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Short communication

Possible transfer of plasmid mediated third generation cephalosporin resistance between *Escherichia coli* and *Shigella sonnei* in the human gut

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ABSTRACT

Choice of antibiotic for treatment of serious bacterial infection is rapidly diminishing by plasmid mediated transfer of antibiotic resistance. Here, we report a possible horizontal transfer of plasmid carrying third-generation-cephalosporin (TGC) resistance between *Escherichia coli* and *Shigella sonnei*. Two different types of colonies were identified in MacConkey agar plate from a faecal specimen collected from a patient with shigellosis. The colonies were identified as *E. coli* and *S. sonnei*. Both of the isolates were resistant to ampicillin, chloramphenicol, co-trimoxazole, erythromycin, azithromycin, nalidixic acid, ceftriaxone, cefixime, ceftazidime, cefotaxime and susceptible to co-amoxiclave, amikacin, imipenam, astreonam, levofloxacin, moxifloxacin, mecillinam. These two strains were positive for extended spectrum β-lactamase. We were able to transfer ESBL producing property from both ceftriaxone-resistant isolates to the ceftriaxone susceptible recipient *E. coli* K12 and *S. sonnei*. Plasmid profile analysis revealed that the first-generation *E. coli* K12 and *S. sonnei* transconjugants harbored a 50 MDa R plasmid, as two-parent ESBL-producing *S. sonnei* and *E. coli* strains. Similar patterns of ESBL producing plasmid and transferable antimicrobial phenotype suggests that the ESBL producing plasmid might transferred between *E. coli* and *S. sonnei* through conjugation in the human gut.

1. Introduction

Antimicrobial therapy for bacterial dysentery, aimed to resolve diarrhea or reducing duration, risk of complications, as well as spread of infection, is routinely used in Bangladesh like many other countries (Radice et al., 2001). Abundant and inappropriate use of antimicrobials causes selection of resistant bacterial strains and may facilitate to develop resistance against the frequently used antibiotics (Tenover, 2006). Thus, multi-drug resistant [MDR; resistant to $\geqslant$3 different classes of antimicrobials such as ampicillin, trimethoprim-sulphamethoxazole (TMP-SMZ), nalidixic acid] *Shigella* isolates are common in many countries (Rahman et al., 2007; Prats et al., 2000; Replogle et al., 2000). Therefore, third generation cephalosporins (TGC) were used for the treatment of MDR *Shigella* infections. Bacterial pathogens resistant to third generation cephalosporin due to the extended spectrum β-lactamase (ESBL) are also resistant to all β-lactams except cephamicins and carbapenems has become a worldwide problem (Bradford, 2001). Following the first report in 1985 in Germany, so far more than 150 different ESBLs have been described. Although various types of ESBLs have been found worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa*, (Bradford, 2001), only a few reports are on ESBL producing *Shigella* (Rahman et al., 2004; Radice et al., 2001; Ahmed and Kundu, 1999; Fortineau et al., 2001; Pai et al., 1960). ESBL producing plasmid was shown to be transferable to *Escherichia coli* K12 and also in the wild type *Shigella* strains in vitro (Rahman et al., 2004). In vivo transfer of resistance factor between enterobacterial populations have been demonstrated in experimental animals by several studies (Salzman and Klemm, 1968; Walton, 1966). In human, a number of investigators have tried but not succeeded to demonstrate the transfer of R plasmid (Anderson, 1975a; Smith, 1969). Therefore, in vivo transfer of ESBL producing R plasmid in the human intestine is an especial interest for the researchers in this field. In the current study, we described a possible natural transfer of ESBL producing plasmid between *E. coli* and *Shigella sonnei* in the human gut with shigellosis.

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2. Materials and methods

2.1. Bacterial Strains

The strains were isolated from faecal samples of a patients with shigellosis in ARI laboratory, International Center for Diarrheal Disease, Bangladesh in 2005. Faecal specimen was subjected to bacteriological culture that produces two different types of colony on MacConkey agar medium. Two colonies were identified as *E. coli* and *S. sonnei* by using standard microbiological, biochemical and serological techniques.

2.2. Antimicrobial susceptibility test

Antimicrobial susceptibility was determined by the Clinical Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines using Muller-Hinton agar, commercial antimicrobial discs (Oxoid, Basingstoke, United Kingdom) and *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) as reference strain (CLSI, 2005). Antimicrobials discs used were ampicillin, amoxicillin + clavulanic acid (AMC), azithromycin, erythromycin, chloramphenicol, trimethoprim-sulphamethoxazole, mecillinam, nalidixic acid, tetracycline, ceftriaxone, cefotaxime, cefixime, cephalothin and ceftazidime (Oxoid, UK). Minimum inhibitory concentration (MIC) of ampicillin, azithromycin, ceftriaxone, nalidixic acid, ciprofloxacin and levofloxacin was determined by *E*-test (AB Biodisk, Solna, Sweden). Nalidixic acid and ceftriaxone powder were obtained from Sigma Chemical Co., St. Louis, MO, USA.

2.3. Detection of ESBL by double disc diffusion synergy test

In the double disc synergy test (DDST), synergy was determined between a disc containing amoxicillin-clavulanate (20 μg amoxicillin and 10 μg clavulanic acid) and 30 μg disc of each oxyimino cephalosporins (third generation cephalosporin; cefotaxime, ceftriaxone, ceftazidime) (3GC). Cefotaxime, ceftriaxone and ceftazidime placed at a distance of 25 mm apart from center of amoxicillin-clavulanate on a lawn culture of the ceftriaxone resistant isolate on Mueller Hinton Agar (Bradford, 2001; Vercauteren et al., 1997). The test organism was considered to produce ESBL (positive), if the zone size around the test antibiotic disc increases towards the amoxicillin-clavulanate disc.

2.4. Transfer of ESBL producing plasmid by conjugation

Conjugation between TGC resistant *Shigella* isolates (donor) and *E. coli* K12 strain (F-, lac$^-$, Nal^R) was performed according to the method of Neu et al. (1975). Transconjugant was selected on MacConkey agar containing ceftriaxone (6 μg/ml) and nalidixic acid (32 μg/ml) that produce lactose-fermenting pink colonies of *E. coli* in contrast to non-lactose-fermenting pale colonies of *Shigella* isolate. For ESBL positive *E. coli*, TGC resistance was transferred to *S. sonnei* (susceptible) and transconjugant was selected on MacConkey agar containing ceftriaxone (6 μg/ml) and nalidixic acid (32 μg/ml). All putative transconjugants were examined for antimicrobial susceptibility and plasmid profiles to obtain transconjugants and were tested for ESBL. In the second transfer of R plasmid by conjugation, ceftriaxone resistance and ESBL production were transferred from transconjugants *E. coli* K12 to ceftriaxone-susceptible and SXT-resistant wild *S. sonnei*. The second-generation *Shigella* transconjugants were selected on MHA plate supplemented with ceftriaxone (6 μg/mL) and trimethoprim (24 μg/mL) and tested for antimicrobial susceptibility, ESBL and R plasmid.

2.5. Plasmid extraction and detection in agarose gel electrophoresis

The ESBL producing clinical strains and the corresponding transconjugants were subjected to plasmid extraction according to the method of Portnoy et al. (1981) and separated by electrophoresis in 0.5% agarose gel at 100 V current for 3 h. Gel was stained with ethidium bromide and visualized. Reference plasmid markers *E. coli* VR_1 was used to determine the size of unknown plasmid.

3. Results

A faecal specimen from a patient with shigellosis was tested by bacteriological culture that produces two different type of colony on MacConkey agar medium. The colonies were identified as *E. coli* and *S. sonnei*. Both *E. coli* and *S. sonnei* were resistant to ampicillin, chloramphenicol, co-trimoxazole, erythromycin, azithromycin, nalidixic acid, ceftriaxone, cefixime, ceftazidime, cefotaxime, astreonam and susceptible to co-amoxiclave, amikacin, imipenam, levofloxacin, moxifloxacin, mecillinam (Table 1). MIC of ceftriaxone, ampicillin, cotrimoxazole, ciprofloxacin, levofloxacin, azithromycin and nalidixic acid were >256/>256, >256/>256, >32/>32, 0.25/0.25, 0.25/0.25, 8/8 and 128/256 respectively for *S. sonnei* and *E. coli* (Table 2). Both strains exhibited decreased susceptibility to ciprofloxacin (MIC = 0.25 μg/mL). The transconjugants strains exhibited similar MIC for beta-lactams as the ESBL containing clinical isolates. However, the MIC of transconjugants and ESBL producing isolates for co-trimoxazole, ciprofloxacin, levofloxacin were different (Table 2).

Both ceftriaxone-resistant isolates were positive by DDST and resistant to β-lactam, but susceptible to β-lactam/β-lactamase inhibitor combination, cephamycin (cefoxitin), monobactam (astreonam) and carbapenem (imipenem) indicating the production of a class A ESBL (Bush group 2; Table 1) (Rahman et al., 2004). We were able to transfer β-lactam resistance and β-lactamase (ESBL) producing plasmid to the recipient *E. coli* K12 and *S. sonnei* (TGC susceptible) from both of the ceftriaxone resistant parent isolates. Subsequently, the TGC resistance and β-lactamase producing plasmid were transferred by second conjugation from *E. coli* transconjugants to wild type co-trimoxazole resistant *S. sonnei*. Plasmid analysis revealed that the first-generation *E. coli* transconjugants harbored a 50 MDa R plasmid, as carried by ESBL-producing two parent isolates *S. sonnei* and *E. coli* (Fig. 1) and their

Table 1
Antimicrobial resistance phenotype transferred from ESBL producing *E. coli* and *S. sonnei* to *E. coli* K12 recipient.

Strain	Susceptibility to			Resistance phenotype	Transferred resistance phenotype to *E. coli* K12
	AMC	CTX	CAZ		
E. coli	S	R	I	AMP, CRO, CTX, CFL, CFM, CAZ, SXT, TET, NAL, AZM	AMP, CRO, CTX CFL, CFM, CAZ
S. sonnei	S	R	I	AMP, CRO, CTX, CFL, CFM, CAZ, SXT, TET, NAL, AZM	AMP, CRO, CTX CFL, CFM, CAZ

S; susceptible, R; resistant, I; intermediate.
Abbreviations: AMP, ampicillin; AMC, amoxicillin-clavulanate; CRO, ceftriaxone; CTX, cefotaxime; CAZ, ceftazidime; CFM, cefixime; CFL, cephalothin; SXT, trimethoprim-sulphamethoxazole; NAL, nalidixic acid; TET, tetracycline; AZM, azithromycin.