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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

Salmonella isolates from urine cultures: serotypes and antimicrobial resistance in hospital settings

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Abstract

This study investigated the serotypes and antimicrobial resistance of *Salmonella* isolates in urine cultures from 38 hospitalized patients. Nine serotypes were detected, and a large proportion was Typhimurium and Enteritidis. The strains presented resistance to 11 different antibiotics. Thirteen isolates (11 from serotype Typhimurium) exhibited multidrug resistance.

Keywords Nontyphoid *Salmonella* · Urinary tract infections · Multidrug resistance

Salmonella is an important pathogen causing approximately 550 million infections in the world per year [1]. Typhoid *Salmonella* (TS) serotypes (Typhi, Paratyphi A and B) are adapted to humans and cause typhoid fever, while nontyphoid *Salmonella* (NTS) serotypes infect a wide range of hosts, causing symptoms ranging from mild gastroenteritis to severe systemic infection. NTS has increasingly impacted human health, and it is responsible for approximately 93.8 million infections with 155,000 deaths per year [1, 2]. The clinical symptoms include diarrhea, fever, headache, and abdominal pain [3]. Infections by invasive NTS (iNTS) strains can also progress to severe life-threatening sepsis, a common cause of bacteremia in children and adults. The diversity in the clinical manifestations is explained by several factors, highlighting the occurrence of a large range of virulence factors in the bacterial strains [3, 4]. Urinary tract infections (UTIs) by *Salmonella* are infrequent in routine hospital practice and rarely reported in the scientific literature, although they may cause serious morbidity in immunocompromised and even in immunocompetent patients [5–8]. The present study describes the serotype identification and antimicrobial resistance of *Salmonella* isolates from urine cultures of hospitalized patients.

A total of 38 *Salmonella* isolates were obtained from urine cultures in different hospitalized patients in Rio Grande do Sul state and sent to a public health laboratory (Laboratório Central do Rio Grande do Sul, LACEN-RS) from 2010 to 2015. Demographic data (such as hospital, city, and date of isolation; age and sex of the patients) were obtained in the clinical forms. The isolates were plated on *Salmonella*/*Shigella* solid media, and presumptive *Salmonella* colonies were cultured in Triple Sugar Iron (TSI), Urea, Citrate, and Lysine Iron Agar (LIA) slants (Probac, São Paulo, Brazil). The isolates were submitted to aerobic incubation at 37 °C for 24 h. Suspected *Salmonella* colonies were partially tested by agglutination with the main polyvalent O and H antisera (Probac, São Paulo, Brazil). Complete antigenic characterization was carried out for serotype identification in the National Reference Laboratory for Cholera and Enteric Diseases in the Institute Oswaldo Cruz (FIOCRUZ, Rio de Janeiro, RJ, Brazil) by the Kauffmann-White-Le Minor scheme [9].

All isolates were also submitted to DNA extraction and real-time polymerase chain reaction (PCR) targeting the *invA*

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gene as previously described [10]. Molecular detection of the two main serotypes (Enteritidis and Typhimurium) was further carried out by testing isolates with a duplex real-time PCR targeted to *safA* and *fliA-IS200* genes, respectively [11]. Resistance to twelve antibiotics (ampicillin, AMP; ciprofloxacin, CIP; nalidixic acid, NAL; nitrofurantoin, NIT; sulfamethoxazole + trimethoprim, SUT; cefoxitin, CFO; chloramphenicol, CLO; streptomycin, STR; gentamicin, GEN; tetracycline, TET; ceftazidime, CAZ; imipenem, IMP) was evaluated for all samples according to the Clinical and Laboratory Standards Institute document M100-S24 (CLSI 2014) [12, 13]. *Escherichia coli* ATCC 25922 was used as a standard control. Isolates with resistance to three or more categories of antibiotics were considered to be multidrug-resistant (MDR) [12, 13]. Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 18.0, Chicago, IL). Pearson's chi-square and/or Fisher's exact tests were used to verify significant differences between qualitative variables, including serotypes and antimicrobial resistance profiles. All statistical tests in this study were two-sided, and p values < 0.05 were considered to be statistically significant.

The sociodemographic data demonstrated that *Salmonella* was more commonly isolated from women (23, 60.5%) and adults older than 45 years (21, 55.3%). All 38 isolates presented positive results for anti-*Salmonella* polyvalent O/H antiserum and for the generic *Salmonella* PCR targeting the *invA* gene. Complete serotyping and real-time PCR analysis showed that all isolates were NTS, and a large proportion were serotypes Typhimurium (21, 55.3%) and Enteritidis (6, 15.8%). Another eight NTS serotypes were also identified: Newport, Infantis, Dublin, Give, Ndolo, Ohio, Saintpaul, and Panama (Table 1).

An antimicrobial susceptibility test was performed for 32 (84.2%) of the NTS isolates. Five (15.6%) isolates were sensitive to all antibiotics, while the other 27 (84.4%) exhibited resistance to the following antibiotics: NAL (17, 53.1%), TET (14, 43.8%), STR (12, 37.5%), AMP (10, 31.3%), NIT (10, 31.3%), GEN (7, 21.9%), CLO (3, 9.4%), SUT (3, 9.4%), CIP (1, 3.1%), CFO (1, 3.1%), and CAZ (1, 3.1%). Twenty (62.5%) of these isolates were from the most frequent serotype (Typhimurium) and exhibited resistance to NAL (13, 65%), TET (13, 65%), STR (10, 50%), AMP (9, 45%), NIT (5, 25%), GEN (5, 25%), CLO (3, 15%), SUT (3, 15%), CIP (1, 5%), CFO (1, 5%), and CAZ (1, 5%) (Supplementary Material).

In addition, 14 (43.7%) isolates were resistant to three or more antibiotic classes and were classified as MDR. These isolates were predominantly from the serotype Typhimurium

Table 1 Sociodemographic variables of patients, serotypes, and MDR frequency in NTS isolated from urine cultures between 2010 and 2015 in southern Brazil

Variables	NTS isolates *n* (%)	Multidrug resistance (MDR) No MDR *n* (%)	MDR *n* (%)	Missing* *n*	*p value***
Sex					
Male	15 (39.5)	7 (53.8)	6 (46.2)	2	0.836
Female	23 (60.5)	11 (57.9)	8 (42.1)	4	
Age range					
0–5	2 (5.3)	0 (0)	0 (0)	2	0.173
6–18	3 (7.9)	1 (50)	1 (50)	1	
19–45	12 (31.6)	9 (81.8)	2 (18.2)	1	
> 45	21 (55.3)	8 (42.1)	11 (57.9)	2	
Serotype					
Typhimurium	21 (55.3)	9 (45)	11 (55)	1	–
Enteritidis	6 (15.8)	2 (66.7)	1 (33.3)	3	
Newport	3 (7.9)	2 (100)	1 (50)	0	
Infantis	2 (5.3)	1 (100)	0 (0)	1	
Dublin	1 (2.6)	1 (100)	0 (0)	0	
Give	1 (2.6)	1 (100)	0 (0)	0	
Ohio	1 (2.6)	1 (100)	0 (0)	0	
Panama	1 (2.6)	1 (100)	0 (0)	0	
Ndolo	1 (2.6)	0 (0)	1 (100)	0	
Saintpaul	1 (2.6)	0 (0)	0 (0)	1	
Total	38	18	14	6	

*Number of isolates not evaluated for MDR

**Chi-square calculation (No MDR vs MDR)

(11, 37.9%), but there was also one (3.1%) Enteritidis isolate, one (3.1%) Newport isolate, and one (3.1%) Ndolo isolate. There was no significant difference between the frequencies of MDR and the variables sex and age (p values > 0.05) (Table 1).

Salmonella isolation in nonenteric samples is not as common in laboratory practice [6]. Two previous studies demonstrated low frequencies (1.4 to 2.8%) of *Salmonella* isolated from urine in comparison with other clinical samples (mainly enteric) in the São Paulo state, Brazil [14, 15]. Despite this low occurrence, both old and recent scientific studies have reported the association of *Salmonella* in urine cultures with UTIs, mainly in immunocompromised and elderly hospitalized patients [16–20]. In the present study, we described 38 *Salmonella* isolates (all of them NTS) obtained from urine cultures of patients from seven different hospitals, five from Porto Alegre (the capital city) and two from two other important and populous cities of the Rio Grande do Sul state (Novo Hamburgo and Santa Maria). NTS was isolated from both sexes and all age groups with predominance in adults over 45 years of age, as observed in other reports [16, 17, 19, 21].

In the serological analysis, most NTS isolates were from serotype Typhimurium (21, 55.3%). This same serotype has been the most frequent serotype isolated from urine in previous reports [5, 17, 20]. Typhimurium has a complete arsenal of virulence genes to remain in certain environments, such as hospital settings. The invasiveness characteristics of this serotype also facilitate bacteremia and the occurrence of UTIs [3, 4]. Other studies have also shown that this serotype is etiologically associated with UTIs in South America [21, 22].

Bacterial resistance was observed to several antibiotics, such as AMP, CIP, NAL, NIT, SUT, CFO, CLO, STR, GEN, TET, and CAZ. In the clinical routine, AMP has been the most commonly employed to treat UTIs, but 31.3% of the *Salmonella* isolates demonstrated resistance to this antibiotic, as previously described [17]. In addition, MDR was observed in several strains, primarily those from serotype Typhimurium. In Brazil, a previous study demonstrated that 59.3% of Typhimurium isolates from human clinical cases exhibited MDR [23]. It was also observed that Typhimurium presented a higher level of resistance than all of the other serotypes. A major concern is the spread of some clones, such as the sequence type 313 (ST313), which has high mortality rates (approximately 25%) and is frequently associated with HIV and/or malaria infection and malnutrition in Sub-Saharan African [4]. This NTS group (ST313) has even already been reported in Brazil [24].

In conclusion, we determined that the Typhimurium serotype was present at a high frequency in urine samples from patients with urinary tract infection in a hospital setting. We also determined that this serotype was the most frequently associated with MDR. Special attention should be given to the prevention and hospital control of urinary tract infections caused by the Typhimurium serotype.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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