Original Article

Salmonella isolates' serotypes and susceptibility to commonly used drugs at a tertiary care hospital in Riyadh, Saudi Arabia

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Abstract

Introduction: Resistance of *Salmonella* to therapeutic agents currently being used for treatment of *Salmonella* infections is emerging as a global problem. This study aimed to assess the prevalence of *Salmonella* serotypes and their susceptibility patterns to commonly used drugs for treatment of *Salmonella* infections including quinolones. Correlation between nalidixic acid susceptibility of these isolates and their ciprofloxacin minimum inhibitory concentrations was also sought.

Methodology; *Salmonella* isolates (n=213) were collected between January 2007 and May 2009 at King Khalid University Hospital in Riyadh, Saudi Arabia. The isolates were serotyped and their susceptibilities to commonly used first-line anti-*Salmonella* drugs (ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, nalidixic acid and ciprofloxacin) were determined using the automated Microscan system, the Kirby-Bauer disk diffusion method, and E-test.

Results: The most frequently detected serotype was D_1 (37%) followed by the serotypes, B (24%) and C_1 (11%). Non-typable *Salmonella* isolates detected using available conventional *Salmonella* anti-sera were (11%). Overall resistance rates to nalidixic acid, ampicillin, trimethoprim/sulfamethoxazole and ceftriaxone were 99/213 (46%), 43/213 (20%), 34/213 (16%) and 7/213 (3%), respectively. Of the total isolates, 117 (55%) had a ciprofloxacin MIC of <0.125 µg/ml and among these 105 (90%) were susceptible to nalidixic acid. The remaining 96 (45%) isolates had a ciprofloxacin MIC of ≥0.125 µg/ml and among them, 83 (86.5%) were resistant to nalidixic acid.

Conclusions: The majority of *Salmonella* isolates in this study were non-typhi serotypes. Significantly higher proportions of *Salmonellae* were resistant to nalidixic acid and ciprofloxacin and a vast majority of nalidixic acid resistant organisms exhibited decreased susceptibility to ciprofloxacin.

Key words: *Salmonella*; nalidixic acid resistance; ciprofloxacin

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Introduction

Resistance of typhi and non-typhi *Salmonellae* to ampicillin, trimethoprim/sulphamexazole and chloramphenicol emerged between the 1970s and 1990s [1-6]. Resistant typhi strains were mainly found in South America, the Indian subcontinent, and Africa [7-8]. As an alternative ciprofloxacin became the antibiotic of choice for *Salmonella* infections, *i.e.*, enteric fever [9]. Increasing numbers of *Salmonella enterica* serotype Typhi [10,11,12] and non-typhi strains [13,14] were later shown to exhibit reduced susceptibility to ciprofloxacin as they were found to be associated with prolonged fever and lack of clinical response despite treatment with the antibiotics [12,15]. In addition, it has been observed that a vast majority of *Salmonella* resistant to nalidixic acid show decreased susceptibility to ciprofloxacin as well [16,17].

This study examines the prevalence of different *Salmonella enterica* serotypes and their susceptibilities to commonly used drugs for treatment of *Salmonella* infections. Correlation was also sought between nalidixic acid resistance and susceptibility to ciprofloxacin.

Methodology

This was a prospective study conducted between 15 January 2007 and 13 May 2009 at the King Khalid University Hospital (KKUH) bacteriology laboratory. KKUH is 850-bed primary, secondary and tertiary care hospital serving about two million people. The study was approved by the Hospital Ethical Committee. Demographic and the clinical data of the patients were collected from a group of 213 patients including 137 (64.3%) males and 76(35.7%) females. The age range of the patients in this study group was

between one month to 81 years where the majority (62%) was either equal to or less than the age of 15 years. Among the patients 81% were Saudi nationals followed by individuals from Pakistan (8%), India (7%) and other nationalities (4%). The majority of the isolates (178) were from faecal specimens, 21 from blood, 4 from urine and 10 were from different body sites. Fecal specimens were collected from patients presenting with diarrhea or other evidence of gastroenteritis; blood samples were obtained from patients presenting with a febrile illness; urine samples were collected from patients with the evidence of urinary tract infection; and swab samples were obtained from infected lesions from various sites. The samples were collected from 62 (29%) patients admitted in the hospital for more than 48 hours and 152 (71%) from patients attending the primary care clinics not requiring hospital admissions.

All the *Salmonella* collected were cultured using conventional bacterial methods. Faecal specimens were cultured on MacConkey, hektoen and xylose lactose deoxycholate medium (XLD). The blood cultures were performed in an automated Bact Alert system (Organon Teknika Corp, Durham, NC, USA). The urine specimens were cultured on Mac Conkey and ClED media, while the general swabs samples were cultured on blood agar and MacConkey agar. All the isolates were identified using the automated Microscan system (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and API 20 system (bioMérieux, Marcy l'Etoile, France). Serotyping was performed by using *Salmonella* antisera (Welcome, KS, USA) using the Kauffmann-White classification system. A single isolate was cultured from each patient in most of the instances. Multiple isolates with similar serotypes cultured from one individual was counted as one event. Susceptibility to ampicillin, ceftriaxone and trimethoprim/sulfamethoxazole was assessed with the automated Microscan system (Dade Behring). Nalidixic acid susceptibility was determined by the Kirby-Bauer disk diffusion comparative method with paper disks containing 30 µg of nalidixic (Oxoid Ltd., Basingstoke, Hampshire UK) using *Escherichia coli* ATCC No 25922 as the control organism. Susceptibility of the isolates was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria [18]. The E-test method (AB Biodisk, Solna, Sweden) was used to determine the ciprofloxacin minimum inhibitory concentrations (MIC). In addition, nalidixic acid susceptibility and ciprofloxacin MIC were also compared.

Results

Table 1 lists the symptoms of patients on presentation. The most common presenting symptom was diarrhea alone (78%) followed by diarrhea and fever (9%). Four percent of the patients were asymptomatic and 3% had diarrhea with abdominal pain. Table 2 shows the serotypes of the *Salmonella* isolates found in the study. The most commonly detected serotypes were D_1 (37%), B (24%), C_1 (11%) and 11% of the isolates could not be typed using recommended sera. Of the enteric fever-causing *Salmonellae*, *S. enterica* serotype Typhi was detected in 2% of patients, while *S. enterica* serotype Para typhi A was present in 1% of cases. The least number of *Salmonella* isolates (3%) were found to be resistant to ciprofloxacin whereas maximum resistance was observed against nalidixic acid (46%). This was mainly due to a high percentage (78%) of resistant D_1 serotype. The overall resistance against ampicillin was 20% where serotype B (49%) had a major contribution. Similarly total resistance against trimethoprim/sulfamethoxazole was 16% where B serotype exhibited the highest resistance (37%). Figure 1 shows data examining nalidixic acid sensitivity among 206 ciprofloxacin sensitive isolates. Of these 114 (55%) isolates had ciprofloxacin MIC of < 0.125 µg/mL and 92 (45%) had ciprofloxacin MIC of ≥ 0.125 µg/mL. These data, when compared with those for nalidixic acid sensitivity, revealed that 103 (90%) isolates with ciprofloxacin MIC of < 0.125 µg/mL were susceptible to nalidixic acid, whereas 79 (86%) isolates with ciprofloxacin MIC of ≥ 0.125 µg/mL were resistant to nalidixic acid.

Discussion

Infections by *S. enterica* cause considerable morbidity and mortality worldwide [13,19]. Outbreaks of strains of *S. enterica* serotype Typhi resistant to all first-line drugs have been reported from the Indian subcontinent, Mexico, South Africa, and the Arabian Gulf [7,8,10,20-22]. Similarly, non-typhoidal *Salmonella* strains resistant to the conventional first-line drugs have also been reported [14].

Among the *Salmonella* isolates detected in the present study serotype D_1 occurred most frequently.

Table 1. Clinical symptoms of patients with *Salmonella* infections

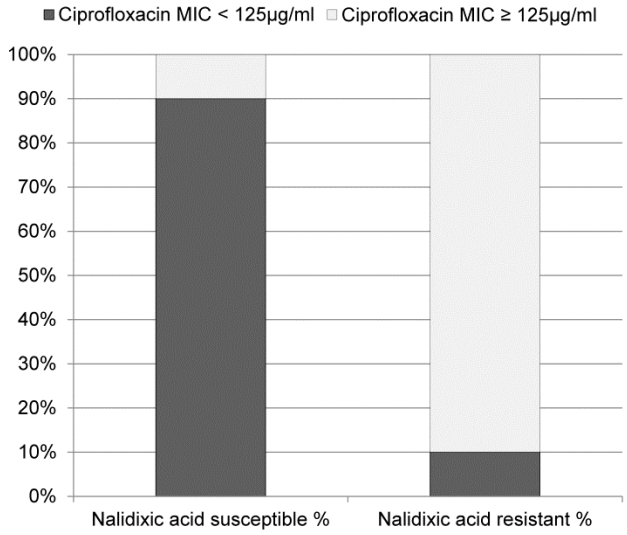
Symptom/s	Number (Percentage)
Diarrhea	168 (78)
Diarrhea and fever	20 (9)
Diarrhea and abdominal pain	7 (3)
Diarrhea and vomiting	2 (2)
Hematuria	2 (2)
Bloody diarrhea and fever	1 (1)
Diarrhea, abdominal pain and Vomiting	1 (1)
Asymptomatic	10 (4)
Total	**213 (100)**

Table 2. Serotype distribution and antibiotic resistance patterns of 213 *Salmonella* isolates

Salmonella serotype	Number (%)	Ampicillin Number (%)	Trimethoprim / Sulpha Number (%)	Ceftriaxone Number (%)	Nalidixic Acid Number (%)
D_1	78 (37)	8 (10)	0 (0)	6 (8)	61 (78)
B	51 (24)	25 (49)	19 (37)	0 (0)	8(16)
Untypeable*	23 (11)	4 (18)	5 (27)	1 (4)	7 (30)
C_1	22 (10)	0 (0)	3 (17)	0 (0)	10 (46)
C_2	13 (6)	4 (31)	3 (23)	0 (0)	4 (31)
E_1	08 (4)	1 (13)	1 (13)	0 (0)	2 (25)
Typhi	05 (2)	1 (20)	1 (20)	0 (0)	3 (60)
E_4	03 (1)	0 (0)	0 (0)	0 (0)	0 (0)
G_1	03(1)	0 (0)	0 (0)	0 (0)	0 (0)
D	03(1)	0 (0)	2 (67)	0 (0)	2 (67)
G	02 (0.9)	0 (0)	0 (0)	0 (0)	1 (50)
Paratyphi A	02 (0.9)	0 (0)	0 (0)	0 (0)	1 (100)
Total	**213**	**43 (20)**	**34 (16)**	**7 (3)**	**99 (46)**

* Isolates that could not be typed using the recommended sera. Figures in parentheses represent percentages

Figure 1. Correlation of ciprofloxacin MIC (µg/ml) with nalidixic acid susceptibility among 206 *Salmonella* isolates.



This is in sharp contrast to the previous report from the same institution where serotypes B and C were reported to be the most commonly detected serotypes [20]. Similarly, the prevalence rate of *S. enterica* serotype Typhi in the present study was only 2% compared to a previous report from the southern region of the Kingdom of Saudi Arabia where *S. enterica* serotype Typhi constituted 65% of the isolates [21]. This discrepancy may be due to the difference in the study populations.

The majority of the isolates in the present study were non-typhoidal *Salmonellae* and resistance to ampicillin and trimethoprim/sulphamexazole was frequently observed. High prevalence of non-typhoidal *Salmonellae* has also been reported from other parts of the world [22-24], indicating that infections with non-typhoidal *Salmonellae* are a global problem. Infections due to multidrug resistant *S. enterica* serotype Typhi have also been regarded as major problems in several parts of the world [25]. These observations indicate regional variations in the prevalence of different *Salmonella* species where the environmental, hygienic, and cultural differences may be important contributing factors.

A sizable proportion of the non-typhoid *Salmonellae* isolated in the present study were found to be resistant to ampicillin and trimethoprim/sulphamexazole. The percentage of these isolates in the present study was higher than those in previous reports from the Kingdom [21,26]. High prevalence of non-typhoid *Salmonella* resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline has been documented in many countries, including the United States and the United Kingdom [27,28,29]. Sensitivity of non-typhoid *Salmonellae* to streptomycin and tetracycline was not determined in the present investigation, as these antibiotics are currently not being used frequently for treatment of enteric fever and other invasive Salmonellosis.

In this study 46% of *Salmonella* isolates were resistant to nalidixic acid. *S. enterica* serotype Typhi resistant to nalidixic acid as high as 83% has been reported from India [30]. However, in India, nalidixic acid resistance among *S. enterica* serotype Typhi was as high as 83% [30]. Figures from Denmark show an increase from (0.8%) in 1995 to (8.5%) in 2000 in the incidence of nalidixic acid-resistant zoonotic *Salmonella* infections [31]. In the United States, *S. enterica* serotype Typhi resistance to nalidixic acid increased from (6.8%) in 1996-1997 to (23.2%) in 2000, indicating a global increase of resistance to nalidixic acid [32]. Reliable data regarding prevalence of nalidixic acid resistant *Salmonellae* in the Kingdom are lacking. It is therefore difficult to perform a comparative analysis and comment on the change in prevalence rates.

Salmonella isolates resistant to nalidixic acid commonly exhibit resistance to ciprofloxacin [33,34]. It is for the same reason that public health surveillance for resistance to nalidixic acid is considered useful to predict resistance against fluoroquinolones. This view is further supported by the fact that treatment with fluoroquinolones has often failed to achieve the desired therapeutic effect in patients infected with nalidixic acid resistant strains of *Salmonella* [35-37]. Similar observations were also made in this study where the majority of the *Salmonella* isolates with a ciprofloxacin MIC of ≥ 0.125 µg/ml were found to be resistant to nalidixic acid. Nalidixic acid-resistant isolates with a ciprofloxacin MIC range of 0.125-0.250 mg/l have already been reported and mutation in DNA topoisomerase has been found to be associated with the increased quinolones resistance [37]. It worth mentioning that the automated Microscan system in the clinical laboratory does not detect actual ciprofloxacin MICs for the organisms tested; it tests break-point susceptibility of *Salmonella* spp to ciprofloxacin with MICs of 1 µg/ml and of 4 µg/ml as the susceptible and resistant breakpoints, respectively. Thus decreased susceptibility of these isolates to ciprofloxacin cannot be detected by this

system and not all laboratories use the E-test to determine MIC. This finding emphasizes the importance of nalidixic acid resistance as a useful marker in predicting *Salmonella* resistance to quinolones thus obviating not only the need for checking susceptibility for quinolones but also avoiding inappropriate use of quinolones for treatment of *Salmonella* infections.

This study fell short of monitoring the clinical outcome of the patients infected with nalidixic acid-resistant and high ciprofloxacin MIC *Salmonellae*. However, it has been reported that patients suffering from typhoid fever infected with nalidixic acid resistant *Salmonellae* tend to have a longer duration of fever and about one third of these patients require further treatment with a higher dosage of quinolones [38,39]. Although there are reports documenting infections with *Salmonella* spp. resistant to nalidixic acid in the Kingdom of Saudi Arabia [20,40,41], large-scale prevalence studies are recommended to investigate the current status of *Salmonella* infections and their susceptibility patterns in the community.

The development in the molecular testing and typing of *Salmonella* enable us to identify the new reservoir of resistant strains. A recent study from Korea demonstrated clonal spread of a *Salmonella* strain harboring genes encoding resistance to nalidixic acid in swine [42], which might have a major role in early detection and prevention of spread of this disease.

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