Indian J Med Res 134, October 2011, pp 487-489

Correspondence

CTX-M15 type ESBL producing *Salmonella* from a paediatric patient in Chennai, India

Sir,

Resistance to 3rd generation cephalosporins in *Salmonella* has been attributed to the production of extended spectrum β-lactamases (ESBLs), but AmpC and KPC or metallo β-lactamase (MBL) mediated resistance have also been reported[1]. ESBLs reported in *Salmonella* spp. are the derivatives of TEM, SHV, CTX-M and PER type β-lactamase families[1]. We isolated *Salmonella enterica* ser. Typhimurium (B9903) from the blood culture of a 6-month old baby admitted to a paediatric hospital in Chennai in October, 2009. The patient was febrile, lethargic and refused intake. The baby was diagnosed with haemophagocytic syndrome with NK cell deficiency, dengue hemorrhagic fever grade IV, sepsis, steroid induced hypertension. The baby responded to ciprofloxacin and co-trimoxazole along with intravenous immunoglobin and steroids. The child was well at the time of discharge after 17 days in hospital. There was no history of travel or any animal contact.

Species identification and antibiotic susceptibility were performed by automated system (VITEK-2, France). The isolate was further confirmed by serotyping using specific antiserum. Antigenic structure of the isolate was identified as O4, 12: H phase 1 – i: phase 2 – 1, 2 at National Salmonella & Escherichia Centre, Central Research Institute, Kasauli, India. The antibiotic susceptibility testing was interpreted according to CLSI guidelines[2]. The isolate was resistant to ampicillin, cefepime, ceftriaxone and ceftazidime but susceptible to piperacillin/tazobactam, ciprofloxacin, co-trimoxazole, and tetracycline. The ESBL phenotype was determined by combined disc synergy test[2] and further confirmed by using E-strip (AB bioMerieux, Solna, Sweden) with cefotaxime and clavulanic acid.

The strain was identified as belonging to Salmonella phage type group E. The phage typing was carried out at National Salmonella Phage Typing Centre at Lady Hardinge Medical College, New Delhi.

PCR-assays for the genes encoding β-lactamases[3] showed that *S.* Typhimurium B9903 was found to carry the ESBL and narrow-spectrum β-lactamase genes namely, $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$. The regions located upstream of the $bla_{\text{CTX-M}}$ were amplified with primers annealing to IS*Ecp1* and to *orf513* of IS*CR1* together with reverse primer of $bla_{\text{CTX-M}}$[4]. The sequences located downstream of the $bla_{\text{CTX-M}}$ gene were amplified with forward primer of $bla_{\text{CTX-M}}$ and reverse primer of IS*903* Bint[4].

Amplification with primers IS*Ecp1*and $bla_{\text{CTX-M}}$ yielded a PCR product of about 1050 bp. Sequencing analysis of PCR products from original isolate and transconjugant revealed that insertion element IS*Ecp1* was located upstream of $bla_{\text{CTX-M-15}}$ gene similar to the *E. coli* isolate previously reported in India (Gen Bank accession number AY044436)[5]. Amplification with primers TEM F and TEM R yielded a 861 bp product with a sequence consistent with that of $bla_{\text{TEM-1}}$.

Plasmid analysis revealed that *S.* Typhimurium B9903 harboured a plasmid with size of 25 kb, using *E. coli* NCTC 50192 harbouring four plasmids of 154, 66, 38 and 7 kb as a reference marker[6]. To determine if the ESBL-phenotype was transferable, liquid mating-out experiment was attempted with azide-resistant *E. coli* J53 as a recipient. Transconjugants were selected on Mac Conkey agar plates containing sodium azide (200 μg/ml) and cefotaxime (4 μg/ml)[6]. The ESBL-phenotype was successfully transferred in conjugation and transconjugation efficiency was very high about 5 x 10^{-1} per donor cells. Plasmids purified from the original strain and transconjugant were typed by PCR-based replicon typing[7].

Table. MICs (µg/ml) of antimicrobial agents for the clinical strain, transconjugant and recipient strain

Antibiotics	*Salmonella* B9903 (TEM-1 & CTX-M15)	*E. coli* J53 B9903 (TEM-1 & CTX-M15)	*E. coli* J53
Ampicillin	>512	>512	<2
Amoxicillin + clavulanic acid	16/8	16/8	<2/1
Cefotaxime	>512	>512	<0.25
Cefotaxime + clavulanic acid	<0.25/4	<0.25/4	<0.25/4
Ceftazidime	>512	>512	<0.25
Ceftazidime + clavulanic acid	<0.25/4	<0.25/4	<0.25/4
Cefpodoxime	>512	>512	0.5
Cefpodoxime + clavulanic acid	1.0/4	0.5/4	0.5/4
Cefoxitin	8	8	8
Piperacillin	>16	>16	<16
Piperacillin + Tazobactam	<4/4	<4/4	<4/4
Nalidixic acid	2	1	1
Ciprofloxacin	0.015	<0.015	<0.015
Co-trimoxazole	<20	<20	<20
Amikacin	<2	<2	<2
Gentamicin	<1	<1	<1
Chloramphenicol	4	8	8
Tetracycline	2	2	2
Ertapenem	<0.5	<0.5	<0.5
Meropenem	<0.25	<0.25	<0.25
Imipenem	<0.25	<0.25	<0.25

The MICs of various antibiotics were determined by using broth macro dilution method according to CLSI guidelines (Table). The MICs of all the β-lactams except carbapenems and cefoxitin for *S.* Typhimurium B9903 and its transconjugant were higher than that of recipient strain. There was a reduction in the MICs of β-lactams when tested with clavulanic acid/tazobactam.

Plasmid analysis revealed that the ESBL-positive transconjugant harboured a 25 kb plasmid that belonged to IncI1 type. It was found to carry the $bla_{\text{CTX-M-15}}$ gene together with $bla_{\text{TEM-1}}$ by PCR.

ESBL producing non-typhoidal Salmonellae are an increasing concern worldwide. CTX-M15 producing *S. enterica* has been reported from Indian and Pakistani patients hospitalized in Kuwait[8]. $bla_{\text{CTX-M-15}}$ gene linked with IncI1 type plasmids have been identified in various strains in different geographic locations around the world viz., *S*. Typhimurium, *S*. Ohio, *S*. Enteritidis, *E. coli* in UK; *S*. Anatum in Pakistan; *E. coli* in France and Australia and *S*. Infantis in Honduras[9-11]. We report here a CTX-M-15 type ESBL producing *Salmonella* isolated from the blood culture of a paediatric patient with haemophagocytic syndrome.

The finding of the $bla_{\text{CTX-M}}$ gene in *Salmonella* in association with IS*Ecp1* is a matter of concern, as this insertion element facilitates the mobilization and expression of CTX-M mediated resistance, thereby limiting the therapeutic options available for typhoid fever and curtailing the use of cephalosporins. Measures such as improved food and personal hygiene could help prevent the potential spread of such resistant strains.

The sequences of the $bla_{\text{CTX-M15}}$ associated with IS*Ecp1* has been submitted to the GenBank database and assigned accession number HM117627.

Acknowledgment

Authors thank Shrimati Priya Goodwill and Shri A. Ramesh, Apollo Hospital, Chennai, for their technical assistance, and National Salmonella & Escherichia Centre, Central Research Institute, Kasauli, India for serotyping. The authors acknowledge the help of Dr Alessandra Carattoli, Department of Infectious, Parasitic, Immune-mediated Diseases, Istituto Superiore di Sanità, Rome, Italy towards plasmid typing in this study.

Karthikeyan K[+], M. Thirunarayan[*] &
Padma Krishnan
Department of Microbiology
Dr ALM PG IBMS
University of Madras &
[*]Department of Microbiology
Apollo Hospitals, Chennai, India
For correspondence:
[+]skk.microbes@gmail.com

References

1. Miriagou V, Tassios PT, Legakis NJ, Tzouvelekis LS. Expanded-spectrum cephalosporin resistance in non-typhoid *Salmonella*. *Int. J Antimicrob Agents* 2004; *23* : 547-55.
2. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing:* 20th Informational Supplement M100-S20. Wayne, PA, USA: CLSI; 2010.
3. Voets GM, Fluit AC, Scharringa J, Cohen Stuart J, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. *Int J Antimicrob Agents* 2011; *37* : 356-9.
4. Lartigue MF, Zinsius C, Wenger A, Bille J, Poirel L, Nordmann P. Extended-spectrum beta-lactamases of the CTX-M type now in Switzerland. *Antimicrob Agents Chemother* 2007; *51* : 2855-60.
5. Karim A, Poirel L, Nagarajan S, Nordmann P. Plasmid-mediated extended-spectrum beta-lactamase (CTX-M-3 like) from India and gene association with insertion sequence IS*Ecp1*. *FEMS Microbiol Lett* 2001; *201* : 237-41.
6. Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob Agents Chemother* 2005; *49* : 71-6.
7. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; *63* : 219-28.
8. Rotimi VO, Jamal W, Pal T, Sovenned A, Albert MJ. Emergence of CTX-M-15 types extended-spectrum beta-lactamase-producing *Salmonella* spp. in Kuwait and the United Arab Emirates. *J Med Microbiol* 2008; *57* : 881-6.
9. Zong Z, Partridge SR, Thomas L, Iredell JR. Dominance of blaCTX-M within an Australian extended-spectrum beta-lactamase gene pool. *Antimicrob Agents Chemother* 2008; *52* : 4198-202.
10. Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A. Replicon typing of plasmids carrying CTX-M or CMY β-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. *Antimicrob Agents Chemother* 2006; *50* : 3203-6.
11. Marcade G, Deschamps C, Boyd A, Gautier V, Picard B, Branger C, *et al*. Replicon typing of plasmids in *Escherichia coli* producing extended-spectrum β-lactamases. *J Antimicrob Chemother* 2009; *63* : 67-71.