Research Article

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 bla 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

Abbreviations

MDR: Multi-Drug Resistant; NTS: Non-Typhoidal *Salmonella*; AMR: Antimicrobial Resistance; ESBL: Extended-Spectrum β Lactamase; FDA: Food Drug Administartion

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

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 37ºC for 24-48 hr. The enriched samples were sub-cultured into MacConkey agar and incubated at 37ºC 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), Ceftazidime (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, 1× master mix (Hot star Taq plus master mix (QIAGEN, Germany) and H_2O up to 20µl. The reaction was amplified in Biometra TAdvanced Thermal cycler (Analytik Jena, Germany) under the following conditions: initial denaturation at 95ºC for 5 min.; followed by 30 cycles of 30 s at 95ºC, 1 min at 44-63 ºC and 90 s at 72ºC and a final extension step of 5 min at 72ºC.

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), *cat*A, *cml*A
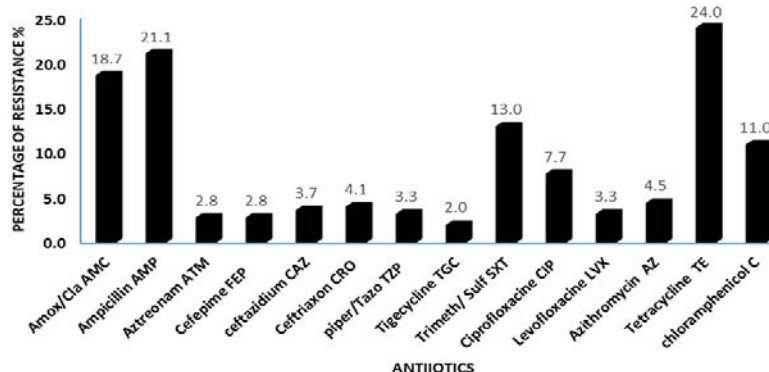
Figure 1: Frequency bar chart illustrating *Salmonella's* phenotypic resistance profile (n=246) isolated from children (age 2 to 18 years) with gastroenteritis. The figure depicts the percentage of isolates with resistance to each of the antibiotics.
AMC: Amoxicillin/Clavulanic Acid; AMP: Ampicillin; CRO: Ceftriaxone; ATM: Aztreonam; FEP: Cefepime; CAZ: Ceftazidime; TZP: Piperacillin/Tazobactam; TGC: Tigecycline; CIP: Ciprofloxaci; LVX: levofloxacin; SXT: Trimethoprim/Sulfamethoxazole; AZ: Azithromycin; TET: Tetracycline; C: Chloramphenicol.

and *flo*R (Chloramphenicol-resistant isolates), *sul*1, *sul*2 and *sul*3 (Trimethoprim–sulfamethoxazole-resistant isolates), ESBL genes ^bla^TEM, ^bla^CTX-M groups and ^bla^SHV (Third generation cephalosporines), *mph*A, *mph*B, *erm*A, *erm*B, *ere*A, *mef*A, and *msr*A (Azithromycin-resistant isolates) and *ampC*, *CMY2* and Class 1 gene cassette (Amoxicillin-clavulanate 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 Jacquard'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 Qatari patient comprises 37.4% (n=92) of this study population, while other nationalities 62.6%. The typhoidal *Salmonella* among Qatari patient was 30.4%/28 (23 Paratyphi B, 2 Paratyphi A and 3 Typhi)

and 38.3%/59 (38 Paratyphi B, 5 Paratyphi A and 16 Typhi) among non-Qatari. On the other hand non-typhoidal *Salmonella* isolates include 64.6% n= 159 (64 Qatari and 95 non-Qatari) including *Salmonella* group B (n=61), *Salmonella* group C1 (n=5), *Salmonella* group C2 (n=9), *Salmonella* group D (n=29), *Salmonella* group E (n=7), *Salmonella* Choleraesuis (n=8), *Salmonella* Enteritidis (n=13), *Salmonella* Typhimorium (n=12) and *Salmonella* group O (n=13).

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 ESBL 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.

Comparative	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)

Table 2: 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
ᵃᵇAMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, TE, C	1	0.4
ᵃᵇAMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, AZ, C	1	0.4
ᵃᵇAMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, C	3	1.2
ᵃᵇAMC, AMP, ATM, FEP, CAZ, CRO, TZP, SXT, CIP, LVX, C	1	0.4
AMC, AMP, C	1	0.4
ᵃᵇAMC, AMP, CAZ, CRO, TE	1	0.4
AMC, AMP, AZ	1	0.4
ᵇAMC, AMP, FEP, CAZ, CRO, TGC, CIP, LVX, TE	1	0.4
ᵃAMC, AMP, SXT, AZ, C	1	0.4
ᵃAMC, AMP, SXT, C	3	1.2
ᵃAMC, AMP, SXT, CIP, TE	1	0.4
AMC, AMP, SXT	4	1.6
ᵃAMC, AMP, SXT, TE	7	2.8
AMC, AMP, TE	2	0.8
ᵃAMC, AMP, TE, C	11	4.5
ᵃᵇAMP, ATM, FEP, CAZ, CRO, TZP, TE	1	0.4
ᵃᵇAMP, CRO, SXT, CIP, LVX, TE	1	0.4
ᵃAMP, 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
ᵃSXT, CIP, LVX, AZ, TE	1	0.4
ᵃSXT, CIP, TE, C	3	1.2
SXT, TE	2	0.8
TE, C	1	0.4
ᵃTGC, CIP, LVX, TE	1	0.4
ᵃTGC, SXT, CIP, TE	1	0.4
TGC, TE	1	0.4
ᵃTZP, TGC, TE	1	0.4
MDR	**40**	**16.2**
ESBL	**10**	**4.1**

ᵃMDR multidrug-resistant.

ᵇESBL 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 *tet*A, *tet*B, *tet*C, *tet*D, *tet*E & *tet*G gene (Figure 2A). *Tet*A gene (64.4%) recorded the highest percentage of all other *tet* genes followed by *tet*C (8.4%), *tet*A, *tet*G & *tet*A 6.8%.

Ten genes underlying ESBL resistance were detected, including ᵇˡᵃTEM, ᵇˡᵃSHV, ᵇˡᵃCTX-M-G-1, 2, 3, 8, 9 & 25, and ᵇˡᵃCTX-M-14 &15 (Figure 2B). Nineteen percentage of ESBL isolates harbored ᵇˡᵃCTX-M-Group genes. ᵇˡᵃCTX-M-G-8 & 25 and ᵇˡᵃSHV were not detected among ESBL isolates. While 60% of ESBL isolates were encoded by ᵇˡᵃTEM gene.

*Sul*1, *sul*2, and *sul*3 were identified among the trimethoprim–sulfamethoxazole-resistant isolates (13%, n=32), Figure 2C. Among these isolates the most common detected gene was *sul*2 (84.4%, n=27), followed by *sul*2 (53.1%, n=18), then *sul*3, (15.6%, n=5/32). These *sul* genes are present as one type or in combination.

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

For azithromycin-resistant, seven genes have been detected among the isolates *mph*A, *mph*B, *erm*A, *erm*B, *ere*A, *mef*A, and *msr*A. PCR results depicted that 1 of the 11 isolates (MIC ≥ 256 μg/mL) harbored *mph*A genes. The *mph*B, *erm*A, *erm*B, *ere*A, *mef*A, and *msr*A genes were negative among all azithromycin-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 *amp*C, 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=73) 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*, *flo*R, *mph A*, ᵇˡᵃ TEM, ᵇˡᵃ CTX-M-G1, ᵇˡᵃ CTX-M-G2, ᵇˡᵃ CTX-M-G3, ᵇˡᵃ CTX-M-G8, ᵇˡᵃ CTX-M-G9, ᵇˡᵃ CTX-M-G14, ᵇˡᵃ CTX-M-G15 & ᵇˡᵃ CTX-M-G25). 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=17/73) were responsible for the predominant tetracycline resistance, followed by A16 (8.2%, n=6), which produces a combination of *tet*A and *sul*2 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, *sul*2, and *cat* A concurrently, then cluster A5 had two
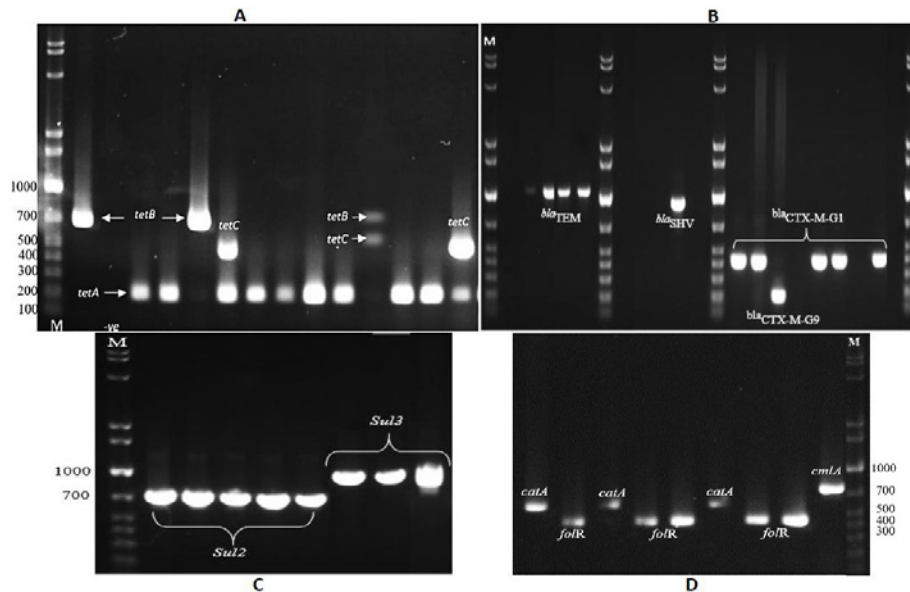
Figure 2: Molecular genotyping profile of antibiotic resistance gene among *Salmonella* isolated from children with gastroenteritis, representative samples are presented. Multiplex PCR was performed for detection of [bla]CTX-M and *Sul* group while Uniplex PCR was used for detection of [bla]SHV, [bla]TEM, *floR*, *catA*, *cml* and *tet* group; A: tetracyclines resistance detected genes- (*tet*A, *tet*B, *tet*C, *tet*D, *tet*E & *tet*G).
B: Chloramphenicol Resistant detected genes; C: trimethoprim-sulfamethoxazole detected genes; D: detection of [bla]SHV, [bla]TEM, and [bla]CTX-M among ESBL producers; M: 1 kb plus ladder marker (Gelpilot, Germany).

combination *sul*1 & *sul*2. Group B includes 2 clusters that express a combination between *tet*A, β lactamase and/or ESBL genes. Group C consists of four clusters with a combination of *tet*A, *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) are 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 *tet*A, 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, *flo*R gene was the most detected gene (44.4%, n=12) followed by *cat* A (37%, n=10) and *cml*A 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 *flo*R 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 *sul*3 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 [bla]CTX-M genes, in which [bla]CTX-M-G-1, [bla]CTX-M15, and [bla]CTX-M-G-3 (50%) were the most common gene. However, the [bla] CTX-M-1 and [bla] CTX-M -9 groups have also been dominant in *S. Typhimurium* isolates in Shanghai [1]. Our results showed that [bla] 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 *mph*A gene was identified in our study. This is in line with Phuc Nguyen et al. [19], who detected *mph*A in 34 of 190 *Escherichia coli*
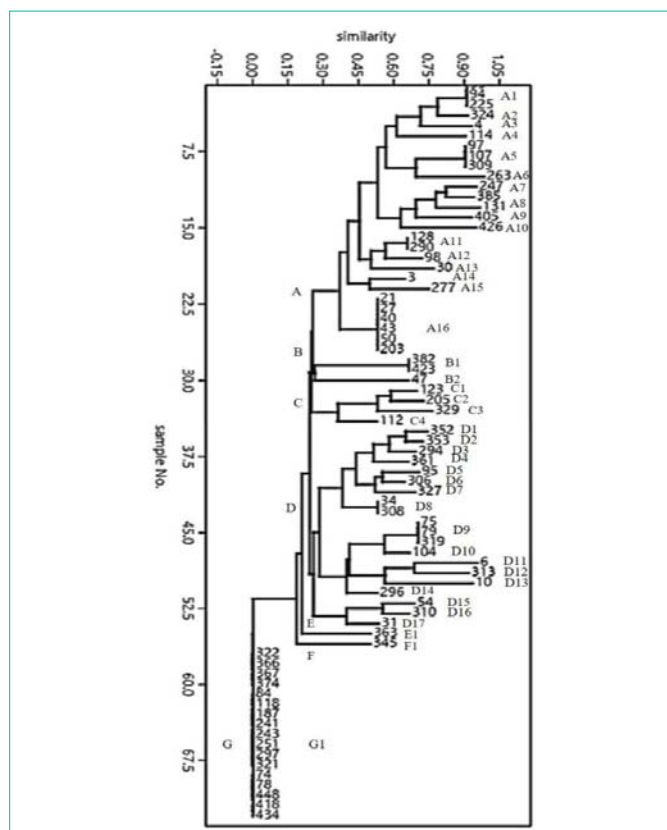
Figure 3: Dendrogram of AMR Genes among *Salmonella* isolates (n=73). Agglomerative hierarchical algorithm illustrating the similarity among the isolates.

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, sul2, cat A*, *bla*TEM, *bla*CTX-M-G-1, *bla*CTX-M-G-3, *bla*CTX-M-15	AMC, SXT, C, (CRO)
A8: *sul2, mph A, cat A*, *bla*TEM, *bla*CTX-M-G-1, *bla*CTX-M-G-3, *bla*CTX-M-15	SXT, (AZM), C, CRO
A9: *amp C, sul1, sul2, catA*, *bla*TEM, *bla*CTX-M-G-1	AMC, SXT, C, CRO
A10: *CMY 2, sul2, cat A*, *bla*TEM, *bla*CTX-M-G-1	AMC, SXT, C, CRO
A11: *tetA, sul1, sul2*	(TET), SXT
A12: *tetA, tetB, sul1, sul2*	TET, SXT
A13: *tetA, sul1, sul2*, *bla*CTX-M-G-9, *bla*CTX-M- 14	TET, SXT, CRO
A14: *amp C, tetA, sul2*	AMC, TET, SXT
A15: *amp C, tetA, sul2, cml A*, *bla*CTX-M-G-9	AMC, TET, SXT, C, CRO
A16: *tetA, sul2*	TET, SXT
B1: *tetA*, *bla*CTX-M-G-3, *bla*CTX-M-G-15	TET, CRO
B2: *CMY 2, tetA*, *bla*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

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 *rlp*D and *rlp*V genes [33].

The most common isolated serovar was *Salmonella* serotyping GroupB and *Salmonella* Paratyphi (24.8%), followed by *Salmonella* serotyping group D (11.8%). This finding contrasts with Iyer et al. [34], who reported that *Salmonella* Typhi was the most abundant type of *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 *Salmonella* Typhi to that caused by *Salmonella* Paratyphi is about 10 to 1.

Conclusion

This is the first study to elucidate the genotypic determinants of resistance among *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 ESBLgenes, *sul*, *tet*, *ampC*, *cat*, *cml* A & *flo*R genes, and some isolates harbored a combination of these genes. The presence of these genes poses a considerable threat to the control of *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

facilities.

References

1. Wang J, et al. Antimicrobial resistance of *Salmonella enterica* serovar Typhimurium in Shanghai, China. Frontiers in microbiology. 2017; 8: 510.

2. Molbak K, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. N Engl J Med. 1999. 341: 1420-1425.

3. Flint JA, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clinical infectious diseases. 2005; 41: 698-704.

4. Threlfall EJ. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food-and water-borne infections. FEMS microbiology reviews. 2002; 26: 141-148.

5. Rahbar M, M Deldari, M Hajia. Changing prevalence and antibiotic susceptibility patterns of different Shigella species in Tehran, Iran. The Internet J Microbiol, 2007.

6. Wellington EM, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. The Lancet infectious diseases, 2013; 13: 155-165.

7. Nataro JP, et al. Escherichia, shigella, and salmonella, in Manual of Clinical Microbiology, 10th Edition. 2011, American Society of Microbiology. 2011; 603-626.

8. Crump JA, Heyderman RS. A perspective on invasive *Salmonella* disease in Africa. Clinical Infectious Diseases. 2015; 61: S235-S240.

9. Haeusler GM, Curtis N. Non-typhoidal *Salmonella* in children: microbiology, epidemiology and treatment. Hot Topics in Infection and Immunity in Children IX. 2013; 764: 13-26.

10. Farag E, et al. A retrospective epidemiological study on the incidence of salmonellosis in the State of Qatar during 2004-2012. Qatar Medical Journal. 2016; 2016.

11. Rowlands REG, et al. Prevalence of drug resistance and virulence features in *Salmonella* spp. isolated from foods associated or not with salmonellosis in Brazil. Revista do Instituto de Medicina Tropical de São Paulo. 2014; 56: 461-467.

12. Eltai NO, Al Thani AA, Al-Ansari K, Deshmukh AS, Wehedy E, Al-Hadidi SH, et al. Molecular characterization of extended spectrum β-lactamases enterobacteriaceae causing lower urinary tract infection among pediatric population. Antimicrobial Resistance & Infection Control. 2018; 7: 90.

13. White DG, et al. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. New England journal of medicine. 2001; 345: 1147-1154.

14. Feria C, et al. Patterns and mechanisms of resistance to β-lactams and β-lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. Journal of Antimicrobial Chemotherapy. 2002; 49: 77-85.

15. Van Pelt W, et al. Explosive increase of *Salmonella* Java in poultry in the Netherlands: consequences for public health. Eurosurveillance. 2003; 8: 31-35.

16. Hudson CR, et al. Genetic Relatedness of *Salmonella* Isolates from Nondomestic Birds in Southeastern United States. Journal of clinical microbiology. 2000; 38: 1860-1865.

17. Ng LK, et al. Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes, 2001; 15: 209-215.

18. Bernal-Bayard J, Ramos-Morales F. *Salmonella* type III secretion effector SlrP is an E3 ubiquitin ligase for mammalian thioredoxin. Journal of Biological Chemistry. 2009; 284: 27587-27595.

19. Nguyen MCP, et al. *Escherichia coli* as reservoir for macrolide resistance genes. Emerging infectious diseases. 2009; 15: 1648-1650.

20. Bora AR. Identification of a single selective enrichment media for the simultaneous recovery of *Salmonella* and *E. coli* O157 on beef hide swabs and fecal samples. 2018.

21. Woodford N. Biological counterstrike: antibiotic resistance mechanisms of Gram-positive cocci. Clinical Microbiology and Infection. 2005; 11: 2-21.

22. Hammer O, Harper DA, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia electronica, 2001; 4: 1-9.

23. Girgis NI, et al. Azithromycin versus ciprofloxacin for treatment of uncomplicated typhoid fever in a randomized trial in Egypt that included patients with multidrug resistance. Antimicrobial agents and chemotherapy, 1999; 43: 1441-1444.

24. Frenck RW, et al. Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. Clinical infectious diseases, 2000; 31: 1134-1138.

25. Nograddy N, et al. Antibiotic resistance and class 1 integron patterns of non-typhoidal human *Salmonella* serotypes isolated in Hungary in 2002 and 2003. Int J Antimicrob Agents. 2005. 26: 126-132.

26. Lu Y, et al. Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. Foodborne pathogens and disease. 2011; 8: 45-53.

27. Fernandes SA, et al. CTX-M-2–producing *Salmonella* Typhimurium isolated from pediatric patients and poultry in Brazil. Microbial Drug Resistance. 2009; 15: 317-321.

28. Antunes P, et al. Dissemination of sulfonamide resistance genes (*sul*1, *sul*2, and *sul*3) in Portuguese *Salmonella enterica* strains and relation with integrons. Antimicrob Agents Chemother. 2005; 49: 836-839.

29. Arlet G, et al. *Salmonella* resistant to extended-spectrum cephalosporins: Prevalence and epidemiology. Microbes Infect. 2006; 8: 1945-1954.

30. Sjolund-Karlsson M, et al. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* isolates from the United States. Antimicrobial agents and chemotherapy. 2011; 55: 3985-3989.

31. Panteix G, et al. *In-vitro* concentration of azithromycin in human phagocytic cells. Journal of Antimicrobial Chemotherapy. 1993; 31: 1-4.

32. Kalonji LM, et al. Invasive *Salmonella* infections at multiple surveillance sites in the Democratic Republic of the Congo, 2011-2014. Clinical Infectious Diseases. 2015; 61: S346-S353.

33. Roberts MC. Update on macrolide–lincosamide–streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiology Letters. 2008; 282: 147-159.

34. Iyer R, et al. Prevalence and trends in the antimicrobial susceptibility pattern of *Salmonella enterica* serovars Typhi and Paratyphi A among children in a pediatric tertiary care hospital in South India over a period of ten years: a retrospective study. European Journal of Clinical Microbiology & Infectious Diseases. 2017; 36: 2399-2404.

35. Ochiai RL, et al. *Salmonella* paratyphi A rates, Asia. Emerging infectious diseases. 2005; 11: 1764-1766.