Original Article

Pattern of increased antimicrobial resistance of *Salmonella* isolates in the Eastern Province of KSA

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Abstract

**Objectives:** The antimicrobial resistance of *Salmonella* species is increasing worldwide. This study was conducted to determine the pattern of antimicrobial susceptibility of *Salmonella* species in a tertiary hospital from 2011 to 2018.

**Methods:** In this retrospective study, the medical records of all patients with *Salmonella* infections were reviewed. The clinical, demographic, and microbiological data of the selected patients were analysed.

**Results:** A total of 752 patients were included. The resistance of *Salmonella* species to antimicrobial drugs increased from 24.6% in 2011 to 37.8% in 2018 (*p* = 0.002). By 2018 all *Salmonella* isolates were completely resistant to cefalotin, cefuroxime, and cefoxitin, while we found some susceptibility to other cephalosporins and ciprofloxacin. The most commonly isolated *Salmonella* serogroups were groups D (36.5%), C (23.5%), and B (11.7%).

**Conclusions:** The incidence of resistance of *Salmonella* to antibiotics is on the rise. The results of this study highlight the need for an active monitoring system of antibiotic usage in humans and domestic animals.

**Keywords:** Antimicrobial resistance; KSA; Non-typhoidal *Salmonella*; *Salmonella*; Typhoid

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Introduction

Salmonella infections are a major public health concern worldwide and have led to a significant number of morbidity and mortalities, particularly in developing countries.1 Gastroenteritis is the most common clinical presentation of Salmonella infection, which can be caused by more than 150 Salmonella serotypes, collectively called non-typhoidal Salmonella (NTS).2 Salmonella typhi and Salmonella paratyphi (typhoidal Salmonella) cause typhoid fever and its milder form, paratyphoid fever, respectively.3 A more complex form of Salmonella infection is the invasive salmonellosis which develops when Salmonella crosses the intestinal mucosa to the blood stream and infects deep organs such as the bones, joints, and meninges.4

Salmonella is a food borne pathogen transmitted to humans through components of the food chain, including ingestion of contaminated poultry, eggs, dairy products, as well as fresh vegetables.5 Salmonella typhi and paratyphi are human-restricted pathogens and can only be transmitted between humans; therefore, the incidence of typhoid fever is lower than non-typhoidal gastroenteritis.6 The World Health Organisation (WHO) estimates the global incidence of typhoidal Salmonella to be 11 to 21 million cases annually, and this leads to approximately 128,000–161,000 deaths.7 In contrast, NTS causes about 1.3 billion illnesses with 3 million deaths annually.8

The incidence of Salmonella infection is rising worldwide due to increased industrialisation of the food supply and globalisation of processed and ready to eat food. Australia reported an approximate two-fold increase in the rate of enteric fever between 1985 and 2010.9 The European Union reported an increasing trend of human salmonellosis since 2013, which was attributed to the rise in Salmonella isolates in egg laying hens by 57% between 2013 and 2016.9 The rising number of salmonellosis cases globally is supported by the acquisition of antimicrobial drug resistance by Salmonella. Drug resistance is most likely due to uncontrolled use and easy accessibility of antibiotics in several countries throughout the world.10,11 Drug resistant Salmonella, including those resistant to clinically significant antibiotics such as ciprofloxacin and ceftriaxone, were responsible for about one tenth of all Salmonella cases in the United States between 2009 and 2011.12 Emergence of drug resistant Salmonella and its clinical significance has been reported from Africa, Asia, and Europe.9,10 Further to the aggravation of the problem, antibiotic resistant clones of Salmonella became more associated with outbreaks leading to more treatment failures and a higher hospitalisation rate.12 Therefore, it is imperative that we pay more attention to the antibiogram of isolated Salmonella strains and follow their resistance profiles over time.

The overall prevalence of NTS in the Middle East and North Africa is about 6.6%. The highest prevalence has been recorded in Morocco and Tunisia and the lowest has been reported in Jordan and Oman.13 Countries located in the Arabian Peninsula, such as Kuwait and Qatar, have reported a high prevalence of fluoroquinolone-resistant Salmonella spp (37.5% and 10% respectively).14 In KSA Salmonella spp have been isolated from environmental samples, meat, and fish.15–18 The prevalence of NTS in the human population in KSA has been reported to be 9 per 1000 acute diarrheal cases.19

Bearing in mind the presence of salmonella in the environment and meat earmarked for human consumption in KSA, we sought, in the current study, to investigate the pattern of Salmonella antimicrobial resistance between 2011 and 2018 at a tertiary hospital in the Eastern Province of KSA.

Materials and Methods

Study type and design

A retrospective study was performed to collect clinical and demographic data for all patients with isolated Salmonella spp from the Medical record system at King Fahd Hospital of the University (KFHU) between August 2011 and October 2018. The total number of Salmonella cases was 752. The demographic data pertaining to each case were collected from electronic patient files, and the microbiological data were collected from the Vitek system. The medical records were retrieved using the search word “Salmonella”. Cases were included in the study if Salmonella spp were isolated from microbial culture of any sample from the patient. Duplicate samples were excluded.

Microbial identification and antimicrobial susceptibility

Stool samples were routinely cultured on MacConkey agar, xylose lysine deoxycholate agar, and Hektoen agar (Oxoid Ltd, Basingstoke, UK). Stool samples were also inoculated into selenite F broth (Oxoid Ltd, Basingstoke, UK). Blood samples were collected in blood culture bottles and incubated in the BD BACTEC™ blood culture system (Becton, Dickinson and Company, Franklin Lakes, USA). A blood culture sample flagged as positive by the system was inoculated on blood agar, chocolate agar, and MacConkey agar. Identification of microbes from urine samples was performed by plating the sample onto MacConkey agar and cystine lactose electrolyte deficient (CLED) agar (Oxoid Ltd, Basingstoke, UK). Samples, other than stool or blood, e.g. urine, abdominal abscess, and surgical wound swabs, were cultivated as per standard operating procedures followed at the microbiology laboratory at KFHU.

Identification and antimicrobial susceptibility testing of all bacterial isolates was performed by the Vitek2 automated card system (bioMérieux Vitek Inc., Hazelwood, MO, USA). When required, an antimicrobial susceptibility test was performed manually using the disc diffusion
method following the Clinical and Laboratory Standards Institute guidelines.\textsuperscript{20} \textit{Salmonella} serogrouping was performed using the BD Salmonella antisera (Becton, Dickinson and Company, Franklin Lakes, USA).

\textbf{Statistical analysis}

SPSS software version 25 was used to calculate frequencies and to design the bivariate tables. The Kruskal–Wallis test in SPSS was used to determine the difference in distribution between the serogroups over time. Chi square for linear trend was calculated using Epi Info Statcalc software. Statistically significant P values were recognised when less than 0.05.

\textbf{Results}

There was an increase in the frequency of isolation of \textit{Salmonella} from the year 2015 compared to previous years (Figure 1). Most \textit{Salmonella} spp were isolated from stool samples (88.6%), followed by blood (7.2%), urine (1.2%), and other samples including abdominal abscesses, and surgical wounds after abdominal surgery (2.5%); 62.1% of the \textit{Salmonella} spp. were isolated from Saudi patients, the remainder were from people of different nationalities.

![Figure 1: Frequency of isolation of \textit{Salmonella} over the study period. Values are presented as percentages.](image-url)

The most prevalent \textit{Salmonella} serogroup was group D (36.5%), followed by groups C (21.5%), B (11.7%), and E (2.8%) (Table 1). \textit{Salmonella} group \textit{typhi} was isolated from seven cases while \textit{Salmonella paratyphi} was isolated from only one isolate (Table 1).

The total resistance of \textit{Salmonella} to all antibiotics rose significantly from 26.4% in 2011 to 37.8% in 2018 (P = 0.002) (Figure 2).

Resistance of \textit{Salmonella} isolates to ampicillin and amoxicillin was 23% and 22% respectively, while resistance to ampicillin/sulbactam and to amoxicillin/clavulanic acid was 16% and 8% respectively. There was no significant difference in the rate of \textit{Salmonella} resistance to this class of antibiotics over time, except for amoxicillin/clavulanic acid (P = 0.001) (Figure 3a).

There was a significant increase in the rate of resistance of \textit{Salmonella} to the first and second generation cephalosporins; cefalotin, cefuroxime and cefoxitin (P < 0.001, <0.001, and 0.002 respectively) but not to other members of the cephalosporins (Figure 3b).

There was also a marked increase in resistance of \textit{Salmonella} isolates to levofloxacin (P = 0.001) while \textit{Salmonella} resistance to ciprofloxacin was around 50% throughout the study period (Figure 3c).

![Figure 2: Increase in \textit{Salmonella} antimicrobial resistance to all tested antibiotics between 2011 and 2018.](image-url)

\begin{table}[h]
\centering
\caption{Frequency of different \textit{Salmonella} groups isolated from patients in KSA from 2011 to 2018.}
\begin{tabular}{lcccccccccc}
\hline
Year & \multicolumn{2}{c}{2011} & \multicolumn{2}{c}{2012} & \multicolumn{2}{c}{2013} & \multicolumn{2}{c}{2014} & \multicolumn{2}{c}{2015} & \multicolumn{2}{c}{2016} & \multicolumn{2}{c}{2017} & \multicolumn{2}{c}{2018} & \multicolumn{2}{c}{Total} \\
\hline
 & % & % & % & % & % & % & % & % & % & % & % & % & % & % & n & % \\
S. enterica arizona & 0 & 2.2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0.1 \\
S. group B & 8.7 & 8.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.1 \\
S. group C & 16.5 & 6.7 & 19.1 & 25.0 & 26.4 & 21.9 & 27.7 & 9.4 & 88 & 11.7 \\
S. groups E/F/H/I/L/M/O/W & 17.3 & 8.8 & 0 & 0 & 4.3 & 1.9 & 4.4 & 4.7 & 1 & 0.1 \\
S. paratyphi A & 0 & 2.2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 8 & 7 & 9 \\
S. Typhi & 0 & 2.2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.8 & 7 & 9 \\
S. species\textsuperscript{a} & 13.0 & 17.8 & 54.4 & 43.8 & 14.5 & 27.7 & 16.4 & 30.1 & 186 & 24.7 \\
\hline
\end{tabular}
\footnotesize{\textsuperscript{a} \textit{Salmonella} species other than typhi and paratyphi. Serogrouping data is not available.}
\end{table}
In addition to the contamination of animal products, *Salmonella* can also be transmitted to humans and cause outbreaks through other food sources such as vegetables and fruit, which can be contaminated with antibiotic resistant *Salmonella* through fecal excretion of livestock. In several studies, authors have reported the frequent isolation of *Salmonella* from meat, fish, environmental samples, and farm animals from KSA. An early study found that 45% of meat at butcheries contained *Salmonella* species.

In a study from Riyadh, *Salmonella* was isolated from the faeces of cattle, goats, camels, and sheep at percentages ranging from 11% to 23%, while the hide of these animals had markedly higher percentages of *Salmonella* ranging from 51% to 80%. Furthermore, *Salmonella* was isolated from about 40% of fresh water fish at retail markets in KSA with high resistance to tetracycline, ampicillin, and amoxicillin/clavulanic acid. All of these sources could constitute significant reservoirs for transmission of *Salmonella* to humans.

The overall resistance of *Salmonella* to antimicrobials has increased significantly over the past eight years. This trend has been reported previously in other studies from KSA and other countries worldwide. Increasing resistance to antimicrobial agents complicates therapy options in salmonellosis and the upsurge in resistance is increasingly attributed to the misuse and overuse of antibiotic prophylaxis in animal feed.

Over the past eight years, *Salmonella* exhibited variable resistance patterns to antibiotic classes and to individual members of these classes. Penicillin and penicillin with β-lactam inhibitors, still exhibit good activity against *Salmonella*. However, *Salmonella*, overtime, has developed complete resistance to half of the tested cephalosporins, including cefalotin, cefoxitin, and ceftaxime. This, in particular, is a serious medical concern as cephalosporins are the drug of choice for the treatment of NTS in children.

Extended spectrum β-lactamases (ESBLs) are a common cause of resistance to this class of antibiotic. However, they were not frequently reported in *Salmonella* species from KSA. One study reported the detection of TEM, CTX-M and OXA-1 genes from NTS isolated from stool and blood samples. A study conducted recently in Riyadh found that most *Salmonella* isolates from clinical and environmental samples were resistant to first and second generation of cephalosporins and aminoglycosides.

Contrastingly, quinolones are the drug of choice for the treatment of invasive salmonellosis in adults. *Salmonella* in our study showed complete resistance to levofloxacin and a high resistance rate to ciprofloxacin. Accumulation of mutations in the quinolone resistance-determining regions (QRDRs) such as the genes encoding gyrase and topoisomerase, are common mechanism of quinolone resistance. About one quarter of the ciprofloxacin sensitive isolates were resistant to nalidixic acid (data not shown) indicating the presence of the Ser83 mutation in the gyrase A gene, which might lead to therapeutic failure. Plasmid-mediated quinolone resistance (PMQR) genes are also implicated in ciprofloxacin resistance in addition to QRDRs. A recent study reported the detection of PMQR genes (*qnrB* and *qnrS*) for the first time from KSA. Fluoroquinolone-resistant *Salmonella* species were reported at variable degrees from the Arabian League. High rates of fluoroquinolone resistance

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**Figure 3:** Antibiotic susceptibility pattern of *Salmonella* spp to different classes of antibiotics between 2011 and 2018. A) Penicillins (P = 0.3 for ampicillin, amoxicillin, and ampicillin/subbactam; P = 0.001 for amoxicillin/clavulanic acid), B) Cephalosporins (P < 0.001 for cefalotin and ceftaxime, 0.8 for ceftriaxone, 0.002 for cefoxitin, 0.4 for cefazidime, and 0.09 for cefepime), C) Quinolones (P = 0.3 for ciprofloxacin and 0.001 for levofloxacin).

**Discussion**

Our data showed an approximately three-fold increase in frequency of *Salmonella* isolation from 2015 onward. This transition in frequency coincides with the total increase in antibiotic resistance and the emergence of cephalosporin and quinolone resistance. Antibiotic resistant *Salmonella* were responsible for a considerable number of cases reported in the United States (US) between 2009 and 2011. *Salmonella* is transmitted to humans through food from animals, and this constitutes a reservoir for the pathogen. *Salmonella* are known to cause a high number of foodborne illnesses as well as hospitalisation. The European Union has reported an increasing trend of human salmonellosis since 2013, which has been attributed to a rise in *Salmonella* isolates in egg laying hens by up to 57% from 2013 to 2016. In KSA the consumption of meat and fish consumption, particularly chicken, has risen since 1985, and this is partly due to affordable pricing. In addition, the global industrialisation of food production and food supply, as well as the increase in popularity of ready to eat meals, has accompanied the rise in *Salmonella* infections.
(higher than 30%) were reported from Iraq, United Arab Emirates, Morocco, and Libya, while countries such as Qatar, Egypt, Tunisia, and Algeria reported a resistance rate lower than 20%.14

The most common Salmonella serogroup in our study was group D, followed by groups C and B. This is consistent with the current prevalence reported from other local and international studies.19,35–38 Previous studies from the same region showed the predominance of the serogroup C.27,39 Most of the isolates in our study belong to the serovar enteritidis, which is mostly associated with broiler and layer flocks, leading to infections associated with consumption of poultry and its products, including eggs.40,41 This observation is supported by the pattern of poultry consumption in KSA.42

Limitations of the study

One of the limitations of the study is that it consists of data from Salmonella typhi together with NTS infections. While technically they are a single species, we are aware that the epidemiology is considerably different. However, given the low numbers identified in our study over the years, we thought to include them in the analysis to provide a general idea about their prevalence and drug resistance.

About 40% of the patients were not Saudi nationals. We expect that these patients have probably acquired their infection during their stay in KSA. However, lack of information about travel history before infection with Salmonella precludes a firm conclusion.

In addition, we lack comprehensive clinical and demographic data as most patients were treated in outpatient clinics.

Conclusions

The antimicrobial resistance of Salmonella isolates is on the rise, and the presence of multiple-resistance to frequently used antibiotics is of concern.

Recommendations

An active monitoring system of antibiotic use in humans and domestic animals is required.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

There is no conflict of interest.

Ethical approval

Ethical approval for the study was obtained from the Institutional Review Board at Imam Abdulrahman Bin Faisal University (IRB-2019-1-176).

Authors contributions

RYA conceived and designed the study, collected and organised data, provided logistic support, participated in writing the final draft and critically revised the manuscript for intellectual content. KRA analysed and interpreted data, and wrote the initial and final draft of the article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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