Turk J Med Sci
32 (2002) 483-486


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# Colicinogeny, Lipopolysaccharide and Outer Membrane Protein Profiles of Multi-Drug Resistant *Salmonella typhimurium* Isolates from Turkey

Received: June 12, 2001

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**Abstract:** A total of 259 clinical isolates of non-repetitive non-typhi salmonellae (NTS) were previously examined for their antibiotic resistance patterns and plasmid contents. Multi-drug resistant strains comprised 19.3% (50/259) of the isolates and almost all were *Salmonella typhimurium*. In the present study, 35 of these multi-drug resistant *S. typhimurium* isolates were further characterized for colicinogeny, lipopolysaccharide (LPS) and outer membrane protein (OMP) profiles. Fourteen of the 35 (40%) isolates were found to be colicin producers. All isolates showed smooth-type LPS. In 26 of the (74.3%) isolates, OMPs of 30.6 and 34.6 kDa were observed whereas three (8.6%) of them were found to carry only a 30.6 kDa protein and six (17.1%) carried 27.2, 30.6 and 34.6 kDa proteins. There was no direct correlation between the plasmid contents, antibiotic resistance patterns, colicinogeny, LPS and OMP profiles of the isolates.

**Key Words:** *Salmonella*, Colicin production, LPS, OMP, plasmid

## Introduction

*Salmonella* infections, which constitute a major public health problem, are the result of transmission from foods of animal origin, especially poultry and poultry products and raw eggs (1). *S. typhimurium* is one of the most common serotypes among *Salmonella* spp. isolated worldwide (2).

The relatedness of *Salmonella* isolates has been evaluated by several methods. These methods include antibiotic susceptibility testing (2,3) colicin typing (4), phage typing (5) and genetic characterizations such as plasmid profile analysis (6) and DNA fingerprint analysis (7). In addition, outer membrane protein (8,9) and lipopolysaccharide profile (10) analyses have proved to be useful techniques in the characterization of these bacteria.

Our former studies with a total of 256 clinical isolates of non-typhi salmonellae from Turkey involved the determination of antibiotic resistance patterns and plasmid contents. The 35 clinical isolates of *S. typhimurium* used in the present study were found to be multi-drug resistant, 21 of these harboring at least one plasmid with sizes ranging between 1.7 and 158 kb (11). We now report on the further characterization of these isolates for their colicin production, lipopolysaccharide (LPS) profiles and outer membrane protein (OMP) profiles.

## Materials and Methods

A total of 35 strains of *S. typhimurium* were isolated from epidemiologically unrelated patients with gastroenteritis in Ankara. Cultures were maintained as frozen stocks in Luria broth supplemented with 20% glycerol at –70 ºC.

Colicin production was detected by using the chloroform-overlaying method (12). The colicin-sensitive test strain was *E. coli* K12. Preparation of LPS was performed by employing the proteinase-K digestion method (13). The OMPs were isolated as in Achtman *et al.* (14). The OMP and LPS contents of *S. typhimurium* isolates were analyzed with a discontinuous SDS-PAGE system (15). For OMP, a 10% separating gel and a 4% stacking gel containing 4 M urea were used whereas a 14% separating gel and a 4% stacking gel were used for LPS. The molecular weights of the OMPs were estimated from calibration curves prepared by using molecular weight standards. The OMP gels were stained with Coomassie Blue (8) and the LPS gels were silver-stained by the method of Tsai and Frasch (16).

## Results and Discussion

The colicinogeny, OMP profiles and LPS profiles of *S. typhimurium* isolates are shown in the Table. Antibiotic resistance patterns and plasmid profiles previously documented by Yıldırmak *et al.* (11) are also included in this table. Fourteen of the 35 isolates (40%) were found to be colicin producers. In the study of Martini *et al.* (17), 45 epidemiologically related *S. typhimurium* PT141 isolates from Rome were all colicinogenic. However, none of the eight *S. typhimurium* and eight *S. dublin* isolates examined by Brockelsberg *et al.* (18) were colicin producers. Considering the sporadic nature of our isolates (11), the finding that colicin production is not a common characteristic was not surprising. Colicins are encoded by both conjugative and non-conjugative plasmids of *Salmonella* (19). *S. typhimurium* contained an

Table. Plasmid patterns, LPS and OMP profiles and colicinogeny of multi-drug resistant *S. typhimurium isolates*.

| Strain | Colicin | LPS [c] | Antibiotic Resistance Group [a, b] | Plasmid Profiles (kb) [a] | OMP (kDa) |
|---|---|---|---|---|---|
| 3 | - | S | AMP, CAR, CHL, AMC, TET, SXT, GEN | 158; 77; 5.2; 2.5; 1.7 | 30.6; 34.6 |
| 34 | - | S | AMP, AMC | 3.4; 2.5 | 30.6; 34.6 |
| 16 | + | S | AMP, CAR | 98.3; 35.5; 12.6; 7.7; 7.2; 6.6 | 30.6; 34.6 |
| 53 | + | S | AMP, CAR, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 125.8; 79.4; 50.1; 15.8; 6.6; 2.3 | 30.6; 34.6 |
| 35 | - | S | AMP, CAR, AMC, TET | | 30,6 |
| 37 | - | S | AMP, CAR, CHL, AMC | | 30.6; 34.6 |
| 6 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 3,8 | 30.6; 34.6 |
| 47 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 158; 6.6; 5.4; 3.4; 2.3 | 30.6; 34.6 |
| 19 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 5.4; 3.8; 2.5 | 30.6; 34.6 |
| 50 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 5.4; 4.5; 3.4; 2.3 | 30.6; 34.6 |
| 7 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 98.3; 6.6; 5.4; 3.0; 2.3 | 30.6; 34.6 |
| 18 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 98.3; 6.6; 5.4; 3.0; 2.3 | 30.6; 34.6 |
| 4 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 98.3; 62.5; 31.6; 6.6; 5.4; 4.1; 3.0; 2.3 | 30.6; 34.6 |
| 42 | + | S | AMP, CAR, CHL, AMC, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 98.3; 62.5; 5.4; 3.8; 3.4; 2.5 | 30.6; 34.6 |
| 40 | - | S | AMP, CAR, CHL, AMC, TET | | 30,6 |
| 23 | - | S | AMP, CAR, CHL, AMC, TET | 98.3 | 27.2; 30.6; 34.6 |
| 26 | - | S | AMP, CAR, CHL, AMC, TET | 98.3 | 27.2; 30.6; 34.6 |
| 27 | - | S | AMP, CAR, CHL, AMC, TET | 98.3 | 27.2; 30.6; 34.6 |
| 24 | - | S | AMP, CAR, CHL, AMC, TET | | 27.2; 30.6; 34.6 |
| 25 | - | S | AMP, CAR, CHL, AMC, TET | | 27.2; 30.6; 34.6 |
| 10 | - | S | AMP, CAR, CHL, AMC, TET | | 30.6; 34.6 |
| 12 | - | S | AMP, CAR, CHL, AMC, TET | | 30.6; 34.6 |
| 13 | - | S | AMP, CAR, CHL, AMC, TET | | 30.6; 34.6 |
| 36 | - | S | AMP, CAR, CHL, AMC, TET | | 30.6; 34.6 |
| 29 | - | S | AMP, CAR, CHL, AMC, TET, SXT | | 27.2; 30.6; 34.6 |
| 30 | - | S | AMP, CAR, CHL, AMC, TET, SXT | 62.5 | 30.6; 34.6 |
| 38 | - | S | AMP, CAR, CHL, AMC, TET, SXT | | 30.6; 34.6 |
| 21 | - | S | AMP, CAR, CHL, AMC, TET, SXT, GM | 98.3; 51.6; 7.7; 5.4; 3.0 | 30.6; 34.6 |
| 22 | + | S | AMP, CAR, CHL, AMC, TET, SXT, GM, CTX, CRO, CAZ, CFM, ATM | 98.3; 51.6; 7.7; 5.4; 3.0 | 30.6; 34.6 |
| 8 | + | S | AMP, CAR, CHL, GM, CTX, CRO, CAZ, CFM, ATM | 6.6; 4.8; 2.3; 1.7 | 30,6 |
| 43 | + | S | AMP, CAR, CHL, GM, CTX, CRO, CAZ, CFM, ATM | 158; 6.6; 5.4; 3.4; 2.5 | 30.6; 34.6 |
| 9 | + | S | AMP, CAR, CHL, GM, CTX, CRO, CAZ, CFM, ATM | 98.3; 6.6; 4.8; 2.3; 1.7 | 30.6; 34.6 |
| 14 | - | S | AMP, CAR, CHL, TET | | 30.6; 34.6 |
| 17 | - | S | AMP, CAR, CHL, TET | | 30.6; 34.6 |
| 20 | - | S | AMP, CAR, CHL, TET | | 30.6; 34.6 |

[a] from Yildirmak *et al.* (1998)

[b] AMP, ampicillin (10 µg); AMC, amoxicillin-clavulinic acid (20:10 µg); ATM, aztreonam (30 µg); CAR, carbenicillin (100 µg); CFM, cefixime (5 µg); CTX, cefotaxime (30 µg); CAZ, ceftazidime (30 µg), CRO, cefriaxone (30 µg); CHL, chloramphenicol (30 µg); GEN, gentamicin (10 µg); TET, tetracycline (30 µg); SXT, trimethoprim-sulfomethoxazole (1,25: 23,75 µg)

[c] S, smooth type LPS

autotransferable large plasmid of 60 MDa that encoded for resistance to three antibiotics and colicin production (17). In another study, it was observed that colicin producing *S. wien* strains have lost the ability to produce colicin upon curing (20). In the present study, all of the colicin producing isolates harbored at least one plasmid (one to eight). However, no direct correlation between colicinogeny and plasmid complements could be established because of the general diversity of plasmid patterns among colicin producers as well as the presence of some non-producer strains with the plasmids of the same size with those of producer strains (15/21). Colicin typing cannot be used in the subdivision of serovars of *Salmonella*, but it is a supplementary method in epidemiological analyses (19). Colicinogenic activity is also considered to be an important factor in the establishment of intestinal infection (17).

LPS analysis was performed using SDS-PAGE, in which the lipopolysaccharides are separated into polysaccharide chains of different lengths to produce a ladder pattern (13,19). Expression of long-chain LPS has been shown to be the major virulence factor (21,22,23). Chart *et al.* (21,22) reported that strains of *S. enteritidis* belonging to phage type 4 that have long-chain LPS and carry a plasmid of 38 MDa were highly virulent for BALB/c mice, and it was revealed that the loss of this plasmid or the inability to express LPS resulted in a million-fold decrease in virulence for mice. In contrast, Gulig and Curtiss (24) have shown that *S. typhimurium* strains possessed complete LPS upon curing. In the present study, all of the isolates (100%) were found to express long-chain LPS giving a characteristic ladder pattern (smooth). It was also shown that the smooth LPS profile of *S. typhimurium* strains was not affected by the presence or absence of plasmids since all of the (40%) plasmid-free isolates expressed long-chain LPS.

The characteristic migration patterns of OMPs during SDS-PAGE have been used for subdividing *Salmonella* serotypes (25) and *S. enteritidis* (26). In the present study, *S. typhimurium* isolates contained OMPs with the molecular masses ranging from 27.2 kDa to 34.6 kDa. This contradicts the report of Helmuth *et al.* (25) who found that *S. typhimurium* strains generally contained OMPs of 37 and 40 and 41.7 kDa. On the other hand, Chart and Rowe (27) demonstrated three major OMPs of 33, 35 and 36 kDa in three *S. enteritidis* strains and one *S. typhimurium* strain. The present work revealed that the majority of *S. typhimurium* isolates (74.3%) contained two OMPs of 30.6 and 34.6 kDa. Six isolates (17.1%) carried three OMPs of 27.2, 30.6 and 34.6 kDa, and three isolates (8.6%) contained only a 30.6 kDa OMP. When OMP profiles were compared to the plasmid profiles of the isolates, there was no direct correlation found. Three plasmid-free and three 98.3 kb plasmid-bearing isolates showed OMPs of 27.2, 30.6 and 34.6 kDa. Two plasmid-free isolates and one 6.6, 4.8, 2.3 and 1.7 kb plasmid-harboring isolate carried only 30.6 kDa OMP. The rest of the plasmid-harboring strains showed OMPs of 30.6 and 34.6 kDa. Of 26 strains with the same OMP profiles of 30.6 and 34.6 kDa, 13 were colicin non-producers and nine of these 13 isolates were plasmid-free. On the other hand, 13 plasmid-containing strains belonging to this OMP group were colicin producers. All of the six isolates containing 27.2, 30.6 and 34.6 kDa OMPs were colicin non-producers, and three of them did not harbour any plasmids. The OMP profile group of 30.6 kDa, on the other hand, was represented by two plasmid-free and non-colicinogenic strains as well as one plasmid-harboring, colicin-producing strain.

In conclusion, our data indicated no direct association between antibiotic resistance patterns, plasmid types, OMPs, LPSs, or colicinogeny of *S. typhimurium* isolates from Turkey.

**Acknowledgment**

This work was financially supported by Middle East Technical University Research Foundation (Project No: AFP-99-06-02-28).

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