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Prevalence, serotyping and antimicrobials resistance mechanism of *Salmonella enterica* isolated from clinical and environmental samples in Saudi Arabia

Mohamed A. El-Tayeb[a], *Abdelnasser S.S. Ibrahim*[a,b,*], *Ali A. Al-Salamah*[a], *Khalid S. Almaary*[a], *Yahya B. Elbadawi*[a]

[a] *King Saud University, College of Science, Department of Botany and Microbiology, Riyadh, Saudi Arabia*
[b] *National Research Center, Pharmaceutical Industries Research Division, Department of Chemistry of Natural and Microbial Products, Cairo, Egypt*

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ABSTRACT

Salmonella is recognized as a common foodborne pathogen, causing major health problems in Saudi Arabia. Herein, we report epidemiology, antimicrobial susceptibility and the genetic basis of resistance among *S. enterica* strains isolated in Saudi Arabia. Isolation of *Salmonella* spp. from clinical and environmental samples resulted in isolation of 33 strains identified as *S. enterica* based on their biochemical characteristics and 16S-rDNA sequences. *S. enterica* serovar Enteritidis showed highest prevalence (39.4%), followed by *S.* Paratyphi (21.2%), *S.* Typhimurium (15.2%), *S.* Typhi and *S.* Arizona (12.1%), respectively. Most isolates were resistant to 1st and 2nd generation cephalosporin; and aminoglycosides. Moreover, several *S. enterica* isolates exhibited resistance to the first-line antibiotics used for Salmonellosis treatment including ampicillin, trimethoprim–sulfamethoxazole and chloramphenicol. In addition, the results revealed the emergence of two *S. enterica* isolates showing resistance to third-generation cephalosporin. Analysis of resistance determinants in *S. enterica* strains ($n = 33$) revealed that the resistance to β-lactam antibiotics, trimethoprim–sulfamethoxazole, chloramphenicol, and tetracycline, was attributed to the presence of *carb-like, dfrA1, floR, tetA* gene, respectively. On the other hand, fluoroquinolone resistance was related to the presence of mutations in *gyrA* and *parC* genes. These findings improve the information about foodborne Salmonella in Saudi Arabia, alarming the emergence of multi-drug resistant *S. enterica* strains, and provide useful data about the resistance mechanisms.

* *Corresponding author at*: King Saud University, College of Science, Department of Botany and Microbiology, Riyadh, Saudi Arabia; National Research Center, Pharmaceutical Industries Research Division, Department of Chemistry of Natural and Microbial Products, Cairo, Egypt.
E-mail: ashebl@ksu.edu.sa (A.S. Ibrahim).

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Introduction

Although the high advances in safety measures taken in food and drinking water, *Salmonella* infections (Salmonellosis) are still recognized as one of most global foodborne diseases with a wide range of hosts. *Salmonella* spp. is facultative anaerobic intracellular gram negative flagellated bacilli that belong to family *Enterobacteriaceae*; and the genus consists of two main species; *S. bongori* and *S. enterica*.[1] The causative agent of salmonellosis is *S. enterica* subsp. *enterica*, which is subdivided into more than 2500 serovars based on antigenic differences in the lipopolysaccharide O antigen and two flagellin structures, most of them are recognized as potential human pathogen.[2] *Salmonella* infections are divided into two main types including (i) invasive typhoidal salmonellosis that caused by *S. enterica* serotype Typhi and Paratyphi A, B and C causing enteric fever gastroenteritis and bacteremia; (ii) non-typhoidal salmonellosis (NTS) caused by *S. enterica* serotype Enteriditis and *S. enterica* serotype Typhimurium, which have a broad vertebrate hosts range and cause various symptoms that usually include diarrhoeal disease.[3,4] Although, typhoidal *Salmonella* caused severe and life-threatening diseases, the non-typhoidal *Salmonella* is associated with self-limiting diseases such as gastroenteritis, with more severe cases reported in immunocompromised individuals.[5] Generally, *Salmonella* infections are transmitted to human via consumption of contaminated water and food particularly the animal products, however typhoidal *Salmonella*, which is restricted to human, is transmitted by fecal oral route or direct contact with the infected persons.[6]

Recently, the selective pressure owing to the misuse of antimicrobial agents in humans and domestic animals led to the emergence of multidrug-resistant *S. enterica* strains, including resistance to quinolone, fluoroquinolones and the third generation of cephalosporin which are the current drugs of choice for salmonellosis treatment in severe cases, representing a significant public health problem throughout the world.[7] There are several evidences underpin that the antibiotics resistance among *Salmonella* strains is attributed to intensive use of antibiotics as growth promoters in animals feeding.[8] Moreover, the intensive use of antimicrobial agents to treat both animal and human infections led to flourish the horizontal resistance genes transfer between bacterial communities.[9] The antimicrobial resistance in *S. enterica* is attributable to various mechanisms such as enzymatic degradation of some antimicrobial agents, blocking the cell permeability to antibiotics, activation of antimicrobial efflux pumps, and alteration the site of drugs actions.[7] The aims of this study were to determine the predominant serotype of *Salmonella* isolated in Saudi Arabia, emergence of antibiotics resistance among *Salmonella* strains and to investigate the genetic basis of antimicrobial resistance among the isolates.

Materials and methods

Clinical samples collection

Different clinical specimens were collected from patients with symptoms suspected to be *Salmonella* infection (King Khalid University Hospital, Riyadh, Saudi Arabia). The clinical samples included stools, urine and blood samples. In addition, various samples were collected from Sewage Treatment Plant in Riyadh (Saudi Arabia). The specimens were collected under sterile conditions and transferred to the laboratory in cold box within 1–2 h for bacterial isolation.

Bacterial isolation and identification

Serial dilutions (10-fold) of the clinical and environmental samples were made in 1% sterile peptone water (Difco, UK). Then 0.1 mL of each dilution was inoculated into *Salmonella* selective medium, Selenite F broth (Oxoid, UK), to enhance the growth of *Salmonella* spp. and inhibit the other contaminants, and incubated at 37 °C for 24–48 h. After enrichment, the growth was transferred to the media recommended for *Salmonella* spp. including: Xylose lysine deoxycholate agar (XLD) (Oxoid, UK) and Deoxycholate citrate agar (DCA) (Oxoid, UK), and incubated at 37 °C for 24 h.[9] *Salmonella* colonies, characterized by producing non-lactose fermenting pale colored colonies with black centers on DCA medium and pink-red colonies with black centers on XLD medium, were picked up and sub-cultured several times on fresh plates until homogeneous colonies were obtained. The colonies were confirmed as Gram negative bacteria using Gram staining procedures, and glycerol cultures of all of the isolates were prepared and stored at −80 °C for further analysis. The isolated bacterial strains were subjected to identification using biochemical tests and Vitek® 2-C15 automated system for bacterial identification (BioMerieux Inc., France), according to manufacturer's instructions. Furthermore, bacterial identification was confirmed by 16S rDNA sequencing analysis.

16Sr rDNA sequencing analysis

The *Salmonella* isolates ($n = 33$) were inoculated into nutrient broth (Merck, UK) and incubated at 37 °C for 18 h. Total bacterial DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen, UK) according to the manufacturer's instructions. The 16S rDNA genes of the isolated *Salmonella* spp. strains ($n = 33$) were PCR-amplified using the universal eubacterial primers[10]: 16F27 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 16R1525 (5′-AAG GAG GTG ATC CAG CCG CA-3′). The PCR amplification was performed using purified genomic DNA of the *Salmonella* spp. strains ($n = 33$) as templates. The PCR reaction (50 μL) contained PCR master mix (Promega, USA) (14 μL), forward primer (4 μL), reverse primer (4 μL), DNA templates (4 μL), and nuclease-free water (13 μL). The PCR reaction was carried

out under the following conditions: initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C (30 s); annealing at 52 °C (30 s); extension at 70 °C (1.5 min), and then, a final extension step at 70 °C (5 min). The PCR products were analyzed by 1% (w/v) agarose gel electrophoresis using a 1 kbp DNA ladder (Qiagen, UK) as molecular size standard. The amplified 16S rDNA products were purified from the agarose using QIAquick gel Extraction Kits (Qiagen, UK). The purified 16S rDNA amplicons were sequenced by an automated sequencer (Macrogen, Korea) using the 16F27 and 16R1525 primers mentioned above. BLAST analysis of the obtained sequences was performed by NBCI online database to determine the phylogenetic grouping of the isolated strains (http://www.ncbi.nlm.nih.gov/genbank/index.html).

Salmonella serotyping

The serotyping of the isolated *Salmonella* ($n = 33$) strains were carried out according to Kauffman–White Scheme[11] by slide agglutination tests using commercially available mono- and poly-O groups *Salmonella* A, B, C, D, E antisera (Remel, Europe Ltd., UK). In addition, polyvalent *Salmonella* antisera phase 1 and phase 2 flagellar H antigens were used for serovars determination of the isolated *Salmonella*. Briefly, a loopful of each isolate grown on Brain Heart Infusion (BHI) agar was suspended in 50 μL of sterile distilled water on a glass slide, and then mixed with one drop of each antiserum. The slide was rotated gently for 1 min, and observed for appearance of any agglutination reaction using indirect lighting over a dark background. However, some strains (*S.* Typhi and *S.* Paratyphi C) may possess capsular polysaccharide antigen, known as Vi, that render the strains non-agglutinable in O-antisera. Therefore, the O-antigen was detected after destruction of Vi antigen by boiling the culture for 10 min. *E. coli* cell suspension was used as negative control.

Antimicrobial susceptibility testing

The isolates identified as *Salmonella* ($n = 33$) were tested for their susceptibility to 26 commonly used antimicrobial agents using disk diffusion assay and Vitek® 2-C15 automated system. The tested antibiotics included (Oxoid Limited Company, UK): kanamycin (k), tetracycline (TE), streptomycin (S), erythromycin (E), neomycin (N), ampicillin sulbactam (SAM), chloramphenicol (C), amikacin (AN), amoxicillin/clavulanic acid (AMC), ampicillin (AM), cefalotin (CF), cefepime (FEP), cefotaxime (CTX), cefoxitin (FOX), cefpodoxime (CPD), ceftazidime (CAZ), cefuroxime (CXM), ciprofloxacin (CIP), gentamicin (GM), meropenem (MEM), nitrofurantoin (FT), norfloxacin (NOR), piperacillin (PIP), piperacillin/tazobactam (TZP), tobramycin (TM) and trimethoprim/sulfamethoxazole (SXT). For disk diffusion assay, the bacterial strains were sub-cultured on fresh Mueller–Hinton agar plates (Difco, UK) for 24 h at 37 °C. After the incubation period, the cells were harvested using a sterile loop and suspended in sterile saline solution to be equivalent to 0.5 McFarland standards. The cell suspensions were inoculated onto Mueller–Hinton agar plates using sterile cotton swabs, and various antibiotic discs were placed on the agar plate surfaces and incubated for 24–48 h at 37 °C.[12] The results were interpreted Clinical and Laboratory Standard Institute (CLSI) guidelines.[13] *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms.

Detection of antimicrobial resistance determinants

Polymerase chain reaction (PCR) was used for detection of various antibiotics resistance genes ($n = 12$) in the isolated *S. enterica* strains ($n = 33$) according to previously reported method with some modifications.[14] The isolates were tested for the presence of the *carb-like, tem*, and *oxa-1* genes, encoding resistance to beta-lactams antibiotics; *floR* gene for chloramphenicol resistance; *tet*A, *tet*G, and *tet*B encoding resistance to tetracycline; *dfrB, dfr*A1 and *dfr*A14 genes encoding trimethoprim resistance; and mutation in *gyrA* and *ParC* for fluoroquinolone resistance. PCR amplification of the resistance genes was carried out using a list of specific primers shown in Table 1.[14] DNA amplification was carried out in PCR thermocycler (Biotech prime thermocycler UK), with the following reaction conditions: initial denaturation for 2 min at 94 °C, followed by 35 cycles of denaturation for 1 min at 94 °C, 30 s at annealing temperature of each primer, and extension at 72 °C for 1.5 min and a final extension for 5 min at 72 °C. The amplified genes were analyzed by 1.5–2% agarose gel electrophoresis. In addition, the PCR products of *gyrA* and *parC* genes were purified from gels by using QIAquick gel extraction kit (Qiagen, UK); the genes were sequenced by automated sequencer services (Macrogen, Korea), and aligned with the known genes available in NBCI online database.

Results

Salmonella isolation and identification

Enrichment and isolation of *Salmonella* spp. from the collected clinical and environmental samples resulted in isolation of 100 non-repetitive bacterial strains. Among the isolates, 33 strains were identified as *Salmonella enterica* based on their metabolic reactions and biochemical characteristics. The isolates ($n = 33$) were oxidase negative, produce H_2S, and able to utilize arginine, lysine, ornithine, citrate (except one isolate), glucose, mannitol, inositol (variable), sorbitol, rhamnose, melibiose, and arabinose. In addition, all isolates were negative with orth-nitro phenyl-β-D-galactopyranoside (ONPG), tryptophan, urea, indole, Voges Proskauer, gelatin, sucrose, and amygdalin tests. In addition to biochemical tests, the identities of the isolates were further confirmed by 16S rDNA genes sequencing analysis. The 16S rDNA genes of different isolates ($n = 33$) were successfully amplified, with expected length of about 1525 bp, purified and sequenced. As shown in Table 2, all isolates ($n = 33$) were affiliated to various strains of *Salmonella enterica* subsp. *enterica* with 96–99% similarities; and the sequences were deposited in the GenBank with accession numbers of KU843835 to KU843866. The phylogenetic tree showing the genetic relatedness among the isolated *S. enterica* strains based on 16S rDNA sequences is shown in Fig. 1.

Table 1 – Primers sequence specific to different antimicrobials resistant determinants in *Salmonella*.

Antibiotic	Gene	Sequence (5′–3′)	Annealing temp (°C)	Amplicon size (bp)
Quinolone	*gyrA*	F-AAATCTGCCCGTGTCGTTGGT R-GCCATACCTACGGCGATACC	55	343
	parC	F-CTATGCGATGTCAGAGCTGG R-TAACAGCAGCTCGGCGTATT	62	270
Tetracycline	*tetA*	F-GATATTCTGAGCACTGTCGC R-CTGCCTGGACAACATTGCTT	57.5	950
	tetB	F-TTGGTTAGGGGCAAGTTTTG R-GTAATGGGCCAATAACACCG	55	600
	tetG	F-GCTCGGTGGTATCTCTGC R-AGCAACAGAATCGGGAAC	55	500
β-Lactams	*Carb*	F-AATGGCAATCAGCGCTTCCC R-GGGGCTTGATGCTCACTCCA	55	586
	tem	F-TTGGGTGCACGAGTGGGTTA R-GACAGTTACCAATGCTTAATCA	55	503
	oxa-1	F-ACCAGATTCAACTTTCAA R-TCTTGGCTTTTATGCTTG	55	598
Chloramphenicol	*floR*	F-CACGTTGAGCCTCTATAT R-ATGCAGAAGTAGAACGCG	55	868
Trimethoprim	*dfrA1*	F-GTGAAACTATCACTAATGG R-TTAACCCTTTTGCCAGATTT	50	474
	dfrB	F-GATCACGTGCGCAAGAAATC R-AAGCGCAGCCACAGGATAAAT	60	141
	dfrA14	F-GAGCAGCTICTITTIAAAGC R-TTAGCCCTTTIICCAATTTT	58	393

Table 2 – Identification of *Salmonella* strains (*n* = 33) based on 16S-rDNA sequencing.

Isolate	Identification	Similarity (%)	Accession number
SA1	*S. enterica* subsp. enterica serovar Typhimurium LT2	99	KU843835
SA2	*S. enterica* subsp. enterica serovar Typhimurium 798	98	KU843836
SA3	*S. enterica* subsp. enterica serovar Typhimurium 138736	99	KU843837
SA4	*S. enterica* subsp. enterica serovar Typhi Ty21a	99	KU843838
SA5	*S. enterica* subsp. enterica serovar Typhimurium 138736	99	KU843839
SA6	*S. enterica* subsp. enterica serovar Typhi CT18	99	KU843840
SA7	*S. enterica* subsp. enterica serovar Paratyphi C7	99	KU843841
SA9	*S. enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843842
SA10	*S. enterica* subsp. enterica serovar Abony 0014,	99	KU843843
SA12	*S. enterica* subsp. enterica serovar Paratyphi C7	99	KU843844
SA14	*S. enterica* subsp. enterica serovar Typhi Ty21a	99	KU843845
SA21	*S. enterica* subsp. enterica serovar Typhi Ty21a,	99	KU843846
SA25	*S. enterica* subsp. enterica serovar Enteritidis EC20110353	99	KU843847
SA26	*S. enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843848
SA28	*S. enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843849
CR	*S. enterica* subsp. enterica serovar Paratyphi B SPB7,	99	KU843850
SAM	*S. enterica* subsp. enterica serovar Paratyphi B SPB7	99	KU843851
Para	*S. enterica* subsp. enterica serovar str. USMARC-S3124.1	99	KU843852
SA35	*S. enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843853
SA36	*S. enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843854
SA37	*S. enterica* subsp. enterica serovar Enteritidis EC20100325	99	KU843855
SA39	*S. enterica* subsp. enterica serovar Typhimurium DT2	99	n/a
SA40	*S. enterica* subsp. enterica serovar Typhimurium DT2	99	KU843856
SA49	*S. enterica* subsp. enterica serovar Abony 0014	99	KU843857
NS1	*S. enterica* subsp. enterica serovar Enteritidis CDC_2010K_0968	99	KU843858
NS2	*S. enterica* subsp. enterica serovar Enteritidis Durban	99	KU843859
NS3	*S. enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843860
NS4	*S. enterica* subsp. enterica serovar Enteritidis EC20100325	99	KU843861
NS5	*Salmonella enterica* subsp. enterica serovar Enteritidis 77-1427	99	KU843862
NS6	*S. enterica* subsp. enterica serovar Typhi CT18	97	KU843863
NS7	*S. enterica* subsp. enterica serovar Tennessee TXSC_TXSC08-19	99	KU843864
NS9	*S. enterica* subsp. enterica serovar Typhimurium LT2	96	KU843865
NS10	*S. enterica* subsp. enterica serovar Typhi CT18	96	KU843866

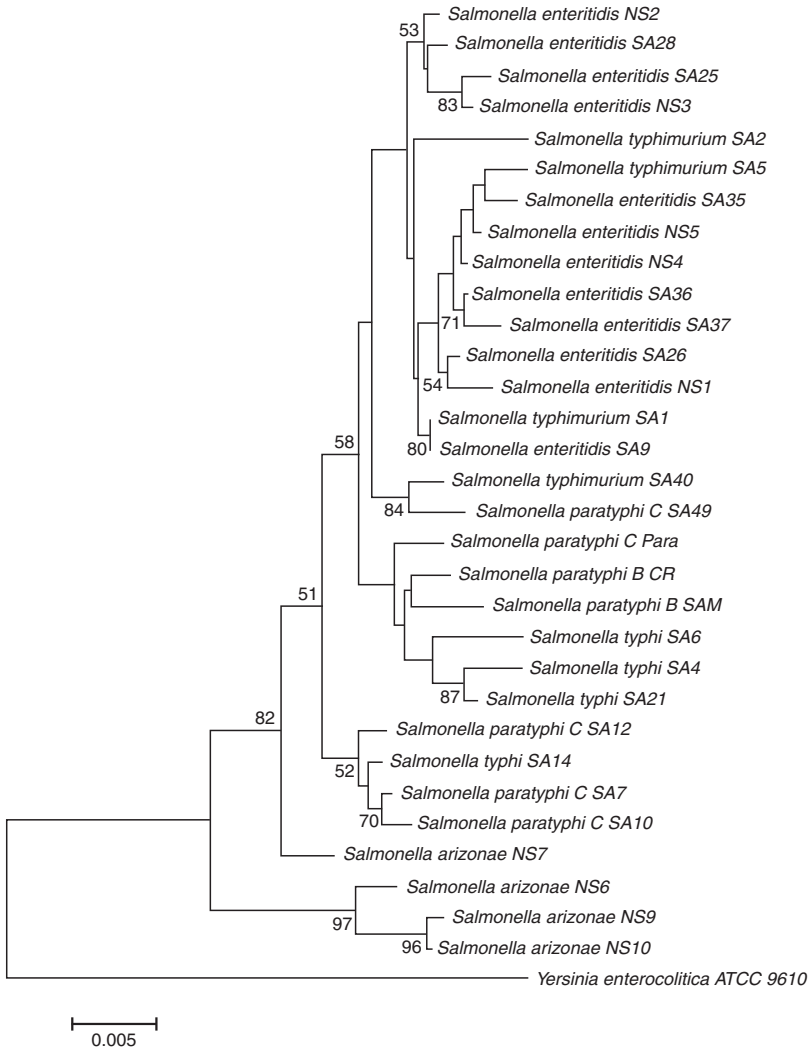

Fig. 1 – Dendrogram showing genetic relatedness among the isolated *Salmonella enterica* strains based on 16Sr DNA sequences analysis.

Salmonella enterica strains serotyping and serogrouping

As shown in Table 3, the serotyping of the *S. enterica* isolates ($n = 33$) revealed that the strains were affiliated to *S. enterica* Paratyphi B ($n = 2$), *S. enterica* Typhimurium ($n = 5$), *S. enterica* Paratyphi C ($n = 5$), *S. enterica* Enteritidis ($n = 13$), *S. enterica* Typhi ($n = 4$) and *S. enterica* Arizonae ($n = 4$). In addition, the serogrouping of *S. enterica* strains ($n = 33$) indicated 51.52%, 21.21%, and 15.15% were classified as serogroups D, B, and C, respectively. No strains were affiliated to serogroups A or E. In addition, four isolates (12.12%) gave unserotypable isolates using the applied antisera. However, these isolates were identified as *S. enterica* serovar Arizonae by VITEK 2-C15 identification system.

Antimicrobial susceptibility testing

Antibiogram of the isolated *S. enterica* strains ($n = 33$) to 26 antimicrobial agents including the commonly used antibiotics for salmonellosis treatment is shown in Fig. 2. It was found that among the isolates ($n = 33$), 26 *S. enterica* strains exhibited multidrug resistance, showing resistance to more than five antibiotics. The results revealed that the highest resistance pattern among all isolates was found to erythromycin (100%), followed by first and second generation of cephalosporin including cefalotin, cefuroxime, cefuroxime axetil (all 90.9%) and cefoxitin (87.9%); and aminoglycosides antibiotics including: gentamicin (90.9%), amikacin and tobramycin (87.9%), respectively. In addition, 57.6% ($n = 19$) of the isolates were resistant to nitrofurantoin, 27.3% ($n = 9$) to streptomycin, 24.2% ($n = 8$) tetracycline, 18.2% ($n = 6$) to trimethoprim–sulfamethoxazole, and 15.2% ($n = 5$) of the isolates were resistant to neomycin. On the other hand, lowest resistance of the *S. enterica* isolates ($n = 33$) was detected towered piperacillin/tazobactam, cefpodoxime, cefotaxime, and norfloxacin, that only 3.1% of the total isolates exhibited resistance to these antibiotics. In addition, most *S. enterica* isolates were susceptible to several β-lactams antibiotics (ampicillin, ampicillin subaclam and piperacillin) and chloramphenicol. Noteworthy, the highest level of resistance against most

Table 3 – Serotyping and serogrouping of the isolated *S. enterica* strains (*n* = 33).

Serotype	Type	No. of isolates	Serogroup
S. enterica serovar Paratyphi	Typhoidal	2	B
S. enterica serovar Typhimurium	NTS	5	
S. enterica serovar Paratyphi	Typhoidal	5	C
S. enterica serovar Enteritidis	NTS	13	D
S. enterica serovar Typhi	Typhoidal	4	
S. enterica serovar Arizonae	NTS	4	n/a

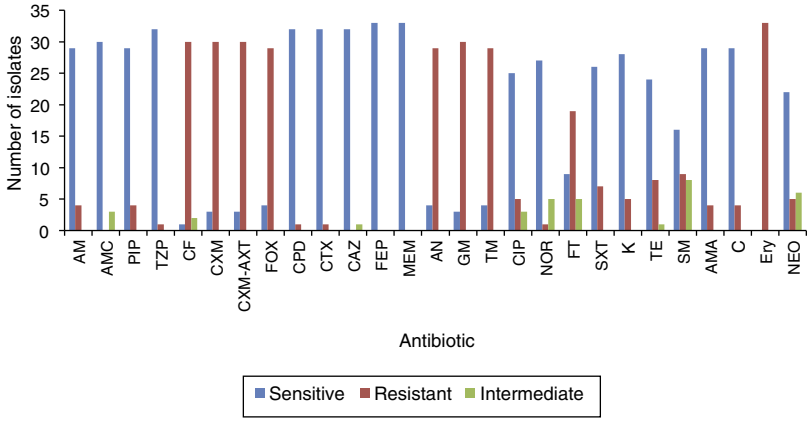

Fig. 2 – Antibiotics susceptibility testing against the *S. enterica* isolates (*n* = 33).

antibiotics was shown among S. Paratyphi C serotype which exhibited high resistance to erythromycin (100% of the isolates), streptomycin (80%), trimethoprim–sulfamethoxazole (80%) tetracycline (80%), neomycin (60%), and kanamycin (60%), followed by S. Paratyphi B which exhibited 100% resistance toward erythromycin, tetracycline, streptomycin, ampicillin-subaclam, and chloramphenicol. Regarding S. Typhi serotype the high level of resistance was shown toward erythromycin (100%), streptomycin (50%), and tetracycline (25%). In addition, five and three isolates exhibited resistance and decreased susceptibility to ciprofloxacin (fluoroquinolone), respectively. Finally, among S. Arizonea isolates (*n* = 4), only one isolate was resistant to kanamycin, tetracycline, trimethoprim–sulfamethoxazole, streptomycin, and ampicillin-subaclam (Table 4).

Molecular mechanisms of antimicrobial resistance

The isolated *S. enterica* strains (*n* = 33) were screened for the presence of some antibiotic resistance genes by PCR including: *tetA*, *tetB* and *tetG* for tetracycline, *carb-like*, *tem-like* and *oxa-1-like* for β-lactam antibiotics, *floR* gene for chloramphenicol, *dfrA1*, *dfrB* and *dfrA14* genes for trimethoprim, and detection of mutation in *gyrA* (gyrase subunit A) and *parC* (topoisomerase IV) genes for quinolone residence (Supplementary data). The results summarized in Table 5 revealed the detection of significant variety of resistance determinants among the isolates. Phenotypically, 87.9–90.9% of isolates were resistant to 1st and 2nd generation of cephalosporin, in addition; only four isolates showed ampicillin and piperacillin resistance, and three isolates gave intermediate pattern to amoxicillin/clavulanic acid. The resistance to those β-lactams antibiotics was attributed mainly to presence of *carb-like* gene which was detected in 22 *S. enterica* isolates, whereas both *tem* and *oxa-1* genes were absent in all isolates. Tetracycline resistance was related mainly to the presence of the *tetA* gene, detected in most of the tetracycline resistant isolates (7/8), whereas *tetG* gene was detected in most isolates. However, *tetB* gene was absent in all isolates. Despite the presence of *floR* gene, which confers resistance to chloramphenicol in the most isolates, only four isolates were resistant to chloramphenicol. On the other hand, while all trimethoprim–sulfamethoxazole resistant isolates (*n* = 8) harbored *dfrA1* gene, both *dfrB* and *dfrA14* genes were not detected in any isolates, indicating that the resistance to trimethoprim–sulfamethoxazole is mediated by *dfrA1* gene. Among the isolated *S. enterica* strains (*n* = 33), only five isolates exhibited fluoroquinolone resistance. Three of them belonged to *S. enterica* serovar Paratyphi C (isolates SA7, SA10, and Para), one *S. enterica* serovar Typhi (SA14) and one *S. enterica* serovar Arizonae (NS 10). Therefore, the amplified *gyrA* and *ParC* genes of those five isolate were purified, sequenced, and the obtained sequences were aligned with reference *gyrA* and *ParC* sequences. The results revealed the presence of point mutations in *gyrA* genes at positions 13 and 24 nucleotides, whereas among *parC* genes point mutations were detected at positions 13, 19 and 28 nucleotides (Supplementary data).

Discussion

Salmonellosis is considered as an immense public health challenge with a reported increase in its incidence.[15] The emergence of multidrug resistant *Salmonella* strains represents a big health challenge and can lead to more acute and invasive infections, in addition to treatment failures owing to resistance would increase the risk of mortality, particularly

Table 4 – Frequency of multidrug-resistance patterns among *Salmonella enterica* isolates ($n = 33$).

Isolate	Serotype	Resistance	Intermediate	Comment
SA7	S. Paratyphi C	CF, CXM, CXA, FOX, AN, GM, TM, FT, SXT	CIP, NOR	Partially quinolones resistance
SA10	S. Paratyphi C	CF, CXM, CXA, FOX, AN, GM, TM, FT, SXT	CIP, NOR	Partially quinolones resistance
SA12	S. Paratyphi C	CF, CXM, CXA, FOX, AN, GM, TM, FT, SXT		Trimetho/sulfa resistance
SA14	S. Typhi	CF, CXM, CXA, FOX, AN, GM, TM, FT, SXT	CIP, NOR	Partially quinolones resistance
CR	S. Paratyphi B	AM, PIP, CF, CXM, CXA, FOX, AN, GM, TM, FT.	AMC	β-Lactams resistance
SAM	S. Paratyphi B	AM, PIP, CF, CXM, CXA, FOX, AN, GM, TM, FT.	AMC	β-Lactams resistance
ParaC	S. Paratyphi C	AM, PIP, CF, CXM, CXA, FOX, AN, GM, TM, SXT.	CIP, NOR, AMC	β-Lactams resistance and partially quinolones resistance
NS 10	S. Arizonae	AM, PIP, CF, CXM, CXA, CPD, CTX, GM, NOR, SXT	CAZ, CIP	β-Lactams resistance and partially quinolones resistance

in the developing countries.[16,17] *Salmonella* spp. is one of the most important pathogen that causes food poisoning in Saudi Arabia, particularly in Umrah and Hajj seasons that a lot of tourists are visiting the holy places in Saudi Arabia.[18] In this study 33 clinical and environmental bacterial strains were isolated and identified as *S. enterica* based on their biochemical characterizations and 16S rDNA genes sequences analysis. It was found that the prevalence of non-typhoidal *Salmonella* ($n = 18$) is more frequent than the typhoid one ($n = 11$). *S.* Enteritidis and *S.* Typhimurium represented 39.4% and 15.2% of the total isolates ($n = 33$) respectively, whereas typhoidal *Salmonella* including *S.* Paratyphi and *S.* Typhi represented 21.2% and 12.1% respectively. In addition, 12.1% of the isolates belonged to *S.* Arizonae. These results were in accordance with various global studies, where *S.* Enteritidis was the most dominant serotype among the isolated *S. enterica* strains.[18–21] However, in a recent study in Belgium reported by Ceyssens et al.,[5] the dominant serotype was *S. enterica* Typhimurium (55%) followed by Enteritidis (19%).

Investigation of susceptibility of the isolated *S. enterica* strains ($n = 33$) toward various antibiotics ($n = 26$), indicated that there was high level of antibiotics resistance among isolated *S. enterica* strains, 26 isolates exhibited multidrug resistance, showing resistance to more than three unrelated antibiotics. Regarding β-lactams antibiotics, 20% of *S.* Paratyphi C isolates were resistant to the first-line antibiotics, ampicillin-subaclam and chloramphenicol, 100% of *S.* Paratyphi B were resistant to those two antibiotics. In addition, Paratyphi C isolates showed high resistance to erythromycin, tetracycline, neomycin and kanamycin. This resistance pattern were in agreement with several previous reports.[22,23] However, in contrast to several studies which reported high resistance of *S.* Typhi strains to all first-line drugs, our results revealed a highest susceptibility among *S.* Typhi isolates (80%) to both ampicillin-subaclam, and chloramphenicol.[24,25] In addition, all isolates ($n = 33/33$) and most isolates ($n = 24/33$) exhibited resistance to erythromycin and nitrofurans, respectively. The high resistance of *Salmonella* to those antibiotics is likely due to the veterinary use of nitrofurans and erythromycin as feed supplement and/or treatment; particularly poultry sector.[26–28] Moreover, the results revealed the emergence of two isolate (6.1%) showing resistance to third-generation cephalosporin antibiotics (Cefpodoxime and Cefotaxime), which is less than a study carried out by Burke et al[7] who reported that 11% of the *S. enterica* isolates exhibiting resistance to third-generation cephalosporin. Among the isolated *S. enterica* isolates ($n = 33$), five and three isolates showed resistance and decreased susceptibility to ciprofloxacin (quinolone), respectively. However, emergence of higher quinolone resistance among *S. enterica* strains to quinolone has been reported.[5]

Analysis of resistance determinants in the isolated *S. enterica* strains ($n = 33$) revealed the detection of *carb-like* gene (carbenicillinase) in the isolates that exhibited resistance or decreased susceptibility to β-lactam antibiotics, suggesting that this resistance is mediated by *carb-like* gene which encoded β-lactamase enzyme. Both *tem* and *oxa-1* genes could not be detected in any isolate which is in contrast to other studies where ampicillin-resistance in *S. enterica* isolates were attributed to bla_{TEM-1} and bla_{oxa-1}.[5,17,23]

Table 5 – Distribution of various antibiotic resistance genes ($n = 11$) in *S. enterica* strains ($n = 33$). P: present; A: absent.

Salmonella isolate	β-Lactamase	Trimethoprim			Chloramphenicol	Tetracycline			Fluoroquinolone		Total
	Carb	*dfrA1*	*dfrA14*	*dfrB*	*floR*	*tetA*	*tetG*	*tetB*	*gyrA* M	*ParC* M	
SA1	P	A	A	A	P	A	P	A	A	A	3
SA2	P	A	A	A	P	A	P	A	A	A	3
SA3	P	A	A	A	P	A	P	A	A	A	3
SA4	P	A	A	A	P	A	P	A	A	A	3
SA5	P	A	A	A	P	A	P	A	A	A	3
SA6	P	A	A	A	P	A	P	A	A	A	3
SA7	A	P	A	A	P	P	P	A	P	P	6
SA9	A	A	A	A	P	A	P	A	A	A	2
SA10	P	P	A	A	P	P	P	A	P	P	7
SA12	P	P	A	A	P	P	P	A	A	A	5
SA14	A	P	A	A	P	P	P	A	P	P	6
SA21	P	P	A	A	P	A	P	A	A	A	4
SA25	P	P	A	A	P	A	P	A	A	A	4
SA26	A	P	A	A	P	A	P	A	P	P	5
SA28	P	P	A	A	P	A	P	A	A	A	4
CR	P	P	A	A	A	A	P	A	A	A	3
SAM	P	P	A	A	P	A	P	A	A	A	4
Para	P	P	A	A	P	P	P	A	P	P	7
SA35	P	P	A	A	P	A	P	A	A	A	4
SA36	P	P	A	A	P	A	P	A	A	A	4
SA37	P	P	A	A	P	A	P	A	A	A	4
SA39	P	P	A	A	P	A	P	A	A	A	4
SA40	P	A	A	A	P	A	P	A	A	A	3
SA49	P	P	A	A	P	A	P	A	A	A	4
NS1	P	A	A	A	P	A	P	A	A	A	3
NS2	P	A	A	A	P	A	P	A	A	A	3
NS3	A	A	A	A	A	A	A	A	A	A	0
NS4	A	A	A	A	P	A	A	A	A	A	1
NS5	A	A	A	A	P	A	A	A	A	A	1
NS6	A	A	A	A	P	A	A	A	A	A	1
NS7	A	A	A	A	A	A	A	A	A	A	0
NS9	A	A	A	A	P	P	A	A	A	A	2
NS10	A	A	A	A	P	P	A	A	P	P	4
Total	22	16	0	0	30	7	26	0	5	5	

It was found that the five isolates that exhibited resistance to trimethoprim–sulfamethoxazole were associated with presence of *dfrA1* gene (*dfrA14* and *dfrB* were not detected in any isolate), indicating it is responsible for the resistance. However, *dfrA1* was not found in resistant S. Arizonae isolate, suggesting that the resistance trimethoprim–sulfamethoxazole in S. Arizonae is attributed to other mechanisms. It was reported that *dfrA1* is the most prevalence in S. *enterica* isolates from Europe, whereas the most common *dfrA* genes in Korea and Australia are *dfrA17* and *dfrA12*, respectively.[23,29–31] The resistance to chloramphenicol is highly associated with the acquisition and expression of efflux pumps that reduce toxic levels of the drug in the bacterial cells. In *Salmonella*, chloramphenicol efflux pumps are encoded by *floR* or *cml*.[27] *floR* gene was detected in most tested isolates ($n = 33$). However, only four S. *enterica* isolates exhibited resistance to chloramphenicol. This finding is supported by other studies that reported the presence of *floR* gene in various S. *enterica* as part of *Salmonella* pathogenicity island-1.[23,32] The resistance to tetracycline is highly associated with the acquisition and expression of efflux pumps, encoded by *tet* genes, that reduce the concentration of the drug inside the bacterial cells. Out of eight of isolates exhibited resistance patterns to tetracycline, seven isolates harbored *tetA* gene. This result was in agreement with the hypothesis said the intestinal tract is a suitable niche for the transfer of *tetA* and *tetB* by horizontal gene transfer thereby these genes are popular among *Enterobacteriaceae*.[33] Quinolones resistance are usually mediated mainly by point mutations in bacterial gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes. These mutations lead to block the binding site of topoisomerase or gyrase targeting by antimicrobial agents.[34,35] In the present study point mutations were detected in all quinolones resistant S. *enterica* isolates ($n = 5$) in both *gyrA* and *parC* with large changes in both Para and NS10 isolates leading to complete frame shift of amino acids sequences of proteins in both topoisomerase and gyrase. Substitutions among SA7, SA10, SA14 isolates occurred in *gyrA* gene in both position 13 and 24 of nucleotides, which led to single amino acid substitution (serine instead of Phenylalanine) in the three isolates while aspartate was replaced by tyrosine in both S. Paratyphi C isolates. On the other hand, a high variation was detected in *parC* gene among the resistant isolates causing major changes in their proteins. The presence of these mutations in both *parC* and *gyrA* renders these isolates to be more resistant to fluoroquinolones. Similar results of point mutations in both *parC* and *gyrA* genes were reported, one mutation in

gyrA (Asp87Asn) and one in parC (Thr54Ser)[17]; and point mutations in GyrA residues Ser83 and Asp87 and ParC_Ser80Ile,[5] that conferred quinolones resistance in *S. enterica*.

Conclusion

In this study, we report epidemiology, antimicrobial susceptibility, and the genetic basis of resistance among *S. enterica* strains isolated in Saudi Arabia. The obtained results alarm the emergence of MDR *Salmonella enterica* isolated in Saudi Arabia, showing resistance to first line drug as well as to third generation cephalosporin in Saudi Arabia. In addition, it describe some details about the molecular mechanism of the resistance which revealed and support the hypothesis that the antimicrobial resistance mechanism in *S. enterica* is varied according to the geographic area and based on the environment of isolation. The obtained data is a basis for further investigation on large scale samples for more understating of the Salmonellosis in Saudi Arabia.

Conflict of interest

The authors have no potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjm.2016.09.021.

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