IJID Regions 11 (2024) 100368

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Clinical, phenotypic, and genotypic characteristics of ESBL-producing *Salmonella enterica* bloodstream infections from Qatar

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ARTICLE INFO

Keywords:
Salmonella
Typhoid fever
ESBL
Gram-negative bacteria (GNB)
Antimicrobial resistance

ABSTRACT

Background: Resistant *Salmonella* infections are a major global public health challenge particularly for multidrug-resistant (MDR) isolates manifesting as bloodstream infections (BSIs).
Objectives: To evaluate clinical, phenotypic, and genotypic characteristics of extended-spectrum beta-lactamase (ESBL) producing *Salmonella enterica* BSIs from Qatar.
Methods: Phenotypic ESBL *Salmonella enterica* from adult patients presenting with positive BSIs were collected between January 2019 to May 2020. Microbiological identification and characterization were performed using standard methods while genetic characteristics were examined through whole genome sequencing studies.
Results: Of 151 episodes of *Salmonella enterica* BSI, 15 (10%) phenotypic ESBL isolates were collected. Recent travel was recorded in most cases (80%) with recent exposure to antimicrobials (27%). High-level resistance to quinolines, aminoglycosides, and cephalosporins was recorded (80-100%) while meropenem, tigecycline and colistin demonstrated universal susceptibility. Genomic evaluation demonstrated dominance of serotype *Salmonella* Typhi sequence type 1 (93%) while antimicrobial resistance genes revealed dominance of aminoglycoside resistance (100%), *qnr*S1 quinolones resistance (80%), $bla_{\text{CTX-M-15}}$ ESBLs (86.7%), and paucity of AmpC resistance genes (6.7%).
Conclusions: Invasive MDR *Salmonella enterica* is mainly imported, connected to patients from high prevalent regions with recent travel and antimicrobial use caused by specific resistant clones. In suspected cases of multidrug resistance, carbapenem therapy is recommended.

Introduction

Throughout human history, infections caused by *Salmonella species* leading to gastroenteritis or typhoid fever are a major global public health concern with significant morbidity and mortality particularly in developing countries in Asia and Africa [1]. According to the World Health Organization (WHO), the estimated global burden of typhoidal disease is about 9 million cases annually with estimated annual mortality reaching 110,000 cases [2]. Since the mode of transmission of the disease is primarily through the consumption of contaminated food and drinks or contact with infected individuals, the burden of the disease is more prevalent in low- and middle-income countries when compared to developed ones adding to the global and regional healthcare constrains [3].

Salmonella species have almost 2500 different serotypes that infect humans and animals but only about 100 subtypes are associated with

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https://doi.org/10.1016/j.ijregi.2024.100368
Received 26 March 2024; Received in revised form 16 April 2024; Accepted 17 April 2024

human diseases. While non-typhoidal *salmonella* tends to cause gastroenteritis with infrequent associated invasive disease, typhoidal *salmonella* is more virulent and aggressive with frequent invasive forms manifesting as classical typhoid fever with associated bloodstream infections (BSIs). Furthermore, among typhoidal *Salmonella* disease spectrum, around 90% of cases are secondary to *Salmonella enterica* serotype typhi (previously *S.* Typhi) which only infects humans while *Salmonella enterica* serotype paratyphi (previously *S.* paratyphi) A, B, and C represents around 10% of the disease burden [3]. In the typhoid spectrum of infections, invasive disease leading to BSI represents a major threat since if left untreated it certainly leads to lethal consequences [1]. Since delaying of appropriate antimicrobials therapy is associated with significant, morbidity and mortality, these highlighted challenges necessitate choosing presumptive antimicrobial therapy ideally at the start of management to avoid subsequent treatment failure [4]. Historically, *Salmonella* infections were susceptible to a wide range of antimicrobials including, chloramphenicol, ampicillin, cotrimoxazole, and quinolones. Over the last decades, infections secondary to multidrug-resistant (MDR) *Salmonella* species emerged as a critical threat with mounting management challenges [5]. Trailing the history of antimicrobial resistance (AMR) for typhoid fever over the past 5 decades, during the 1970s-1980s, ampicillin and cotrimoxazole replaced the historic chloramphenicol but faced with mounting increased resistance while during the 1990s, quinolones became the *sine qua non* for the management of typhoidal diseases, particularly, they were associated with rapid resolution of symptoms and faster defervesce when compared to other therapeutic agents [6]. At the turn of the 21st century, there were growing and escalating resistance challenges to quinolones particularly in Asia when compared to Africa hence third-generation cephalosporins overtook quinolones as the presumptive management for suspected resistant cases. Over the last decade, MDR *Salmonella,* particularly extended-spectrum beta-lactamase (ESBL) phenotypic types that exhibit resistance to third-generation cephalosporins class, became increasingly reported from different global regions. Warningly, recent years witnessed reporting, of extensively drug-resistant isolates (XDR) that are resistant to a multitude of antimicrobials including advanced carbapenems [7]. The Gulf countries is a dynamic region of diverse populations from different countries particularly the Middle East region, Asia, and Africa with frequent travel to home countries which represent a hazard for imported infectious diseases and AMR. The current study aimed to explore the epidemiology, clinical, microbiological, and genomic characteristics of ESBL *Salmonella enterica* BSI in adults admitted to Hamad Medical Corporation in Qatar between 2018-2019.

Material and methods

The accredited central microbiology department at Hamad Medical Corporation (HMC) handles all clinical samples processed from 14 acute and specialized hospitals with a total capacity of almost 2500 beds. A prospective cohort study was conducted between 1 January 2019 and 1 May 2020 for all adult patients >14 years of age presenting with symptoms of typhoidal disease associated with non-repetitive invasive BSI that exhibit ESBL phenotypic patterns.

Study definitions

BSI was defined as at least one positive blood culture for *Salmonella enterica* from inpatients with clinical symptoms and signs of infection. In patients with persistent BSIs caused by the same organism, only the first episode was included. Empirical antibiotic therapy was defined as antimicrobials initiated before the detection of BSIs and reporting antibiotic susceptibility results while appropriate antimicrobial therapy was defined as suitable antimicrobials active against ESBL-producing *Salmonella enterica* with adequate dosage >48 hours from detection of BSI and within 5 days from disease onset.

Data collection

Following case identification, patients' hospital electronic records were reviewed including demographics, clinical presentation, and management and outcomes in addition to microbiological characteristics. Of 151 cases of *Salmonella* BSIs identified, 15 phenotypic ESBL-producing *Salmonella enterica* fulfilling inclusion criteria were identified and all were included for genetic analysis.

Microbiological Identification, antimicrobial susceptibility testing, and serological tests

Microbiological identification and Antimicrobial Susceptibility Tests (AST) were performed using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), Bruker Daltonics MALDI Biotyper (Billerica, MA, USA), and BD Phoenix system using the NMIC/ID-94 panel according to manufacturer's instructions. All isolates evaluated positive for ESBL by Phoenix or showed an minimum inhibitory concentration of >2 μg/mL for ceftriaxone and ceftazidime, were subsequently confirmed by a double-disk potentiation test with ceftriaxone and ceftazidime, with or without amoxicillin/clavulanic acid disks and interpreted accordingly. Following repeating AST through E-test, the interpretation of results was according to the Clinical and Laboratory Standards Institute cut-off at the time of testing [8].

Molecular and genomic evaluation through whole genome sequencing

Bacterial deoxyribonucleic acid (DNA) was extracted by the Wizard Genomic DNA Purification Kit (Promega, Madison, USA). The DNA libraries were prepared using NExtera XT kit (Illumina) and sequenced on the Illumina NextSeq 550 platform using 2 × 150 PE at SeqCenter (Pennsylvania, USA) [9]. The raw reads were assembled de novo using SPAdes v.3.9.0 [10] implemented in shovill (https://github.com/tseemann/shovill) [11]. Sequence types (ST), plasmid replicons and AMR genes were predicted from the assembled contigs using multi-locus sequence typing (MLST) (https://github.com/tseemann/mlst), Plasmidfinder v2.1 [12] and ResFinder v3.2 databases implemented in ABRicate v0.9 (https://github.com/tseemann/abricate), based on >70% coverage and 80% sequence identity. SeroSeq https://cge.food.dtu.dk/services/SeqSero/ was used to detect the serotype of the studied isolates. Resistance-associated mutations were predicted using AMRFinderPlus [13]. Relationship among isolates was constructed using Mash [14] and Parsnp (based on core genome SNPs and visualized together with associated AMR data using iTOL) [15].

Statistical analysis

Descriptive data were analyzed using Microsoft Excel software. Categorical variables were expressed as frequencies and percentages and continuous variables were expressed as medians.

Results

Over the study period, 151 episodes of *Salmonella* BSIs were recorded, 15 phenotypic ESBL were detected (10%) demonstrating male predominance (N = 10, 67%) and a median age of 20 years (range 15-36 years) as depicted in Table 1. History of recent travel to high-prevalence countries for MDR *Salmonella* was the commonest risk factor recorded in 12 cases (80%) followed by antimicrobial use within the last 3 months in four cases (27%). The most common associated symptoms were fever (100%) and gastrointestinal symptoms (N = 12, 80%). Bacteremia was cleared in almost one-third of the cases (N = 4, 27%) at a median of

Table 1
Demographic and clinical characteristics of patients diagnosed with extended-spectrum beta-lactamase-producing *Salmonella* species bloodstream infections isolated between 2019-2020.

Variable	Total (N = 15)
Age	Mean 20 (range 15-36)
Gender	M 67%; F 33%
Nationality by WHO region of origin	
Eastern Mediterranean Region	93%
African Region	7%
Pitt Bacteremia Score	1 (1)
Risk factors for Salmonella BSI acquisitions	
History of travel to risk countries	80%
Renal replacement therapy	0
Antibiotic with in the last 3 months	27%
Immunosuppression	13%
Fever	100%
Gastrointestinal symptoms	80%
Outcomes	
Clearance of bacteremia	27%
Blood cultures not repeated	20%
Clearance of bacteremia (days)	7 (2.75)
Recurrence of bacteremia	0
30-day mortality by day 30	0

BSI, bloodstream infections; WHO, World Health Organization.

Table 2
Antimicrobial susceptibility tests for extended-spectrum beta-lactamase producing-*Salmonella* species isolated from bloodstream infections.

Antimicrobials	% susceptibility
CST	100
FOS	100
ERT	100
MER	100
TIG	100
DOX	87
LVX	27
CIP	20
TZP	0
AMK/GEN	0

AMK, amikacin; CIP, ciprofloxacin; CST, colistin; DOX, doxycycline; ERT, ertapenem; FOS, fosfomycin; GEN, gentamycin; LVX, levofloxacin; MEM, meropenem; TIG, tigecycline; TZP, piperacillin-tazobactam.

7 days (range 1-15 days) with no recorded 30 days mortality as outlined in Table 1. Of the 15 phenotypic ESBL *Salmonella species*, microbiological characteristics demonstrated universal antimicrobial susceptibility to meropenem, ertapenem, colistin, tigecycline, and fosfomycin at 100%. Generally, there was a low level of antimicrobial activity for quinolones at 27% and 20% for levofloxacin and ciprofloxacin, respectively. In contrast, there was utmost AMR to broad-spectrum βlactam βlactamase inhibitors (BLBLIs) represented by piperacillin-tazobactam, aminoglycosides such as amikacin and gentamycin with susceptibility rates of 0% as outlined in Table 2.

Serotypes and resistance gene distribution

Among the 15 phenotypic ESBL isolates, *Salmonella* Typhi sequence type 1 (ST1) is the predominant identified serotype (93.3%, 14/15), together with a single isolate of *Salmonella enterica* serovar Typhimurium ST10 as outlined in Table 3. All 14 ST1 isolates belonged to serovar typhi, and their genetic distance was small (<10 cgSNPs). The remaining isolate (S4) belonged to serovar Dublin as outlined in Figure 1. Evaluating the genomic diversity of antibiotics resistance genes (ARGs), encoding narrow-spectrum β-lactamase were represented by $bla_{\text{-TEM}}$, identified in 86.7% of cases, while bla_{CTX} variants were equally detected with a clear predominance of the ESBL $bla_{\text{CTX-M-15}}$. Only one isolate (S4) harbored AmpC β-lactamases represented $bla_{\text{CMY-2}}$ (6.6%) as outlined in

Table 3
Sequence types and antibiotics resistance genes of extended-spectrum beta-lactamase-producing *Salmonella* species isolated from bloodstream infections.

MLST		N	Frequency%
ST 1		14	93.3%
ST10		1	6.7%
ARG genes	**Drug class**		
$bla_{\text{CTX-M-15}}$	Extended-spectrum beta-lactamase	13	86.7%
$bla_{\text{TEM-1B}}$	Narrow-spectrum beta -lactamases	13	86.7%
$bla_{\text{CMY-2}}$	AmpC beta-lactamases	1	6.7%
*qnr*S1	Quinolone	12	80%
aph(6)-Id	Aminoglycoside	14	93.3%
aph(3″)-Ib	Aminoglycoside	14	93.3%
aac(6′)-Iaa	Aminoglycoside	15	100%
*dfr*A7	Trimethoprim	13	86.7%
*sul*1	Sulfonamide	13	86.7%
sul2	Sulfonamide	14	93.3%
*flo*R	Phenicol	1	6.7%

ARG, antibiotics resistance genes; MLST, Multi-locus sequence typing; ST, sequence typing.

Table 3 and Figure 1. Examining other related ARGs, aminoglycoside resistance was detected in all isolates with a predominance of *aac* (6′)-Iaa (, 100%) and aph (6)-Id (93.3%), while quinolone resistance genes qnrS1 was identified in 12 isolates (80%). Additionally, trimethoprim resistance gene *dfr*A7 was identified in 86.7% of isolates while sulphonimide *sul*1 and *sul*2 were detected in 93.3% (14/15). A point mutation conferring resistance to quinolones was detected in *gyrA* [S83F] in all ST1 isolates as outlined in Table 3 and Figure 1.

Discussion

Salmonella infection (Salmonellosis) is a global foodborne enteric disease that can be traced from ancient times of different human civilizations to the present with a significant burden, particularly for developing countries [1]. The spectrum of disease ranges from self-limiting gastroenteritis caused by non-typhoidal *Salmonella* to the more serious enteric fever caused by the distinct typhoidal *Salmonella*. Typhoidal *Salmonella* is mainly caused by *Salmonella enterica* serotype typhi which tends to cause more profound and predominant disease in the majority of cases while *Salmonella enterica* serotype para-typhi (*Salmonella paratyphi A, B, and C*) accounts for less serious presentation in the minority of cases [6]. In the susceptible population, besides the clear manifestation as an invasive form of the disease, *Salmonella* BSI can also seed into many body organs leading to distant diseases causing bones, joints, and endovascular infections with serious consequences [16]. Therefore, prompt recognition and initiation of appropriate management is vital to prevent distant complications. To complicate clinical presentation, over the past years, there have been rising trends of AMR in Gram-negative bacteria (GNB) including *Salmonella species* which limits presumptive and tailored therapeutic options [7]. As a consequence, the global threat of MDR *Salmonella* infection represents a massive challenge because of the scale of the affected population in endemic areas together with the substantial cost of management [17].

One of the striking features of the study is the significant scale of the problem since in almost 10% of the screened cohort of 151 episodes, phenotypic ESBL was identified with significant AMR spanning broad-spectrum antimicrobials such as third-generation cephalosporins, advanced BLBLIs such as piperacillin-tazobactam and quinolones as outlined in Table 2. These broad-spectrum antimicrobials are usually initiated in serious GNB BSI which prompts a cautious approach in case of suspected invasive MDR *Salmonella* infection. Of note, from the review of the clinical presentation of resistant cases, recent travel to drug-resistant endemic areas and the recent use of previous antimicrobial therapy are recognized risk factors for MDR disease, outlined in Table 1. These two prominent risk factors are associated with imported infections secondary to MDR *Salmonella* infection which prompted the specific rec-

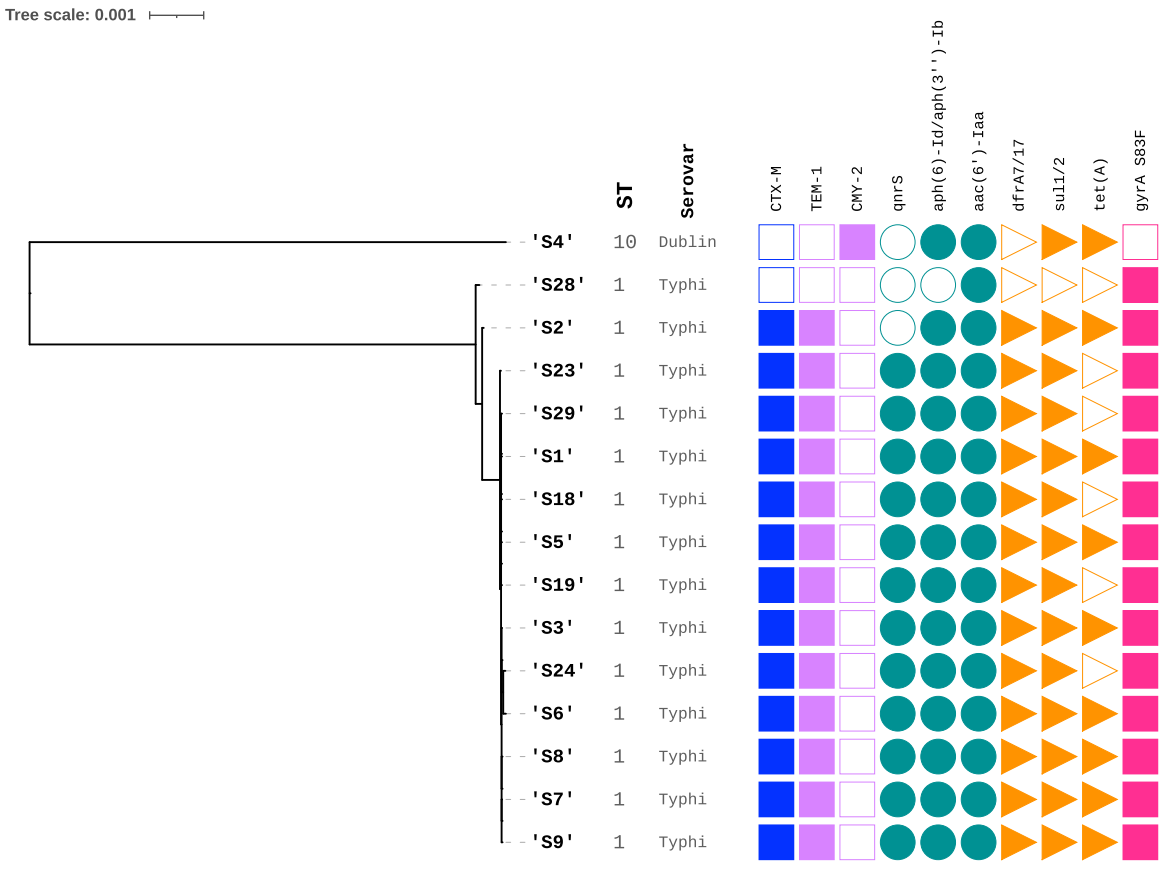

Figure 1. Phylogenetic tree of 15 extended-spectrum beta-lactamase *Salmonella* species bloodstream infection with color-shaded antibiotic resistance genes.

ommendations for the initiation of targeted antimicrobial therapy for imported infections to overcome potential resistance [18]. Accordingly, invasive *Salmonella* BSIs associated with noticeable AMR are certainly regional being specifically common in South Asia when compared to the rest of the world [19].

While studying the underlying serotypes associated with MDR profiles, there is a clear predominance of ST1 in 93.33% of cases, outlined in Table 3, which has been associated with endemic and resistant diseases linked to the Indian subcontinent. The fact that most of our cohort was affected by this pandemic-resistant clone raises the focused need for developing targeted vaccinations to face resistant clones [20]. Correlating with the observation that the majority were young males from the Indian subcontinent with a history of recent travel. Additionally, the close relatedness of 14 isolates from the constructed phylogenetic tree might suggest a common source or outbreak situation although these cases were sporadic throughout the year affecting unrelated patients as outlined in Figure 1. Conversely, the MDR clones exhibit a multitude of closely related ARGs that affect penicillin, tetracycline, cotrimoxazole, quinolone, and aminoglycosides antimicrobial classes. Detailed genomic characterization of our ESBL *Salmonella* isolates revealed a predominance of $bla_{\text{CXM-15}}$ ARGs. These well-established groups of ARGs are at the forefront of the underlying mechanism of ESBL resistance in *Enterobacterales* including *Salmonella species* both globally and regionally [21,22]. The emergence of these notorious ESBL genes since the 1990s overshadowed other historic narrow-spectrum β-lactamases such as bla_{TEM} which has been observed in almost 90% of the collection [21,23]. These genes are responsible for the displayed phenotypic patterns including resistance to penicillin, cephalosporins, and BLBLIs. Since ESBLs are not capable of hydrolyzing carbapenems such as meropenem, imipenem, and ertapenem, therefore, they are the recommended therapeutic options, particularly for invasive ESBL disease including invasive MDR *Salmonella* BSIs. Consequently, the current recommendation for the treatment of serious and invasive MDR GNB including ESBL such as BSI, is to administer carbapenem specifically meropenem for critical disease and only downgrade when clinical and microbiological parameters are favorable [24]. Other agents that displayed universal *in vitro* activity against ESBL *Salmonella* infection include tigecycline, colistin, and fosfomycin, outlined in Table 2. Despite the universal susceptibility for tigecycline, from the pharmacodynamic properties of the agent, the fact that it achieves low bloodstream levels with high tissue volume of distribution makes it unsuitable for serious invasive BSI if there are other available effective alternatives. Such observation can be extrapolated to similar novel agents from the same class such as eravacycline and omadacycline that demonstrated *in vitro* activity against ESBL GNB [25]. Furthermore, despite fosfomycin having a reliable record in the treatment of ESBL-related urinary tract infections, its application in MDR GNB invasive diseases such as BSIs is limited albeit been used successfully as part of combination therapy for the treatment of MDR *Salmonella*. Randomized clinical trials showed it is inferior to comparators while its evaluation in critically ill patients with MDR GNB infection showed it is safe and effective but only as a combination therapy with other effective agents. Additionally, colistin is usually reserved as the last resort for MDR GNB that exhibit advanced resistance, but it is well known to correlate with significant adverse events such as acute kidney injury and challenges in achieving effective therapeutic levels which limited its wider previous use [24]. Of note, it is worth highlighting the interface between colistin with global *Salmonella* infection since it is one of the leading antimicrobials in veterinary and husbandry medicine which has an impact on animal-human shared pathogens that can be passed upon the animal-human food chain. From multiple studies, colistin-associated resistance genes such as the described *mcr*-like genes have been linked to enteropathogenic *Salmonella* which carries risks of propagation of AMR

to humans [26,27]. This might be related to our study since one of the identified resistant isolates (*Salmonella enterica serovar* Typhimurium) is a crossover pathogen from animals to humans [28].

Among the identified ARGs, aminoglycoside-resistant genes were the most identified among all isolates in agreement with the displayed phenotypic pattern of utmost resistance to amikacin and gentamycin (Table 2). Because of the poor activity of the class against *Salmonella* infections, they are not recommended for disease control and management including warnings from the WHO [6]. Similarly, isolates carried the plasmid-mediated quinolone resistance genes represented by *qnr*S1 which is inhibitory to quinolones topoisomerase group of integral cellular enzymes leading to the observed phenotypic resistance (20% susceptibility to ciprofloxacin and 27% for levofloxacin). The disparity between the two closely related agents can be explained since despite embedded genotypic patterns, different quinolones from the same class might display different phenotypic patterns which was examined more closely in related Enterobacterales such as *E.coli* [29]. Intriguingly, despite the most widely described mechanism of resistance in MDR GNB are ESBL and AmpC group of βlactamases, among the collection there was paucity of AmpC ARG (only one $bla_{\text{CMY-2}}$) identified in *Salmonella enterica* serovar Typhimurium (Dublin S10) and was completely absent in *Salmonella enterica* serovar typhi (S1) Table 3. *Salmonella enterica* serovar Typhimurium is cattle-adapted salmonella that can cause both gastrointestinal and enteric human diseases with well-recognized records of AMR. Previous research affirms the presence but paucity of AmpC ARGs isolated from *Salmonella species* while the carbapenem mechanism of resistance is thought to be mediated through mutations of the porin channels rather than through the production of carbapenemases [30]. Importantly, there was a complete absence of identifiable carbapenem resistance both phenotypically and genotypically, which is a significant disease-surveillance benchmark for the country.

Despite the study presenting some interesting results, it has some limitations. Only 10% (15) of the identified collection were closely examined in detail. If the total cohort was similarly examined some observed clinical information such as travel, or use of antimicrobials might be more reliably related. Additionally, we did not test the MDR *Salmonella* specifically for azithromycin, one of the recommended antimicrobials to overcome the growing resistance. Lastly, genomic evaluation demonstrated close relatedness of 14 of 15 isolates which merits more investigation for source(s) identification which was not feasible because of time lag. Nevertheless, the study improved our local understanding of the microbiological and genetic characteristics of the important disease which can be extrapolated to other nearby regions.

Conclusions

Over almost 15 months of the study period, we recorded 151 episodes of *Salmonella enterica* BSI with 10% phenotypic ESBL prevalence that belongs to *Salmonella enterica serotype* typhi *sequence type 1*, primarily linked to travel to endemic areas with significant AMR displaying ARGs for all the main antimicrobials classes. In our settings, carbapenems remain the advisable presumptive therapy for suspected typhoidal ESBL resistance since they remain universally active with the therapeutic limitations of other agents.

Declaration of competing interest

All authors have no conflicts of interest in relation to this academic research and publication.

Funding

The research study was funded by the Medical Research Centre (MRC) of Hamad Medical Corporation (Ref: MRC 01/20/379).

Data management and Ethical considerations

The study and collaboration were approved by the Medical Research Centre (MRC) of Hamad Medical Corporation which abides by local and international research standards. The study also received ethical approval from the Institution Review Board of HMC - after demonstrating needed standards of data management and sharing including limited access to nominated primary investigators, data anonymity, and governance. For academic interest, raw sequencing reads are available from the National Center for Biotechnology Information under the accession number PRJNA990977.

Authors contribution and statement

WG, MSA and HAH conceived the idea of the research projects. CKT interpret the genomic data including detailed methodology. GA, MSN, KS collected and analyzed clinical data while EI led microbiological part including expert advice, AS and SS supervised and coordinated all genomic tests. WG, CKT and HAH prepared the original manuscript which was edited after feedback from all authors. All authors reviewed the final draft and agreed on published content.

Acknowledgment

We would like to express our gratitude to all involved staff, specifically to Mr. Jemal A. Hamid from the Department of Laboratory Medicine and Pathology at Hamad Medical Corporation and the Department of Microbiology and Immunology at Weill Cornell Medicine, Qatar for processing specimens and performing needed technical tests. The publication of academic research has been facilitated through collaboration with the Medical Research Centre of HMC. Additionally, in some parts, computational work was supported through resources (Sockeye) and services provided by Advanced Research Computing at the University of British Columbia, Canada.

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