## Susceptibility patterns and serotypes of non-typhoidal salmonella in Trinidad

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## ABSTRACT

**Objective:** To study the serotype distribution of nontyphoidal salmonella isolated from clinical specimens and to evaluate their susceptibility patterns to the most commonly prescribed antimicrobial agents used in this region.

**Methods:** A retrospective study during the period 1993– 1999 was carried out at the Eric Williams Medical Sciences Complex Microbiology Laboratory, a teaching hospital in the northwestern part of Trinidad. Strains of non-typhoidal salmonella were serogrouped with polyvalent *Salmonella O* antiserum and sent to the local Public Health Laboratory for confirmation and further serotyping.

**Results:** A total of 412 strains of non-typhoidal salmonella were serotyped. Of the 13 serotypes, *Salmonella enteritidis* was the most prevalent 57%, followed by *Salmonella typhimurium*, 25%, *Salmonella virchow* 5% and *Salmonella heidelberg*, 4%. Three hundred and sixty-two (87.5%) non-typhoidal salmonella were isolated from stool, 38 (9%) from blood, 8 (2%) from urine and 4 about 1% from infected wounds. Of the 13 serotypes only *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella virchow* and *Salmonella heidelberg* were simultaneously isolated from blood and stool. One serotype, *Salmonella aberdeen*, was

encountered for the first time in 1993. All isolates were fully sensitive to chloramphenicol, gentamicin, cefuroxime and ceftrioxone. Resistance to ampicillin, augmentin and co-trimoxazole was less than 4%. One third of all nontyphoidal salmonella isolates were resistant to tetracyclines.

Conclusion: The most prevalent serotypes were Salmonella enteritidis and Salmonella typhimurium which accounted for 82% of all isolates. The prevalence of resistance of non-typhoidal salmonella to antibiotics was very low (5%). Therefore, due to this low prevalence of resistance, we did not provide details of resistance in relation to serotype. However, it was observed that of the 137 non-typhoidal salmonella resistant to tetracycline, more than 80% were Salmonella enteritidis and Salmonella typhimurium strains. Of the 9 antibiotics, tetracycline was the only drug to which one-third of the non-typhoidal salmonella were resistant. Due to the risk of resistance continued surveillance of susceptibility patterns of non-typhoidal salmonella to antibiotics must be maintained and encouraged.

Keywords: Non-typhoidal Salmonella, antibiotic resistance.

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**N** on-typhoidal Salmonella (NTS) continues to be a major cause of morbidity and mortality worldwide.<sup>1-3</sup> These organisms have been associated with a variety of extra-intestinal infections. For example, NTS is a frequent cause of septicemia in developing countries,<sup>4-5</sup> and has also been associated with wound infections, meningitis, and urinary tract infections.<sup>6</sup> The global Acquired immune deficiency syndrome (AIDS) epidemic has added a new

dimension to the importance of NTS infections in that, recurrent infections due to these organisms have now become an early marker for AIDS in young infected persons.<sup>7</sup> Gilks and co-workers<sup>8</sup> have reported that NTS bacteremia was detected in 11% of Human immuno-deficiency virus (HIV)-seropositive patients on admission to a Kenyan hospital. To decrease morbidity and mortality due to systemic salmonellosis, antimicrobial therapy must be started

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immediately using one of the 3 commonly used antibiotics in tropical countries for the treatment of systemic NTS infections, that is, ampicillin, cotrimoxazole and chloramphenicol. Recently however, there have been several reports of multi-drug resistant Salmonella organisms emerging in the Middle East,<sup>9,10</sup> South Africa,<sup>11</sup> the United Kingdom,<sup>12</sup> Spain<sup>2</sup> and Southeast Asia.<sup>13</sup> Trinidad and Tobago frequently import from many of these countries, all categories of workers and therefore, it is possible for the importation of multi-drug resistant bacteria particularly Salmonella, into the country. The emergence of resistance to these antibiotics among Salmonella would present serious problems for the country, as alternative therapies such as the fluroquinolones are more expensive. The purpose of this retrospective study was to identify the prevalent NTS serogroups and to assess and document their susceptibility patterns to the most commonly used antimicrobial agents used in this region.

**Methods.** All cases of Salmonella infections isolated from blood, stool, urine or wound cultures from January 1993-December 1999 at the Eric Williams Medical Sciences Complex (EWMSC) were included in this study. The EWMSC is a 560-bed tertiary teaching hospital complex located in the northwestern part of Trinidad. Trinidad is the larger of the twin-island Republic of Trinidad and Tobago, located approximately 11 kilometers off the northern coast of Venezuela in South America. The population of the republic is approximately 1.25 million people. Identification of Salmonella was performed using standard biochemical tests according to procedures previously described.<sup>14</sup> Salmonella isolates were then serologically grouped with polyvalent *Salmonella O*-

 
 Table 1 - Frequency of non-typhoidal Salmonella serotypes isolated at the Eric Williams Medical Science Complex (EWMSC), 1993-1999.

Isolate	1993	1994	1995	1996	1997	1998	1999
S antonitidia	22	26	20	42	20	42	20
Stophimurium	18	18	10	42	10	42 22	10
S.iypninurium S.iypninurium	6	2	2	14	10	4	10
S.virchow S.haidalbara	2	2 4	2 4	$\frac{2}{2}$	2	4	
Sagona	$\frac{2}{2}$	$\vec{0}$	2			4	0
Shraendesun	$\frac{2}{2}$	0	õ	0	0	2	4
S javiana	õ	0	0	0	2	$\frac{2}{4}$	ō
S thompson	ŏ	Ő	Ő	Ő	$\frac{2}{2}$	2	ő
S hadar	õ	0	0	2	õ	õ	ő
S saint-naul	ŏ	ő	ő	1	ő	ő	1
S ohio	1	ŏ	1	Ô	ŏ	ŏ	Ô
S aherdeen	1	ŏ	Ô	ŏ	1	ŏ	ŏ
S.kentucky	2	Ő	Ő	Ő	Ō	Ő	Ő
Total	56	60	51	63	51	84	47

antiserum (S.antiserum) and then sent to the local Public Health Laboratory for confirmation and further subtyping. Antimicrobial susceptibility testing carried out on Mueller-Hinton. (BBL was Microbiology Systems, Cockeysville, Maryland, United States of America) agar using the disc diffusion technique and the following antimicrobial discs and concentrations: 10mg, Ampicillin Augmentin (Amoxicillinclavulanic acid) 20/10mg, Ceftrioxone 30mg, Chloramphenicol 30mg Co-trimoxazole (Trimethoprim-sulfamethoxazole) 30mg, Gentamicin 10mg and Tetracycline 30mg.

**Results.** During the period 1993-1999, 412 strains of non-typhoidal Salmonella (NTS) were isolated from clinical sources. The most prevalent which accounted for 234 (57%) of all isolates. This followed by Salmonella typhimurium was (S.typhimurium), 102 (25%), Salmonella virchow (S.virchow), 22 (57%) and Salmonella heidelberg (S.heidelberg), 18 (47%). The other 9 NTS each accounted for less than 2% of isolates (Table 1). The frequency of isolation of all serotypes was relatively stable throughout the study period. Of the 412 NTS serotypes 362 (87.5%) were identified from stool and 38 (97%) were recovered from blood cultures. Eight (2%) serotypes were isolated from urine of patients diagnosed with upper urinary tract infection and 4 about 1% from infected wounds. Salmonella enteritidis and S. typhimurium were the only serotypes isolated from urine, 6 and 2. All isolates from infected wound were S.enteritidis. Blood cultures were positive in 17 (45%) for S.typhimurium infections,  $\overline{12}$  (32%) isolates for S.enteridis infections, 7 for (18%) S.virchow infections and one

 Table 2 - Clinical source of 412 non-typhoidal Salmonella serotypes isolated at the Eric Williams Medical Sciences Complex (EWMSC), 1993-1999.

Isolate	Stool	Blood	Urine	Wound			
S enteritidis S Jyphimurium S.virchow S Jeaidelberg S.agona S Javiana S Javiana S Jadar S Jadar S Saint-paul S Jabio S Jaberdeen S Jkentucky	212 85 15 15 8 8 5 4 2 2 2 2 2 2	12* 17* 7* 1* - 1 - - - -	6 2 - - - - - - - - - -	4			
Total	362	38	8	4			
*=Serotype was also recovered from stool, S=Salmonella							

Antibiotic	n (%) Sensitive	n (%) Resistant	Total Isolates Tested			
Ampicillin Augmentin <sup>b</sup> Tetracycline Co-trimoxazole <sup>c</sup> Chloramphenicol Cefuroxime Gentamicin Ceftrioxone	397 (96) 402 (98) 275 (67) 402 (98) 412 (100) 361 (100) 412 (100) 412 (100)	15 (4) 10 (3) 137 (33) 10 (3)    	412 412 412 412 412 361 412 412 412			
Augmentin <sup>b</sup> =amoxicillin-clavulanic acid,Co-trimoxazole <sup>c</sup> =trimethoprim- sulfamethoxazole, n=number						

 Table 3 - Susceptibility patterns of Salmonella isolates at the Eric Williams Medical Sciences Complex (EWMSC), 1993-1999.

(3%) in each of *S. heidelberg* and *Salmonella javiana* (*S. javiana*) infections (Table 2). The pattern of antimicrobial susceptibility of the NTS isolates from clinical sources shown in Table 3. About one 3rd of all isolates were resistant to tetracycline, while resistance to ampicillin, augmentin and co-trimoxazole was less than 4%. More than 80% of tetracycline resistance was seen among *S. enteritidis* and *S. typhimurium* strains isolated from stool. All isolates were fully sensitive to chloramphenicol, gentamicin, cefuroxime and ceftrioxone.

**Discussion.** Only 13 non-typhoidal Salmonella (NTS) serotypes accounted for Salmonellosis in Trinidad between 1993-1999. The most prevalent serotypes were S.enteritidis and S.typhimurium which accounted for approximately 82% (336 of 412) of all isolates in the study. This seems to be the pattern in other developing countries,<sup>15-17</sup> as well as the United States of America,18 where S.enteritidis and S.typhimurium were reported to be the predominant NTS serotypes from clinical sources. Non-typhoidal salmonella infections are most often associated with food products and such infections are the most frequently identified cause of foodbourne disease outbreaks,19 although other vehicles of transmission have been implicated in human disease.<sup>20,21</sup> Changes in food consumption and the rapid growth of international trade in agricultural products have facilitated the dissemination of new Salmonella serotypes.<sup>22</sup> In 1993 the Public Health Laboratory of this country along with the Reference Laboratory, The Caribbean Epidemiology Center, a branch of Pan american Health Organization (PAHO)/World Health Organisation (WHO), reported for the first time, the isolation of a new Salmonella serotype, S. aberdeen, never before seen in the Caribbean. This particular serotype was first isolated from a visitor who developed a diarrhoeal illness during the Carnival

celebrations that year. The risk of importation of multi-resistant organisms poses serious problems for the Public Health Authority and the country. In reports from the Kingdom of Saudi Arabia and Kuwait that have a high immigrant population, resistance to chloramphenicol, co-trimoxazole and ampicillin was more commonly seen in patients coming from India and other non-Middle Eastern countries than the native population.9,23 In fact, antimicrobial resistance among human NTS has been increasing worldwide even in countries with very high public health and hygeinic standards.<sup>24</sup> Familiarity with resistance patterns from different parts of the world may be useful due to the frequency and rapidity of overseas travelling and emigration behavior of populations.

The present study shows that apart from gastrointestinal infections being the most common NTS infections (88%), 12% of our patients had a variety of extra-intestinal infections as reported elsewhere.<sup>6</sup> The most common extra-intestinal site in our study was the bloodstream, 9%, followed by the urinary tract 2% and wounds 1%. There is a lot of variability involving the proportion of NTS serogroups and serotypes identified from any particular geographic area.<sup>25</sup> These different serogroups and serotypes have been shown to have different resistant patterns, with serogroups B and C being more resistant than serogroup D.<sup>2,26</sup> This pattern was not seen in our study where the overall prevalence of resistance was less than 4% (except for tetracycline which was 33%). Also in the present study resistance to ampicillin was 4%. In other countries, ampicillin resistance ranged from less than 2% in New Zealand,<sup>27</sup> to 67% in Ethiopia.<sup>15</sup> Cotrimoxazole resistance was only approximately 2% in our study, and this was comparable to reports of 2% from Spain<sup>2</sup> and represents a good oral alternative against ampicillin-resistant NTS strains. Higher resistant rates to co-trimoxazole have been reported from Kenya (46%)<sup>28</sup> and Saudi Arabia (36.5%).<sup>9</sup> The antibiotics chosen are among the cheapest and the ones most frequently used in this country.

Resistance to tetracycline was the highest seen in this study. The reason for this is not altogether clear, but maybe due in part to the relative ease with which this drug is obtained over the counter without a prescription. The results also showed that all NTS were fully susceptible serotypes to the cephalosporins-cefuroxime and ceftrioxone, as well gentamicin chloramphenicol. and as Chloramphenicol resistance among NTS is common worldwide with high rates of 26% in Kenya and 32% seen in the Kingdom of Saudi Arabia.<sup>9,28</sup> Because the overall prevalence of resistance was very low approximately 5%, we did not provide details of resistance in relation to serotype. However, it was observed that of the 137 NTS resistant to

tetracycline, more than 80% were *S.enteritidis* and *S. typhimurium* strains.

In conclusion, the overall resistance of NTS to commonly prescribed antimicrobial agents in Trinidad is very low. However, continued surveillance of susceptibility patterns of NTS to these drugs must continue and prudent use of all antimicrobial agents must be encouraged and maintained.

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