Salmonella Infections among Pediatric Population in Qatar: Phenotypic Resistance and Associated Genotypic Determinants

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Abstract

Salmonella is a significant public health burden worldwide and being the most common bacterial diarrheal illness among infants and young children. In the last few years, Qatar reports a high incidence of salmonellosis outbreaks coupled with a significant increase of Multidrug-Resistant (MDR) among pediatric populations every year. This study aims to elucidate the molecular mechanisms underlying resistance to ceftriaxone, cefepime, amoxicillin-clavulanate tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, and azithromycin among Salmonella isolated from the pediatric population. A total of 246 Salmonella isolates were collected from children under 18 years old admitted to the Pediatric Emergency Center (PEC), Hamad Medical Corporation (HMC) from Jan. 2018 to Dec 2019 with gastroenteritis. Isolates were tested for antibiotic susceptibility against nineteen relevant antibiotics using E-test. Resistance was confirmed using PCR-specific primers for 38 genes. Resistance was detected against 14 antibiotics, and 38.2% of isolates were resistant to at least one antibiotic. Overall, we reported 23.9%, resistance to tetracycline 21.1%, ampicillin 18.7%, AMC, and 13% sulfamethoxazole-trimethoprim. Further, 16.2% of the isolates were Multidrug-Resistant (MDR), with 4.1% being Extended-Spectrum β Lactamase (ESBL) producers. 90% of ESBL producers harbored one of 10 CTX-M-Group. Class 1 AMC resistant samples showed the highest resistance to different antibiotics. Our results indicate a high antimicrobial resistance pattern of Salmonella and the presence of Class (1) cassette that involves the transmission and expression of the resistance among AMC resistance isolates, which might lead to increased multi-drug resistance. This study provides evidence guidance to activate and implement the pillars of an antimicrobial stewardship program in human health to reduce MDR salmonellosis.

Keywords: Salmonella; MDR; salmonellosis; pediatric; Qatar

Introduction

Salmonella is one of the most common etiological agents of foodborne diarrheal illness and typhoid fever worldwide, leading to mortality in complicated cases [1]. Importantly, children less than 5-years old account for approximately a third of deaths attributed to foodborne diseases (WHO, 2015). There is an increasing concern regarding this pathogen due to the emergence and spread of antibiotic-resistance and potentially more pathogenic strains [2]. Drug-resistance among gastrointestinal pathogens preferentially cause illness in persons receiving antimicrobial drugs for any medical condition. Consequently, emerging resistance in foodborne pathogens may result in increased burdens of illness and outbreaks in settings where patients are treated with antimicrobial drugs [3]. Antimicrobial therapy is not indicated in uncomplicated cases of gastrointestinal illness in patients without underlying illness. However, therapy is needed, and maybe lifesaving in selected patients with the prolonged febrile course of suspected underlying invasive illness [3]. Non-Typhoidal Salmonella (NTS) can be invasive and cause paratyphoid fever, which requires immediate treatment with antibiotics. Extended-spectrum cephalosporins being used favorably to treat salmonellosis in children [4]. The treatment of the Enterobacteriaceae family, including Salmonella, has been increasingly complicated by the emergence of resistant strains to first-line antimicrobial agents [5,6].

The gram-negative bacterial genus Salmonella is divided into two species, Salmonella enterica, and Salmonella bongori. Only the Salmonella enterica subspecies enterica is of clinical relevance for humans [7], and is further classified into more than 2,600 serovars. Salmonella can be broadly categorized as typhoidal and Non-Typhoidal Salmonella (NTS). The typhoidal Salmonella includes serovar Typhi, and the closely related serovar Paratyphi (A-C) that cause enteric fever [8]. The much larger NTS group causes infectious diarrhea worldwide and primarily induces acute, self-limiting

Abbreviations

MDR: Multi-Drug Resistant; NTS: Non-Typhoidal Salmonella; AMR: Antimicrobial Resistance; ESBL: Extended-Spectrum β Lactamase; FDA: Food Drug Administration
gastroenteritis [9]. NTS can also cause various other disorders, including bacteremia, meningitis, and osteomyelitis, particularly among immunocompromised patients.

*Salmonella* is emerging as a priority public health hazard in Qatar, being the most reported bacterial diarrheal illness among infants and young children, alarmingly associated with multiple incidences of salmonellosis outbreaks among this pediatric population each year. *Salmonella* is listed among the four commonly isolated Enterobacteriaceae from Hamad Medical Corporation (HMC, Personal communication). A previous study showed a high incidence of salmonellosis in Qatar, especially in children less than five years of age, with an overall annual incidence rate ranging between 12.3 and 30.3 per 100,000 population in 2004-2012 [10]. Nonetheless, this previous study does not characterize the molecular level of resistance to understand better the relatedness, typing of different isolates, and understanding their resistance mechanism. Besides, there are no data available after 2012 on salmonellosis incidences in the State of Qatar. The spread of resistant bacteria is linked to the misuse of antibiotics in human and animal populations and the consumption of contaminated food with resistant bacteria [11].

There are limited published data on antibiotic resistance profile and its associated genetic determinants in Qatar among *Salmonella* for the pediatric population. Therefore, a study is required to define and analyze the antimicrobial resistance and molecular epidemiology of *Salmonella*. Obtaining such information is crucial to tackle and mitigate the salmonellosis problem at the clinical, public health, and economic levels. The recurrent *Salmonella* outbreaks in Qatar and the increasing number of salmonellosis cases mandate rapid regulatory and monitoring reforms at the State level. This study aimed to profile the phenotypic resistance of *Salmonella* to the relevant antibiotics. Furthermore, to elucidate the molecular determinants underlying resistance to the most worldwide used antibiotics, including ceftriaxone, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, and azithromycin.

This is the first study among Qatar’s pediatric population that demonstrates the correlation between genetic and phenotypic trends of antimicrobial resistance that would influence the development and implementation of stewardship programs to reduce pathogenic resistant bacteria’s burden on the community.

**Materials and Methods**

**Clinical isolates**

Ethical approval for this study was obtained from the Medical Research Centre, HMC, Doha, Qatar, protocol no. MRC-01-17-198 and Qatar University approval # QU-IBC-2019/008. A total of 246 *Salmonella* isolates were collected between January 2018 and December 2019 from children (2-18 years of age) of different nationalities presented to the Pediatric Emergency Center (PEC), HMC, the primary provider of healthcare services in Qatar with symptoms mainly, fever and gastroenteritis. For each patient, demographic data such as age, nationality, and gender were reported.

**Bacterial culture**

Pathogens were isolated from human samples using the standard bacteriological procedure. Briefly, 1 g of the collected stool samples was diluted with 3ml of phosphate-buffered saline (PBS, pH 7.2; Sigma, St. Louis, MO, USA), and 500μl of this dilution was added to 5 ml of Selenite broth (Oxoid, Basingstoke, Hampshire, UK) for the enrichment and incubated at 37°C for 24-48 hr. The enriched samples were sub-cultured into MacConkey agar and incubated at 37°C for 24 hr. The non-lactose fermenter colonies tested negative with oxidase and produced hydrogen sulfide gas were sub-cultured into Hektoen Enteric Agar (Biolife-Italia). Suspected colonies (transparent green colonies with black centers) except *S. paratyphi* A, whose colonies appear without black centers, were identified by Matrix-Assisted laser Desorption/Ionization, MALDI-TOF (Bruker Daltonik GmbH, Leipzig, Germany). On the other hand, blood culture samples were incubated on automated BACTEC FX blood culturing instrument (B.D., U.S.). Gram stain was performed on blood cultures flagged positive. Then, cultured isolates demonstrating Gram-negative bacteria were inoculated into MacConkey agar and blood agar. Suspected colonies were automatically identified using MALDI-TOF. *Salmonella* serology was performed using (Difco® *Salmonella* O Antiserum) and (Difco® *Salmonella* H Antiserum).

**Phenotypic antibiotic susceptibility testing**

The antibiotic susceptibility testing was conducted using Phoenix (the NMIC/ID-5 panel, B. D. Biosciences, Heidelberg, Germany) per the manufacturer’s recommendations. This panel includes 15 antibiotics, namely Amoxicillin/Clavulanic Acid (AMC), Ampicillin (AMP), Ceftriaxone (CRO), Aztreonam (ATM), Cefepime (FEP), Ceftazidine (CAZ), Meropenem (MEM), Imipenem (IPM), Ertapenem (ETP), Piperacillin/tazobactam (TZP), Tigecycline (TGC), Ciprofloxacin (CIP), Levofloxacin (LVX) and trimethoprim/sulfamethoxazole (SXT). At the same time, E-test (Liofilchem, Germany) was performed for Azithromycin (AZM), Fosfomycin (FOS), Tetracycline (TET), and Chloramphenicol (C) as they were not included in the Phoenix automated panel. MICs were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute CLSI (CLSI, 2017). *Escherichia coli ATCC 25922* strain was used as a quality control organism. Isolates were confirmed as ESBL producers via the Double Disc Synergy Test (DDST) as previously described by Eltai et al. [12] *AmpC* β-lactamase production was measured using cefoxitin; a zone diameter of ≥18 mm was considered as *AmpC*-positive (CLSI, 2017). All intermediate resistant isolates were considered susceptible.

**Molecular characterization of antibiotic resistance**

Whole-genomic DNA was extracted from *Salmonella* bacterial cultures using QIAamp UCP pathogen Minikit (QIAGEN, Germany). Extracted DNA was then used to run PCR for 32 genes using previously published primers [11-21]. The conditions used for reactions were as follows: PCR mixture was made in the volume of 20μl containing 0.5μM of each primer, 1μl of DNA, 1x master mix (Hot star Taq plus master mix (QIAGEN, Germany) and H₂O up to 20μl. The reaction was amplified in Biotema TAdvanced Thermal cycler (Analytik Jena, Germany) under the following conditions: initial denaturation at 95°C for 5 min.; followed by 30 cycles of 30 s at 95°C, 1 min at 44-63°C and 90 s at 72°C and a final extension step of 5 min at 72°C.

The 32 genes that responsible for the resistance of the most prescribed antibiotics were amplified. These genes include *tet* A, B, C, D, E and G (Tetracycline resistant isolates), *catA*, *cmlA*,
and floR (Chloramphenicol-resistant isolates), sul1, sul2 and sul3 (Triamterhopen–sulfamethoxazole-resistant isolates), ESB L2 genes TEM, CTX-M groups and SHV (Third generation cephalosporines), mphA, mphB, ermA, ermB, ereA, mefA, and msrA (Azithromycin-resistant isolates) and ampC, CMY2 and Class 1 gene cassette (Amoxicillin-clavulunate resistant isolates).

The only clear bands were considered for the further fingerprinting scoring. The scores ‘1’ and ‘0’ were given for the presence and absence of genes ‘amplicon’s band, respectively. The data obtained by scoring the Antimicrobial Resistance (AMR) genes were subjected to cluster analysis. A similarity matrix was constructed using Jaccard’s coefficient, where the similarity values were used for cluster analysis. Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering was performed using the Unweighted Pair Group Method with the Arithmetic Averages (UPGMA) method. Data were then analyzed using Past software version 3.26 [22] based on Hamming similarity index with 100 bootstraps.

Statistical analysis

Data were introduced into Microsoft Excel 2010 (Microsoft Corporation, New York, USA) to generate figures and run the initial analysis. The relation between the resistance, nationality, age, and gender grouping was calculated using the Pearson test (GraphPad Software, San Diego, California, USA). A probability value (P-value) less than 0.05 was considered statistically significant. Past software, version 1.91 was used to construct a hierarchical clustering dendrogram, and Jaccard’s coefficient was applied to generate the similarity values for the cluster analysis.

Results

Demography of the study population

In this study, we tested 246 Salmonella isolates from children between 2-18 years of age. Among these, 220 (89.4%) were isolated from stool and 26 (10.6%) from blood. The demographic profile of the studied population was summarized in Table 1. The male: female ratio was 1.4:1 and 194 (78.9%) were under five years of age. The studied population was summarized in Table 1. The male: female ratio was 1.4:1 and 194 (78.9%) were under five years of age. The highest resistance was reported to tetracycline (23.9%) followed by (21.1%) ampicillin, (18.7%) AMC, and (13%) trimethoprim-sulfamethoxazole. The highest phenotypic combination of resistance was detected against AMC, AMP, TE & C (4.5%). Furthermore, 16.2% of the isolates were Multidrug-Resistant (MDR), with 4.1% being ESB L2 producers (Table 2).

Phenotypic profile of Salmonella isolates

The antibiotic resistance profile of 246 Salmonella isolates is depicted in Figure 1. Resistance was detected against 14 antibiotics. 38.2% of isolates were resistant to at least one antibiotic. Overall, the highest resistance was reported to tetracycline (23.9%) followed by (21.1%) ampicillin, (18.7%) AMC, and (13%) trimethoprim-sulfamethoxazole. The highest phenotypic combination of resistance was detected against AMC, AMP, TE & C (4.5%). Furthermore, 16.2% of the isolates were Multidrug-Resistant (MDR), with 4.1% being ESB L2 producers (Table 2).

Molecular genotyping profile of Salmonella isolates

| Table 1: Demographic profile of the study population (n = 246) with Salmonella among pediatric (2 to 18 years old) in the State of Qatar. |
|---------------------------------------------------------------|
|          | Qatari n=92 (37.4%) | Non-Qatari n=154 (62.6%) | Total no./ percentage (n=246) |
| Gender  |                           |                           |                           |
| Male    | 46 (50%)                   | 84 (54.5%)                 | 130 (52.8)                |
| Female  | 46 (50%)                   | 70 (45.5%)                 | 116 (47.2)                |
| Total no./percentage (n=246) 92 (37.4%) | 154 (62.6%)               | 246 (100)                |
| Age group |                           |                           |                           |
| 5-Feb   | 74 (80.4%)                  | 120 (78%)                  | 194 (78.9)                |
| 18-Jun  | 18 (19.6%)                  | 34 (22%)                   | 52 (21.1)                 |
| Total no./percentage 92 (100) | 154 (100)               | 246(100)                  |
| Salmonella species |                           |                           |                           |
| Non-typhoidal Salmonella | 64 (69.6%)               | 95 (61.7%)                 | 159 (64.6)                |
| Typhoidal Salmonella | 28 (30.4%)                  | 59 (38.3%)                 | 87 (35.4)                 |
| Total no./percentage 92 (100) | 154 (100)               | 246 (100)                  |
phenotypic resistant profiles of Salmonella isolates from hospitalized pediatric in Qatar (n=246).

| Resistant phenotype | Frequency | Percentage % |
|---------------------|-----------|---------------|
| No resistance       | 152       | 61.8          |
| Resistant to only one antibiotic | 32 | 13 |
| AMC, AMP            | 2         | 0.8           |
| +AMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, TE, C | 1 | 0.4 |
| +AMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, AZ, C | 1 | 0.4 |
| +AMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, C | 3 | 1.2 |
| +AMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, CIP, LVX, C | 1 | 0.4 |
| AMC, AMP, AZ        | 1         | 0.4           |
| +AMC, AMP, AZ, C    | 1         | 0.4           |
| +AMC, AMP, SXT, AZ, C | 1 | 0.4 |
| +AMC, AMP, SXT, C   | 3         | 1.2           |
| +AMC, AMP, SXT, CIP, TE | 1 | 0.4 |
| AMC, AMP, SXT       | 4         | 1.6           |
| +AMC, AMP, SXT, TE  | 7         | 2.8           |
| AMC, AMP, TE        | 2         | 0.8           |
| +AMC, AMP, TE, C    | 11        | 4.5           |
| +AMP, ATM, FEP, CAZ, CRO, TZP, TE | 1 | 0.4 |
| +AMP, CRO, SXT, CIP, LVX, TE | 1 | 0.4 |
| +AMP, SXT, AZ, C    | 1         | 0.4           |
| AMP, TE             | 1         | 0.4           |
| CIP, AZ             | 2         | 0.8           |
| CIP, LVX            | 3         | 1.2           |
| SXT, CIP            | 1         | 0.4           |
| +SXT, CIP, LVX, AZ, TE | 1 | 0.4 |
| +SXT, CIP, TE, C    | 3         | 1.2           |
| SXT, TE             | 2         | 0.8           |
| TE, C               | 1         | 0.4           |
| +TGC, CIP, LVX, TE  | 1         | 0.4           |
| +TGC, SXT, CIP, TE  | 1         | 0.4           |
| TGC, TE             | 1         | 0.4           |
| +TZP, TGC, TE       | 1         | 0.4           |
| MDR                 | 40        | 16.2          |
| ESBL                | 10        | 4.1           |

- MDR multidrug-resistant.
- ESBL extended-spectrum beta-lactamase producer.
- AMC: amoxicillin/clavulanic acid, AMP: ampicillin, CRO: ceftriaxone, ATM: aztreonam, FEP: cefepime, CAZ: ceftazidime, TZP: piperacillin/tazobactam, TGC: tigecycline CIP: ciprofloxacin, LVX: levofloxacin, SXT: trimethoprim/sulfamethoxazole, AZM: azithromycin, TET: tetracycline, C: chloramphenicol.

The detected phenotypic resistance was verified by molecular detection of relevant genetic determinants. Tetracycline resistance has been confirmed by the existence of tetA, tetB, tetC, tetD, tetE & tetG gene (Figure 2A). TetA gene (64.4%) recorded the highest percentage of all other tet genes followed by tetC (8.4%), tetG & tetA 6.8%.

Ten genes underlying ESBL resistance were detected, including \(^{13}\)TEM, \(^{14}\)SHV, \(^{13}\)CTX-M-G1, 2, 3, 8, 9 & 25, and \(^{14}\)CTX-M-14 &15 (Figure 2B). Nineteen percentage of ESBL isolates harbored \(^{13}\)CTX-M Group genes. \(^{14}\)CTX-M-G-8 & 25 and \(^{15}\)SHV were not detected among ESBL isolates. While 60% of ESBL isolates were encoded by \(^{14}\)TEM gene.

*Sul1, sul2, and sul3* were identified among the trimethoprim-sulfamethoxazole-resistant isolates (13%, n=32), Figure 2C. Among these isolates the most common detected gene was sul2 (84.4%, n=27), followed by sul2 (53.1%, n=18), then sul3, (15.6%, n=5/32). *Sul* genes are present as one type or in combination.

Among chloramphenicol resistant isolates (11%, n=27), the most predominant gene was flor 44. 4% (n=12), whereas the other genes catA and cmlA detected in 37% (n=10) and 11.1% (n=3), respectively. (Figure 2D). In addition to two combination, namely cmlA & flor and catA & flor, which detected among 3.7% (n=1).

Amoxycillin-resistant, seven genes have been detected among the isolates mphA, mphB, ermA, ermB, ereA, mefA, and msrA. PCR results depicted that 1 of the 11 isolates (MIC ≥ 256 μg/mL) harbored mphA genes. The mphB, ermA, ermB, ereA, mefA, and msrA genes were negative among all amoxycillin-resistant isolates. Class (1) gene cassette spotted in (78.5%) isolates. Whereas class 2 gene cassette was not detected. Among AMC resistant isolates, 25% (n=12) harbored ampC, while only two isolates (4.3%) had CMY2 gene.

**Statistical correlation**

There was no significant relationship among all the studied isolates, between antibiotic resistance, age, gender, and nationality variables, through the Pearson test of independence, probability value (P>0.05) using (GraphPad Prism version 9.0.2 for Windows, GraphPad Software, San Diego, California USA). Calculated using the Pearson test, followed by multiple comparisons test was performed using Probability value (P>0.05) No significant differences were detected by nationality, age, and gender (P>0.05) in relation to resistance.

**Clustering AMR genes of Salmonella isolates**

Cluster analysis was used to study similarity among individual of *Salmonella* isolates that harbored at least one of the studied genes (n=246) based on the presence and absence of 24 genes (amp C, CMY 2, sul 1, sul 2, sul 3, tet A, tet B, tet C, tet D, tet E, tet G, cat A, cml A, flor A, mph A, \(^{13}\)TEM, \(^{14}\)SHV, \(^{13}\)CTX-M-G1, 2, \(^{14}\)CTX-M-G2, \(^{14}\)CTX-M-G3, \(^{14}\)CTX-M-G8, \(^{14}\)CTX-M-G9, \(^{14}\)CTX-M-G14, \(^{14}\)CTX-M-G15 & \(^{14}\)CTX-M-25). Data were analyzed using an agglomerative hierarchical algorithm that revealed forty-three clonal clusters among the 73 tested *Salmonella* isolates. Clonally related strains of cluster G1 that harbors only tet A (23.3%, n=1773) were responsible for the predominant tetracycline resistance, followed by A16 (8.2%, n=6), which produces a combination of tetA and sul2 type genes that express tetracycline and sulfamethoxazole resistance, respectively. Sixteen clonal clusters were detected among group A with the main cluster A16 followed by cluster A1 producing a combination of three genes, sul 1, sul2, and cat A concurrently, then cluster A5 had two
combination sul1 & sul2. Group B includes 2 clusters that express a combination between tetA, β-lactamase and/or ESBL genes. Group C consists of four clusters with a combination of tetA, sul, and chloramphenicol genes. Group D had seventeen clusters that mainly express different tet and chloramphenicol genes.

**Discussion**

Out of 246 *Salmonella* isolated from blood and stool samples obtained from pediatric patients (2-18 years) who presented with gastroenteritis symptoms, 61.8% (n=152) of the isolates exhibit no resistance against the tested antibiotics. While 13% (n=32) were resistance to only one antibiotics, 4.1% (n=10) were ESBL producers and 16.2% (n=40) were MDR.

Tetracycline resistance was the highest observed (24%) among the isolates investigated in this study. 64.4% of tetracycline resistance isolates harbor the gene tetA, which is often considered part of transposon Tn1721. This gene can be associated with conjugative and transmissible plasmids with a high capability of moving from one bacteria to the other, contributing to the spreading and increase of tetracycline resistance [23,24]. Among the 11% (n=27) chloramphenicol resistant isolates, floR gene was the most detected gene (44.4%, n=12) followed by catA (37%, n=10) and cmlA gene (11.1%, n=3). This agrees with Nogrady et al. [25], who reported that (46.4%) of *Salmonella* isolated from humans in their study harbored the floR gene.

Besides, we reported resistance of 21% and 18.7% to ampicillin and amoxicillin/clavulanic, respectively. These two antibiotics are hugely prescribed to treat respiratory infectious diseases in chickens and cattle [26]. This use of antibiotics for therapeutic purposes in veterinary medicine and as growth promoters in food-producing animals is speculated to be a significant cause of the development of resistance in *Salmonella*, presenting a potential risk to public health [27]. The most common genetic determinant of trimethoprim-sulfamethoxazole resistant isolates was the sul2 gene, which was detected among (84.4%, n=27/32), followed by the sul1 gene 53.1% (n=18/32) then sul3 15.6% (n=5/32). In contrast to our findings, Antunes et al. reported that the sul1 gene was the most prevalent gene among *Salmonella* resistant to sulfamethoxazole [28]. This is probably endorsed to the variance in the source of samples wherein the previous study; the samples were collected from environmental Portuguese *Salmonella enterica*. The same study reported that 7% of *Salmonella* isolates harbored sul3 gene being the lowest reported as in our study.

In this study, 90% (n=10) of the extended cephalosporin-resistant isolates harbored β-CTX-M genes, in which β-CTX-M-1, β-CTX-M-15, and β-CTX-M-3 (50%) were the most common gene. However, the β-CTX-M-1 and β-CTX-M-9 groups have also been dominant in *S. Typhimurium* isolates in Shanghai [1]. Our results showed that β-CTX-M-9 and 14 were demonstrated by 20% and 10%, respectively, among ESBL isolates. Most of the ESBL genes are usually harbored in a bacterial plasmid, which gives them the ability to distribute easily among different bacteria species contributing to the increase of antibiotic resistance [29].

Our findings reveal that 11 (4.47%) of the isolates were resistant to azithromycin. Food and Drug Administration (FDA) recommended using azithromycin to treat invasive *Salmonella* infections [30] because this antibiotic proved an excellent ability to accumulating at high intracellular concentrations [31]. Resistant to azithromycin were also recorded in other countries [1,30,32]. One high-level azithromycin-resistant isolate (MIC ≥256 μg/mL) that harbored the mphA gene was identified in our study. This is in line with Phuc Nguyen et al. [19], who detected mphA in 34 of 190 *Escherichia coli*
isolated from human feces with MICs 256 mg/L to >1,024 mg/L. The resistance against azithromycin among the other ten isolates in this study can be underlined by other possible mechanisms, such as mutations in the \textit{rlpD} and \textit{rlpV} genes [33].

The most common isolated serovar was \textit{Salmonella} \textit{serotyping} \textit{GroupB} and \textit{Salmonella} \textit{Paratyphi} (24.8%), followed by \textit{Salmonella} \textit{serotyping group D} (11.8%). This finding contrasts with Iyer et al. [34], who reported that \textit{Salmonella Typhi} was the most abundant type of \textit{Salmonella} spread between children of less than five years but in concordance with Ochaia et al. [35], who stated the overall ratio of disease caused by \textit{Salmonella Typhi} to that caused by \textit{Salmonella Paratyphi} is about 10 to 1.

**Conclusion**

This is the first study to elucidate the genotypic determinants of resistance among \textit{Salmonella} isolates from Qatar’s pediatric population. The highest resistance was depicted against tetracycline, ampicillin, and amoxicillin-clavulanate. We identified various transferrable antimicrobial-resistance genes among the MDR isolates, such as ESBL genes, \textit{tet}, \textit{ampC}, \textit{cat}, \textit{cml A} \\& \textit{floR} genes, and some isolates harbored a combination of these genes. The presence of these genes poses a considerable threat to the control of \textit{Salmonella} infection locally and globally. Overall, this work provides baseline data for the Prevalence of the genetic determinants underlying the phenotypic resistance among Qatar’s pediatrics populations. These findings can help update the local antimicrobial policy and inform the antimicrobial stewardship program to be implemented in health.

**Figure 3:** Dendrogram of AMR Genes among \textit{Salmonella} isolates (n=73).

Agglomerative hierarchical algorithm illustrating the similarity among the isolates.

| A1: sul1, sul2, cat A | (SXT), (C) |
| A2: ampC, sul1, sul2, cat A | (AMC), SXT, C |
| A3: sul2, cat A | SXT, C |
| A4: sul2 | SXT |
| A5: sul1, sul2 | SXT |
| A6: sul1 | SXT |
| A7: ampC, sul1, cat A, $\beta$-TEM, $\beta$-CTX-M-1, $\beta$-CTX-M-3, $\beta$-CTX-M-15 | AMC, SXT, C, (CRO) |
| A8: sul2, mph A, cat A, $\beta$-TEM, $\beta$-CTX-M-1, $\beta$-CTX-M-3, $\beta$-CTX-M-15 | SXT, (AZM), C, CRO |
| A9: amp C, sul1, sul2, catA, $\beta$-TEM, $\beta$-CTX-M-1, $\beta$-CTX-M-15 | AMC, SXT, C, CRO |
| A10: CMY 2, sul2, cat A, $\beta$-TEM, $\beta$-CTX-M-1 | AMC, SXT, C, CRO |
| A11: tetA, sul1, sul2 | (TET), SXT |
| A12: tetA, tetB, sul1, sul2 | TET, SXT |
| A13: tetA, tetB, sul1, sul2, $\beta$-CTX-M-1, $\beta$-CTX-M-15 | TET, SXT, CRO |
| A14: amp C, tetA, sul2 | AMC, TET, SXT |
| A15: amp C, tetA, sul2, cml A, $\beta$-CTX-M-1, $\beta$-CTX-M-9 | AMC, TET, SXT, C, CRO |
| A16: tetA, sul2 | TET, SXT |
| B1: tetA, $\beta$-CTX-M-3, $\beta$-CTX-M-15 | TET, CRO |
| B2: CMY 2, tetA, $\beta$-TEM | AMC, TET, CRO |
| C1: tetA, sul3, cml A | TET, SXT, C |
| C2: tetA, sul1, sul3, cml A | TET, SXT, C |
| C3: tetA, tetE, sul1, sul3, cml A, floR | TET, SXT, C |
| C4: tetA, sul3 | TET, SXT |
| D1: ampC, tetA, tetB, tetC, tetE, floR | AMC, TET, C |
| D2: tetA, tetB, tetC, tetE, floR | TET, C |
| D3: tetA, tetC, tetE, floR | TET, C |
| D4: tetA, tetC, tetE | TET |
| D5: tetA, tetC, cat A, floR | TET, C |
| D6: tetA, tetC, floR | TET, C |
| D7: amp C, tetA, tetC, floR | AMC, TET, C |
| D8: tetA, tetC | TET |
| D9: amp C, tetA, tetG, floR | AMC, TET, C |
| D10: tetA, tetG, floR | TET, C |
| D11: tetB | TET |
| D12: tetB, floR | TET, C |
| D13: floR | C |
| D14: tetA, floR | TET, C |
| D15: tetA, tetB, tetD | TET |
| D16: tetA, tetC, tetD | TET |
| D17: tetA, tetB | TET |
| E1: tetA, tetE | TET |
| F1: amp C, tetA | TET |
| G1: tetA | TET |
facilities.

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