Diarrhea in an infant due to *Shigella flexneri* 1 carrying multiple cephalosporinase-encoding genes

M. John Albert1*, Prashant Purohit2, Laurent Poirel3,4,5, Glen Carter6 and Dieter Bulach6

**Abstract**

**Background:** Infections caused by multidrug-resistant shigellae resistant to broad-spectrum cephalosporins are becoming more prevalent in the Middle East. We report a case of severe diarrhea due to a multiresistant *Shigella flexneri* 1 strain carrying four different β-lactamase genes.

**Case presentation:** A one-year-old Syrian infant presented with severe acute diarrhea, vomiting and dehydration. She did not respond to empirical treatment with amoxicillin-clavulanic acid followed by cefotaxime. Later, stool culture revealed *S. flexneri* 1 resistant to both these drugs. The patient was successfully treated with meropenem to which *S. flexneri* 1 was susceptible. The isolate was resistant to eight classes of antibiotics, and the whole genome sequence (WGS) identified four β-lactamase genes (*bla*CTX-M-15, *bla*EC-8, *bla*OXA-1, and *bla*TEM-1) along with genes mediating resistance to seven other antibiotic classes. The WGS also identified several virulence genes including *senA* that encodes ShET-2 which induces watery diarrhea. Phylogenetically, the isolate was closely related to isolates from South Asia.

**Conclusions:** This report highlights the emergence of extremely resistant *Shigella* that has acquired multiple resistance genes to cephalosporins rendering these drugs ineffective.

**Keywords:** *S. flexneri* 1, Diarrhea, Multidrug-resistance, Cephalosporinase

**Background**

Antibiotics are the cornerstone therapy for treating shigellosis [1]. However, over the years, shigellae have developed resistance to numerous antimicrobials making treatment difficult [2–4]. *Shigella* isolates in many countries have acquired resistance traits leading to the inefficacy of cephalosporins for treating patients [5–9]. Resistance to cephalosporins is mostly mediated by genes located on plasmids and encoding the so-called AmpC or extended-spectrum β-lactamases (ESBLs) [4, 10, 11]. There are reports of *Shigella* isolates carrying two or three β-lactamases [8, 12–14]. We describe here a case of shigellosis in an infant in Kuwait due to a multi-drug-resistant *Shigella flexneri* 1 that carried four different β-lactamase genes along with many other antibiotic resistance genes, resulting in a highly drug-resistant strain. We also performed a phylogenetic analysis of the isolate to find out its relationship with the isolates from other parts of the world.

**Case presentation**

A one-year-old female Syrian infant was admitted at the Al-Sabah hospital, Kuwait, in April 2019, with an acute onset of diarrhea and vomiting, both 8–10 times a day, and fever. There was no macroscopic evidence of blood in the stool. She did not have a history of travel. She was severely dehydrated with a reduced skin turgor and sunken eyes, lethargic and tachypneic, with a body temperature of 40 °C, and a blood pressure of 97/58 mm. She...
was rehydrated with intravenous fluid containing saline, dextrose and KCl, and treated with intravenous amoxicillin-clavulanic acid (500 mg every 12 h). Her stool, urine and blood samples were analyzed for bacterial culture and susceptibility. Even though her general condition significantly improved after 24 h, fever (39.1 °C) and diarrhea continued. Amoxicillin-clavulanic acid was stopped and intravenous cefotaxime (275 mg every 6 h) was started. Fever (39.7 °C) and diarrhea continued through to the fourth day, when the stool culture and susceptibility report was received (see below). Based on the report, cefotaxime was stopped and intravenous meropenem was started (200 mg every 8 h). After 48 h, the child became afebrile and the frequency of diarrhea was reduced. After the completion of a 7-day course of meropenem, the child was discharged with a complete recovery from diarrhea. Urine and blood cultures were sterile (see below).

Stool, urine and blood samples were cultured for bacterial pathogens as described previously [15]. *S. flexneri* 1 (further designated as SF1) was cultured from stool with no bacteria cultured from other samples. The organism was tested for susceptibility to several antibiotics (HiMedia) by disk diffusion method and interpreted according to CLSI criteria [16]. The isolate was resistant to streptomycin, ampicillin, piperacillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, aztreonam, tetracycline, trimethoprim, sulfamethoxazole, cotrimoxazole, ciprofloxacin, chloramphenicol, cephalothin, cephalozolin, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, and cefepime, but susceptible to gentamicin, amikacin, piperacillin/tazobactam, azithromycin, colistin, fosfomycin, ertapenem, meropenem, imipenem, cefoxitin, and tigecycline. These results were corroborated with Etests for all antibiotics except for cephalothin and glycolglycline for which Etests were not available. The isolate was, therefore, multi-resistant to eight classes of antibiotics.

Agar-based double-disk synergy testing showed that the *S. flexneri* 1 isolate produced an extended-spectrum β-lactamase (ESBL) [17].

Chromosomal DNA from *S. flexneri* 1 was extracted by QIAamp Fast DNA Stool Mini Kit (Qiagen). Extraction of plasmids from *S. flexneri* 1 was done with the Plasmid Mini Kit (Qiagen). Plasmids were separated by horizontal gel electrophoresis. Plasmid sizing was done by comparison with plasmids present in *E. coli* V517 (35.8, 4.8, 3.7, 2.6, 2.0, 1.8 and 1.4 kbps). *S. flexneri* 1 had plasmids of sizes 36, 2.6, 2, 1.4 and 0.8 kbp respectively (Fig. 1).

Chromosomal DNA was sequenced. Sequencing libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina) and the sequence read data were produced on the Illumina NextSeq instrument (paired end, 150 base reads). The reads were assembled using Skesa 2.3.0 [18]. The draft assembly contained 418 contigs and 4,412,722 total bases. The sequence type (ST) of the isolate was 245 which confirmed it as *S. flexneri* [19, 20]. Comparative core genome phylogenetic analysis was performed using Nullarbor (https://github.com/tseemann/nullarbor). Abricate (https://github.com/tseemann/abricate) was used to detect virulence genes by scanning with VFDB [21] and antibiotic resistance genes by scanning with the Resfinder database [22] and the National Database of Antibiotic Resistant Organisms (NDARO) (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/). Read data produced for the isolate, SF1 has been submitted to NCBI under BioProject, PRJNA556301.

The details of phylogroup 1 *Shigella* isolates [23] used for core genome phylogenetic analysis are shown in Additional file 1: Table S1. The phylogenetic tree is shown in Fig. 2. The isolate SF1 clustered with the isolates that had been recovered from Pakistan and Bangladesh. The resistance genes found in the WGS were
aadA1, aph(3\')-1b, aph (6)-1d, catA1, dfrA1, qnrS1, sul2, tet(A), tet(B), blacTX-M-15, blacEC-8, blaoXA-1, and blatem-1. No mutation in gyrA, gyrB, parC and parE known to confer resistance to quinolone [24] was identified. The resistance genes of the isolates are shown on the right of Fig. 2. The number of resistance genes among the isolates from developing countries appeared to be higher than that in developed countries. A number of virulence genes were identified in SF1, namely ipaA-ipaD, spa29, ipaH, sepA and senA.

Conjugation experiments for resistance transfer were done by both liquid mating [25] and filter mating [26] with sodium azide-resistant E. coli J53 as the recipient strain. Bacterial suspension from the filter and culture after liquid mating were streaked on three MacConkey agar (Oxoid) plates, each one with one of the drug combinations, (sodium azide [200 mg/L] + cefotaxime [2 mg/L], sodium azide [200 mg/L] + ampicillin [50 mg/L], and sodium azide + cefotaxime + ampicillin) to get isolated colonies. No colonies grew on these three selective agars after liquid and filter mating showing a lack of transfer of resistance.

Discussion and conclusions

Although shigellosis is a typically dysenteric illness with blood, pus and mucus in the stool [27], children often present with diarrhea only [28]. Our patient had watery diarrhea. Shigella enterotoxin 1 (ShET-1) and ShET-2 alter water and electrolyte transport in the small intestine, and cause diarrhea and dehydration [29]. Our isolate possessed the senA gene that encodes ShET-2 which likely contributed to the severe watery diarrhea. We detected ipaH and spa29 genes that are involved in the invasion of the colonic epithelial cells [30]. The genome also contained the sepA gene, which encodes serine protease A autotransporter, which is responsible for barrier disruption [31].

There was a good correlation between the resistance genotypes and phenotypes. The genome contained aadA1, aph(3\')-1b, and aph(6)-1d all encoding resistance to aminoglycosides, including streptomycin [32, 33]. The isolate also had catA1, dfrA1, qnrS1, sul2, and tetA & tetB genes that mediate resistance to chloramphenicol [34], trimethoprim [35], ciprofloxacin [36], sulfamethoxazole [37], and tetracycline [38] respectively. Phenotypically, the isolate was resistant to all these antibiotics.

The isolate was unusual as it had four different genes encoding β-lactamases belonging to Ambler class A (blacTX-M-15, blatem-1), Ambler class C (blacEC-8) and Ambler class D (blaoXA-1) [39], blacTX-M mediates resistance to cefotaxime and ceftriaxone [40, 41], blacEC-8 mediates resistance to cephalosporins including third (ceftazidime) and fourth generation (cefepime) cephalosporins [42], and blatem-1 mediates resistance to ampicillin [43]. Accordingly, the isolate possessed phenotypic resistance to these antimicrobials. blaoXA-1 gene was present in the isolate. This gene mediates resistance to aminopenicillin (ampicillin), ureidopenicillin (penicillin), and cephalosporin (cepheme) [42]. There is a co-occurrence of blaoXA-1 with blacTX-M-15 in E. coli [44]. This co-occurrence makes the isolate resistant to β-lactam–β-lactamase inhibitor combination antibiotics, such as amoxicillin-clavulanic acid and ampicillin-sulbactam [42]. Indeed, this isolate was resistant to these inhibitor combination antibiotics.

The blacTX-M-15 gene is present in the chromosome or plasmid in shigellae [45, 46]. The blacEC-8 gene was reported in Escherichia coli with the apparent location of the gene in the chromosome [47]. blaoXA-1 has been found in plasmid and integron in shigellae [48, 49]. The blatem-1 is present in plasmid or the chromosome [6, 50]. We could not transfer any of the cephalosporinase genes to the recipient E. coli by mating, indicating that their locations might be in the chromosome, even though SF1 had plasmids.

Although the child was of Syrian origin, her family had been living in Kuwait long-term with no history of recent travel outside of Kuwait, indicating that the infection was acquired locally. Phylogenetic analysis showed that SF1 was closely related to the isolates from Pakistan and Bangladesh. An expatriate population
from these countries resides in Kuwait. It appears that the clone(s) circulating in South Asia is circulating in Kuwait. The study of Connor et al. [23] suggested local acquisition of antimicrobial resistance determinant(s) by isolates in each country, and no evidence of intercontinental spread of antimicrobial resistant strains. However, our study suggested the possible spread of a resistant clone from South Asia to the Middle East. The present day intercontinental migration of refugees portends a widespread transfer of resistant strains.

The occurrence of multiple β-lactamase-encoding genes in *Shigella* is novel which will render cephalosporin antibiotics ineffective for treating the disease.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13099-021-00413-9.

**Additional file 1: Table S1.** The 77 phylogroup 1 *S. flexneri* 1 isolates used for construction of phylogenetic tree in Fig. 2 are listed. These isolates were taken from Connor et al. [23].

**Acknowledgements**

We thank Susan Silpikurian for performing some laboratory work.

**Authors' contributions**

MA and PP developed the concept of the project; MA and GC performed the experiments; DB, LP and MA analyzed and interpreted the results; MA wrote the manuscript. All authors read and approved the final manuscript.

**Funding**

This work did not receive specific support from any funding body.

**Availability of data and materials**

Sequence data are available in the NCBI BioProject database under the accession number, PRJNA556301. All other data are available in the manuscript.

**Declaration**

**Ethics approval and consent to participate**

Since the laboratory samples were part of the routine investigation for patient care, ethical approval was waived by the Ethics Committee, Al Sabah Hospital, Kuwait. Father of the infant gave informed written consent to participate. Local, national and international guidelines were followed in the conduct of this study.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Christopher PR, David KV, John SM, Sankarapandian V. Antibiotic therapy for *Shigella* dysentery. Cochrane Database Syst Rev. 2010;8:CD006784.

2. Abbasi E, Abtahi H, v. Belkum A, Ghasanavi-Rad E. Multidrug-resistant *Shigella* infection in pediatric patients with diarrhea from central Iran. Infect Drug Resist. 2019;12:535–44.

3. Puzari M, Sharma M, Chetta P. Emergence of antibiotic resistant *Shigellosis* a matter of concern. J Infect Public Health. 2018;11:451–4.

4. Ranjan R, Farahani A. *Shigella*: Antibiotic-resistance mechanisms and new horizons for treatment. Infect Drug Resist. 2019;12:3137–67.

5. Jamal W, Rotimi VO, Pål T, Sonnevend A, Dimitrov TS. Comparative in vitro activity of ticarcilin and other antimicrobial agents against *Shigella* species from Kuwait and the United Arab Emirates. J Infect Public Health. 2010;3:35–42.

6. Matar GM, Jaafar R, Sabra A, Hart CA, Corkill JE, Dbaibo GS, et al. First detection and sequence analysis of the bla-CTX-M-15 gene in Lebanese isolates of extended-spectrum-beta-lactamase-producing *Shigella sonnei*. Ann Trop Med Parasitol. 2007;101:511–7.

7. Rahman M, Shoma S, Rashid H, Siddique AK, Nair GB, Sack DA. Extended-spectrum beta-lactamase-mediated third-generation cephalosporin resistance in *Shigella* isolates in Bangladesh. J Antimicrob Chemother. 2004;54:846–7.

8. Liu G, Qian H, Tang B, Chen Y, Kang H, Jiang F, et al. Prevalence and characterisation of third-generation cephalosporin-resistant *Shigella* isolates from Jiangsu Province, China, 2013–2015. J Glob Antimicrob Resist. 2018;15:283–7.

9. Tajbaksh M, Garcia Migura L, Rahbar M, Svendsen CA, Mohammadzadeh M, Zali MR, et al. Antimicrobial resistant *Shigelloides* infections from Iran: an overlooked problem? J Antimicrob Chemother. 2012;67:1128–33.

10. Potz NAC, Hope R, Warner M, Johnson AP. Prevalence and mechanisms of resistance in *Enterobacteriaceae* in London and South-East England. J Antimicrob Chemother. 2006;58:320–6.

11. Livermore DM. Mechanisms of resistance to cephalosporin antibiotics. Drugs. 2012;34:64–88.

12. Liu Y, Cheng Y, Yang H, Hu L, Cheng J, Ye Y, et al. Characterization of extended-spectrum β-lactamase genes of *Shigella flexneri* Isolates with fosfomycin resistance from patients in China. Ann Lab Med. 2017;37:415–9.

13. Bian F, Yao M, Fu H, Yuan G, Wu S, Sun Y. Resistance characteristics of CTX-M-type *Shigella flexneri* China. Biosci Report. 2019;39:852019174.

14. Zhang CL, Liu QZ, Wang J, Chu X, Shen LM, Guo YY. Epidemic and virulence characteristic of *Shigella* spp with extended-spectrum cephalosporin resistance in Xiaoshan District, Hangzhou China. BMC Infect Dis. 2014;14:4260.

15. Alkouzian W, Bulach D, Izumiya H, AlBassam K, Sheik S, Alnuzaa’aan N, et al. Carbuncle due to *Salmonella* Enteritidis: a novel presentation. Gut Pathog. 2017;9:59.

16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disc susceptibility tests, M100S, 29th Ed., CLSI Vol. 39 No 1, Jan 2019.

17. Dioneux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β-lactamase production in *Enterobacteriaceae*: Review and bench guide. Clin Microbiol Infect. 2008;14(Suppl 1):90–103.

18. Souborov A, Agarwal A, Lipman DJ. SKESA: strategic k-mer extension for scrupulous assemblies. Gen Biol. 2018;19:3715–9.

19. Taihunlu A, García-Migura L, Rahbar M, Svendsen CA, Mohammadzadeh M, Zali MR, et al. Antimicrobial resistant *Shigelloides* infections from Iran: an overlooked problem? J Antimicrob Chemother. 2012;67:1128–33.

20. Potz NAC, Hope R, Warner M, Johnson AP. Prevalence and mechanisms of resistance in *Enterobacteriaceae* in London and South-East England. J Antimicrob Chemother. 2006;58:320–6.

21. Liu G, Qian H, Tang B, Chen Y, Kang H, Jiang F, et al. Prevalence and characterisation of third-generation cephalosporin-resistant *Shigella* isolates from Jiangsu Province, China, 2013–2015. J Glob Antimicrob Resist. 2018;15:283–7.

22. Zankari E, Hasman H, Cosentino S, Westergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67:2640–4.

Received: 5 December 2020 Accepted: 11 March 2021 Published online: 20 March 2021
23. Connor TR, Barker CR, Baker KS, Weil FX, Talukder KA, Smith AM, et al. Species-wide whole genome sequencing reveals historical global spread and recent local persistence in Shigella flexneri. eLife. 2015;4:e07335.

24. Coreia S, Poeta P, Hebraud M, Capelo JL, Igrejas G. Mechanism of quinolone action and resistance: where do we stand? J Med Microbiol. 2017;66:551–9.

25. Al Hashem G, Rotimi VO, Albert MJ. Antimicrobial resistance of serial isolates of Acinetobacter baumannii colonizing the rectum of adult intensive care patients in a teaching hospital in Kuwait. Microb Drug Resist. 2021;27:64–72.

26. Wang Y, Liu H, Wang Q, Du X, Yu Y, Jiang Y. Coexistence of blaOXM-51, blaOXY, and mph-1- IncX4 plasmids in a ST48 Escherichia coli strain in China. J Glob Antimicrob Resist. 2020;23:149–53.

27. Bennish ML, Albert MJ. Shigellosis. In: Selendy JMH, editor. Water and sanitation-related diseases and the environment: challenges, interventions and preventive measures. New Jersey: Wiley-Blackwell; 2011. p. 187–204.

28. Huskens WC, Griffiths JK, Farouque AS, Bennish ML. Shigellosis in neonates and young infants. J Pediatr. 1994;125:14–22.

29. Gu B, Fan W, Qin T, Kong X, Dong C, Tan Z, et al. Existence of virulence genes in clinical Shigella sonnei isolates from Jiangsu Province of China: a multicenter study. Ann Transl Med. 2017;5(14):305.

30. Martock E, Blocker AJ. How do the virulence factors of Shigella work together to cause disease? Front Cell Infect Microbiol. 2017;7:64.

31. Maldonado-Conteras A, Bitterley JR, Roll E, Zhao Y, Mumy KL, Toscano J, et al. Shigella depends on SepA to destabilize the intestinal epithelial integrity via coflin activation. Gut Microbes. 2017;8:544–60.

32. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev. 1993;57:138–63.

33. Lambert T, Rudant E, Bouvet P, Courvalin P. Molecular basis of aminoglycoside resistance in Acinetobacter spp. J Med Microbiol. 1997;46:731–5.

34. Parent R, Roy PH. The chloramphenicol acetyl transferase gene Tn2424: a new breed of Tn. J Bacteriol. 1992;174:2891–7.

35. Brolund A, Sundqvist M, Kahlmeter G, Grape M. Molecular characterisation of trimethoprim resistance in Escherichia coli and Klebsiella pneumoniae during a two-year intervention on trimethoprim use. PLoS ONE. 2010;5(2):e9233.

36. M. Closed genome and comparative phylogenetic analysis of the new breed of cat 2424: a coside resistance in Spp. J Med Microbiol. 1997;46:731–5.

37. Jiang H, Cheng H, Liang Y, Yu S, Yu T, Fang J, et al. Diverse mobile genetic elements and conjugal transferability of sulfonamide resistance genes (sul1, sul2, and sul3) in Escherichia coli isolates from Penaeus vannamei and pork from large markets in Zhejiang China. Front Microbiol. 2019;17:1787.

38. Bryan A, Shapir N, Sadowsky MJ. Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected, and nonclinical Escherichia coli strains isolated from diverse human and animal sources. Appl Environ Microbiol. 2004;70:2503–7.

39. Sawá T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care. 2020;8:13.

40. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. CTX-M-type β-lactamases: an emerging group of extended-spectrum enzymes. Int J Antimicrob Agents. 2000;14:137–42.

41. Cantón R, Coque TM. The CTX-M β-lactamase pandemic. Curr Opin Microbiol. 2006;9:466–75.

42. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrob Agents Chemother. 2010;54:24–38.

43. Alpay-Karaoglou S, Ozgumus OB, Sevim E, Kolayli F, Sevim A, Yesilgil P. Investigation of antibiotic resistance profile and TEM-type β-lactamase gene carriage of ampicillin-resistant Escherichia coli strains isolated from drinking water. Ann Microbiol. 2007;57:281.

44. Coque TM, Novais A, Carattoli A, Poirel L, Pitout JD, Peixe L, et al. International dissemination of clonally related Escherichia coli strains expressing the extended-spectrum β-lactamase CTX-M-15. Emerg Infect Dis. 2008;14:195–200.

45. Ranjarb R, Ghazi FM, Farshad S, Giammanco GM, Aleo A, Owlia P, et al. The occurrence of extended-spectrum beta-lactamase producing Shigella spp. in Tehran, Iran. Iran J Microbiol. 2013;5:108–12.

46. Kim JS, Kim J, Jeon SE, Kim SJ, Kim NO, Hong S, et al. Complete nucleotide sequence of the IncI1 plasmid pSh4469 encoding CTX-M-15 extended-spectrum beta-lactamase in a clinical isolate of Shigella sonnei from an outbreak in the Republic of Korea. Int J Antimicrob Agents. 2014;44:533–7.

47. Mammen H, Poirel L, Fortineau N, Nordmann P. Naturally occurring extended-spectrum cephalosporinases in Escherichia coli. Antimicrob Agents Chemother. 2006;50:2573–6.

48. Allue-Guardia A, Koenig SSX, Quiros P, Muniesa M, Bono JL, Eppinger M. Closed genome and comparative phylogenetic analysis of the clinical multidrug resistant Shigella sonnei strain 866. Genome Biol Evol. 2018;10:2241–7.

49. Schumacher H, Nic M, Mansa B. Grassy A. beta-lactamases in Escherichia coli strains isolated from patients with bacillary dysentery in Lebanon. J Insect Dev Cyt. 2009;3(4):300–5.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.