

Antimicrobial-resistant *Shigella* infections from Iran: an overlooked problem?

Mercedeh Tajbakhsh¹, Lourdes García Migura^{2,3}, Mohammad Rahbar⁴, Christina Aaby Svendsen⁵, Mona Mohammadzadeh⁶, Mohammad Reza Zali¹, Frank M. Aarestrup⁵ and Rene S. Hendriksen^{5*}

¹Research Center for Gastroenterology and Liver Disease, Shahid Beheshti University, Medical sciences, Tehran, Iran; ²Centre de Reserca en Sanitat Animal, Campus UAB, edifici CReSA, Barcelona, Spain; ³Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Barcelona, Spain; ⁴Department of Microbiology, Iranian Reference Health Laboratory, Tehran, Iran; ⁵WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens and European Union Reference Laboratory for Antimicrobial Resistance, National Food Institute, Technical University of Denmark, Kgs. Lyngby, Denmark; ⁶Department of Microbiology, Milad Hospital, Tehran, Iran

*Corresponding author. National Food Institute, Technical University of Denmark, Kemitorvet, Bygning 204, DK-2800 Kgs. Lyngby, Denmark. Tel: +45-35-88-60-00; Fax: +45-35-88-60-01; E-mail: rshe@food.dtu.dk

Received 11 October 2011; returned 6 January 2012; revised 10 January 2012; accepted 17 January 2012

Objectives: In this study, we wanted to assess the level of antimicrobial resistance, the presence of genes encoding resistance to cephalosporins and plasmid-mediated quinolone resistance (PMQR), and genetic relatedness among *Shigella* isolates obtained from Iranian patients.

Methods: A total of 44 *Shigella* isolates were collected from Iranian patients admitted to Milad Hospital, Tehran, Iran, during 2008–10. Of these, 37 were serotyped and characterized by MIC determination. A subset of eight suspected extended-spectrum β -lactamase (ESBL) producers (six *Shigella sonnei* phase II and two *Shigella flexneri* type 1b) were examined for the presence of genes encoding cephalosporin resistance. The presence of PMQR was assessed in one *S. flexneri* isolate exhibiting low-level resistance to ciprofloxacin and susceptibility to nalidixic acid. PFGE was performed on 25 *S. sonnei* phase II isolates.

Results: Of the isolates, 25 (68%) were *S. sonnei* phase II, with 5 (14%) *S. flexneri*, 5 (14%) *Shigella dysenteriae* type 2, and 2 (5%) *Shigella boydii* type 2. Resistance to at least three classes of antimicrobials was detected in all species. The presence of *bla*_{CTX-M-15} and the AmpC β -lactamase producer *bla*_{CMY-2} was confirmed in five and one *S. sonnei* phase II isolates, respectively. One of the two *S. flexneri* type 1b that contained *bla*_{CTX-M-15} also harboured a *qnrS1* gene. PFGE identified seven PFGE profiles; the main cluster included 15 of the strains, suggesting low genetic diversity between isolates or the presence of an endemic clone in Iran.

Conclusions: This is the first known description of ESBL-producing and AmpC β -lactamase-producing *Shigella* and of PMQR *Shigella* in Iran. The emergence of CTX-15, CMY-2 and *qnrS1* genes may compromise the treatment of shigellosis. Strategies to minimize the spread of ESBL-producing and AmpC- β -lactamase-producing *Shigella* should be implemented.

Keywords: ESBLs, AmpC, PMQR, antimicrobial resistance, PFGE

Introduction

Shigella is an important cause of acute diarrhoeal disease. Worldwide, there are ~164.7 million cases yearly, and 1.1 million people are estimated to die from *Shigella* infections.¹ A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involve children <5 years of age.¹ In Iran, shigellosis is one of the major causes of childhood morbidity associated with diarrhoea.^{2,3}

All four species of *Shigella* (*Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*) can cause shigellosis.

S. flexneri is endemic in many developing countries and causes higher mortality than other *Shigella* species.⁴ The most common serotype is *S. flexneri* serotype 2a, but other serotypes are also of major importance.¹ In Iran, *S. flexneri* was the most frequent cause of shigellosis up to 2003,^{5,6} when a shift in highest prevalence towards *S. sonnei* was observed.

A range of antimicrobials is effective for the treatment of shigellosis, although the options have become limited due to the emergence of multidrug resistance. Thus, sulphonamides, tetracycline, ampicillin and co-trimoxazole are no longer recommended for empirical treatment.⁷ An increase in the occurrence

of antimicrobial resistance, including resistance to fluoroquinolones and third- and fourth-generation cephalosporins, among *S. sonnei* has been observed in several countries.^{8–15} To date, *bla*_{CTX-M-15}, *bla*_{CTX-M-14}, *bla*_{CTX-M-3} and *bla*_{CMY-2} genes have been reported in *S. sonnei* isolates. A few articles have described the presence of plasmid-mediated quinolone resistance (PMQR) determinants mainly associated with *S. flexneri*, where the *qnrS1* gene appears to be dominant.^{16–18}

The emergence of antimicrobial resistance is a matter of concern¹⁹ and poses major difficulties in the determination of appropriate antimicrobial treatment due to shifts in the prevalences of the different serogroups and changes in the resistance patterns.⁷ Knowledge of the occurrence of different serotypes in different countries and geographic regions may assist in the recognition and tracing of emerging pathogens and in the implementation of correct treatment and control strategies.¹

In this study, we have determined the serotype distribution and antimicrobial resistance profiles as well as the genes conferring resistance to quinolones, extended-spectrum β -lactamase (ESBL) and AmpC β -lactam antimicrobials among *Shigella* spp. collected from patients admitted to Milad Hospital, Tehran, Iran, during 2008–10. Additionally, we have investigated the molecular relatedness of *S. sonnei* isolates using PFGE.

Materials and methods

Sample collection

A total of 848 patients admitted to Milad Hospital, Tehran, Iran, between September 2008 and March 2010 suffered from acute diarrhoea and gastroenteritis. Only patients without a history of antimicrobial consumption submitted stool samples and were included in the study. From the 848 patients, 44 *Shigella* isolates were obtained and further analysed.

The strains were isolated directly from fresh stool samples following plating on selective agar and biochemical testing. The selective agar plates [MacConkey agar, *Salmonella-Shigella* agar and XLD agar (Merck, Hamburg, Germany)] were incubated at 37°C for 18–24 h. Presumptive *Shigella* colonies were identified based on the following biochemical tests: triple sugar iron agar, motility-indole-urea agar and IMVIC (indole, methyl red, Voges-Proskauer and citrate). A subset of 37 viable isolates (the other 7 isolates did not survive storage) were included in this study and sent to the National Food Institute, Technical University of Denmark (DTU Food) for further analysis.

Serotyping

Presumptive *Shigella* isolates were sent to the WHO National Salmonella and Shigella Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand, and serotyped based on serological slide agglutination, using the polyvalent antisera A, A1, B, C, C1, C1-3 and D and the following monovalent antisera: *S. dysenteriae* type 1–12; *S. flexneri* type I–VI and group factor (3)4, 6 and 7(8); *S. boydii* type 1–18; and *S. sonnei* phase I–II (S & A Reagents Ltd, Bangkok, Thailand). The serotypes were interpreted according to the Manual of Clinical Microbiology.²⁰

Antimicrobial susceptibility

MIC testing was performed at DTU Food using the following antimicrobials: ampicillin, amoxicillin/clavulanic acid, apramycin, cefotaxime, ceftiofur, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin,

nalidixic acid, neomycin, spectinomycin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim. For ESBL-producing and/or AmpC- β -lactamase-producing isolates, susceptibility to the following β -lactams was also tested: cefazolin, cefepime, cefoxitin, cefpodoxime, ceftazidime, ceftriaxone, cefalotin, imipenem and meropenem. Epidemiological cut-off values according to current EUCAST recommendations (<http://www.eucast.org>) were applied, other than for amoxicillin/clavulanic acid, apramycin, ceftriaxone, cefazolin, cefepime, spectinomycin and sulfamethoxazole where CLSI standards and clinical breakpoints were used.^{21–23} As the quality control, *E. coli* ATCC 25922 was used according to the CLSI standards.^{21,23}

Detection of resistance genes

Of the 37 *Shigella* isolates, eight strains exhibiting resistance to ceftiofur and cefotaxime were examined for the presence of *bla*_{TEM}, *bla*_{CTX}, *bla*_{SHV}, *bla*_{CMY-1} and *bla*_{CMY-2} as previously described.^{24,25} One isolate showing reduced susceptibility to ciprofloxacin and susceptibility to nalidixic acid was tested by PCR for the presence of PMQR genes [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac(6')-Ib*] as previously described.^{25–27}

PCR products were purified using a GFXTM PCR DNA Kit (GE Healthcare, Chalfont St Giles, UK) and submitted to Macrogen Inc. (Seoul, Korea) for sequencing. Sequence analysis and alignments were performed using Vector NTI suite 9 (InforMax Inc., Bethesda, MD, USA). The resulting nucleotide sequences were compared with sequences from public databases (www.ncbi.nlm.nih.gov and www.lahay.org/studies/).

PFGE

All 25 *S. sonnei* isolates were analysed for genetic relatedness by PFGE using XbaI (Fermentas, Glen Burnie, MD, USA) according to the US CDC PulseNet protocol.²⁸ Electrophoresis was performed with a CHEF-DR III System (Bio-Rad Laboratories, Hercules, CA, USA) using 1% SeaKem Gold agarose in 0.5× Tris/borate/EDTA at 6 V with an angle of 120° (one phase 2.2–54.2 s, run time 20 h).

Results

Patient data

The age of infected patients ranged from 2 to 56 years (median 7 years). The ratio between genders was 1:0.85 (females/males).

Serotyping

All four species of *Shigella* were represented among the 37 isolates. Of these, 25 (68%) were *S. sonnei* phase II, with 5 (14%) *S. flexneri*, 5 (14%) *S. dysenteriae* type 2, and 2 (5%) *S. boydii* type 2. In addition, three different serotypes of *S. flexneri* were detected: 2 (5%) type 1b (I: 4, 6), 1 (3%) type 2b (II: 7, 8), and 2 (5%) untypeable (Table 1).

Antimicrobial resistance

All *Shigella* species showed multidrug resistance to at least three classes of antimicrobial agent. However, susceptibility to the different antimicrobials appeared to differ depending on the species and serotype (Table 1). The 25 *S. sonnei* phase II isolates exhibited resistance to the highest number of antimicrobials, followed by *S. flexneri* type 1b. In general, these 27 isolates were resistant to 13 of the 17 antimicrobials tested, but differences between the isolates were observed. Resistance to streptomycin, sulfamethoxazole, tetracycline and trimethoprim was common

Table 1. Frequency of resistance among *Shigella* serotypes from patients admitted to Milad Hospital, Tehran, Iran during 2008–10

Species and serotype	No. (%) of isolates	Number (%) of isolates resistant to various antimicrobial agents ^a at the indicated breakpoints ^b														
		AMC ≥32	AMP >8	XNL >2	CHL >16	CIP		GEN >2	CTX >0.5	NAL >16	NEO >4	SPT >64	STR >16	SMX >256	TET >8	TMP >2
<i>S. sonnei</i> phase II	25 (68)	1 (4)	8 (32)	6 (24)	0	16 (64)	2 (8)	6 (24)	16 (64)	1 (4)	2 (8)	24 (96)	24 (96)	24 (96)	25 (100)	
<i>S. flexneri</i> type 1b	2 (5)	0	2 (100)	2 (100)	0	1 (50)	0	2 (100)	0	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	
<i>S. flexneri</i> untypeable	2 (5)	0	2 (100)	0	2 (100)	0	0	0	0	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	
<i>S. flexneri</i> type 2b	1 (3)	0	1 (100)	0	1 (100)	0	0	0	0	0	1 (100)	1 (100)	0	1 (100)	1 (100)	
<i>S. dysenteriae</i> type 2	5 (14)	0	5 (100)	0	1 (20)	0	1 (20)	0	0	1 (20)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	
<i>S. boydii</i> type 2	2 (5)	0	0	0	0	2 (100)	0	0	2 (100)	0	2 (100)	2 (100)	2 (100)	0	2 (100)	
Total	37	1 (3)	18 (49)	8 (22)	6 (16)	0	19 (51)	3 (8)	8 (22)	18 (49)	2 (5)	14 (38)	36 (97)	35 (95)	34 (92)	37 (100)

AMC, amoxicillin/clavulanic acid; AMP, ampicillin; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; CTX, cefotaxime; NAL, nalidixic acid; NEO, neomycin; SPT, spectinomycin; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim.

^aAll strains were susceptible to apramycin, carbapenems (imipenem and meropenem), colistin and florfenicol.

^bMIC breakpoints in mg/L.

for *S. sonnei* (96%–100%, $n=24$), *S. flexneri* serotype 1b (100%, $n=2$) and *S. dysenteriae* type 2 (100%, $n=5$). In addition, 18 isolates (16 *S. sonnei* and 2 *S. boydii* type 2) exhibited resistance to nalidixic acid and low-level resistance to ciprofloxacin. In contrast, one isolate of *S. flexneri* serotype 1b exhibited low-level resistance to ciprofloxacin but was susceptible to nalidixic acid. Resistance to β -lactam antimicrobials ceftiofur, cefazolin, cefalotin, cefpodoxime, ceftriaxone and cefotaxime was observed in all six (24%) of the *S. sonnei* phase II isolates. Of these, five (83%) also exhibited resistance to ceftazidime and one (17%) also exhibited resistance to ceftoxitin. In addition, the two *S. flexneri* type 1b isolates were resistant to β -lactam antimicrobials with the exception of ceftoxitin. None of the strains was resistant to apramycin, carbapenems (imipenem and meropenem), colistin or florfenicol (Table 1).

Identification of genes conferring resistance to third- and fourth-generation cephalosporins and PMQR determinants

Among the six isolates of *S. sonnei* phase II resistant to third-generation cephalosporins, five harboured $bla_{CTX-M-15}$ and one harboured bla_{CMY-2} . The two *S. flexneri* type 1b were also positive for the presence of $bla_{CTX-M-15}$. Additionally, one of the *S. flexneri* type 1b that displayed the PMQR phenotype harboured a $qnrS1$ gene.

PFGE

A subset of 25 *S. sonnei* phase II isolates were subtyped by PFGE, and seven unique XbaI PFGE patterns were observed. The three XbaI PFGE clusters contained 15, 4 and 2 isolates, with distinct profiles (Figure 1). Within each of the clusters containing four or two isolates, the strains exhibited the same antimicrobial resistance profile (Figure 1). Furthermore, in the main cluster, 10 of the 15 isolates displayed the same antimicrobial resistance profile. No correlation was detected between the PFGE profile and age or gender of the patient or time of infection.

Discussion

Over the past decade, an emergence of ESBL-producing *Shigella* spp. has been observed in the Middle East, South-East Asia, China and Argentina, where the presence of $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-3}$ genes has been reported.^{8–15} However, worldwide only a few reports have described the presence of AmpC β -lactamases (bla_{CMY-2}) and PMQR ($qnrS1$, $qnrB6$ and $qnrB19$) in *Shigella* spp.^{16–18,29,30}

In 2010, the first characterization of ESBL-producing *Salmonella enterica* harbouring $bla_{CTX-M-15}$, $bla_{CTX-M-88}$ and $bla_{TEM-169}$ from Iran was described.³¹ This study reports the first known description of ESBL producers (harbouring $bla_{CTX-M-15}$), AmpC β -lactamase producers (harbouring bla_{CMY-2}) and PMQR (harbouring $qnrS1$) in *S. sonnei* phase II and *S. flexneri* serotype 1b isolated from patients in Iran.

This is indeed a worrisome development in antimicrobial resistance, especially as the *S. sonnei* phase II isolates were highly resistant to many of the antimicrobials tested, including decreased susceptibility to ciprofloxacin, complicating the

Dice (Opt:1.00%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]

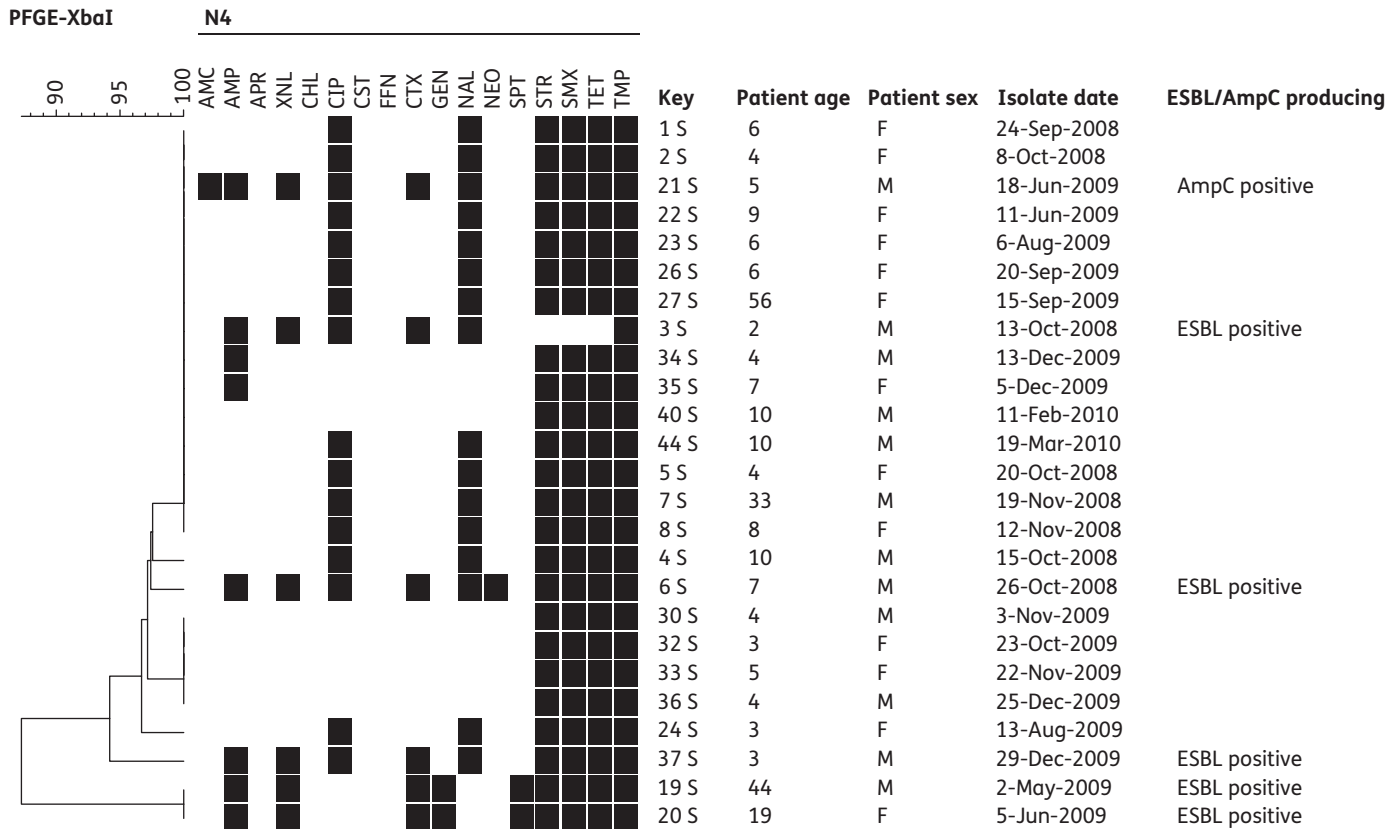


Figure 1. PFGE (XbaI) analysis and antimicrobial resistance patterns of 25 *S. sonnei* serotypes from patients admitted to Milad Hospital, Tehran, Iran during 2008–10. The scale is a similarity index in percentages, the key represents the isolate numbers, and the three clusters of identical isolates are identified by 100% similarity. Black squares represent resistance. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; APR, apramycin; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; CST, colistin; FFN, florfenicol; CTX, cefotaxime; GEN, gentamicin; NAL, nalidixic acid; NEO, neomycin; SPT, spectinomycin; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim.

treatment of the patients. Interestingly, the patients selected for this study claimed not to have been subjected to any antimicrobial treatment prior to their hospitalization. This underlines the extensive selection pressure present in the Iranian community, where self-medication due to ‘over the counter’ antimicrobial consumption and abuse of prescribed antimicrobials is widespread.³² Our results emphasize the need for prudent and controlled use of antimicrobials, especially third-generation cephalosporins and fluoroquinolones, in the treatment of invasive infections to avoid further acquisition of resistance to these antimicrobials, which have been designated by the WHO as critically important for human health.³³ The detection of ESBL producers, AmpC β-lactamase producers and PMQR in *Shigella* spp. for the first time in Iran (to our knowledge) was surprising and indicates a lack of data on the prevalence of these types of resistance genotype, and illustrates the need to establish a proper antimicrobial resistance surveillance system^{34,35} to monitor the emergence and development of antimicrobial resistance. Furthermore, it is important to initiate strategies for prevention and control, such as those mentioned by Niyogi¹⁹ provision of safe and abundant water, effective faeces disposal, education on how to avoid faecal contamination of food and water, awareness programmes focusing on hand

washing and breast feeding, promotion of oral rehydration therapy to patients suffering from acute diarrhoea and supply of additional nutrients to children recovering from acute diarrhoea or dysentery.^{7,36}

A high genetic homogeneity was observed among *S. sonnei* phase II isolates, of which many clustered with similar antimicrobial susceptibility patterns. Based on the isolates in this study, it is uncertain whether this is the result of sporadic outbreaks or clonally related endemic isolates present in Iran. Limited PFGE data are available in Iran; however, Ranjbar *et al.*³⁷ suggested endemic circulation of *S. sonnei* in Tehran, Iran. Additionally, the PFGE profile of the main cluster seems to correspond to both the antimicrobial resistance pattern and PFGE profile of the second largest PFGE cluster obtained during the 18 years of antimicrobial resistance surveillance in Belgium.³⁶ The hypothesis of endemic circulation of *S. sonnei* cannot yet be confirmed due to the limited PFGE data available as well as the low discriminatory power of PFGE for *Shigella* spp.³⁸ Further studies aimed at understanding the clonal relatedness of *S. sonnei* strains need to be initiated in order to investigate the hypothesis raised in this study and by Ranjbar *et al.*³⁷

In conclusion, this study describes the presence of the first ESBL producers (*bla*_{CTX-M-15}), AmpC β-lactamase producers

(*bla*_{CMY-2}) and PMQR (*qnrS1*) in *S. sonnei* and *S. flexneri* isolated from patients in Iran. Antimicrobial susceptibility testing revealed high levels of antimicrobial resistance in all *Shigella* species detected. Based on PFGE, a high genetic homogeneity was observed among *S. sonnei* phase II isolates, many of which clustered with similar antimicrobial susceptibility patterns. Additionally, this is the first known description of serotype distribution among *Shigella* spp. encountered in a hospital setting in Tehran, Iran, and it revealed that *S. sonnei* was by far the most common *Shigella* species, followed by *S. flexneri* and *S. dysenteriae*. Since there is a lack of data on the epidemiology of *Shigella* spp. in Iran, we encourage the Iranian authorities to establish a rigorous antimicrobial resistance surveillance system to monitor the emergence and development of antimicrobial resistance and to initiate strategies for prevention and control to reduce the burden of shigellosis in Iran.

Funding

This work was supported by the WHO Global Foodborne Infections Network (GFN).

Transparency declarations

None to declare.

References

- Kotloff KL, Winickoff JP, Ivanoff B et al. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull World Health Organ* 1999; **77**: 651–66.
- Ranjbar R, Soltan Dallal MM, Talebi M et al. Increased isolation and characterization of *Shigella sonnei* obtained from hospitalized children in Tehran, Iran. *J Health Popul Nutr* 2008; **26**: 426–30.
- Jafari F, Garcia-Gil LJ, Salmanzadeh-Ahrabi S et al. Diagnosis and prevalence of enteropathogenic bacteria in children less than 5 years of age with acute diarrhea in Tehran children's hospitals. *J Infect* 2009; **58**: 21–7.
- Bennish ML, Wojtyniak BJ. Mortality due to shigellosis: community and hospital data. *Rev Infect Dis* 1991; **13**: 245–51.
- Nikkah J, Mehr-Movahead A. Antibiotic resistance among *Shigella* species isolated in Tehran, Iran. *Ann Trop Med Parasitol* 1988; **82**: 481–3.
- MoezArdalan K, Zali MR, Dallal MM et al. Prevalence and pattern of antimicrobial resistance of *Shigella* species among patients with acute diarrhoea in Karaj, Tehran, Iran. *J Health Popul Nutr* 2003; **21**: 96–102.
- Niyogi SK. Shigellosis. *J Microbiol* 2005; **43**: 133–43.
- Sabra AH, Araj GF, Kattar MM et al. Molecular characterization of ESBL-producing *Shigella sonnei* isolates from patients with bacillary dysentery in Lebanon. *J Infect Dev Ctries* 2009; **3**: 300–5.
- Vasilev V, Japheth R, Yishai R et al. Extended-spectrum β -lactamase-producing *Shigella* strains in Israel, 2000–2004. *Eur J Clin Microbiol Infect Dis* 2007; **26**: 189–94.
- Xiong Z, Li T, Xu Y et al. Detection of CTX-M-14 extended-spectrum β -lactamase in *Shigella sonnei* isolates from China. *J Infect* 2007; **55**: 125–8.
- Zhang R, Zhou HW, Cai JC et al. Serotypes and extended-spectrum β -lactamase types of clinical isolates of *Shigella* spp. from the Zhejiang province of China. *Diagn Microbiol Infect Dis* 2011; **69**: 98–104.
- Acikgoz ZC, Koseoglu EO, Kocagoz S. CTX-M-3 type β -lactamase producing *Shigella sonnei* isolates from pediatric bacillary dysentery cases. *Jpn J Infect Dis* 2008; **61**: 135–7.
- Acikgoz ZC, Gulay Z, Bicmen M et al. CTX-M-3 extended-spectrum β -lactamase in a *Shigella sonnei* clinical isolate: first report from Turkey. *Scand J Infect Dis* 2003; **35**: 503–5.
- Hong SJ, Lee CH, Wang JH et al. [Clinical characteristics of extended-spectrum β -lactamase producing *Shigella sonnei* infection outbreak in Chungju area.] *Korean J Lab Med* 2006; **26**: 168–73.
- Rapoport M, Monzani V, Pasteran F et al. CMY-2-type plasmid-mediated AmpC β -lactamase finally emerging in Argentina. *Int J Antimicrob Agents* 2008; **31**: 385–7.
- Hata M, Suzuki M, Matsumoto M et al. Cloning of a novel gene for quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. *Antimicrob Agents Chemother* 2005; **49**: 801–3.
- Folster JP, Pecic G, Bowen A et al. Decreased susceptibility to ciprofloxacin among *Shigella* isolates in the United States, 2006 to 2009. *Antimicrob Agents Chemother* 2011; **55**: 1758–60.
- Pu XY, Pan JC, Wang HQ et al. Characterization of fluoroquinolone-resistant *Shigella flexneri* in Hangzhou area of China. *J Antimicrob Chemother* 2009; **63**: 917–20.
- Niyogi SK. Increasing antimicrobial resistance—an emerging problem in the treatment of shigellosis. *Clin Microbiol Infect* 2007; **13**: 1141–3.
- Bopp CA, Brenner FW, Fields PI, et al. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Jorgensen JH, et al., eds. *Manual of Clinical Microbiology*. 8th edn. Washington, DC: ASM Press, 2003; 654–71.
- Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Seventh Edition: Approved Standard M07-A7*. CLSI, Wayne, PA, USA, 2006.
- Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals—Third Edition: Approved Standard M31-A3*. CLSI, Wayne, PA, USA, 2008.
- Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement M100-S16*. CLSI, Wayne, PA, USA, 2006.
- Hasman H, Mevius D, Veldman K et al. β -Lactamases among extended-spectrum β -lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother* 2005; **56**: 115–21.
- Hendriksen RS, Mikoleit M, Kornschöber C et al. Emergence of multidrug-resistant *Salmonella* Concord infections in Europe and the United States in children adopted from Ethiopia, 2003–2007. *Pediatr Infect Dis J* 2009; **28**: 814–8.
- Cavaco LM, Hasman H, Xia S et al. *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother* 2009; **53**: 603–8.
- Périchon B, Courvalin P, Galimand M. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob Agents Chemother* 2007; **51**: 2464–9.
- Ribot EM, Fair MA, Gautom R et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis* 2006; **3**: 59–67.
- Huang IF, Chiu CH, Wang MH et al. Outbreak of dysentery associated with ceftriaxone-resistant *Shigella sonnei*: First report of plasmid-mediated

CMY-2-type AmpC β -lactamase resistance in *S. sonnei*. *J Clin Microbiol* 2005; **43**: 2608–12.

30 Livermore DM, Mushtaq S, Nguyen T *et al.* Strategies to overcome extended-spectrum β -lactamases (ESBLs) and AmpC β -lactamases in shigellae. *Int J Antimicrob Agents* 2011; **37**: 405–9.

31 Hamidian M, Tajbakhsh M, Walther-Rasmussen J *et al.* Emergence of extended-spectrum β -lactamases in clinical isolates of *Salmonella enterica* in Tehran, Iran. *Jpn J Infect Dis* 2009; **62**: 368–71.

32 Ashtiani MT, Monajemzadeh M, Kashi L. Trends in antimicrobial resistance of fecal *Shigella* and *Salmonella* isolates in Tehran, Iran. *Indian J Pathol Microbiol* 2009; **52**: 52–5.

33 Collignon P, Powers JH, Chiller TM *et al.* World Health Organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. *Clin Infect Dis* 2009; **49**: 132–41.

34 Aarestrup FM. Monitoring of antimicrobial resistance among food animals: principles and limitations. *J Vet Med B Infect Dis Vet Public Health* 2004; **51**: 380–8.

35 Bager F, Aarestrup FM, Jensen NE *et al.* Design of a system for monitoring antimicrobial resistance in pathogenic, zoonotic and indicator bacteria from food animals. *Acta Vet Scand Suppl* 1999; **92**: 77–86.

36 Vrints M, Mairiaux E, Van Meerveene E *et al.* Surveillance of antibiotic susceptibility patterns among *Shigella sonnei* strains isolated in Belgium during the 18-year period 1990 to 2007. *J Clin Microbiol* 2009; **47**: 1379–85.

37 Ranjbar R, Aleo A, Giammanco GM *et al.* Genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002–2003. *BMC Infect Dis* 2007; **22**: 62.

38 Xia S, Xu B, Huang L *et al.* Prevalence and characterization of human *Shigella* infections in Henan Province, China, in 2006. *J Clin Microbiol* 2011; **49**: 232–42.