

# *Salmonella* in Food Environments in Canteens: A Focus on Antibiotic and Disinfectant Resistance Patterns

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**Abstract.** The objective of this study was to determine the molecular characterization and antibiotic and disinfectant resistance potential of *Salmonella* isolates from food related areas in canteens. This study was performed in food related areas as well as food handlers in student canteens in University campus to trace source of *Salmonella* contamination with a special focus on antibiotic and disinfectant resistance of the isolates. *Salmonella* isolates were identified by conventional and molecular techniques. Genetic similarity, antibiotic and disinfectant resistance patterns of the isolates were performed by sequence analyzing, disc diffusion and PCR, respectively. *Salmonella* contamination was determined from hand (Newport and Infantis), knife handle (Newport) and grilled chicken (Koessen) samples. The identity of the 16S rRNA sequence of two Newport isolates (knife handle and hand) from Canteen 4 and Canteen 6 were found 100% identical. Multidrug resistance (MDR) was observed among all *Salmonella* isolates with resistance to at least five or more antibiotics. *sugE* and *qacEΔ1* disinfectant genes were determined in 2 and 1 isolate respectively while cross-resistance to antibiotics and disinfectants in three isolates were detected. The results of this study indicate that the food-handlers and food preparation equipments may serve as reservoirs for cross-resistant *Salmonella*, a potential public health concern. Therefore, periodic training programmes should effectively be implemented for food handlers. Owing to the growing concern that antibiotic resistant mutants could be induced by improper disinfectant use, unnecessary and misuse of disinfectants should be avoided, especially on food contact surfaces.

**Key words:** antibiotic resistance, disinfectant resistance, food environments, public health, *Salmonella*

## Introduction

Every year, approximately 3 million children die of diarrhea, and hundreds of millions of people suffer from diarrhea attacks and complications mainly caused by food-borne pathogens, among which *Salmonella* is one of the most commonly isolated (1, 2). At the present, over 2500 *Salmonella* serotypes have been identified; more than 50% of these serotypes belong to *Salmonella* Enterica Subsp. Enterica and many of these serovars are able to cause illness such as enteric fever, bacteremia, gastroenteritis in both humans and animals

(3, 4) *S. Enterica* Subsp. *Enterica* Serovar Enteritidis (*S. Enteritidis*) and *S. Enterica* Subsp. *Enterica* Serovar Typhimurium (*S. Typhimurium*) are the most common causative agents of food-borne gastroenteritis (5).

Due to surveillance, prevention and treatment costs, antibiotic resistant *Salmonella* infections are considered among the major public health concerns worldwide. It has been reported that human antibiotic resistant *Salmonella* infections are often caused by contaminated food (6). It is suggested that *Salmonella* could survive on surfaces and equipment used for preparing food after conventional cleaning and sanitation

practices (7, 8, 9). Ability of *Salmonella* in food environment could to adhere and form biofilm on different food contact surfaces, allowing resistance to different conditions including disinfectants (10). Disinfectants, especially quaternary ammonium compounds (QACs), are commonly used for food contact surfaces and other equipment in the food industry to prevent contamination of raw materials and products with pathogens and spoilage microorganisms (11, 12). In recent years, concerns about disinfectant tolerance have gradually increased mainly based on the fact that disinfectant use may induce evolution of cross-resistance to antibiotics (13) which in turn may lead to a great challenge in the control of pathogens. The aim of this study was to determine evaluate antimicrobial susceptibility and disinfectant resistance of *Salmonella* isolates obtained from food environments at canteens as well as their genetic relationships. The genetic characteristics of pathogens provide information about their sources and the paths they follow in transmission.

## Materials and Methods

### Sampling

In this study, a total of 212 samples (100 foods, 45 hand, 27 cutting board and 40 knife handle surface) taken from six different canteens inside university campus were examined during the period of 2017-2018. The food samples studied were consisted of 10 grilled chicken (Ready to eat; RTE), 5 meatball (raw), 5 chicken meat (raw), 5 chicken saute (raw), 5 chicken shish (raw), 5 chicken skewers (raw), 10 soujouk (RTE), 10 Adana kebab (raw), 10 Urfa kebab (raw), 20 chicken doner (RTE), 10 vegetables (RTE) and 5 pancake (RTE). At least 100 gr food samples were taken into sterile bags. Forefingers and thump samples from 45 food handlers were taken during working hours without previous notification.

Cutting board and knives were sampled by swaps (premoistened sterile swab in 10 mL of sterile 0.1%-peptone water) after bordering the surface of sampling area by a 15-cm<sup>2</sup> sterile template. The food contact surfaces were wiped three times in three directions with the swaps (14). All samples were transported

to the laboratory in a cool box under 2 °C and were immediately analyzed.

### Isolation of *Salmonella* from Samples

RODAC plates containing selective agar (XLD, CM 0469) was used to sample the thumb and forefinger of both hands of the personnel.

Swaps taken from cutting boards and knife handles were placed in sterile tubes containing 10 ml buffer peptone water (OXOID, CM0509) and used directly for pre-enrichment and enrichment stage.

The isolation and phenotypic identification of *Salmonella* from all samples were carried out as referred by ISO 6579 (15). Presumptive *Salmonella* isolates were verified with polyvalent O (somatic) and H (flagellar) antisera (Difco 2264-47-2).

### PCR and Sequencing

The total genomic DNA was extracted by using the commercial spin column kit (Thermo Fisher Scientific, K0722) according to the manufacturer's recommendations. *rrsE 16S rRNA* and *sun 16S rRNA* (cytosine (967)-C(5))-methyltransferase genes were used to identify *Salmonella* strains. The isolates were analysed for *qacE*, *qacEΔ1*, and *sugE* genes for the determination of disinfectant resistance (DR). Fast PCR Professional 6.1.2 package program was used for primers design (16) as shown in table 1.

Melting temperature (T<sub>m</sub>) was determined using gradient PCR with the following PCR mixture (each 20 μl) consisted of 1X PCR buffer, 2.0m M MgCl<sub>2</sub>, 0.2Mm dNTP, 0.25mM forward and reverse primer, 1U Platinum Taq DNA polymerase (Invitrogen,10966034) and 20ng template DNA. All PCR amplification was carried out initial denaturation at 95°C for 2 min, 35 cycles of denaturation 45 sec at 94°C, 1 min primer annealing at 60-62°C, 1 min extension at 72°C and a final extension at 72°C for 10 min. Amplified PCR products were analyzed by gelelectrophoresis on 2% agarose gel followed by Red Safe (Intron, 21141) staining.

Subsequently, all PCR products were purged with exonucleaseI (Thermo Fisher Scientific, EN0581) and Fast AP thermosensitive alkaline phosphatase

**Table 1.** Primers designed to determine *Salmonella* strains and DR genes

| Gene              | Forward (5'→3')         | Revers (5'→3')         | Tm(°C) | Base pair | GenBank No  |
|-------------------|-------------------------|------------------------|--------|-----------|-------------|
| <b>rrsE16sRNA</b> | ACCAAGTCTCAAGAGTGAACACG | TCACAAAGTGGTAAGCGCCCTC | 60     | 1540      | NC_003197.2 |
| <b>sun16sRNA</b>  | ATGCTTACCCACACAGTGGCA   | AGGTGGCGTAAACCAGCGTCC  | 62     | 660       | NC_003197.2 |
| <b>sugE</b>       | TTCTTCCGGGCAAAAATGCCA   | TCCTTTCACGGACCCCTACTA  | 62     | 528       | NC_010259.1 |
| <b>qacE</b>       | AACAATTCGTTCAAGCCGACG   | GATCCGACTCGCAGCATTTTCG | 62     | 615       | NC_013437.1 |
| <b>qacEΔ1</b>     | GCACATAATTGCTCACAGCCA   | GATCCGACTCGCAGCATTTTCG | 62     | 552       | NC_003292.1 |

(Thermo Fisher Scientific, EF0652). PCR Sequencing was performed according to Big Dye Terminator 3.1 kit protocol. The products obtained at the end of the sequenced PCR were purified by ethanol/EDTA/sodium acetate precipitation method. Hi Diformamide was added to 15 µl to each well and loaded onto the DNA Sequence Analyzer (ABI3500). Sequence chromatograms were edited and defined by alignment of forward and reverse *sequences* using Sequencher® 5.4.6 DNA sequence analysis software (Gene Codes Corporation, Ann Arbor, MIUSA) and Bioedit sequence alignment editor analysis program (17). All sequences were edited (GeneCode, Sequencher 5.4.6) and aligned with the Bioedit sequence alignment editor analysis program (17) and amplification product was read 360 bp. Genotypic results were compared, and similarity searches were performed using MEGA 4 (18).

#### *Antimicrobial susceptibility testing*

The isolates were tested against 24 antimicrobial agents including Imipenem (10 µg, Bioanalyse), Marbofloxacin (5 µg, Bioanalyse), Vancomycin (30 µg, Bioanalyse), Erythromycin (15 µg, Bioanalyse), Streptomycin (10 µg, Bioanalyse), Rifampin (5 µg, Bioanalyse), Cloxacillin (5 µg, Oxoid), Lincomycin/Neomycin (75 µg, Oxoid), Tetracycline (30 µg, Oxoid), Cefoperazone (75 µg, Oxoid), Cefuroxime (30 µg, Bioanalyse), Chloramphenicol (30 µg, Oxoid), Amoxicillin/Clavulanic acid (20/10 µg, Bioanalyse), Gentamicin (10 µg, Bioanalyse), Ciprofloxacin (5 µg, Oxoid), Neomycin (10 µg, Oxoid), Penicillin/Novobiocin (10/30 µg, Bioanalyse), Sulphamethoxazole/

Trimethoprim (25 µg, Oxoid), Kanamycin (30 µg, Oxoid), Amoxicillin (25 µg, Oxoid), Ceftazidime (30 µg, Oxoid), Tobramycin (10 µg, Oxoid), Amikacin (30 µg, Oxoid), Nalidixic acid (30 µg, Oxoid) by using the Kirby-Bauer disc diffusion method (19). The resistance levels were defined as described by the Clinical and Laboratory Standards Institute (20).

#### **Results**

Overall, 6 (2.7%) of 212 samples yielded *Salmonella* including 1(1%) grilled chicken, 4 (8.8%) food handlers' hand and 1 (2.5%) knife handle samples. A total of 4 serotypes were identified from *Salmonella* contaminated samples and the serotype distribution of the isolates are listed in table 2.

According to sequencing results; two Newport (knife handle and hand) from Canteen 4 and Canteen 6 were found 100% identical and were also 100% identical to *S. Newport* obtained from Genbank. Moreover, 16S rRNA sequence of other strains obtained from this study also showed similar pattern (99.0–100.0%) with five other sequences provided from GenBank (Table 2).

Among the DR genes, *sugE* was detected in one hand and one grilled chicken isolates while *qacEΔ1* was detected in 2 hand isolates. None of the isolates found to harbour *qacE* gene (Table 3).

As shown in table 4, all six isolates were found 100% resistant to penicillin/novobiocin, tetracycline, cloxacillin and rifampin. Also, evident resistance to sulphamethoxazole / trimethoprim (83.3%), vancomycin,

**Table 2.** Genetic similarity of the isolates

| Sample | Source          | Closest 16S rRNA NCBI isolate and accession  | Sequence similarity to isolate (%) | Canteen   |
|--------|-----------------|--|------------------------------------|-----------|
| 43     | Hand            | <i>Salmonella</i> <i>supsb. enterica</i> serovar Infantis strain L41/ CP038516.1                             | 100                                | Canteen 1 |
| 6a     | Hand            | <i>Salmonella</i> <i>supsb. enterica</i> serovar Infantis strain L41/ CP038516.1 strain NCTC8272/ LR134149.1 | 100                                | Canteen 2 |
| 6b     | Grilled chicken | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Koessen strain S-1501/ CP019412.1                  | 99                                 |           |
| 39a    | Hand            | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Newport strain USDA-ARS-USMARC-1925/ CP025232.1    | 100                                | Canteen 4 |
| 39b    | Hand            | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Newport strain NCTC129/ LR134140.1                 | 99                                 |           |
| 45     | Knife handle    | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Newport strain USDA-ARS-USMARC-1925/ CP025232.1    | 100                                | Canteen 6 |

**Table 3.** DR genes of *Salmonella* isolates

| Source          | Species   | Disinfectant resistance genes |               |             |
|-----------------|---|-------------------------------|---------------|-------------|
|                 |   | <i>sugE</i>                   | <i>QacEΔ1</i> | <i>qacE</i> |
| Hand            | <i>Salmonella</i> <i>supsb. enterica</i> serovar Infantis         | -                             | -             | -           |
| Hand            | <i>Salmonella</i> <i>supsb. enterica</i> serovar Infantis         | +                             | +             | -           |
| Grilled chicken | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Koessen | +                             | -             | -           |
| Hand            | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Newport | -                             | -             | -           |
| Hand            | <i>Salmonella enterica</i> <i>supsb. enterica</i>                 | -                             | +             | -           |
| Knife handle    | <i>Salmonella enterica</i> <i>supsb. enterica</i> serovar Newport | -                             | -             | -           |

**Table 4.** Antimicrobial resistance percentages of *Salmonella* isolates tested

| Antibiotics                             | No. of <i>Salmonella</i> spp. isolates (n= 6) |       |
|---|---|-------|
|   | S (%)   | R (%) |
| Imipenem (10 µg)                        | 100   | 0     |
| Marbofloxacin (5 µg)                    | 100   | 0     |
| Vancomycin (30 µg)                      | 66.7  | 66.7  |
| Erythromycin (15 µg)                    | 33.3  | 66.7  |
| Streptomycin (10 µg)                    | 33.3  | 66.7  |
| Rifampin (5 µg)                         | 33.3  | 100   |
| Cloxacillin (5 µg)                      | 0   | 100   |
| Lincomycin/Neomycin (75 µg)             | 0   | 33.3  |
| Tetracycline (30 µg)                    | 66.7  | 100   |
| Cefoperazone (75 µg)                    | 0   | 0     |
| Ceftrofur (30 µg)                       | 100   | 0     |
| Chloramphenicol (30 µg)                 | 100   | 0     |
| Amoxicillin/ Clavulanic acid (20/10 µg) | 100   | 16.7  |

| Antibiotics                            | No. of <i>Salmonella</i> spp. isolates (n= 6) |       |
|--|---|-------|
|  | S (%)   | R (%) |
| Gentamicin (10 µg)                     | 83.3  | 0     |
| Ciprofloxacin (5 µg)                   | 100   | 0     |
| Neomycin (10 µg)                       | 100   | 0     |
| Penicillin/Novobiocin (10/30 µg)       | 100   | 100   |
| Sulphamethoxazole/Trimethoprim (25 µg) | 0   | 83.3  |
| Kanamycin (30 µg)                      | 16.7  | 33.3  |
| Amoxicillin (25 µg)                    | 66.7  | 66.7  |
| Ceftazidime (30 µg)                    | 33.3  | 33.3  |
| Tobramycin (10 µg)                     | 66.7  | 33.3  |
| Amikacin (30 µg)                       | 66.7  | 33.3  |
| Nalidixic acid (30 µg)                 | 66.7  | 0     |

**Table 5.** Multidrug-resistant (MDR) *Salmonella* isolates from canteens in Afyon –Turkey

| Serotype | Source             | Antibiotics (n)  | Antimicrobial resistance percentages |
|----------|--------------------|--|--------------------------------------|
| Infantis | C1-Hand            | VA, E, S, RA, CX, TE, P/NV, SXT, K, CAZ, TOB, AK (12)  | 50                                   |
|          | C2-Hand            | VA, E, S, RA, CX, TE, P/NV, SXT, K, CAZ, TOB, AK (12)  |                                      |
| Koessen  | C2-Grilled chicken | VA, E, S, RA, CX, L/N, TE, AM/C, N, P/NV, SXT, AX (12) |                                      |
| Newport  | C4-Hand            | RA, CX, TE, P/NV, AX (5)                               | 20.8                                 |
|          | C4-Hand            | VA, E, S, RA, CX, L/N, TE, P/NV, SXT, AX (10)          | 41.6                                 |
|          | C6-Knife handle    | RA, CX, TE, P/NV, SXT, AX (6)                          | 25                                   |

VA: Vancomycin; E: Erythromycin; S: Streptomycin; RA: Rifampin; CX: Cloxacillin; L/N: Lincomycin/Neomycin; TE: Tetracycline; AMC: Amoxicillin/ Clavulanic acid; P/NV: Penicillin/Novobiocin; N: Neomycin; SXT: Sulphamethoxazole/Trimethoprim; K: Kanamycin; AX: Amoxicillin; CAZ: Ceftazidime; TOB: Tobramycin; AK: Amikacin

erythromycin, amoxicillin and streptomycin (66.7%), lincomycin, kanamycin, ceftazidime, tobramycin and amikacin (33.3%) and amoxicillin / clavulanic acid (16.7 %) were observed.

All isolates exhibited resistance to at least five or more antimicrobial agents used and considered as multidrug resistant (MDR) (Table 5).

## Discussion

Causes of most of food-borne diseases are commonly related to cross-contamination caused by unsanitary food handling, contaminated raw material, inadequate cooking, heating and cooling as well

as insufficient cleaning of food contact surfaces and manipulators hands (21). In this study, 2.8 % of samples found to be contaminated with *Salmonella*; 1(1%) grilled chicken, 4 (8.8%) food handlers' hand and 1 (2.5%) knife handle samples. *Salmonella* strains can be transmitted to the food chain by food-handler with asymptomatic disease, especially during catering practices (22, 23). It was noteworthy that among the six positive samples obtained from this study, 4 (66.6%) were isolated from manipulators hands. Food-handlers are reported to be the potential cause of serious outbreaks of food-borne diseases due to lack of hygiene knowledge (24). Food handlers, unconsciously and perhaps habitually touching their own body parts, can transfer pathogenic microorganism to food. Regarding

the food contact surfaces, in mass consumption places, one of the leading causes of foods contamination is reported to be peeling, slicing, shredding and chopping (25, 26, 27).

Although *S. Typhimurium* and *S. Enteritidis* are the most prevalent *Salmonella* serotypes in the global health arena with over 70% of human infections, (28, 29, 30) in our study, *S. Newport*, *S. Infantis* and *S. Koessan* Serovars were obtained. *S. Newport* was listed among the top ten serovars in human salmonellosis across the EU, causing 1.0% of all reported cases in 2015 (31). This change in serotype distribution noted in this study could be related to the implementation of several *Salmonella* control programmes including vaccination against *S. Typhimurium* and *S. Enteritidis*, which may have support new serotypes. These *Salmonella* diversities of different serotypes highlight the importance of periodic monitoring of this agent in food environments.

In the present study, the isolates indicated 100% resistance to penicillin/novobiocin, tetracycline, cloxacillin and rifampin. In addition, evident resistance to sulfamethoxazole/trimethoprim (83.3%), vancomycin, erythromycin, amoxicillin and streptomycin (66.7%) was observed. It was noteworthy that the resistance pattern of *Salmonella* *Infantis* and *Koessen* isolates from food handlers and grilled chicken, respectively were higher (50%) than *Newport* isolates in our study. Overall, we observed MDR among all *Salmonella* isolates with resistance to at least five or more antibiotics. The increase in the prevalence of MDR *Salmonella* could cause this pathogen to become a super bacteria (32) that cause public health concern. The circulation of MDR *Salmonella* strains in food environments not only complicate the control of the pathogen, but also contribute to the transmission of antimicrobial resistance to other *Enterobacteriaceae* (33). Our results, indicating frequent involvement of antibiotic-resistant *Salmonella* in food chain, underlines the necessity of the exploration of novel non-antibiotic interventions to combat against pathogens.

Among the DR genes tested in our study, *sugE* and *qacEΔ1* genes were detected from 33.3% of isolates (Table 4). *sugE* and *qacEΔ1* genes in *Salmonella* isolates have also been reported in previous studies (34, 35, 36, 37). Although *qacE*, is reported to be very

common in gram-negative bacteria<sup>13</sup> none of the isolates tested in our study is found to harbour *qacE* gene. However, Fernandez Marquez et al. (36) detected *qacE* gene in 9.5% of *Salmonella* isolates. The development of disinfectant resistance in pathogenic bacteria is related to their common applications in human medicine and the food industry (38). The widespread use of disinfectants has led to their dissemination into the environment which could lead to bacterial adaptation and result in cross-resistance to antibacterial agents (39, 40). Likewise, hand and grilled chicken isolates found to harbour *sugE* and *qacEΔ1* genes and were MDR in our study. Similarly, Deng et al. (37) reported that 92.8% of 152 *Salmonella* isolates obtained from retail foods of animal origin were resistant to at least one antibiotic among which 8.6% contained *qacEΔ1* gene. Tetracycline and sulfonamide resistance genes (*tet*, *sul*) are reported to be highly correlated with disinfectant resistance genes of *qacF* and *qacEΔ1* (37).

In this study phylogenetic analysis revealed that *Infantis* (hand) and *Koessen* (grilled chicken) isolates were genetically identical. Furthermore, close genetic relationship between *Newport* strains obtained from hand and knife handle were observed, indicating food-handler to be the source of contamination. FDA recommends avoiding bare-hand contact to RTE food as washed hands still could transmit the pathogens to food or food environments (41).

## Conclusion

This study demonstrates that food-handlers and food preparation equipments may serve as reservoirs for cross-resistant *Salmonella* in food chain. Increased tolerance to disinfectants could probably facilitate the survival of pathogenic organisms and contribute to the emergence of persistent strains. The increasing prevalence of disinfectant and MDR *Salmonella* strains isolated from food environments could pose potential risk for public health. Therefore, periodic food safety training programmes should effectively be implemented for food handlers and cross-resistant pathogens should be monitored in food environments. As disinfectant tolerance could facilitate the antibiotic resistant strains in food related areas, attention should be paid while

selecting appropriate disinfectant for food preparing surfaces.

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