Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Epidemiology, Serogroups and Resistance of *Salmonella* During a 15-Year Period (2006–2020) in Kuwait

Wafaa Jamal[1]
Fatema Bibi Khodakhast[1]
Manuel John Albert[1]
Vincent Rotimi[2]

[1]Department of Microbiology, Faculty of Medicine, Kuwait University, Jabriya, Kuwait; [2]Department of Medical Microbiology and Parasitology, College of Medicine, Lagos State University, Ikeja, Nigeria

Purpose: The aim of the study was to investigate the changing pattern in serogroup distribution and antimicrobial resistance of all *Salmonella* spp. isolated from patients attending the Mubarak Al Kabeer Hospital (MAK), Kuwait from 2006 to 2020.

Patients and Methods: A retrospective study of all enrolled patients attending the MAK with culture-positive *Salmonella* spp. was undertaken. Data on age, gender, culture sample and serogroup were obtained from the laboratory information system. A prospective antimicrobial susceptibility of all stock isolates was carried out using E test. The trend rates of *Salmonella* serogroups and antimicrobial resistance were compared among 5 periods: 2006–2008, 2009–2011, 2012–2014, 2015–2017, and 2018–2020.

Results: A total of 700 isolates were identified. The majority of the isolates were from the stool (77.6%), followed by the blood (16.4%). The most common serogroups were serogroup D (37.6%) and B (23.4%). There was a significant rise in ciprofloxacin resistance from 32.2% during 2006–2008 to 54.3% during 2018–2020 and from 32.5% during 2009–2011 to 54.3% during 2018–2020 (P=0.0001, respectively). The resistance trend to cefotaxime was at relatively low levels ranging from 0% to 3.4% through 2006–2008 to 2018–2020. There was a significant drop of the resistance to ampicillin from 23.6% in 2015–2017 to 12.3% in 2006–2008 to 2018–2020 (P=0.03). Trimethoprim/sulfamethoxazole resistance dropped significantly from 14.5 to 3.6% (P=0.002) during 2006–2008 to 2018–2020 and then from 13.5 to 3.6% (P=0.02) during 2015–2017 to 2018–2020. One hundred and seventeen (16.7%) isolates were multidrug-resistant.

Conclusion: Continuous surveillance of *Salmonella* and its antimicrobial resistance is important for antibiotic policy formulation for invasive *Salmonella* infections.

Keywords: salmonella, susceptibility, serogroups, resistance, state of Kuwait

Introduction

Salmonellae are motile, Gram-negative bacilli, belonging to the family Enterobacterale. The genus *Salmonella* consists of 3 species: *Salmonella bongori, Salmonella enterica* and *S. subterranean*.[1] *S. bongori* (subspecies V) causes diseases in reptiles and rarely causes disease in humans, while *S. enterica* consists of over 2600 serotypes or serovars that have been identified up to date and causes disease in humans.[1] *S. enterica* itself is composed of six subspecies: *entericae* (subspecies I), *salamae* (subspecies II), *arizonae* (subspecies IIIa), *diarizonae* (subspecies IIIb), *houtanae* (subspecies IV), *indica* (subspecies VI).[1] *S. enterica* serovar is divided into typhoidal and nontyphoidal strains. Almost all serotypes can cause disease in

Correspondence: Wafaa Jamal
Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat, Jabriya, 13110, Kuwait
Tel +965 2463 3509
Fax +965 2533 2719
Email wafaa.jamal@ku.edu.kw

Received: 28 September 2021
Accepted: 13 November 2021
Published: 25 November 2021

humans. A few are host-specific and can be found in few animal species, eg *S. enterica* serotype Dublin in cattle and *S. enterica* serotype Choleraesuis in pigs.[2]

Salmonella infection is an important cause of food-borne-disease and gastroenteritis worldwide. According to the World Health Organization (WHO), it is regarded as 1 of the 4 key global causes of diarrheal diseases.[2] Most cases are mild but some can be life threatening according to the host factors and serotype of salmonella. It causes a considerable morbidity and mortality, especially in developing countries.[3] WHO has estimated that every year, almost 1 out of 10 people become sick and 33 million of healthy life years are lost. The Centers for Disease Control and Prevention (CDC), USA estimates that approximately 1.35 million illnesses, 26,500 hospitalizations and 420 deaths occur due to non-typhoidal *Salmonella* each year in the US, resulting in an estimated $400 million in direct medical costs.[4]

There is no vaccine available for controlling invasive non-typhoidal *Salmonella* infection. Although antimicrobial agents are not recommended in the treatment of salmonella gastroenteritis, it is, however, recommended for extra-intestinal invasive infections, especially in the extreme of age, immunosuppressed patients and those with underlying diseases, eg, meningitis, septicemia, septic arthritis, and osteomyelitis.[5,6] The management of these invasive infections entails using antimicrobial agents. However, antimicrobial resistance in *Salmonella* spp. is a serious issue all over the world especially against the first-line drugs, eg, ampicillin, chloramphenicol, and cotrimoxazole. Third-generation cephalosporins and fluoroquinolones have become the standard first-line empirical therapy. A CDC report stated that antibiotic-resistant non-typhoidal *Salmonella* infections are on the rise approaching an estimated 10% for ciprofloxacin, 3% for ceftriaxone and 1% for azithromycin.[4] Prolonged hospitalization and increased risk of bloodstream infections, treatment failure and excess mortality have been associated with antimicrobial drug resistant non-typhoidal *Salmonella* infections.[6–9] Previous reports from Kuwait have shown that resistance to *Salmonella* spp. in Kuwait is high.[10,11] According to a previous report from Kuwait, the commonest serogroups in both adults and children were serogroup B followed by serogroup C and D.[9] Thus, it is important to do a continuous surveillance of salmonella antimicrobial resistance and serogroups for patient management in order to reduce the occurrence of complications and mortality.

The aim of this study was to investigate the changing pattern in serogroup distribution and antibiotic resistance among *Salmonella* isolates from patients attending the Mubarak Al Kabeer Teaching Hospital, Kuwait, during a period of 15 years from 2006 to 2020.

Materials and Methods

Bacterial Isolates

All *Salmonella* species isolated from inpatients and outpatients attending the Mubarak Al Kabeer Hospital from 2006 to 2020 were sent to Anaerobe/Hospital Infection Laboratory, Department of Microbiology, Faculty of Medicine, Kuwait University, where they were stored at −80°C freezer in CryoBank beads (Mast Group Limited, Merseyside, UK). The specimens were obtained from the following sites as requested by the clinical diagnosis: stool, blood, extra-intestinal samples, eg, urine, CSF, pus, tissue, pleural fluid, bed sore, synovial fluid, bile, eye swab, ascetic fluid, wound, and fine needle aspirates. Only one isolate per patient per site was collected. The isolates were stored at −80°C freezer as above until used for study. Data on patient's age, gender, culture site, date of collection and serogroups were obtained from the laboratory information system (LIS).

Culture Method

Stool specimens were cultured on the following media: MacConkey agar (Oxoid, Basingstoke, UK), *Salmonella-Shigella* (SS) agar (ThermoFisher Scientific, Waltham, MA, USA), *Campylobacter* agar (ThermoFisher Scientific) and Selenite F broth (Becton Dickinson, Franklin Lakes, NJ, USA) which was subcultured on SS gar after 18 h incubation. If the stool was watery, it was also cultured on *Aeromonas* selective agar (Sigma-Aldrich, St. Louis, MO, USA) and Sorbitol-MacConkey agar (Oxoid).[12]

The following automated blood culture systems were used: BACTEC 9240 (Becton Dickinson) from 2006 to 2014, and BD BACTEC FX (Becton Dickinson) and BACT/ALERT VIRTUO (bioMérieux, Marcy-l'Etoile, France) from 2015.

Identification and Serogrouping

All *Salmonella* isolates were re-identified and serogrouped by standard VITEK II system (bioMerieux) and slide agglutination test during 2021. Isolates with low scores on VITEK II were subjected to further identification on Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) (bioMerieux). For serogrouping, the isolates were inoculated into triple sugar iron (TSI) agar (Hardy Diagnostics, Santa

Maria, CA, USA) and slide agglutination test was carried out using *Salmonella* polyvalent and group-specific antisera (Denka Seiken, Tokyo, Japan; SSI Diagnostics, Hillerod, Denmark; and Remel, Thermo Fisher Scientific) according to manufacturer instructions.

Antimicrobial Susceptibility Testing

Susceptibility testing of all isolates was carried out during 2021, in the Anaerobe/Hospital Infection Laboratory by determining the minimum inhibitory concentrations (MIC). The antibiotics tested and their breakpoints were the following: ampicillin (8 µg/mL), cefotaxime (1 µg/mL), chloramphenicol (8 µg/mL), ciprofloxacin (0.06 µg/mL), ertapenem (0.5 µg/mL), gentamicin (4 µg/mL), meropenem (1 µg/mL), tigecycline (0.5 µg/mL) and trimethoprim/sulfamethoxazole (2/38 µg/mL) using E-test method (bioMerieux). The breakpoints were used according to the interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI), except for tigecycline, which was done according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST).[13,14] *Escherichia coli* ATCC 25922 strain was used as a quality control strain in each batch of test.

To look for changes in resistance over time, the duration of study was broken into five periods: A (2006 to 2008), B (2009 to 2011), C (2012 to 2014), D (2015 to 2017), and E (2018 to 2020).

Statistical Analysis

The difference between proportions was compared by a two-tailed Chi square test. A P value of ≤0.05 was considered as significant.

Ethical Approval

Institutional ethical approval was obtained from the Health Sciences Centre Ethical Committee, Health Sciences Centre, Kuwait University (permit number VDR/EC/3779). Collection of the specimens was conducted according to the Declaration of Helsinki and with particular institutional ethical and professional standards. No additional specimens were collected from the patients for this study and the patient identities were kept anonymous.

Results

Demographics and Epidemiology

As shown in Table 1, a total of 700 isolates were collected of which 543 (77.6%) were from stool, 115 (16.4%) from blood, 18 (2.6%) from urine, 1 (0.1%) from cerebrospinal fluid (CSF), and 21 (3%) from other extra-intestinal sites (7 isolates from pus, 4 tissue, 2 pleural fluids, 2 ascetic fluids, 1 bedsore, 1 synovial fluid, 1 bile, 1 eye swab, 1 wound and 1 fine needle aspirate). The most common serogroup was group D (263; 37.6%), followed by group B (164; 23.4%), group C (154; 22%), *Salmonella* Typhi (46; 6.5%), group E (30; 4.3%), miscellaneous groups (17; 2.4%), group G (14; 2%), and *Salmonella* Paratyphi A, B, and C (12; 1.7%). The miscellaneous group include the following: group F (6; 0.85%), group A (3; 0.4%), group I (2; 0.28%), group O (2; 0.28%), group J (2; 0.28%), group H (1; 0.14%), and *Salmonella enterica* subsp. *diarizonae* (1; 0.14%). Among the bloodstream infections, group D (45; 39.1%) was the commonest followed by *S.* Typhi (40; 34.8%), group B (11; 9.6%) and *S.* Paratyphi A, B and C (6; 5.2%) and group C (6; 5.2%). Only one case of meningitis due to *Salmonella* serogroup D was reported during

Table 1 Clinical Samples from Which *Salmonella* Was Isolated

Salmonella Groups/Species	No. of Isolates in						Total No. of Isolates
	Stool	Blood	Urine	CSF	Other Samples [b]	Unknown Samples	
Salmonella grp. B	140	11	3	0	9	1	164
Salmonella grp. C	140	6	1	0	6	1	154
Salmonella grp. D	203	45	10	1	4	0	263
Salmonella grp. E	27	1	2	0	0	0	30
Salmonella grp. G	11	3	0	0	0	0	14
Salmonella Typhi	4	40	2	0	0	0	46
Salmonella Paratyphi (A, B, C)	6	6	0	0	0	0	12
Miscellaneous [a]	12	3	0	0	2	0	17
Total *Salmonella* spp.	543	115	18	1	21	2	700

Notes: [a]Miscellaneous: *Salmonella* group F (6); *Salmonella* group A (3); *Salmonella* group I (2); *Salmonella* group O (2); *Salmonella* group J (2); *Salmonella* group H (1); and *Salmonella enterica* subsp. *diarizonae* (1). [b] Other samples: pus (7), tissue (4), pleural fluid (2), ascetic fluid (2), bedsore (1), synovial fluid (1), bile (1), eye swab (1), wound (1), and fine needle aspirate (1).
Abbreviation: CSF, cerebrospinal fluid.

Table 2 Age and Gender Distribution of Patients from Whom *Salmonella* Was Isolated

Salmonella Group/Species	Total No. of Isolates	Gender		Age [b]	
		Male	Female	Mean (Years)	Range
Total Salmonella spp.[a]	700	393	306	28.3	23 Days- 90Y
Salmonella grp. D [a]	263	151	111	28.6	23 Days - 90Y
Salmonella grp. B	164	91	73	30.2	3 months- 86Y
Salmonella grp. C	154	86	68	30.3	1 month - 78 Y
Salmonella Typhi	46	23	23	18.6	2 Y - 75 Y
Salmonella grp. E	30	14	16	29.6	6 months- 46Y
Miscellaneous	17	9	8	23	1 Y - 80 Y
Salmonella grp. G	14	11	3	16	1 Y - 51 Y
Salmonella Paratyphi	12	7	5	19.7	11 months - 44 Y

Notes: [a]Gender was unknown for one sample. [b] Ages were not available for: Total *Salmonella* spp. (232), *S.* group B (70); *S.* group C (55); *S.* group D (49); *S.* Typhi (23); *S.* group E (19); miscellaneous group (5); *S.* group G (9); *S.* Paratyphi (2).
Abbreviations: M, month; Y, years.

the study period. As shown in Table 2, more than half of the isolates were obtained from male patients (393; 56.2%) compared to (306; 43.9%) from female patients. The age ranged between 23 days to 90 years. The age stratification according to the groups is shown in Table 2.

Changing Trends in Resistance Rates Among Different Years

As shown in Figure 1, there was a significant drop in the isolation rates of resistant isolates against ampicillin from 23.6% to 12.3% (P=0.03) between periods 2015–2017 and 2018–2020, respectively. While trimethoprim/sulfamethoxazole resistance dropped significantly from 14.5 to 3.6% (P=0.002) for periods between 2006–2008 and 2018–2020 and from 13.5 to 3.6% (P=0.02) for periods 2015–2017 and 2018–2020. There was a significant rise in resistance to gentamicin from 3.5% during 2006–2008 to 10.1% during 2015–2017 (P=0.05). There was a significant rise in ciprofloxacin resistance from 32.2% during 2006–2008 to 54.3% in 2018–2020, and again from 32.5% during 2009–2011 to 54.3% in 2018–2020 (P=0.0001 for both comparisons). Tigecycline resistance dropped significantly from 60.5% during 2006–2008 to 31.9% during 2018–2020, from 52.6% during 2009–2011 to 31.9% during 2018–2020, and from 59.6% during 2015–2017 to 31.9% in 2018–2020 (P=0.0001 for all three comparisons) and from 59.6% during period 2012–2014 to 31.9% during 2018–2020 (P=0.0008). Third-generation cephalosporins, cefotaxime, had an excellent activity against all isolates with resistance rates ranging from 0% to 3.4% during 2015–2017. Meropenem and ertapenem demonstrated excellent activities against all isolates with 0% resistance in the 15-year study period. Resistance to chloramphenicol was relatively high at 10.5% during the initial 6 years, then dropped by nearly one-third (3.4% to 3.9%) and finally spiked to 5.1% during the last 3 years of the study.

Resistance Rates for Different Years for Different *Salmonella* Groups Against Ampicillin

As shown in Figure 2, there was a significant drop in ampicillin resistance in *Salmonella* group B from 27.5% to 10.6% in 2006–2008 to 2009–2011 (P=0.03). But there was a significant rise in resistance for group D from 11.1% during 2009–2011 to 28.6% during 2015–2017 (P=0.02) followed by a significant drop from 28.6% for the period 2015–2017 to 11.4% in 2018–2020 (P=0.02).

Resistance Rates of Different *Salmonella* Groups Against Ciprofloxacin in Different Years

As shown in Figure 3, isolates belonging to *Salmonella* Group D demonstrated a significant rise of ciprofloxacin resistance from 27.6% during 2006–2008 to 67.1% during 2018–2020 (P=0.0004), from 17.5% (2009–2011) to 40.7% (2012–2014) (P=0.03), from 17.5% in 2009–2011 to 67.1% in 2018–2020 (P=0.01), and from 39.3% (2015–2017) to 67.1% (2018–2020) (P=0.002). There were no significant changes in resistance pattern for other groups.

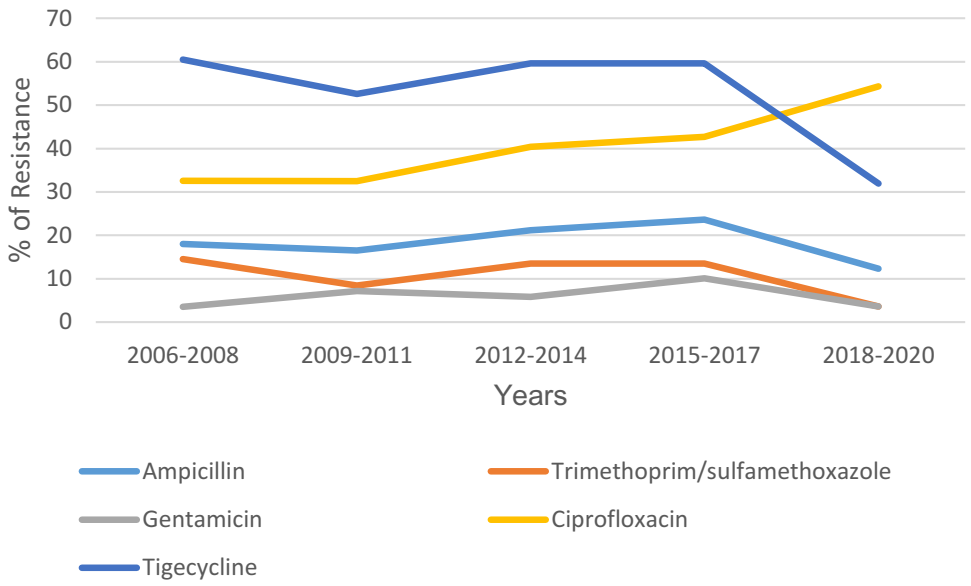

Figure 1 Resistance of all *Salmonella* groups during different years. Significant difference between periods for prevalence of resistance for antibiotics is as follows: 2015–2017 vs 2018–2020 for ampicillin (*P*=0.03); 2006–2008 vs 2018–2020 (*P*=0.002) and 2015–2017 vs 2018–2020 (*P*=0.02) for trimethoprim; 2006–2008 vs 2015–2018 (*P*=0.05) for gentamicin; 2006–2008 vs 2018–2020 (*P*=0.0001) and 2009–2011 vs 2018–2020 (*P*=0.0001) for ciprofloxacin; 2006–2008 vs 2018–2020 (*P*=0.0001), 2009–2011 vs 2018–2020 (*P*=0.0001), 2015–2017 vs 2018–2020 (*P*=0.0001), and 2012–2014 vs 2018–2020 (*P*=0.0008) for tigecycline.

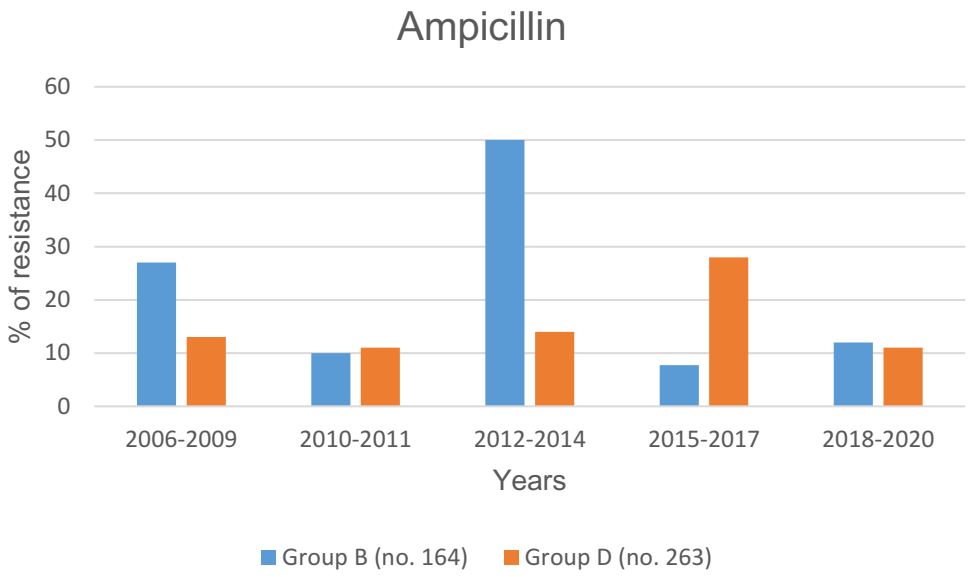

Figure 2 Resistance of *Salmonella* groups B and D for the five periods against ampicillin. Significant difference between periods for prevalence of resistance for ampicillin is as follows: 2006–2008 vs 2009–2011 (*P*=0.03) in *Salmonella* group B; and 2009–2011 vs 2015–2017 (*P*=0.02) and 2015–2017 vs 2018–2020 (*P*=0.02) in *Salmonella* group D.

Resistance Rates for Different Years for Different *Salmonella* Groups Against Tigecycline

As shown in Figure 4, there was a significant drop of resistance to tigecycline in *Salmonella* group B from 76.5% (2006–2008) to 56.1 (2009–2011) (*P*=0.031) and from 76.5% (2006–2008) to 41.7% (2018–2020) (*P*=0.005). Among *Salmonella* group C, the resistance rate to tigecycline dropped significantly from 77.5% (2006–2008) to 65.3% (2009–2011) (*P*=0.002) and from 65.3% (2009–2011) to 33.3% (2018–2020) (*P*=0.01). Among *Salmonella* group D, the resistance level increased significantly from 44.8% (2006–2008) to 47.6% (2009–2011) (*P*=0.04). However, the resistance level dropped

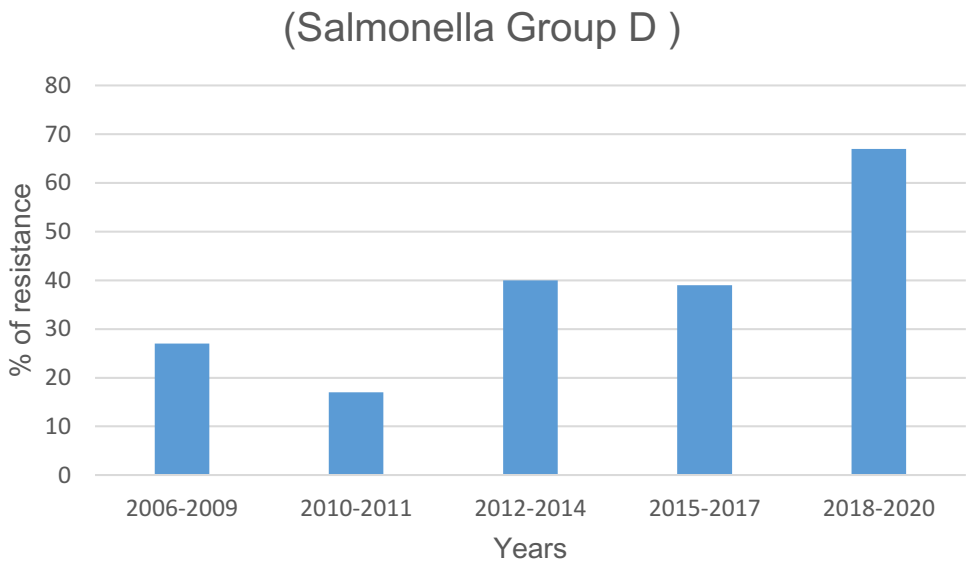

Figure 3 Resistance of *Salmonella* group D for the five periods against ciprofloxacin. Significant difference between periods for prevalence of resistance for ciprofloxacin is as follows: 2006–2008 vs 2018–2020 (*P*=0.0004); 2009–2011 vs 2012–2014 (*P*=0.03); 2009–2011 vs 2015–2017 (*P*=0.01); 2009–2011 vs 2018–2020 (*P*=0.0001); 2012–2014 vs 2015–2017 (*P*=0.02); and 2015–2017 vs 2018–2020 (*P*=0.0002).

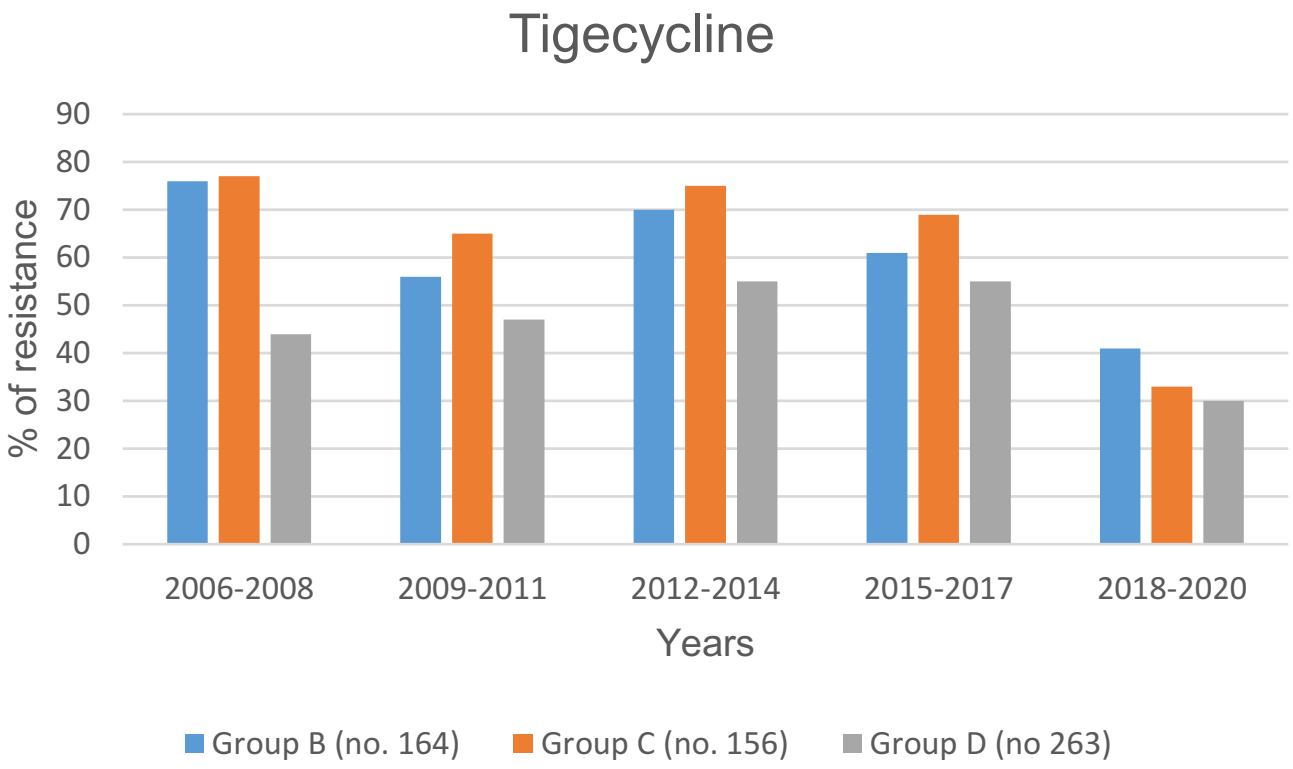

Figure 4 Resistance of *Salmonella* groups B, C and D for the five periods against tigecycline. Significant difference between periods for prevalence of resistance for tigecycline is as follows: 2006–2008 vs 2009–2011 (*P*=0.03) and 2006–2008 vs 2018–2020 (*P*=0.0005) in *Salmonella* group B; 2006–2009 vs 2018–2020 (*P*=0.002) and 2009–2011 vs 2018–2020 (*P*=0.01) in *Salmonella* group C; and 2006–2008 vs 2018–2020 (*P*=0.04), 2012–2014 and 2018–2020 (*P*=0.02) and 2015–2017 vs 2018–2020 (*P*=0.005) in *Salmonella* group D.

significantly from 55.6% (2012–2015) to 30.7% (2018–2020) (P=0.02) and from 55.4% (2015–2017) to 30.7% (2018–2020) (P = 0.0005).

Resistance Rates for Different Years for Different *Salmonella* Groups Against Chloramphenicol, Trimethoprim/Sulfamethoxazole, Gentamicin and Cefotaxime

There were no significant differences in the resistance rates for chloramphenicol, trimethoprim/sulfamethoxazole, gentamicin and cefotaxime in different years for the different salmonella groups (data not shown).

Multidrug Resistance Phenotypes

The resistance phenotypes of all 700 isolates are shown in Table 3. A total of 492 isolates (70.28%) were resistant to one or more antibiotics. One hundred and seventeen isolates (16.7%) were multidrug-resistant isolates (ie, resistant to 3 or more different classes of antibiotics). Fifty-one (7.2%), 48 (6.85%), 14 (2%), and 4 (0.57%) isolates were resistant to 3, 4, 5 and 6 antimicrobial agents, respectively. Insight into the origin of multi-resistant strains is given in Table S1. There were 27 patterns of multi-resistance. The most prevalent patterns were: ampicillin, ciprofloxacin, and tigecycline (16 isolates) > ampicillin, gentamicin, ciprofloxacin, and tigecycline (15 isolates) > chloramphenicol, ampicillin, and ciprofloxacin (11 isolates) > chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, and ciprofloxacin (10 isolates). The involvement of antibiotics in multi-resistant was tigecycline (22 isolates) > ampicillin and ciprofloxacin (20 isolates) > trimethoprim-sulfamethoxazole (15 isolates) > chloramphenicol (14 isolates) > gentamicin (13 isolates). Many multi-resistant isolates were spread across *Salmonella* groups B, C and D. Multi-resistance strains were present across all five periods.

Table 3 Resistance Phenotypes of All 700 *Salmonella* Isolates

Resistance Phenotypes	No. of Isolates
TGC	173
Cip	82
Cip, TGC	79
Amp, Cip, TGC	16
Amp, Gn, Cip, TGC	15
Chl, Amp, Cip	11
Chl, Amp, TS, Cip	10
Amp, TGC	9
TS, TGC	8
TS, Cip, TGC	8
Amp	7
Chl, Cip, TGC	6
Amp, TS, Cip, TGC	6
Amp, Cip	5
Amp, TS, TGC	5
Chl, Amp, TS, TGC	5
TS, Cip	4
Chl, Amp, Cip, TGC	4
Amp, TS, Gn, Cip, TGC	4
Amp, TS	3
Chl, Amp, TS, Gn, TGC	3
Chl, TS, Gn, Cip, TGC	3
Chl, Amp, TS, Gn, Cip, TGC	3
Chl, TS, TGC	2
Amp, Gn, Cip	2
Gn, Cip, TGC	2
Chl, Amp, CT, Cip	2
Amp, TS, Gn, TGC	2
Amp, CT, Cip, TGC	2
Chl, Amp, Gn, Cip, TGC	2
Chl	1
Chl, TGC	1
Amp, CTX	1
Amp, Gn, TGC	1
TS, Gn, Cip, TGC	1
Chl, TS, Gn, Cip	1
Chl, Amp, TS, Cip, TGC	1
Amp, Gn, CT, Cip, TGC	1
Chl, Amp, TS, Gn, CT, TGC	1

Abbreviations: Chl, chloramphenicol; Amp, ampicillin; Cip, ciprofloxacin; TGC, tigecycline; TS, trimethoprim/sulfamethoxazole; CTX, cefotaxime; Gn, gentamicin.

Discussion

Salmonella infections are an important public health issue all over the world. There is a geographic difference in distribution of *Salmonella* serogroups in children and adults in different countries including Kuwait. In a previous study from Kuwait, it was reported that serogroup B was more prevalent than serogroups C.[10] However, in this study, serogroup D was the most prevalent followed by serogroup B, a finding similar to previous reports from Saudi Arabia, Turkey, Taiwan, and Malawi.[15–19]

Our Salmonella isolates demonstrated a relative drop in the resistance to the first-line drugs during the 15-year period. For instance, resistance to chloramphenicol dropped from 10.5% to 5%, likewise resistance to ampicillin (18% to 12.3%) and trimethoprim/sulfamethoxazole (14.5% to 3.6%). This observation may be explained, in part, by the fact that there has been steady replacement of the conventional drugs by the quinolones and third-generation cephalosporins over time in our hospitals and

community. Our observation is concordant with a similar report from Europe where there was a decline in the occurrence of resistance to chloramphenicol from 14% to 8%.[20] However, unlike our finding of decline in resistance to the first-line drugs, reports from Saudi Arabia and Taiwan have actually demonstrated an increase in resistance to the first-line drugs.[15,18]

Resistance to the third-generation cephalosporin such as cefotaxime and ceftriaxone had been reported in *Salmonella* spp. since 1991.[21] This resistance appeared to be due to plasmid mediated AmpC or ESBL genes. In a study reported from Kuwait, CTX-M-15 with insertion sequence *ISEcpl* gene was identified in several *Salmonella* serotypes belonging to serogroups B and C.[22] In the current study, although cefotaxime resistance was detected in 0.4%, 3.6% and 3.4% during 2009–2011, 2015–2017, and 2018–2020, respectively, there were no significant differences in the resistance during these 3 periods. In the current study, resistance to cefotaxime was lower than previously reported in Kuwait, Saudi Arabia and Europe.[15,20,22] However, this is unlike a previous study from Taiwan, where the resistance to cefotaxime increased from <5% to >10% in serogroup B and *S. Choleraesuis* from 1999 to 2010.[23]

In our study, there was a gradual increase in ciprofloxacin resistance from 32.2% to 54.3% during the study period and there was a significant rise in resistance between periods of 2006–2008 vs 2018–2020, and 2009–2011 vs 2018–2020. This rise in the ciprofloxacin resistance is of concern as it is the drug of choice in Kuwait to treat invasive salmonella infections. Ciprofloxacin resistance is mediated by mutations in *gyrA* and *gyrB* genes that lead to mutations in the quinolone resistance determining region.[24] This probably explains our observation as there has been a persistent increase in the use and misuse of this drug in the healthcare centers. It is even mandatory to give people on pilgrimage to holy sites in Saudi Arabia a capsule of the antibiotic as a prophylaxis against communicable diseases. These measures exert undue pressure on the bacteria to develop resistance.

Carbapenem is the drug of choice for treating patients with MDR-salmonella infections when the third-generation cephalosporin and quinolones are ineffective. Although none of our isolate, in this study, was resistant to carbapenems, resistance to this group of antibiotics has been reported previously.[25] Should resistance to this class of drugs develop in the future, it would further complicate the treatment of invasive salmonella infections.

It was noted that tigecycline resistance dropped during the 15-year period from 60.5% to 31.9% with statistically significant differences among several periods. This is unlike the situation in Taiwan in 2008, where 1.6% of *Salmonella* serotype Typhimurium isolates, and 1.6% of *Salmonella* serotype Choleraesuis isolates were not susceptible to tigecycline using the EUCAST breakpoints.[26] The reason for this high resistance level among our isolates is probably due to the overuse of tigecycline at the onset of its introduction to the country, but whose use gradually tapered off with time and development of resistance among other species of the family Enterobacterales.[27]

Since the 1990s, the prevalence of multidrug-resistant *Salmonella* spp. has been on the increase worldwide, including Kuwait, the UK, and USA.[11,28,29] Our previous report in 2008 showed that 28 out of 287 isolates were MDR representing 9.8% of the total isolates. In this report, the MDR rate was 16.7%, nearly doubling over the previous rate.[11] This observation is discordant with the report from Turkey, Taiwan and Europe where the rate of MDR dropped over time.[16,18,20] Multi-resistant strains were present in several groups and in many periods. This is not surprising since the antibiotics tested were in use for treatment before we started the study.

Although the susceptibility testing of the isolates was prospective, the patient data collection was retrospective with its own limitations. Other limitations include: involvement of a single center, lack of access to clinical data including mortality and morbidity rates, and absence of investigation of molecular mechanisms of resistance in the resistant isolates.

Conclusion

There was a significant rise in ciprofloxacin resistance and a significant drop in ampicillin and trimethoprim-sulfamethoxazole resistances in *Salmonella* during the 15-year study period. Resistance to cefotaxime was low and steady, and multi-resistance was below 20%. Continuous surveillance of *Salmonella* and its antimicrobial resistance provided information on these changing trends. This type of study is required for formulation of antibiotic policy to treat serious invasive infections.

Abbreviations

MAK, Mubarak Al Kabeer Hospital; WHO, World Health Organization; CDC, Center for Disease Control and Prevention; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; TSI, triple sugar iron; MIC,

minimum inhibitory concentration; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

Disclosure

All authors declare no competing interests in this work.

References

1. Su LH, Chin C-H. Salmonella: clinical importance and evolution of nomenclature. *Chang Gung Med J*. 2007;30(3):210–219.
2. WHO. Salmonella (non-typhoidal); 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal). Accessed November 18, 2021.
3. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal Salmonella gastroenteritis. *Clin Infect Dis*. 2010;50(6):882–889. doi:10.1086/650733
4. CDC. *Antibiotic Resistance Threats in the United States, 2019*. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.
5. Gordon MA. Invasive non-typhoidal Salmonella disease- epidemiology, pathogenesis and diagnosis. *Curr Opin Infect Dis*. 2011;24(5):484–489. doi:10.1097/QCO.0b013e32834a9980
6. Gilchrist JJ, Maclennan CA. Invasive nontyphoidal *Salmonella* disease in Africa. *EcolSalPlus*. 2019;8(2):254.
7. Verma JK, Molbak K, Barrett TJ, et al. Antimicrobial-resistant nontyphoidal Salmonella is associated with excess bloodstream infections and hospitalizations. *J Infect Dis*. 2005;191(4):554–561. doi:10.1086/427263
8. Helms M, Vastrup P, Gerner-Smidt P, Molbak K. Excess mortality associated with antimicrobial drug-resistant *Salmonella typhimurium*. *Emerg Infect Dis*. 2002;8(5):490–495. doi:10.3201/eid0805.010267
9. Molbak K, Baggesen DL, Aarestrup FM, et al. An Outbreak of Multidrug-Resistant, Quinolone-Resistant Salmonella enterica Serotype Typhimurium DT104. *N Engl J Med*. 1999;341(19):1420–1425. doi:10.1056/NEJM199911043411902
10. Jamal WY, Pal T, Rotimi VO, Chugh TD. Serogroups and antimicrobial susceptibility of clinical isolates of *Salmonella* species from a teaching hospital in Kuwait. *J Diarrhoeal Dis Res*. 1998;16(3):180–186.
11. Rotimi VO, Jamal W, Pal T, Sonnevend A, Dimitrov TS, John Albert M. Emergence of multidrug-resistant *Salmonella* species and isolates with reduced susceptibility to ciprofloxacin in Kuwait and the United Arab Emirates. *Diag Microbiol Infect Dis*. 2008;60(1):71–77. doi:10.1016/j.diagmicrobio.2007.07.007
12. Thomson RB, Miller JM. Specimen collection, transport, and processing: bacteriology. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. *Manual of Clinical Microbiology*. Washington DC: ASM Press; 2003:286–330.
13. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. 30th ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2020.
14. EUCAST. Breakpoints tables for interpretation of MICs and zone diameters 2019. Available from: www.eucast.org. accessed December 18, 2019.
15. Elhadi N, Aljindan R, Aljeldah M. Prevalence of nontyphoidal *Salmonella* serogroups and their antimicrobial resistance patterns in a university teaching hospital in Eastern Province of Saudi Arabia. *Infect Drug Resist*. 2013;6:199–205. doi:10.2147/IDR.S51184
16. Ince OT, Yalcin SS, Yardakok K, et al. *Salmonella* gastroenteritis in children (clinical characteristics and antibiotic susceptibility): comparison of the years 1995–2001 and 2002–2008. *Turk J Pediatric*. 2012;54(5):465–473.
17. Chang Y-J, Chen Y-C, Chen N-W, et al. Changing antimicrobial resistance and epidemiology of non-typhoidal *Salmonella* infection in Taiwanese children. *Front Microbiol*. 2021;12:648008. doi:10.3389/fmicb.2021.648008
18. Lo H-Y, Lai F-P, Yang Y-J. Changes in epidemiology and antimicrobial susceptibility of nontyphoid *Salmonella* in children in southern Taiwan, 1997–2006. *J Microbiol Immunol Infect*. 2020;53(4):585–591. doi:10.1016/j.jmii.2018.06.004
19. Gordon MM, Graham SM, Walsh AL, et al. Epidemics of invasive *Salmonella* enterica serovar enteritidis and *S*. enterica serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis*. 2008;46(7):963–969. doi:10.1086/529146
20. Meakins S, Fisher IST, Berghold C, et al. Antimicrobial drug resistance in human nontyphoidal *Salmonella* isolates in Europe 2000–2004: a report from Enter-net International Surveillance network. *Microb Drug Resist*. 2008;14(1):31–35. doi:10.1089/mdr.2008.0777
21. Poupart M-C, Chanal C, Sirot D, Labia R, Sirot J. Identification of CTX-2, a novel cefotaximase from a *Salmonella mbandaka* isolate. *Antimicrob Agents Chemother*. 1991;35(7):1498–1500. doi:10.1128/AAC.35.7.1498
22. Rotimi VO, Jamal W, Pal T, Sovenned A, Albert MJ. Emergence of CTX-M-15 type extended–spectrum beta lactamase–producing Salmonella spp. in Kuwait and the United Arab Emirates. *J Med Microbiol*. 2008;57(Pt 7):881–886. doi:10.1099/jmm.0.47509-0
23. Su L-H, Teng W-S, Chen C-L, et al. Increasing ceftriaxone resistance in Salmonellae, Taiwan. *Emerg Infect Dis*. 2011;17(6):1086–1090. doi:10.3201/eid/1706.101949
24. Cloeckaert A, Chaslus-Dancla E. Mechanisms of quinolone resistance in Salmonella. *Vet Res*. 2001;32(3–4):291–300. doi:10.1051/vetres:2001105
25. Su LH, Wu TL, Chiu CH. Development of carbapenem resistance during therapy for non-typhoid Salmonella infection. *Clin Microbiol Infect*. 2012;18(4):E91–94. doi:10.1111/j.1469-0691.2012.03767.x
26. Liu C-Y, Huang Y-T, Liao C-H, Hsueh P-R. In vitro activities of tigecycline against clinical isolates of *Aeromonas, Vibrio, Salmonella* species in Taiwan. *Antimicrob Agents Chemother*. 2008;52(7):2677–2679. doi:10.1128/AAC.00002-08
27. Jamal WY, John Albert M, Rotimi VO. High prevalence of New Delhi metalo-β-lactamase-1 (NDM-1) producers among carbapenem-resistant *Enterobacteriaceae* in Kuwait. *PLoS One*. 2016;11(3):e0152638. doi:10.1371/journal.pone.0152638
28. Threlfall EJ, Ward LR, Skinner JA, Rowe B. Increase in multiple antibiotic resistance in nontyphoidal salmonellas from humans in England and Wales: a comparison of data for 1994 and 1996. *Microb Drug Resist*. 1997;3(3):263–266. doi:10.1089/mdr.1997.3.263
29. Hosek G, Leschinsky DD, Irons S, et al. Multidrug-resistant Salmonella serotype Typhimurium–United States, 1996. *Morb Mortal Wkly Rep*. 1997;46(14):308–310.