Acta Microbiologica et Immunologica Hungarica, 49 (4), pp. 433–444 (2002)

SEROTYPES, VIRULENCE FACTORS, ANTIBIOTIC SENSITIVITY, BETA-LACTAMASE ACTIVITY AND PLASMID ANALYSIS OF *SALMONELLA* FROM CHILDREN WITH DIARRHEA IN TRIPOLI (LIBYA)

A. EL-GHODBAN[1], K. S. GHENGHESH[2*], K. MÁRIALIGETI[1] AND S. ABEID[2]

[1]Department of Microbiology, Eötvös Loránd University, Pázmány Péter sétány 1/c, H–1117 Budapest, Hungary, [2]Department of Medical Microbiology, Faculty of Medicine, Al-Fateh University of Medical Sciences, P.O. Box 80013, Tripoli, Libya

(Received: 30 June 2000; accepted: 4 October 2000)

A total of 21 *Salmonella* strains isolated in Libya (16 from children with diarrhea and 5 from healthy controls) were serotyped and studied for their cell invasive ability, production of cytotoxin, antibiotic susceptibility, β-lactamase activity and plasmid profiles. Eight different serotypes of *Salmonella* were identified: 6 *S. saintpaul*, 4 *S. wien* (1 from control), 2 *S. newport*, 2 *S. muenchen* (1 from control), 2 *S. typhimurium* (1 from control), 2 *S. hadar* (1 from control), 2 *S. reading* (1 from control), 1 *S. kottbus*. Twenty (95%) were positive in the invasiveness assay using HeLa cells, and all (100%) were negative for cytotoxin production in HT29 cells. More than 40% were resistant to ampicillin, cefalexin, cefamandole, cefoperazone, chloramphenicol, gentamicin, mezlocillin and trimethoprim-sulphamethoxazole and 100% were susceptible to the new quinolones. Most (67%) of the strains harbored plasmids and 43% produced β-lactamase. A strong association was observed between the presence of more than one plasmid, β-lactamase activity, and multiple-resistance to antimicrobial agents and serotypes *S. saintpaul* and *S. wien*. Curing experiments with acridine orange showed that 2 plasmids (33 and 1.4 megadaltons) might be responsible for the resistance to chloramphenicol and gentamicin. The present study demonstrated that multiple-resistant salmonellae are widespread in Libya and the resistance is mainly plasmid mediated.

Keywords: *Salmonella*, virulence factors, antibiotic sensitivity

*Corresponding author

1217-8950/2002/$ 5.00

Introduction

Non-typhoid salmonellae are major causes of diarrhea in children and pose important public health problem all over the world [7, 34, 37, 40, 41, 46, 48]. In the animal model, mucosal invasion is a necessary factor for the intestinal fluid loss in salmonellosis [14]. The use of mammalian tissue culture assays has facilitated the study of invasiveness of *Salmonella* [15, 21, 23, 28]. In addition to cholera-like enterotoxin, *Salmonella* species have been shown to produce a cytotoxin that inhibits protein synthesis [6, 27]. However, the role of these toxins in salmonellosis is not conclusive [26]. In the 70s *Salmonella* species were the major bacterial agents of diarrhea in Libya and the neighboring countries, with serotypes *S. wien* and *S. muenchen* predominating [10]. Since then few studies were carried out on the role of these organisms in diarrhea in the North African region. Furthermore, reports on the virulence factors of non-typhoid salmonellae from this region are rare if not lacking. The aims of the present study were to determine the serotypes of non-typhoid salmonellae involved in children diarrhea in Tripoli area, their determinants of pathogenecity, susceptibility to antimicrobial agents, ability to produce β-lactamase and plasmid profiles.

Materials and methods

Strains: Included in the present study were 16 *Salmonella* strains isolated from Libyan children with diarrhea aged between a few days and 3 years and 5 *Salmonella* strains from age- and sex-matched children without diarrhea (controls).

Serotyping: The strains were serotyped with antisera to 0- and H-antigens (Welcome Diagnostics, UK) as described previously [11].

Invasion assay: *Salmonella* strains were inoculated into LB broth (Difco Laboratories, USA) and incubated at 37 °C for 24 hours. Cultures were then diluted and tested for their ability to invade monolayer of HeLa cells in microplates according to the method of Giannella et al. [15]. Cytotoxicity of strains was also recorded by microscopic control of invasiveness.

Cytotoxicity assay: Strains of *Salmonella* were inoculated into LB broth and incubated at 37 °C for 24 hours. Cultures were filtered through 0.45 μm membrane filters (Sartorius, Germany) and the sterile filtrates, after dilution, were tested for cytotoxic activity in monolayer of HT29 cells in microplates. The methods described previously [43] were otherwise adapted.

The invasion and cytotoxicity assay results were read after 8 and 24 hours, all tests were made in duplicates including both positive and negative controls.

Antibiotic susceptibility: *Salmonella* strains were tested for their susceptibility to antimicrobial agents in vitro by the method of Bauer and Kirby [2]. The following antibiotics (Oxoid, UK) were used: amikacin, ampicillin, cefalexin, cefamandole, cefoperazone, chloramphenicol, ciprofloxacin, gentamicin, mezlocillin, netilmicin, ofloxacin, pefloxacin, polymyxin, tetracycline and trimethoprim-sulphamethoxazole.

β-lactamase production: β-lactamase was detected by the iodometric method [42].

Plasmid analysis: The method of Birnboim and Doly [5] was applied. *Escherichia coli* strain V517 [32] was used as a standard for determining molecular sizes of plasmids.

Plasmid curing (elimination): acridine orange was used as described previously [8].

Results

Serotyping: Eight different serotypes of *Salmonella* were identified as follows: 6 *S. saintpaul*, 4 *S. wien* (1 from control), 2 *S. newport*, 2 *S. muenchen* (1 from control), 2 *S. typhimurium* (1 from control), 2 *S. hadar* (1 from control), 2 *S. reading* (1 from control), 1 *S. kottbus* (Table I).

Invasiveness: The *Salmonella* strains were tested with HeLa cells and results were read after 8 and 24 hours incubation at 37 °C. Of the 16 *Salmonella* strains from children with diarrhea 11 (69%) invaded HeLa cells after 8 hours incubation, while all (100%) strains were positive in this assay after 24 hours incubation. Of the 5 *Salmonella* strains from controls 2 (40%) and 4 (80%) were invasive to HeLa cells after 8 and 24 hours incubation, respectively. The results of the invasiveness assay are shown in Table I.

Cytotoxin production: All of the 21 *Salmonella* strains proved to be negative (Table II).

Antibiotic resistance: Of the 21 *Salmonella* strains 52% were resistant to ampicillin, chloramphenicol and mezlocillin, 48% to cefoperazone and trimethoprim-sulphamethoxazole, 43% to cefalexin, cefamandole and gentamicin and 14% to tetracycline. All isolates were susceptible to amikacin, ciprofloxacin, nalidixic acid, netilmicin, ofloxacin and pefloxacin.

Of the 16 *Salmonella* strains isolated from children with diarrhea 11 (69%) showed resistance to at least one antibiotic against the 15 tested ones (Table II). Ten (91%) of these 11 strains were resistant to four or more antimicrobials. The remaining strain was resistant to tetracycline only. On the other hand, of the 5 *Salmonella* strains isolated from controls 2 (40%) were resistant, one of these 2 strains was multiple-

resistant and the other was resistant only to tetracycline. Of the 11 (10 from children with diarrhea) multiple-resistant isolates, 5 were *S. saintpaul* and 4 were *S. wien* serotypes. According to their antibiotic susceptibility, the *Salmonella* strains tested can be divided into 5 resistance profiles as shown in Table II. As can be seen from this table, profile I includes only *S. saintpaul* and *S. wien* isolates.

Table I

Serotypes, invasiveness and cytotoxin production of Salmonella strains isolated in Tripoli, Libya

Strain No.	Serotype	Invasiveness*	Production of cytotoxin*
19A†	*S. kottbus*	+	–
24A	*S. saintpaul*	+	–
27A	*S. saintpaul*	+	–
32A	*S. muenchen*	+	–
34A	*S. wien*	+	–
36A	*S. saintpaul*	+	–
59A	*S. saintpaul*	+	–
64A	*S. saintpaul*	+	–
70A	*S. wien*	+	–
73A	*S. saintpaul*	+	–
100A	*S. reading*	+	–
108A	*S. wien*	+	–
124A	*S. newport*	+	–
144A	*S. newport*	+	–
147A	*S. typhimurium*	+	–
165A	*S. hadar*	+	–
10B‡	*S. typhimurium*	+	–
30B	*S. wien*	+	–
128B	*S. muenchen*	+	–
143B	*S. muenchen*	–	–
165B	*S. hadar*	+	–

*Results taken after 24 hours of incubation.
†A=from children with diarrhea.
‡B=from controls.

β-lactamase production: Eight (50%) of the 16 salmonellae isolated from diarrheic children and 1 (20%) of 5 salmonellae from controls produced β-lactamase. Production of β-lactamase was detected in 9 (82%) of the 11 multiple-resistant strains

(10 from children with diarrhea) and in none of the strains that were susceptible to all antimicrobial agents tested or only to tetracycline (Table II).

Table II

Antibiotic resistance pattern and β–lactamase activity in Salmonella strains isolated in Tripoli, Libya

Strain	Resistance profile	Antimicrobial resistance								β-l
S. saintpaul 36A†	I	Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts*	+
S. saintpaul 59A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. wien 30B‡		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. saintpaul 24A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	-
S. saintpaul 64A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. saintpaul 73A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. wien 34A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. wien 70A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. wien 108A		Ap	Gm	Cm	Cl	Cd	Cp	Mc	Ts	+
S. muenchen 32A	II	Ap	Cm	Mc	Tc	Ts				+
S. typhimurium 147A	III	Ap	Cm	Mc	Cp					–
S. hadar 165A	IV	Tc								–
S. hadar 165B		Tc								–
S. kottbus 19A	V	Susceptible**								–
S. saintpaul 27A		Susceptible								–
S. reading 100A		Susceptible								–
S. newport 124A		Susceptible								–
S. newport 144A		Susceptible								–
S. typhimurium 10B		Susceptible								–
S. muenchen 128B		Susceptible								–
S. reading 143B		Susceptible								–

†A=Isolated from children with diarrhea.
‡B=Isolated from children without diarrhea (control).
*Abbreviations: Ap=ampicillin, Gm=gentamicin, Cm=chloramphenicol, CI=cefalexin, Cd=cefamandole, Cp=cefoperazone, Mc=mezlocillin, Tc=tetracycline, Ts=trimethoprim-sulfamethoxazole, β-l=production of β-lactamase
**Susceptible to all antimicrobials tested in the present study.

Plasmid profiles: Of the 16 *Salmonella* strains from diarrheic children 12 (75%) harbored plasmids. On the other hand, only 2 (40%) strains from controls did so. The number of plasmids varied from 1 to 9 per strain. The molecular weights of plasmid

DNA ranged from 1.2 to 80 megadaltons. Results of plasmid analysis are shown in Table III.

Table III

Plasmid profiles of Salmonella strains isolated in Tripoli, Libya

Strain*	Plasmid profile (plasmid size in megadaltons)												
S. saintpaul 24A†	33			3.7			2.5		2.1	1.8	1.5		
S. saintpaul 36A					3.3		2.5			1.8	1.5		1.2
S. saintpaul 59A					3.3		2.5		2.1	1.8	1.5		
S. saintpaul 64A						2.8	2.5		2.1	1.8	1.5		
S. saintpaul 73A						2.8	2.5		2.1	1.8	1.5		
S. saintpaul 27A	21.5												
S. wien 34A	33				3.3	2.8	2.5		2.1	1.8	1.5	1.4	1.2
S. wien 70A	41					2.8	2.5		2.1	1.8	1.5	1.4	1.2
S. wien 108A				3.7			2.5		2.1	1.8	1.5		
S. wien 30B‡	33				3.3	2.8	2.5			1.8	1.5		
S. muenchen 32A	80	5.5	4.1		3.3	2.8	2.5	2.3	2.1				
S. muenchen 128B		5.5			3.3				2.1		1.5		
S. reading 100A	67												
S. newport 124A					3.3			2.3	2.1				

*In strains *S. typhimurium* 147A and 10B, *S. hadar* 16A and 165B, *S. reading* 143B, *S. newport* 144A, and *S. cottbus* 16A no plasmids were detected.
†A=Isolated from children with diarrhea.
‡B=Isolated from children without diarrhea (control).

Of the 11 *Salmonella* strains that showed multiple-resistance to antimicrobial agents 10 (91%) harbored more than one plasmid. On the other hand, out of the 10 strains that were resistant to one drug only or to none at all, 3 (30%) harbored more than one plasmid. With exception of strain *Salmonella reading* 100A, plasmids with molecular weights more than 30 megadaltons were detected in multiple-drug resistant isolates only.

Of the 14 *Salmonella* strains (2 from controls) that harbored plasmids, only two showed identical plasmid profiles. These two strains were *S. saintpaul* 64A and *S. saintpaul* 73A and both were resistant to the same set of antibiotics (Table II). Also 2 other strains (*S. hadar* 165A and *S. hadar* 165B), that harbored no plasmids, were susceptible to all antimicrobials tested. With exception of *S. saintpaul* 27A, all *S. saintpaul* and *S. wien* strains shared at least 3 plasmids similar in size (i.e. 2.5, 1.8, and

1.5 megadaltons). In addition, both *S. muenchen* strains shared a plasmid with approximately 5.5 megadaltons. This plasmid was found in these two isolates only.

Plasmid curing: Plasmid curing experiments were carried out with *S. wien* 34A isolated from a child with diarrhea. This strain possessed 9 plasmids (Table III) with molecular weights ranging from 33 to 1.2 megadaltons. The curing experiments were repeated several times. Individual colonies were selected at the end of the experiments and tested for loss of plasmids. From *S. wien* 34A two derivatives were obtained that lost plasmids after treatment with acridine orange. In one culture the following plasmids were eliminated; 33, 2.8, and 1.4 megadaltons and in the other, plasmids 33, 2.1 and 1.4.

Susceptibility of the cured derivatives of S. wien 34A to antibiotics: The 2 cured strains mentioned above were tested for their susceptibility to the same antibiotics that were used before curing. Only resistance to chloramphenicol and gentamicin was eliminated from both strains regardless of the slight difference in plasmids eliminated.

Discussion

Serotyping of our *Salmonella* strains revealed the predominance of serotype *S. saintpaul* and *S. wien* (29% and 19% of the strains, respectively). Although eight different serotypes of *Salmonella* were detected in the present study, serotype *S. enteritidis* was not included. In the last decade *S. enteritidis* has been reported as the most common serotype in non-typhoid salmonellae from humans from different parts of the world [45]. This increase is believed to be due to mishandling of raw or undercooked eggs before ingestion [4, 36] and a strong association has been found between *S. enteritidis* outbreaks and consumption of food containing raw whole-shell eggs [36], a practice that is not common in Libya. These differences in feeding habits may explain the absence of serotype *S. enteritidis* among our *Salmonella* strains. Furthermore, it is interesting to note that *S. kottbus* has never been reported before from Libya.

Of the virulence factors reported for *Salmonella*, invasion of the gastrointestinal mucosa is an essential step in the pathogenesis of these organisms [14]. HeLa cells in vitro are used as a convenient model for assessing strains for invasive potential [15]. It has been suggested that gene products of a 60 megadalton plasmid are necessary for invasion of epithelial cells by *S. typhimurium* [22], but others reported that this plasmid is probably more closely associated with serum resistance and survival within macrophages [16, 19]. Only 6 of the 20 *Salmonella* strains that were invasive in HeLa harbored a large plasmid (>33 megadaltons). This is in line with other studies, which

indicate that the invasive phenotype is encoded by chromosomal genes in *Salmonella* species, but this marker has not been mapped [17].

In the early seventies Giannella *et al.* [15] were the first to report that some strains of *S. typhimurium*, that invaded HeLa cells, exhibited the additional property of cytotoxicity to the HeLa cell monolayer after invasion. Although 95% of our *Salmonella* strains invaded HeLa cells, none were cytotoxic to HeLa cell monolayer after 24 hours incubation. Furthermore, no cytotoxicity was observed using broth culture supernatants of our strains and HT 29 cells. In contrast to this finding, others reported the production of cytotoxin by *Salmonella* species in tissue culture [1]. These differences may be due to different methods used and to the difference in the serotypes of salmonellae studied. In the light of the studies by Wallis *et al.* [47] enterotoxins have a possible role in salmonellosis) too. Unfortunately, we had no chance to test culture extracts in RILT (rabbit ileal-loop test) [14]. It is worth mentioning that Ketyi *et al.* [25] found heat-labile (LT) and heat-stable (ST) enterotoxin production in a *S. enteritidis*, however in the same assay serotypes *S. kapemba* and *S. thompson* were LT and ST negative, but exhibited cytotoxic effect using AV-3 and CHO cell-lines.

There is a rapid rise in multidrug-resistant strains of *Salmonella* species responsible for both typhoid and non-typhoid salmonellosis all over the world. Multiple-drug resistance (resistance to 4 or more antibiotics) was observed in more than 50% of *Salmonella* strains studied. Although the rates of resistance, obtained in the present work, to the commonly used antimicrobial agents were very high compared with those reported from the Middle East and Europe [33, 35, 39], they are not as high as those reported previously from Tripoli [10]. This divergent finding may be due to the difference in the serotypes included in the present study and those included in the above-mentioned ones. The high rate of resistance to the commonly used antimicrobials tested is probably the result of the misuse of these drugs in Libya where unrestricted use of over-the-counter drugs without medical supervision is common. In addition, the use of antibiotics in animal fodder may play a role in the spread of these organisms [7, 20, 45].

It has been suggested that new quinolones may be the first effective treatment for *Salmonella* enteritis [9, 29, 30, 38]. All the Libyan *Salmonella* strains were sensitive to the new quinolones, i.e. ciprofloxacin, ofloxacin and pefloxacin. However, unless some degree of regulation of antimicrobial use is implemented, this may not be for long as these drugs were only introduced into the country in the last two years. Rivera *et al.* [39] detected β-lactamase activity in most of their beta-lactam resistant *Salmonella* strains and found that this activity is mainly plasmid encoded. Similar to their finding we observed a strong association between multiple-drug resistant strains and β-lactamase production. Furthermore, most (67%) of our strains harbored

plasmids. A strong association was also found between the presence of more than one plasmid and multiple-resistance to antimicrobial agents. Cefoperazone resistance in *S. typhimurium* has been bound to a CTX-M-type extended spectrum β-lactamase encoded by 12 Kb plasmids in twelve strains isolated from cases of gastroenteritis during 1996 to 1998 in Greece, Hungary and Russia [44] .Our cefoperazone resistant *S. typhimurium* strain, did not harbor detectable plasmids. Plasmid curing experiments, carried out in the present study with *S. wien* 34A, support the role played by plasmids in multiple-drug resistance. Antibiotic susceptibility testing of two derivatives from this strain showed that two plasmids of 33 and 1.4 megadaltons might be responsible for the resistance of *S. wien* 34A to chloramphenicol and gentamicin.

Plasmid profile analysis has proven to be a useful epidemiological tool in outbreaks of salmonellosis caused by different *Salmonella* serotypes [3, 18]. Studies have shown that plasmid profiles are most useful when they are combined with other methods for screening or serotyping [13, 23]. In the present study, analysis of plasmid profiles of *Salmonella* strains revealed that antimicrobial resistance determination in itself cannot be used as an epidemiological marker. This has clearly been shown by the presence of different plasmid profiles in isolates with similar antibiograms.

It has been proposed that plasmid profile analysis in conjunction with antimicrobial resistance determination can be useful for subtyping resistant *Salmonella* isolates [39]. In the present work two *Salmonella* strains (*S. saintpaul* 64A and *S. saintpaul* 73A) with similar antibiograms had similar plasmid profiles. These two isolates were isolated from children with diarrhea and both children lived in the same neighborhood (Janzour) in Tripoli area.

Although most multiple-drug resistance in enteric bacteria is plasmid-mediated, genes located on the bacterial chromosome [12] can determine important types of resistance. Three (23%) of the 13 Libyan *Salmonella* strains, that were resistant to antibiotics, had no plasmids. Using similar methods that were used in the present work, other investigators [39] found all their resistant *Salmonella* strains to harbor plasmids. This variation may be due to the difference in serotypes isolated from different geographical locations. Moreover, megaplasmids (not tested in this study) may also carry pathogenecity markers. It may also be due to the fact that plasmids can change rapidly, since many plasmids carry antibiotic resistance determinants contained within mobile genetic elements (transposons) that can be readily acquired or deleted [31].

In conclusion, the present study demonstrated that the problem of multiple-resistant *Salmonella* is widespread in Libya and this resistance is mainly plasmid mediated. In addition, nearly all isolates were invasive in HeLa cells and none produced cytotoxin, which supports the view that the latter character may not be important in the virulence of these organisms. The worldwide problem of multiple-drug

resistance among *Salmonella* species and – as demonstrated by the present study – in the Tripoli area shows urgent need for more rational use of antibiotics in humans and animals.

References

1. Ashkenazi,S., Cleary,T.G., Murray,B.E., Wanger,A., Pickering,L.K.: Quantitative analysis and partial characterization of cytotoxin production by *Salmonella* strains. Infect Immun **56**: 3089–3094 (1988).
2. Bauer,A.W., Kirby,W.M.M., Sherris,I.C., Turck,M.: Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol **45**: 493–496 (1966).
3. Bezanson,G.S., Khakhria,R., Pagnutti,D.: Plasmid profiles of value in differentiating *Salmonella muenster* isolates. J Clin Microbiol **17**: 1159–1160 (1983).
4. Binkin,N., Scuderi,G., Novaco,F.: Egg-related *Salmonella enteritidis*, Italy, 1991. Epidemiol Infect **110**: 227–237 (1993).
5. Birnboim,H.C., Doly,J.A.: A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acid Res **7**: 1513–1523 (1979).
6. Caprioli,A., D'Agnolo,G., Falbo,V., Roda,L.G., Tomasi,M.: Isolation of *Salmonella wien* heat-labile enterotoxin. Microbiology **5**: 1–10 (1982).
7. Centers for Disease Control. Multidrug-resistant *Salmonella* serotype *typhimurium* – United States, 1996. MMWR **46**: 308–310 (1997).
8. Crosa,J.H., Falkow,S.: Plasmids. In: Gerhardt,P.H. (ed.) Manual and Methods for General Bacteriology. ASM, Washington, 1991. pp. 266–282.
9. DuPont,H.L., Corrado,M.L., Sabbaj,J.: Use of norfloxacin in the treatment of acute diarrheal disease. Am J Med **82**: S79–S83 (1987).
10. EI-Nageh,M.: Salmonella isolations from humans in Tripoli, Libya. Trans R Soc Trop Med Hyg **82**: 324–326 (1988).
11. Ewing,W.H.: Identification of Enterobacteriaceae. 4th ed. New York, Elsevier, 1986.
12. Farrar,W.E.: Antibiotic resistance in intestinal bacteria. Clin Gastroenterology **8**: 803–826 (1979).
13. Gadó,I., László,V.G., Nagy,B., Milch,H., Drin,I., Awad-Masalmeh,M., Horváth,J.: Phage restriction and the presence of small plasmids in *Salmonella enteritidis*. Zentbl Bacteriol **287**: 509–519 (1998).
14. Giannella,R.A., Formal,S.B., Dammin,G.J., Collins,H.: Pathogenesis of salmonellosis: studies of fluid secretion, mucosal invasion, and morphologic reaction in the rabbit ileum. J Clin Invest **52**: 441–453 (1973).
15. Giannella,R.A., Washington,O., Gemski,P., Formal,S.B.: Invasion of HeLa cells by *Salmonella typhimurium*: a model for study of invasiveness of *Salmonella*. J Infect Dis **128**: 69–75 (1973).
16. Hackett,J., Kotlarski,I., Mathan,V., Franki,K., Rowley,D.: The colonisation of Peyer's patches by a strain of *Salmonella typhimurium* cured of the cryptic plasmid. J Infect Dis **153**: 1119–1125 (1986).
17. Hale,T.L., Formal,S.B.: Virulence mechanisms of enteroinvasive pathogens. In: Roth,J.A. (ed.) Virulence mechanisms of bacterial pathogens. Washington, 1988. pp. 61–69.
18. Hansfield,T.L., Monson,M.H., Wachsmuth,I.K.: An outbreak of antibiotic-resistant *Salmonella enteritidis* in Liberia, West Africa. J Infect Dis **151**: 790–795 (1985).

19. Helmuth,R., Stephan,R., Bunge,C., Hoog,B., Steinbeck,A., Bulling,E.: Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infect Immun **48**: 175–182 (1985).
20. Jansson,C., Franklin,A., Skold,O.: Trimethoprim resistance arising in animal bacteria and transferring into human pathogens. J Infect Dis **167**: 785–787 (1993).
21. Jones,B.D., Lee,C.A., Falkow,S.: Invasion of *Salmonella typhimurium* is affected by the direction of flagellar rotation. Infect Immun **60**: 2475–2480 (1992).
22. Jones,G.W., Rabert,D.K., Svinarich,D.M., Whitfield,H.J.: Association of adhesive, invasive, and virulent phenotypes of *Salmonella typhimurium* with autonomous 60-Mdal plasmids. Infect Immun **35**: 1110–1118 (1982).
23. Jones,G.W., Richardson,L.A., Uhlman,D.: The invasion of HeLa cells by *Salmonella typhimurium*: reversible and irreversible bacterial attachment and the role of bacterial motility. J Gen Microbiol **127**: 351–360 (1981).
24. Kapperud,G., Lassen,J., Dommarsnes,K., Kristiansen,B.E., Caygant,D.A., Ask,E., Jahkola,M.: Comparison of epidemiological marker methods for identification of *Salmonella typhimurium* isolates from an outbreak caused by contaminated chocolate. J Clin Microbiol **27**: 2019–2024 (1989).
25. Ketyi,I., Pácsa,S., Emődy,L., Verténvi,A., Kocsis,B., Kuch,B.: *Shigella dysenteriae*-like cytotoxic enterotoxins produced by *Salmonella* strains. Acta Microbiol Acad Sci Hung **26**: 217–223 (1979).
26. Keusch,G.T., Thea,D.M.: Invasive and tissue-damaging enteric bacterial pathogens: bloody diarrhea and dysentery. In: Schaechter,M., Medoff,G., Eisenstein,B.I. (eds) Mechanism of microbial disease. 2nd ed. Williams and Wilkins, Baltimore 1993. pp. 250–278.
27. Koo,F.C.W., Peterson,J.W., Houston,C.W., Molina,N.C.: Pathogenesis of experimental salmonellosis: inhibition of protein synthesis by cytotoxin. Infect Immun **43**: 93–100 (1984).
28. Lee,C.A., Falkow,S.: The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. Proc Natl Acad Sci USA **87**: 4304–4308 (1990).
29. Lolekha,S., Patanachareon,S., Thanangkul,B., Vibulbandhitkit,S.: Norfloxacin versus co-trimoxazole in the treatment of acute bacterial diarrhoea: a placebo controlled study. Scand J Infect Dis Suppl **56**: 35–45 (1988).
30. Lopez-Brea,M., Jimenez,M.L., Lopez Lavid,M.C., Padila,B., Isasia,T.: Norfloxacin vs. trimethoprim-sulfamethoxazole in the treatment of *Salmonella* gastroenteritis. Rev Infect Dis **11**: S1153–S1154 (1989).
31. Lupski,J.R.: Molecular mechanisms for transposition of drug-resistance genes and other movable genetic elements. Rev Infect Dis **9**: 357–368 (1987).
32. Macrina,F.L., Kopecko,D.J., Jones,K.R., Ayers,D.J., McCowan,S.M.: A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid **1**: 417–420 (1978).
33. Malik,G.M., Al-Wabel,A.A., Ahmed,M.E.K.: Pattern of antibiotic sensitivity of *Salmonella* isolates in Asir region, Southern part of Saudi Arabia. Saudi Med J **15**: 48–51 (1994).
34. Mikhail,I.A., Hyams,K.C., Podgore,J.K.: Microbiologic and clinical study of acute diarrhea in children in Aswan, Egypt. Scan J Infect Dis **21**: 59–65 (1989).
35. Milch,H., László,V.G., Nikolnikov,S.: Epidemiological analysis of *Salmonella typhimurium* infections on the basis of laboratory methods II. Resistance to antibiotics and R-plasmid carrier state in *Salmonella typhimurium* isolated in Hungary in the period 1974 to 1981. Acta Microbiol Hung **32**: 87–98 (1985).

36. Palmer,S.P.: The investigation of food poisoning. Lett Appl Microbiol **12**: 146–148 (1991).
37. Pang,T., Bhutta,Z.A., Finlay,B.B., Altwegg,M.: Typhoid fever and other salmonellosis: a continuing challenge. Trends Microbiol **3**: 253–255 (1995).
38. Pichler,H.E.T., Diridl,G., Sticler,K., Wolf,D.: Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. Am J Med **82**: S329–S332 (1987).
39. Rivera,M.J., Rivera,N., Castillo,J., Rubio,M.C., Gomez-Lus,R.: Molecular and epidemiological study of *Salmonella* clinical isolates. J Clin Microbiol **29**: 927–932 (1991).
40. Rodrigue,D.C., Tauxe,R.V., Rowe,B.: International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol Infect **105**: 21–27 (1990).
41. Ryan,C.A., Nickles,M.K., Hargrett-Bean,N.T.: Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. J Am Med Ass **258**: 3269–3274 (1987).
42. Skyes,E.J.: Methods for detecting beta-lactamases. In: Reeves,D.S., Phillips,I., Williams,J.D., Wise,R. (eds) Laboratory methods in antimicrobial chemotherapy. Churchill Livingstone, Edinburgh 1978. pp. 125–198.
43. Sullivan,N.M., Pellet,S., Wilkins,D.D.: Purification and characterization of toxins A and B of *Clostridium difficile*. Infect Immun **35**: 1032–1040 (1982).
44. Tassios,P.T., Gazouli,M., Trelepi,E., Milch,H., Kozlova,N., Sidorenko,S., Legakis,J., Tzouvelekis,L.S.: Spread of *Salmonella typhimurium* clone resistant to expanded-spectrum cephalosporins in three European countries. J Clin Microbiol **37**: 3774–3777 (1999).
45. Threlfall,E.J.: Antibiotics and the selection of food-borne pathogens. J Appl Bacteriol **73**: S96–S102 (1992).
46. Threlfall,E.J., Frost,J.A., Ward,L.R., Rowe,B.: Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet **347**: 1053–1054 (1996).
47. Wallis,T.S., Starkey,W.G., Stephen,J., Haddon,S.J., Osborne,M.P., Candy,D.C.A.: Enterotoxin production by *Salmonella typhimurium* strains of different virulence. J Med Microbiol **21**: 19–23 (1986).
48. Wong,S.S.Y., Yuen,K.Y., Yam,W.C., Lee,T.Y., Chau,P.Y.: Changing epidemiology of human salmonellosis in Hong Kong, 1982–93. Epidemiol Infect **113**: 425–434 (1994).