275 bp), while 72.4% and 79.3% were positive for presence of *stn* gene (617 bp) and *hilA* gene (845 bp), respectively (Table, 2). Detection of *invA* gene in all *Salmonella* isolates surveyed in our study was similar to those reported in previous investigations carried out in Egypt **[4]**, Malaysia **[23]** and China **[24]**. Conversely, **[25]**, who detected *invA* gene in 36.3% of *Salmonella* spp. from poultry samples in India. In this study, *stn* gene was detected in 72.4% of *Salmonella* isolates. While, a previous study **[4]** detected stn gene in all examined *Salmonella* isolates from beef samples in Egypt. High detection rate of *hilA* gene (79.3%) among our *Salmonella* isolates is compatible with **[23]**, who detected *hilA* gene in 82.61% of *Salmonella* spp. from beef samples in Malaysia. Presence of these genes that aid the organisms to interact with the host cells and may indicate the virulence potential of *Salmonella* spp.

### *3.4. Antimicrobial susceptibility and distribution among Salmonella strains.*

In the present study, all *Salmonella* isolates (n=58) showed resistance to at least one antibiotic with MAR index ranged from 1 to 0.071 (Table, 3). This finding is consistent with previous studies published in Algeria **[12]**, India **[26]** and Malaysia **[23]**. The emergence and spread of multidrug resistant among *salmonella* serotypes has become a public health threat as it also tends to be more virulent when compared with non-multidrug resistant isolates **[4, 23]**. MAR index value higher than 0.2, is considered a high risk, while value lower than 0.2 indicates low risk **[27]**.

The antimicrobial susceptibility testing showed 100% resistance of *Salmonella* isolates (n=58) against erythromycin followed by streptomycin (98.2%), clindamycin (87.9%), cefepime (77.6%), nalidixic acid (65.5%), sulphamethoxazol (56.9%), ampicillin (41.4%), tetracycline (32.8%), enrofloxacin (27.6%) and ciprofloxacin (18.9%). Lower resistant rates were observed for cefotaxime, gentamicin, ipipenem and amikacin with an incidence of 8.6%, 6.9%, 3.4%, and1.7%, respectively

(Table, 4). These higher resistances of *Salmonella* spp. to erythromycin and nalidixic acid were reported previously in Egypt, which revealed 100% and 70% resistance to erythromycin and nalidixic acid, respectively **[4]**. On the contrary, all *Salmonella* isolates from buffalo meat were sensitive to ciprofloxacin, streptomycin and gentamicin in Bangladesh **[20]**. The higher MAR index value among *Salmonella* serovars in our results can be explained by extensive use and misuse of antimicrobial agents in animal husbandry as growth promoters or disease prevention.

## Conclusion

Virulent *Salmonella* and multidrug resistance (MDR) serotypes is highly prevalent in buffalo meat sold in Mansoura City, Egypt. Serotyping of isolated *Salmonella*, showed predominance of *S*. Enteritidis and *S*. Typhimurium in the tested samples, but other ten serotypes were also determined. These results indicated that Egyptian buffalo meat can constitutes a potential risk for public health and efforts must be exerted to control *Salmonella* contamination at slaughter and butcher shops, in order to prevent *Salmonella* from reaching foodstuffs.

## Authors contributions

Takwa Mohammed performed the lab work and wrote the first draft, Amira Zakaria designed the study, management and coordination responsibility for the research activity planning and execution, reviewing and editing the article. Samir Abd-Elghany and Khalid Sallam revised the final version. All authors have read and approved the final version of the manuscript for publication.

## Research Ethics Committee permission

The research was conducted according to standards of Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University

**Table 2:** Prevalence of different virulence genes among *Salmonella* serovars isolated from buffalo meat.

| Serovars (number) | | *Salmonella* serovars positive for virulence genes tested | | |
|---|---|---|---|---|
| | | *Stn* | *hilA* | *invA* |
| ***Salmonella*** **Enteritidis** | **(10)** | + | + | + |
| ***Salmonella*** **Enteritidis** | **(1)** | - | + | + |
| ***Salmonella*** **Enteritidis** | **(1)** | + | - | + |
| ***Salmonella*** **Typhimurium** | **(8)** | + | + | + |
| ***Salmonella*** **Typhimurium** | **(1)** | - | + | + |
| ***Salmonella*** **Typhimurium** | **(1)** | + | - | + |
| ***Salmonella*** **Montevideo** | **(4)** | + | - | + |
| ***Salmonella*** **Montevideo** | **(3)** | + | + | + |
| ***Salmonella*** **Rissen** | **(6)** | - | + | + |
| ***Salmonella*** **Infantis** | **(4)** | + | + | + |
| ***Salmonella*** **Infantis** | **(2)** | - | + | + |
| ***Salmonella*** **Virchow** | **(3)** | - | + | + |
| ***Salmonella*** **Virchow** | **(2)** | + | - | + |
| ***Salmonella*** **Essen** | **(3)** | + | - | + |
| ***Salmonella*** **Essen** | **(1)** | + | + | + |
| ***Salmonella*** **Dublin** | **(3)** | - | + | + |
| ***Salmonella*** **Anatum** | **(2)** | + | + | + |
| ***Salmonella*** **Chester** | **(1)** | + | + | + |
| ***Salmonella*** **Derby** | **(1)** | + | + | + |
| ***Salmonella*** **Saintpaul** | **(1)** | + | - | + |
| **Total** | **(58)** | **42(72.4%)** | **46 (79.3%)** | **58 (100%)** |

*invA*: Invasion gene. *hilA*: Hyper-invasive locus gene. *Stn*: Enterotoxin gene.

(+): positive (-) : negative

**Table 3:** Antimicrobial resistance profile and multi antibiotic resistance (MAR) index of *Salmonella* strains isolated from buffalo meat (n=58).

| *Salmonella* strains | Antimicrobial resistance profile | MAR index |
|---|---|---|
| ***S.* Enteritidis** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP, CF, G, IPM, AK | 1 |
| ***S.* Typhimurium** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP, CF, G, IPM | 0.928 |
| ***S.* Enteritidis** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP, CF, G | 0.857 |
| ***S.* Montevideo** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP, CF, G | 0.857 |
| ***S.* Rissen** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP, CF | 0.785 |
| ***S.* Typhimurium** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP | 0.714 |
| ***S.* Infantis** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP | 0.714 |
| ***S.* Rissen** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP | 0.714 |
| ***S.* Virchow** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP | 0.714 |
| ***S.* Essen (n=2)** | E, S, CL, FEP, NA, SXT, AM, T, EN, CP | 0.714 |
| ***S.* Enteritidis** | E, S, CL, FEP, NA, SXT, AM, T, EN | 0.642 |
| ***S.* Typhimurium** | E, S, CL, FEP, NA, SXT, AM, T, EN | 0.642 |
| ***S.* Montevideo** | E, S, CL, FEP, NA, SXT, AM, T, EN | 0.642 |
| ***S.* Dublin** | E, S, CL, FEP, NA, SXT, AM, T, EN | 0.642 |
| ***S.* Anatum** | E, S, CL, FEP, NA, SXT, AM, T, EN | 0.642 |
| ***S.* Infantis** | E, S, CL, FEP, NA, SXT, AM, T | 0.571 |
| ***S.*Enteritidis (n=2)** | E, S, CL, FEP, NA, SXT, AM, T | 0.571 |
| ***S.*Typhimurium(n=2)** | E, S, CL, FEP, NA, SXT, AM | 0.500 |
| ***S.* Rissen** | E, S, CL, FEP, NA, SXT, AM | 0.500 |
| ***S.* Virchow (n=2)** | E, S, CL, FEP, NA, SXT, AM | 0.500 |
| ***S.* Enteritidis (n=2)** | E, S, CL, FEP, NA, SXT | 0.428 |
| ***S.* Infantis** | E, S, CL, FEP, NA, SXT | 0.428 |
| ***S.* Essen** | E, S, CL, FEP, NA, SXT | 0.428 |
| ***S.* Montevideo (n=2)** | E, S, CL, FEP, NA, SXT | 0.428 |
| ***S.* Enteritidis** | E, S, CL, FEP, NA, SXT | 0.428 |
| ***S.*Typhimurium(n=2)** | E, S, CL, FEP, NA, SXT | 0.428 |
| ***S.* Enteritidis** | E, S, CL, FEP, NA | 0.357 |
| ***S.* Montevideo (n=2)** | E, S, CL, FEP, NA | 0.357 |
| ***S.* Infantis** | E, S, CL, FEP, NA | 0.357 |
| ***S.* Virchow** | E, S, CL, FEP, NA | 0.357 |
| ***S.* Enteritidis** | E, S, CL, FEP | 0.285 |
| ***S.* Rissen** | E, S, CL, FEP | 0.285 |
| ***S.* Infantis (n=2)** | E, S, CL, FEP | 0.285 |
| ***S.* Dublin (n=2)** | E, S, CL, FEP | 0.285 |
| ***S.* Derby** | E, S, CL, FEP | 0.285 |
| ***S.* Enteritidis (n=2)** | E, S, CL | 0.214 |
| ***S.* Typhimurium** | E, S, CL | 0.214 |
| ***S.* Rissen (n=2)** | E, S, CL | 0.214 |
| **S. Essen** | E, S, CL | 0.214 |
| ***S.* Saintpaul** | E, S, CL | 0.214 |
| ***S.*Typhimurium(n=2)** | E, S | 0.142 |
| ***S.* Montevideo** | E, S | 0.142 |
| ***S.* Virchow** | E, S | 0.142 |
| ***S.* Anatum** | E, S | 0.142 |
| ***S.* Chester** | E | 0.071 |
| | **MAR index Average 0.459** | |

E: erythromycin; S: streptomycin; CL: clindamycin; FEP: cefepime; NA: nalidixic acid; SXT: sulphamethoxazol; AM: ampicillin; T: tetracycline; EN: enrofloxacin; CP: ciprofloxacin; CF: cefotaxim; G: gentamicin; IMP: ipipenem; AK: amikacin

**Table 4:** Antimicrobial susceptibility of *Salmonella* strains (n = 58).

| Antimicrobial agents | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| **Erythromycin (E)** | 0 (0%) | 0 (0%) | 58 (100%) |
| **Streptomycin (S)** | 0 (0%) | 1 (1.8%) | 57 (98.2%) |
| **Clindamycin (CL)** | 2 (3.4%) | 5 (8.6%) | 51 (87.9%) |
| **Cefepime (FEP)** | 9 (15.5%) | 4(6.9%) | 45 (77.6%) |
| **Nalidixic acid (NA)** | 16 (27.6%) | 4 (6.9%) | 38 (65.5%) |
| **Sulphamethoxazol (SXT)** | 19 (32.7%) | 6 (10.3%) | 33 (56.9%) |
| **Ampicillin (AM)** | 31 (53.4%) | 3 (5.2%) | 24 (41.4%) |
| **Tetracycline (T)** | 35(60.3%) | 4 (6.9%) | 19 (32.8%) |
| **Enrofloxacin (EN)** | 42 (72.4%) | 0 (0%) | 16 (27.6%) |
| **Ciprofloxacin (CP)** | 40 (68.9%) | 7 (13.8%) | 11 (18.9%) |
| **Cefotaxime (CF)** | 48 (82.8%) | 5 (8.6%) | 5 (8.6%) |
| **Gentamicin (G)** | 50 (86.2%) | 4 (6.9%) | 4 (6.9%) |
| **Ipipenem (IPM)** | 53(91.4%) | 3 (5.2%) | 2 (3.4%) |
| **Amikacin (AK)** | 57 (98.2%) | 0 (0%) | 1 (1.7%) |

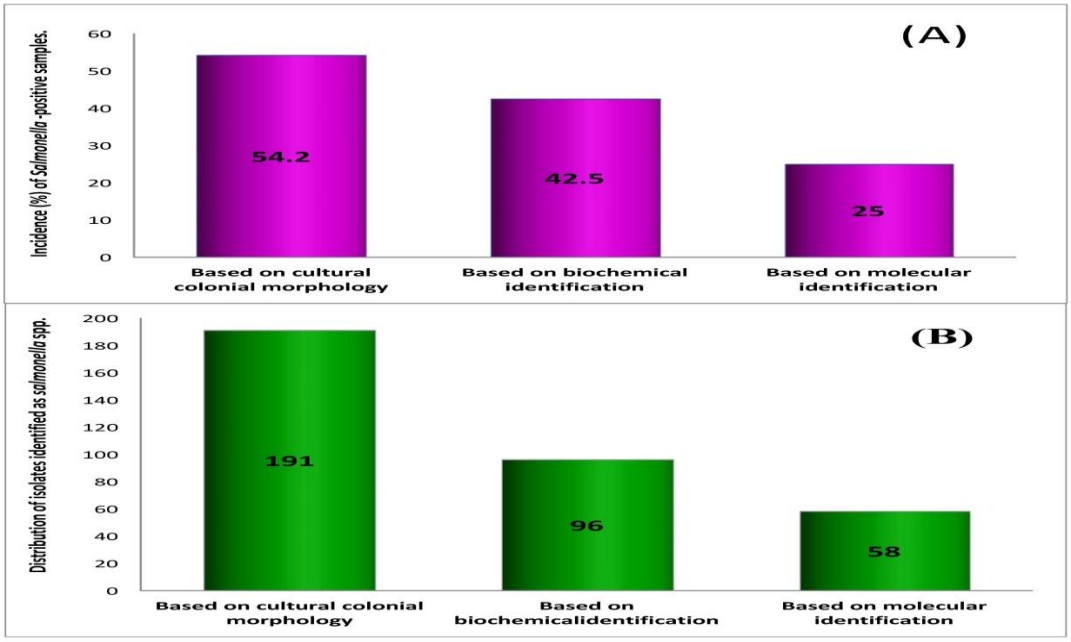


**Figure 1.** Prevalence of *Salmonella*-positive samples among buffalo meat samples examined (A), and distribution of *Salmonella* isolates based on cultural colonial morphology, biochemical, and molecular identifications (B).

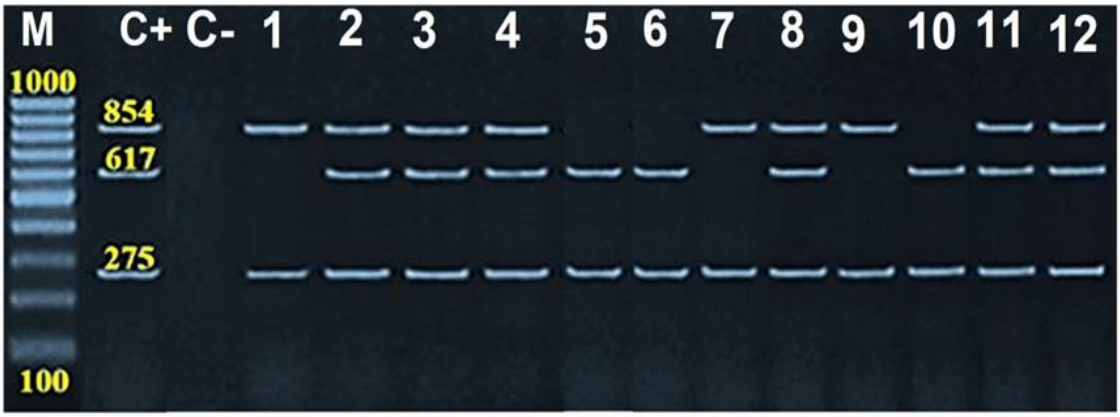


**Figure 2:** Agarose gel electrophoresis of multiplex PCR of *invA* (275 bp), *stn* (617 bp) and *hilA* (854 bp) virulence genes of *Salmonella* spp. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive strain for *invA, stn,* and *hilA* genes. Lane C-: Control negative. Lanes 2 (*S*. Enteritidis), 3 (*S*. Typhimurium), 4 (*S*. Infantis), 8 (*S*. Dublin), 11 (*S*. Anatum), & 12 (*S*. Derby): Positive strains for *invA*, *stn* and *hilA* genes. Lanes 5 (*S.* Montevideo), 6 (*S*. Essen), & 10 (*S.* Chester): Positive strains for *invA* and *stn* genes. Lanes 1 (*S*. Rissen), 7 (*S*. Virchow), & 9 (*S*. Saintpaul): Positive strains for *invA* and *hilA* genes.

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