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Correspondence

Genetic environment of OXA-2 beta-lactamase producing Gram-negative bacilli from a tertiary referral hospital

Sir,

OXA-2 type beta lactamses belong to Ambler molecular class D and functional Group 2d. These types of beta lactamases are characterized by their high hydrolytic spectrum of activity against cloxacillin and oxacillin, and are poorly inhibited by clavulanic acid. Presence of this gene was first reported in *Pseudomonas* in France[1], in *Escherichia coli* from Israel[2], and in India it was reported in *E. coli* in 2007[3]. However, there is no knowledge regarding genetic environment and gene location of this resistant determinant from this part of the world. Our study reports presence of $bla_{\text{OXA-2}}$ within IncF plasmid in a tertiary referral hospital of north-east India.

This study was conducted in the department of Microbiology, Assam University, Silchar. A total number of 476 consecutive, non-duplicates, Gram-negative rods consisting of members of Enterobacteriaceae family and non-fermenting Gram-negative rods were isolated from different clinical specimens spanning a period of 12 months (March 2012 to February 2013) from different Wards/Clinics of Silchar Medical College and Hospital, Assam, India (Table). Screening and confirmation for extended spectrum beta lactamases (ESBLs) was done as per Clinical Laboratory Standards Institute (CLSI) guidelines[4]. Multiplex PCR was performed to characterize ESBL genes[1]. Reactions were run under the following conditions: initial denaturation 94°C for 5 min, 33 cycles of 94 °C for 35 sec, 51°C for one min,

Table. Details of $bla_{\text{OXA-2}}$ harbouring isolates

Sl. No.	Age (yr)	Sex	Wards/OPD	Clinical sample	Organisms	Other ESBL gene	Integron
1	70	F	Female Burn Unit	Urine	*Escherichia coli*	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$	Class 2
2	48	M	Surgery	Pus	*P. aeruginosa*	$bla_{\text{SHV-148}}$	Class 1
3	2	F	Paediatrics	Urine	*P. aeruginosa*	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$	Class 1
4	5	F	ENT	Oral swab	*E. coli*	$bla_{\text{SHV-148}}$,$bla_{\text{CTX-M-15}}$	Class1
5	40	M	Medicine	Urine	*E. coli*	$bla_{\text{CTX-M-15}}$	Class 1
6	40	F	Gynaecology	Urine	*E. coli*	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$	Class 1&2
7	45	F	Gynaecology	Urine	*Klebsiella* spp.	$bla_{\text{SHV-148}}$	Class 1
8	30	F	Surgery	Pus	*E. coli*	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$	Class 1
9	48	M	Medicine	Sputum	*E. coli*	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$	Class 1
10	3	F	Paediatrics	Pus	*E. coli*	$bla_{\text{CTX-M-15}}$	Class 1
11	2 month	M	Paediatrics	Urine	*P. aeruginosa*	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$	Class 1
12	22	F	Gynaecology	Urine	*Klebsiella* spp.	$bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, $bla_{\text{SHV-148}}$	Class 1
13	5	M	Paediatrics	Pus	*E. coli*	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-148}}$	Class 1
14	30	M	Medicine	Urine	*E. coli*	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-148}}$, $bla_{\text{TEM-1}}$	Class 1
15	30	M	Medicine	Urine	*Klebsiella* spp.	$bla_{\text{SHV-148}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$	Class 1

72°C for one min and final extension at 72°C for seven min. PCR product was purified (Gene Jet Purification kit, Lithuania) and sequencing was done. For detection of class 1 and class 2 integron, integrase gene PCR was performed[5]. Two PCR reactions were carried out, one with HS287 and bla_{OXA-2} reverse, another with HS286 and bla_{OXA-2} forward[1,6]. The amplified products were further sequenced. Plasmids were purified by Gene Jet plasmid Miniprep kit (Thermo scientific, Lithuania). Transformation was carried out using *Escherichia coli* JM107 as recipient. Transformants were selected on cefotaxime (0.5 mg/l) containing Luria-Bertani agar (Hi-Media, Mumbai, India) plates. Conjugation experiments were carried out between clinical isolates as donors and a streptomycin resistant *E. coli* recipient strain B (Genei, Bangalore), transconjugants were selected on cefotaxime (0.5 mg/l) and streptomycin (800 mg/l) agar plates. For plasmid profiling, 1.5 µl of each sample was used and analyzed by agarose gel electrophoresis (1% agarose, Hi-Media, Mumbai, India), gel was run at 40V for 8 h at 18°C. PCR based replicon typing was carried out targeting 18 different replicon types, to perform five multiplex and three simplex PCRs as described previously[7]. Antimicrobial susceptibility was determined by Kirby Bauer disc diffusion and minimum inhibitory concentration (MIC) method[4]. Typing of isolates was done by enterobacterial repetitive intergenic consensus (ERIC) PCR[8].

A total of 15 isolates were harbouring OXA-2 gene which was further confirmed by sequencing. Co-existence of other ESBL genes was also noticed in all 15 isolates (Table). Class 1 integron was found in 13 isolates whereas one isolate carried class 2 integron and the remaining isolate carried class 1 and 2 both (Table). Sequencing results confimed that bla_{OXA-2} was found to be located within class I integron in 14 isolates while presence of this gene in class2 integron could not be established. Transformation results disclosed that in 13 isolates bla_{OXA-2} was located within the 20 kb plasmid which was also conjugatively transferable in *E. coli* strain B. Incompatibility typing of plasmids demonstrated that diverse Inc group types namely I1/Iγ, FIA, FIB, FIC, Y, FrepB, K and B/o were present in all bla_{OXA-2} harbouring isolates. But plasmid IncF was found to be common in all isolates as well as in their transformants and transconjugants. Tigecycline (n= 13; 86.66%) was the most effective antibiotics followed by imipenem (n=12; 80%) and meropenem (n=12; 80%). High MICs was observed against different groups of cephalosporins (≥256 µg/ml; n =15) and monobactam (≥256 µg/ml; n=15). All the OXA-2 producing isolates were clonally unrelated.

This study indicates propagation of the bla_{OXA-2} by horizontal gene transfer additionally facilitated by integron mediated gene capture mechanism. Presence of this rare type of ESBL gene in diverse group of organisms and its carriage in integrons may restrict therapeutic options.

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