

NONTYPHOIDAL SALMONELLOSIS: MONITORING AND ANALYSIS OF SALMONELLA SEROVARS AND THEIR SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

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Abstract

A total of 201 nontyphoidal *Salmonella* strains were analysed, of them 191 strains were isolated from faecal specimens of patients with enterocolitis syndrome, and 10 strains were isolated from other materials taken from patients with nontyphoidal *Salmonella* bacteraemia and localized infections. Leading etiological agents in nontyphoidal *Salmonella* gastroenterocolitis were the serovars *Salmonella enteritidis* (82.19%), *Salmonella typhimurium* (8.90%) and *Salmonella choleraesuis* (3.14%), and in nontyphoidal *Salmonella* extraintestinal infections were *Salmonella enteritidis* and *Salmonella choleraesuis*. No causative agents of enteric fever were isolated during this study. A total of 201 nontyphoidal *Salmonella* strains manifested high susceptibility to the following three antimicrobial agents: ampicillin, TMP/SMZ, and ciprofloxacin – 83.58, 93.53 and 99.00%, respectively. Two serovars (*Salmonella enteritidis* and *Salmonella choleraesuis*) showed high susceptibility to cephalosporins 3rd generation and chloramphenicol. Multiple drug resistant strains were not isolated, as well as strains producers of extended-spectrum β -lactamases.

Key words: nontyphoidal *Salmonella*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella choleraesuis*, antimicrobial susceptibility

Salmonella-induced infections are an important medical problem in many countries all over the world [1,2]. In the last decades a trend to an increase in the relative share of nontyphoidal salmonellosis was observed at the background of a decrease in the incidence of enteric fevers. Nontyphoidal *Salmonella* (NTS) serovars are common etiological agents of bacterial enterocolitis both in the developing and in the developed countries [2-5]. They are a serious problem for patients with impaired immunity, newborn babies and infants as causative agents of severe generalized infections [1,2,5].

According to data from the Center for Disease Control in the U.S.A. (CDC, Atlanta, U.S.A.), in the last decade about 45 000 cases of nontyphoid salmonellosis have been registered, of them 400–600 with lethal exit.

Recently, a number of papers were published concerning the enhanced resistance of NTS to antibacterial agents [3,4]. Presence of extended-spectrum β -lactamases (ESBLs)

has been found, as well as multiple drug resistance (MDR) in clinical isolates from different places of the world [1,3,4,6,7].

No cases of extraintestinal infections caused by NTS have been so far reported for our country.

The aim of the present study was to determine the serotype composition, frequency of isolation and in vitro susceptibility to antimicrobial agents of *Salmonella* serovars causing intestinal and extraintestinal infections recorded at regional level in the Institute Hospital in Pleven (Bulgaria).

Materials and methods. This study involved a period of six years (1996–2001) dealing with the problem of *Salmonella* as causative agents of intestinal infections – gastroenteritis, as well as another 16-year period (1985–2001) related to the problem of *Salmonella* as causative agents of extraintestinal infections.

A total of 201 strains of *Salmonella* were isolated. Of them 191 strains were isolated from faecal specimens of patients manifesting enterocolitis syndrome (intestinal *Salmonella*-induced infections), and 10 strains were isolated from other clinical materials – blood, wound secretion, urine, cerebrospinal fluid (CSF), testicular punctate from patients with extraintestinal *Salmonella*-induced infections.

Samples from all materials collected from extraintestinal sources were used to make Gram- and methylene blue-stained smears for microscopic analysis.

All clinical materials taken from sterile parts: CSF, bloodculture, urine, wound secretion and testicular punctate were cultured on blood agar, chocolate agar and Levine medium. The faecal samples were cultured on Levine medium, apocholate-citrate agar and selenite broth.

Salmonella strains were biochemically identified with the aid of an algorithm on the basis of enzyme productivity recorded by a set of 9 tests including media for demonstration of indole production, breakdown of glucose and lactose, formation of sulphuretted hydrogen (polytropic nutrient medium of Kligler-Bader) phenyl alanine desaminase activity, urease activity, utilization of citrate as the only source of carbon (Simons medium), medium for degradation of mannitol and mobility recording, lysine and ornithine decarboxylase activity, breakdown of dulcitol.

Salmonella strains were also serologically identified by the agglutination test of Gruber type with hyperimmune agglutinating sera: polyvalent OA-OE; group-specific O, as well as with H₁ and H₂ *Salmonella* sera. The Kauffman White scheme was used to determine the serovar of these *Salmonella* strains.

Susceptibility of *Salmonella* strains to antibacterial agents was recorded by the disk-diffusion method of Bauer-Kirby [8]. The results were interpreted in accordance with the criteria published by the National Committee for Clinical Laboratory Standards (NCCLS) 2000 [8]. ESBLs production in *Salmonella* isolates from bloodcultures, CSF, punctate and urine was measured by the double-disk synergistic test (DDST) [9].

Results and discussion. Frequency of isolation and susceptibility to antimicrobial agents of 191 *Salmonella* strains isolated from stool samples of patients with enterocolitis syndrome are shown in Table 1.

Our study identified 9 serovars, causing intestinal infections (Table 1) despite the great serotype diversity of *Salmonella*. *S. enteritidis* (82.19%) was the most frequently isolated serovar followed by the second significant serovar *S. typhimurium* (8.90%) and the third – *S. choleraesuis* (3.14%).

In the period 1995–1998 at the Hospital de la Santa Creu 1 Sant Pau in Barcelona, Spain, the frequency of isolation of *Salmonella* serovars was as follows: *S. enteritidis* (46%), *S. typhimurium* (44%), *S. hadar* (5%) [10], while in the period of 1985–1987 it was 60% for *S. enteritidis* and 7% for *S. typhimurium*, respectively [10].

The results from our study revealed high susceptibility of *S. enteritidis* and *S. typhimurium* serovars to ciprofloxacin (98.72 and 100%, respectively), to nalidixic acid

T a b l e 1

Frequency of isolation and susceptibility to antimicrobial agents of 191 *Salmonella* strains isolated from patients with enterocolitis syndrome

Antimicrobial agents	No (%) of susceptible <i>Salmonella serovars</i>										No (%) of Salmonella isolates		
	Group B			Group C				Group D	Group E				
	<i>S. agona</i> <i>n</i> = 4 (2.09)	<i>S. heidelberg</i> <i>n</i> = 1 (0.52)	<i>S. typhimurium</i> <i>n</i> = 17 (8.90)	<i>S. choleraesuis</i> <i>n</i> = 6 (3.14)	<i>S. isangi</i> <i>n</i> = 3 (1.57)	<i>S. istanbul</i> <i>n</i> = 1 (0.52)	<i>S. loanda</i> <i>n</i> = 1 (0.52)	<i>S. enteritidis</i> <i>n</i> = 157 (82.19)	<i>S. anatum</i> <i>n</i> = 1 (0.52)	S	IS	R	
Ampicillin	4	1	11 (64.70)	5	3	0	1	135 (85.98)	0	160 (83.76)	2 (1.04)	2 (1.04)	29 (15.18)
Chloramphenicol	4	1	15 (88.23)	6	3	1	1	147 (93.63)	1	179 (93.71)	2 (1.04)	2 (1.04)	10 (5.23)
Ciprofloxacin	4	1	17 (100)	6	3	1	1	155 (98.72)	1	189 (98.95)	0	0	2 (1.04)
Nalidixic acid	4	1	16 (94.12)	5	3	1	1	146 (92.99)	1	178 (93.19)	2 (1.04)	2 (1.04)	11 (5.76)
TMP/SMZ	4	1	16 (94.12)	6	3	1	1	145 (92.35)	1	178 (93.19)	0	0	13 (6.80)

TMP/SMZ – trimethoprim/sulfamethoxazole; S – susceptible; IS – intermediately susceptible; R – resistant

(92.99 and 94.12%, respectively), to trimetoprim/sulfamethoxazole (TMP/SMZ) (92.35 and 94.12%, respectively). Eleven out of 17 *S. typhimurium* strains and 85.98% of *S. enteritidis* strains were susceptible to ampicillin.

Despite the high values of susceptibility to the above-mentioned antibacterial agents recorded by us, there is a world-wide tendency to an increase in the level of resistance among NTSalmonella. In Taiwan the resistance of *S. typhimurium* to tetracycline, chloramphenicol and ampicillin has been 94, 90 and 88%, respectively.

A study performed in Spain on the susceptibility of intestinal *S. typhimurium* isolates revealed MDR to ampicillin, tetracycline and chloramphenicol [10]. The same authors, however, pointed out that there is high susceptibility of *S. enteritidis* serovar to nalidixic acid (95%) and ciprofloxacin (99.7%) which coincides with the results from our investigation.

During our 16-year long study on extraintestinal Salmonella infections a total of ten Salmonella strains were isolated which belonged to both serovars: *S. enteritidis* and *S. choleraesuis* – causative agents of NTS bacteraemia and NTS localized infections (Tables 2 and 3).

Nontyphoidal Salmonella (NTS) bacteraemia. Cases Nos 1, 2, 3, 4 and 10 (Tables 2 and 3). We recorded five cases of NTS bacteraemia. In this category of seriously sick patients five Salmonella strains were isolated only from bloodcultures: four belonged to *S. enteritidis* (patients Nos 1, 2, 3, 4) and one to *S. choleraesuis* (patient No 10) (Tables 2 and 3).

Cases Nos 2, 3 and 10 were infants at ages < 2 years with clinical diagnoses: bronchopneumonia, pleuropneumonia and sepsis, respectively.

Case No 1 was a man at age of 51 years with diagnosis: sepsis.

Case No 4 was a man at age of 51 years with impaired immunity and diagnosis: status post transplantationem renum, subjected to immunosuppressive therapy.

In these five patients Salmonella strains were isolated from bloodcultures only; this fact indisputably supports the microbiological and clinical diagnosis: NTS bacteraemia.

In cases Nos 1, 2, 4 and 10 there was an extraintestinal Salmonella monoinfection manifested as NTS bacteraemia whereas in case No 3 the infection was mixed showing microbial association of *S. enteritidis* isolated from bloodculture with *Haemophilus influenzae* type *b* isolated from pleural punctate, which is a unique case.

In a ten-year long study, performed at the same time when we carried out our investigation, Israeli authors have demonstrated that NTS bacteraemia in adults was due to group D Salmonella (78%) and serovar *S. enteritidis* – 22 strains, whereas in children it was due to group C Salmonella (46%) – 15 *S. virchow* strains followed by group D Salmonella (34%) – 5 *S. enteritidis* strains [11].

NTS localized infections. In this category of seriously sick patients the cases were 5 including patients with NTS: osteomyelitis, pyelonephritis, meningitis, orchitis, infection of the urinary tract (Table 2).

NTS OSTEOMYELITIS. Case No 5, a woman at age of 58 years with diagnosis osteomyelitis sterni. In the course of 2 months the same etiological agent – *S. enteritidis*, was isolated from 5 consecutively collected clinical materials (wound secretions taken from the focus of the process – the sternum). The etiological role of *S. enteritidis* in NTS osteomyelitis (Table 2) demonstrated by us, is confirmed by studies of other authors [12].

NTS PYELONEPHRITIS. Case No 6, a woman at age of 81 years with diagnosis nephrolithiasis chronica and pyelonephritis chronica. *S. enteritidis* serovar was isolated from three consecutively collected urine samples with pyuria and microbe number 10^5 CFU/ml and on this basis it was accepted as etiological agent (Table 2). In the period of 1992–1996 American authors reported 799 NT Salmonella strains isolated from urine

Table 2
Cases of extraintestinal infections caused by *Salmonella enteritidis* strains

Category	Year	Case No	Lab. No	Patients	Sex	Age	Material	Clinical diagnosis	Type of infection
Nontyphoidal	1995	1	3650	PMS	M	51 yrs	blood (bloodculture)	sepsis	Mo
Salmonella	1997	2	2391	IIN	M	1 yr 3 m.	blood (bloodculture)	bronchopneumonia	Mo
(NTS)	1998	3	3058	SSS	M	10 mon.	blood (bloodculture)	sepsis pleuropneumonia	Mx
bacteraemia	1999	4	2400	IVK	M	51 yrs	blood (bloodculture)	sepsis status post transplantationem renum	Mo
NTS	1989	5	21646	ZhKK	F	58 yrs	wound secretion	osteomyelitis sterni	Mo
localized	1994	6	15684	NPM	F	81 yrs	urine	nephrolithiasis chronica pyelonephritis chronica	Mo
infections	1997	7	1974	RBM	M	2 mon.	cerebrospinal fluid(CSF)	enterocolitis acuta meningitis purulenta	Mo
	1999	8	2942	PIP	M	74 yrs	testicular punctate	orchiepididymitis acuta sinistra, orchiectomy sinistra	Mx
	2001	9	5755	HAM	M	70 yrs	urine	myelodysplastic syndrome, UTI	Mo

Mo – monoinfection; Mx – mixed infection; UTI urinary tract infection; M – male; F – female

Table 3
A case of extraintestinal infection caused by *Salmonella choleraesuis*

Category	Year	Case No	Lab. No	Patients	Sex	Age	Material	Clinical diagnosis	Type of infection
NTS bacteraemia	1999	10	3023	VMS	M	1 yr 2 m.	blood (bloodculture)	bronchopneumonia	Mo

samples taken from patients with infections of the urinary tract, of them 136 isolates being of serogroup D [13].

NTS MENINGITIS. Case No 7, a baby at age of 2 months with clinical diagnosis meningitis purulenta and enterocolitis acuta. In this case *S. enteritidis* was isolated only from one clinical source – CSF, and on this basis it was accepted as etiological agent of NTS meningitis (Table 2).

In the available medical literature there are no descriptions of cases of NTS meningitis caused by *S. enteritidis*. In 1997 Z. A. Bhutta in Pakistan reported a single case of meningitis in a newly-born baby caused by quinolone-resistant *S. paratyphi* B strain.

NTS ORCHITIS. Case No 8, a man at age of 74 years with diagnosis orchiepididymitis acuta sinistra, orchiectomy sinistra. Serovar *S. enteritidis* was isolated from testicular punctate which was the basis to accept it as etiological agent. *Klebsiella pneumoniae* was also isolated from the punctate of the same patient which defined the infection of a mixed type in microbial association of *S. enteritidis* and *K. pneumoniae* (Table 2). There are no cases of NTS orchitis caused by *S. enteritidis* reported so far in the available medical literature.

NTS URINARY TRACT INFECTION (UTI). Case No 9, a man at age of 70 years with diagnosis myelodysplastic syndrome (MDS) having impaired immunity. Serovar *S. enteritidis* was isolated only from two consecutively collected urine samples with pyuria and microbe number 10^5 CFU/ml which was the basis to accept it as etiological agent of UTI in this patient suffering from haematological disorders with MDS who has been subjected to haemotransfusion therapy (Table 2).

No Salmonella strains were isolated from the intestinal tracts of these 10 clinical cases of extraintestinal NTS infection. These results confirm the etiological role of *S. enteritidis* and *S. choleraesuis* in the development of Salmonella extraintestinal infections.

Table 4 shows data on the susceptibility of strains of *S. enteritidis* and *S. choleraesuis*, isolated from patients with extraintestinal infections, to 12 antibacterial agents. NT Salmonella strains isolated by us manifested high susceptibility to the strategic drugs used for management of extraintestinal Salmonella infections: ampicillin, cefalosporins 3rd generation, chloramphenicol, ciprofloxacin, TMP/SMZ (Table 4).

Nine Salmonella strains isolated from bloodcultures, CSF, punctate and urine were not found to be producers of ESBLs. With respect to *S. enteritidis*, up to now ESBLs production was registered only in one strain of this serovar isolated from bloodculture of a 2-year old girl [9].

Swedish authors recommend the application of quinolones (ciprofloxacin or norfloxacin) for treatment of salmonellosis [14]. This empirical therapy is based on the high susceptibility of NTS to ciprofloxacin which was also demonstrated by us.

Conclusion.

1. No causative agents of enteric fevers were isolated from patients treated at the Institute Hospital in Pleven for a period of 16 years.

2. Leading etiological agents in patients with enterocolitis syndrome were the serovars *S. enteritidis* (82.19%), *S. typhimurium* (8.90%) and *S. choleraesuis* (3.14%). Serovars *S. agona*, *S. isangi*, *S. heidelberg*, *S. loanda* and *S. anatum* displayed frequency of isolation < 3%, and a new for this region serovar *S. istanbul* (0.52%) was isolated. High susceptibility of the tested strains was recorded to ampicillin, nalidixic acid, TMP/SMZ, chloramphenicol, and ciprofloxacin – 83.76, 93.19, 93.19, 93.71 and 98.95%, respectively.

3. In cases of Salmonella-induced extraintestinal infections, *S. enteritidis* was the leading etiological agent of NTS: bacteraemia, meningitis, osteomyelitis, pyelonephritis, orchitis, urinary tract infection, followed by *S. choleraesuis*, causative agent of NTS bacteraemia. Both serovars manifested high susceptibility to all tested antimicrobial agents: cefalosporins 3rd generation, chloramphenicol, quinolones, TMP/SMZ.

Table 4

Frequency of isolation and susceptibility to antimicrobial agents of 10 Salmonella strains, causative agents of extraintestinal infections

Antimicrobial agents	Sources of isolation of											
	<i>S. enteritidis</i>										<i>S. choleraesuis</i>	
	bloodculture		wound secretion		urine		CSF		punctate		bloodculture	
	(n = 4)		(n = 1)		(n = 2)		(n = 1)		(n = 1)		(n = 1)	
	S	R	S	R	S	R	S	R	S	R	S	R
Ampicillin	4		1		1	1	1			1	1	
Cefazidime	4		1		2		1		1		1	
Ceftriaxone	4				2		1		1		1	
Cefotaxime	4				2		1		1		1	
Cefepime	4				1		1		1		1	
Amoxicillin/ Clavulanic acid	4				1		1		1		1	
Chloramphenicol	4		1		2		1		1		1	
Ciprofloxacin	4		1		2		1		1		1	
Ofloxacin	4				1		1		1		1	
Pefloxacin	4				1		1		1		1	
Tremetoprim/ Sulfametoxazole	4		1		2		1		1		1	
Nitroxolin	4				2							

S – susceptible; R – resistant

4. During the period of our study no MDR Salmonella strains were isolated, as well as strains producers of ESBLs.

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