Prevalence and characterisation of extended spectrum β-lactamases genes in *Shigella* isolates, in Wenzhou, Southern China

Dear Editor,

Shigellosis remains a public health concern throughout the world.[1] However, the emergence of multiple-drug-resistant (MDR) strains of *Shigella* spp. has made the treatment for shigellosis more difficult.[2] Third-generation cephalosporins so far have been commonly used to treat the infections caused by MDR isolates. However, since the first report of SHV-11-type β-lactamases in *Shigella dysenteriae* from India in 1999,[3] *Shigellae* species expressing extended-spectrum β-lactamases (ESBLs) have emerged globally, and this has further narrowed the choice of effective antimicrobials.[4] The objectives of the present study were to determine the antibiotic susceptibility patterns, evaluate the distribution of the serotypes of *Shigella* spp. and investigate the drug-resistant genes of ESBLs in *Shigella* in Wenzhou, southern China.

A total of 62 non-duplicated *Shigella* strains (42 *Shigella flexneri*, 16 *Shigella sonnei*, 3 *Shigella dysenteriae* and 1 *Shigella boydii*) were isolated from stool samples of patients with diarrhoea in six hospitals of Wenzhou from June 2006 to

June 2009. Serotypes for *Shigella* isolates were determined by serum agglutination test by slide agglutination. Eight serotypes were identified, that is *S. sonnei* (n = 16, 25.8%), *S. flexneri* serotype f4c (n = 15, 24.2%), *S. flexneri* serotype f2a (n = 8, 12.9%), *S. flexneri* serotype f1a/f2b (n = 5, 8.1%), *S. flexneri* serotype f4a (n = 4, 6.5%), *S. flexneri* serotype f4b (n = 3, 4.8%) and *S. flexneri* serotype f1b (n = 2, 3.2%). The predominant serotype was *S. flexneri* serotype f4. In developing countries, *S. flexneri* serotype f4 is not a commonly identified serotype, the predominant serotype of *S. flexneri* is 2a, followed by 1b, 3a, 4a and 6. However, our current study indicates that S. flexneri serotype f4 is common in Zhejiang, China.

Antimicrobial susceptible testing was performed by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2010). Production of ESBLs was determined by double disc synergy and inhibitor-potentiated disc diffusion tests. ESBLs genes were detected by PCR using primers described previously.[5] Both *S. flexneri* and *S. sonnei* isolates have high resistance against ampicillin and nalidixic acid and high sensitivity to piperacillin/tazobactam and cefepime. More than 37% of *Shigella* isolates showed resistance to ceftriaxone or cefotaxime. However, susceptibility to the different antimicrobials appeared to differ depending on the species. The prevalence of ceftriaxone (or cefotaxime) and gentamicin resistance of *S. sonnei* isolates (12/16, 75%; 12/16, 75%) was significantly higher than that (9/42, 21.4%; 4/42, 9.5%) of *S. flexneri* isolates ($P < 0.005$, $\chi^2 = 14.4$; $P < 0.005$, $\chi^2 = 14.92$). Twenty-two isolates were positive in phenotypic confirmatory tests. ESBLs genes were detected in 22 of 62 clinical *Shigella* isolates, which involved the $bla_{\text{CTX-M-14}}$ gene from 12 *S. sonnei*, 7 *S. flexneri* and 2 *S. dysenteriae*, the $bla_{\text{CTX-M-15}}$ gene from 1 *S. dysenteriae* and 2 *S. flexneri*, the $bla_{\text{CTX-M-65}}$ gene from 1 *S. sonnei* and $bla_{\text{SHV-12}}$ gene from 1 *S. dysenteriae* [Table 1]. Obviously, $bla_{\text{CTX-M-14}}$ was the major ESBLs gene. Conjugation experiments were performed using rifampin-resistant *Escherichia coli* C600 as the recipient strain. ESBLs genes were successfully transferred from 15 of 22 ESBL-producing isolates to *E. coli* C600. The 15 ESBL-producing transconjugants showed 128- to 1024-fold increases in the MIC ceftriaxone and 256- to 1064-fold increases in the MIC cefotaxime relative to those of the recipient. These data provide evidence that ESBLs can be disseminated through horizontal gene transfer to other *Shigella* pathogens. Pulsed-field gel electrophoresis (PFGE) typing was performed for all the isolates as described previously,[5] and a high genetic homogeneity was observed among nine ESBL-producing *S. sonnei* isolates. Therefore, more active surveillance is clearly needed to minimise the spread of ESBL-producing *Shigella* isolates. These data further emphasised the necessity of strict antimicrobial application control in developing nations.

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