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Multiple drug resistance patterns and plasmid profiles of non-typhi salmonellae in Turkey

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SUMMARY

A total of 259 clinical isolates of nonrepetitive non-typhi salmonellae (NTS) were examined for antibiotic resistance patterns and plasmid content. The antibiotics used were amoxicillin-clavulanic acid (AMC), ampicillin (AM), aztreonam (ATM), carbenicillin (CB), cefixime (CFM), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CRO), chloramphenicol (C), ciprofloxacin (CIP), gentamicin (GM), imipenem (IPM), ofloxacin (OFX), tetracycline (TE), trimethoprim-sulfomethoxazole (SXT). Multi-drug resistant (MDR) strains comprised 19·3% of the total isolates (50/259) and almost all were *S. typhimurium* (49/50). Fifteen different patterns of resistance was observed, AM/CB/C/AMC/TE and AM/CB/C/AMC/SXT/GM/CTX/CRO/CAZ/CFM/ATM being the most frequent patterns. Twenty-eight out of 50 multiresistant isolates were found to contain at least one plasmid (mean five) and the size of the plasmids ranged between 1·7 and 158 kb. Plasmid profiles of multi-resistant NTS strains were heterogenous as 21 different profiles were detected in a total of 28 plasmid-bearing isolates. No direct correlation was established between antibiotic resistance patterns and plasmid profiles.

INTRODUCTION

Non-typhoidal salmonellosis is an important public health problem in many parts of the world. The incidence of non-typhi salmonellae (NTS) has gradually increased in United States; more than 40000 culture confirmed infections are reported each year and it is known that this number represents only 1–5% of the actual incidence of NTS infections [1]. In England, over 30000 salmonellae are reported annually [2]. Particularly in developing countries, infections with NTS are of greater importance [3]. Although 2–5% of patients develop invasive infections, more severe complications may occur especially in immunocompromised hosts [4]. A general increase in the frequency of antibiotic resistant NTS isolates is another dimension of the matter [5–8].

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In Turkey, because of the lack of national reference centre, reliable statistical data on NTS are not available. The present study not only documents the antibiotic resistance and plasmid patterns among NTS in Turkey, but also constitutes the first report describing the plasmid profiles of clinical isolates of *S. typhimurium* from the country.

METHODS

A total of 259 strains of NTS were isolated from epidemiologically unrelated patients with gastroenteritis in Ankara, over a 28-month period, from September 1994 to December 1996. Thirty-two of these isolates were from blood, the rest were from diarrhoeic stools.

Cultivation of the isolates and their biochemical characterization were performed as described in

standard protocols [9]. The identification was confirmed by serogrouping and serotyping with commercial antisera (Difco Laboratories, Detroit, Michigan). Control strains were obtained from Refik Saydam Central Institute of Hygiene. The isolates were stored on tryptic soy agar (Difco) slopes at 4 °C.

Antimicrobial sensitivity was determined by the disk-diffusion method [10]. Sixteen different antibiotics included in the tests and disk contents were AM (10 μg), CB (100 μg), AMC (20:10 μg), GM (10 μg), C (30 μg), TE (30 μg), SXT (1·25:23·75 μg), ATM (30 μg), CRO (30 μg), CAZ (30 μg), CTX (30 μg), CFM (5 μg), FOX (30 μg), IPM (10 μg), OFX (5 μg), CIP (5 μg) (Difco). Sensitive and resistant strains are defined as based on reference zone diameter interpretive standards [11]. The isolates with intermediate zones of inhibition were scored as resistant and those resistant to two or more antibiotics were considered to be multi-drug resistant (MDR). All MDR strains were screened by double-disk synergy test to detect extended-spectrum β-lactamases [12].

Plasmid DNA was extracted from NTS using the method of Birnboim and Doly [13], electrophoresed on horizontal 0·7% agarose gels and stained with ethidium bromide. Plasmid molecular weights were determined by co-electrophoresis with plasmids of known molecular weight from *E. coli* V517.

RESULTS

The antibiotic susceptibilities of the NTS isolates determined by the disk-diffusion method are presented in Table 1. As can be seen, multi-drug resistance was observed in 50 strains (19·3%) and resistance to single antibiotic was recorded in three strains. All of the multi-resistant NTS had been cultured from the stool specimens. As represented by 166 out of 259 isolates (64·1%), serogroup B predominated. The other serogroups were D1 and C1 which were represented by 82 and 9 strains, respectively. Serotype determination was done for all MDR strains as well as the blood-borne isolates. This showed that almost all MDR strains (49/50) were *S. typhimurium* while only one (1/50) was *S. enteritidis*. Blood-borne strains were mostly (84·3%) identified as *S. typhimurium*, the rest belonged to serogroups D_1 or C_1.

Although 15 different multi drug resistance patterns were observed among 50 isolates, 50% of them grouped in two major patterns: AM/CB/C/AMC/TE (34%) and AM/CB/C/AMC/SXT/GM/CTX/CRO/CAZ/CFM/ATM (16%), corresponding to the groups I and II, respectively (Table 2). It is to be noted that all blood-borne strains were susceptible to all antibiotics tested. The incidence of resistance to old-generation antibiotics was higher than that to newer ones. Except two isolates, the strains were resistant to AM and CB. On the other hand, none of the isolates were resistant to IPM, OFX, CIP or FOX. Extended-spectrum β-lactamases (ESBL) were detected in 14 MDR strains and all such strains but one were susceptible to TE. In turn, of 36 ESBL-non producing strains, 31 were TE-resistant.

Table 1. *Frequency of antimicrobial resistance among 259 NTS isolates from Turkey. September 1994 to December 1996*

Antimicrobial agent	Number of isolates	Percentage of isolates
AM	49	19
CB	48	18
C	44	17
AMC	41	16
TE	32	13
SXT	21	8
GM	20	8
CTX	14	5
CRO	14	5
CAZ	13	5
CFM	13	5
ATM	13	5
FOX	0	0
IPM	0	0
OFX	0	0
CIP	0	0

The plasmids harboured by multi-resistant NTS are shown in Table 3. Twenty-eight out of 50 isolates were found to contain at least one plasmid (mean five), the plasmid profiles differed greatly among the strains when more than one plasmid was present, and 22 isolates did not harbour any plasmid DNA. The plasmids from plasmid-bearing isolates grouped into 21 different profiles. The size of the plasmids ranged between 1·7 and 158 kb. No single plasmid was common to all or even most of the strains. Yet the 98·3, 6·6, 5·4 and 2·3 kb plasmids were more prevalent than the others and harboured by 26, 24, 20 and 20% of the MDR isolates, respectively. The 5·4 kb small plasmid was mostly detected in ESBL-producing strains. Spontaneous loss of resistance to third-generation cephalosporins was detected in some ESBL-producing strains during consecutive transfers.

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Table 2. *Multi-drug resistance patterns in NTS isolates*

Multi-drug resistance pattern	Number of isolates	Percentage of multi-drug resistant isolates
I (AM, CB, C, AMC, TE)	17	34
II (AM, CB, C, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM)	8	16
III (AM, CB, C, AMC, TE, SXT)	5	10
IV (AM, CB, C, AMC, TE, SXT, GM)	4	8
V (AM, CB, C, GM, CTX, CRO, CAZ, CFM, ATM)	3	6
VI (AM, CB, C, TE)	3	6
VII* (AM, CB)	2	4
VIII (AM, CB, C, AMC, TE, SXT, GM, CTX, CRO, CAZ, CFM, ATM)	1	2
IX (AM, CB, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM)	1	2
X (AM, CB, C, AMC, SXT, GM, CTX, CRO)	1	2
XI (AM, CB, C, AMC, SXT, GM)	1	2
XII (AM, CB, AMC, TE)	1	2
XIII (AM, CB, C, AMC)	1	2
XIV (TE, GM)	1	2
XV (AM, AMC)	1	2

* This group harboured the sole *S. enteritidis* isolate.

Plasmid rearrangements were evident in such strains (data not shown).

DISCUSSION

In the present study, almost all MDR isolates were *S. typhimurium* while multi-resistance was uncommon in *S. enteritidis*. Multiple resistance has also been shown to be extremely rare in *S. enteritidis* isolates from England and Wales [6], and also from United States [5]. On the other hand, B was the predominant serogroup among our isolates and in this respect, our findings agree well with a former report on the prevalence (59%) of group B among NTS isolates from Turkey [14].

When compared to the resistance reports from some other countries [7, 8, 15], the rate of resistance has been shown to be rather low in Turkey. A previous study conducted between 1989 and 1990 revealed that the incidence of resistance of NTS to older antibiotics (AM, C, SXT and TE) was around 85% [16], a percentage significantly higher than those determined by Arman in 1993 (22–33%) [17] and also by the present investigation (8–19%). The decline in resistance to these antibiotics has been most likely due to the widespread clinical use of quinolones and third-generation cephalosporins in Turkey since 1990. NTS isolates had been susceptible to third-generation drugs before this year [16], thus the use of these drugs should have also accounted for the emergence of extended-spectrum β-lactamases producing pathogens resistant to third-generation cephalosporins (5%; Table 1). On the other hand, despite the extensive usage of quinolones, none of the isolates were found to be resistant to these antibiotics. The general decrease in resistance to antimicrobial drugs might also be explained by the ability of these drugs to eliminate R-plasmids and to inhibit conjugal transfer [18].

Plasmid profile data indicated no strict association between antibiotic resistance pattern and plasmid type although the molecular characterization of these plasmids has not yet been made. Furthermore, our finding that the loss of resistance to third-generation cephalosporins was associated with plasmid re-arrangements did not permit us to rule out the involvement of specific plasmids. Plasmid-bearing NTS strains remain to be tested for their ability to transfer the resistance determinants. The heterogeneity of the plasmid profiles is thought to reflect the sporadic and non-repetitive nature of the isolates.

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Table 3. *Plasmid profiles of multi-drug resistant non-typhi salmonellae*

Isolate (stock number)	Multi-resistance group	Plasmid profiles, size in kilobases											
5	I	—	—	62·5	—	—	—	—	—	—	—	—	—
10	I	—	—	—	—	—	—	—	—	—	—	—	—
12	I	—	—	—	—	—	—	—	—	—	—	—	—
13	I	—	—	—	—	—	—	—	—	—	—	—	—
15	I	—	—	—	—	—	—	—	—	—	—	—	—
23	I	—	98·3	—	—	—	—	—	—	—	—	—	—
24	I	—	—	—	—	—	—	—	—	—	—	—	—
25	I	—	—	—	—	—	—	—	—	—	—	—	—
26	I	—	98·3	—	—	—	—	—	—	—	—	—	—
27	I	—	98·3	—	—	—	—	—	—	—	—	—	—
36	I	—	—	—	—	—	—	—	—	—	—	—	—
40	I	—	—	—	—	—	—	—	—	—	—	—	—
44	I	158	—	—	—	—	—	—	—	—	—	—	—
45	I	—	—	—	—	—	—	—	—	—	—	—	—
52	I	—	—	—	—	—	—	—	—	—	—	—	—
54	I	—	—	—	—	—	—	—	—	—	—	—	—
55	I	—	—	—	—	—	—	—	—	—	—	—	—
4	II	—	98·3	62·5	31·6	—	—	6·6	5·4	4·1	3·0	2·3	—
6	II	—	—	—	—	—	—	—	—	—	3·8	—	—
7	II	—	98·3	—	—	—	—	6·6	5·4	—	3·0	2·3	—
18	II	—	98·3	—	—	—	—	6·6	5·4	—	3·0	2·3	—
19	II	—	—	—	—	—	—	—	5·4	—	3·8	2·5	—
42	II	—	98·3	62·5	—	—	—	—	5·4	—	3·8; 3·4	2·5	—
47	II	158	—	—	—	—	—	6·6	5·4	—	3·4	2·3	—
50	II	—	—	—	—	—	—	—	5·4	4·5	3·4	2·3	—
29	III	—	—	—	—	—	—	—	—	—	—	—	—
30	III	—	—	62·5	—	—	—	—	—	—	—	—	—
38	III	—	—	—	—	—	—	—	—	—	—	—	—
39	III	—	—	—	—	—	—	—	—	—	—	—	—
46	III	158	—	—	—	—	—	—	—	—	—	—	—
3	IV	158	—	—	—	—	7·7	—	5·2	—	—	2·5	1·7
21	IV	—	98·3	51·6	—	—	7·7	—	5·4	—	3·0	—	—
48	IV	158	—	—	—	—	—	6·6	5·2	4·5	3·4	2·3	—
49	IV	—	98·3	—	—	—	—	—	5·2	—	3·4	2·3	—
8	V	—	—	—	—	—	—	6·6	—	4·8	—	2·3	1·7
9	V	—	98·3	—	—	—	—	6·6	—	4·8	—	2·3	1·7
43	V	158	—	—	—	—	—	6·6	5·4	—	3·4	2·5	—
14	VI	—	—	—	—	—	—	—	—	—	—	—	—
17	VI	—	—	—	—	—	—	—	—	—	—	—	—
20	VI	—	—	—	—	—	—	—	—	—	—	—	—
16	VII	—	98·3	—	35·5	12·6	7·7; 7·2	6·6	—	—	—	—	—
41*	VII	—	—	—	—	—	—	—	—	—	—	—	—
22	VIII	—	98·3	51·6	—	—	7·7	—	5·4	—	3·0	—	—
53	IX	125·8	79·4	50·1	—	15·8	—	6·6	—	—	—	2·3	—
1	X	—	—	—	—	—	7·7	6·6	5·2	—	—	—	—
2	XI	—	98·3	—	—	—	7·7	6·6	5·2	—	—	2·5	1·7
35	XII	—	—	—	—	—	—	—	—	—	—	—	—
37	XIII	—	—	—	—	—	—	—	—	—	—	—	—
11	XIV	—	—	—	—	—	—	—	—	—	—	—	—
34	XV	—	—	—	—	—	—	—	—	—	3·4	2·5	—

* Identified as *S. enteritidis*.

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