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Correspondence

A report on the presence of GES-5 extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* associated with urinary tract infection from north-east India

Sir,

Guiana extended spectrum (GES) beta lactamase belongs to molecular class A[1] frequently found in Gram-negative rods by and large in *Pseudomonas aeruginosa* in addition to other members of *Enterobacteriacae*[2]. Till date, only a few studies on epidemiology and environmental burden of GES-type ESBLs have been published[1,2].

We present the occurrence of bla_{GES-5} harbouring *Pseudomonas aeruginosa* isolated from human urine specimen from north-east India.

The study was conducted in the department of Microbiology, Assam University, Silchar, Assam, India, from January to December 2012. The first isolate (AM 328) was obtained from the urine sample of a 4 month old female in May 2012 and the second isolate (AM 438) was recovered from the urine of a 39-year-old male in July 2012 (Table). These patients attended the Out Patient Department of Silchar Medical College and Hospital, Silchar, Assam and diagnosed with urinary tract infection. The selection of the samples was based on the initial screening of isolates for the presence of ESBL[3]. Antimicrobial susceptibility was determined by Kirby Bauer disc diffusion method on Muller-Hinton agar plates[3]. The following antibiotics were used for antimicrobial susceptibility: cefopodoxime (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), trimithoprim/dulphamethoxazole (1.25/23.75 µg), tigecycline (15 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), ceftriaxone (30 µg), aztreonam (30 µg) and cefoxitin (30 µg). Minimum inhibitory concentrations (MIC) of various antibiotics [cefotaxime, ceftazidime, Ceftriazone, cefepime, imipenem, meropenem, ertapenem and aztreonam (Hi-Media, Mumbai, India] were determined on Muller Hinton agar plates containing 2,4, 8, 16, 32, 64, 128, 256 mg/l of antibiotics, by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines[3].

Table. Clinical information and molecular details of bla_{GES-5} carrying *P. aeruginosa* urinary isolates

ID. No.	Male/ Female	Age (y)	Integron	Replicon type
AM-328	Female	4	Class-1	I1/Iγ, FIA, FIB, W, FIC, FrepB and K
AM-438	Male	39	Class-1	I1/Iγ, FIA, FIB, W, FIC and FrepB

For amplification and characterization of bla_{ESBL} genes, a set of six primers were used: bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{OXA-2}, bla_{OXA-10} and bla_{GES} as described previously[1]. Reactions were run under the following conditions: initial denaturation 94°C for 5 min, 33 cycles at 94°C for 35 sec, 51°C for one min, 72°C for one min and final extension at 72°C for seven min. PCR product was purified by Gene Jet PCR purification Kit (Thermo Scientific, Lithuania); 30 µl of purified products were used for sequencing along with bla_{GES}. Efflux pump activity of the isolates was phenotypically detected by double disc synergy test[4] using imipenem (10 µg), ertapenem (10 µg) and CCCP (100mM) (carbonyl cyanide m-chlorophenylhydrazone) (Hi-Media, Mumbai, India) as described earlier[4]. MIC reduction assay was performed using imipenem and ertapenem alone and in combination with CCCP at a concentration 20 µg/ml[5]. For detection of class 1 and class 2 integron, integrase gene PCR were performed as described previously[6]. For detection of association of gene cassette with bla_{ESBL} gene, two PCR reactions were carried out, one with HS287 and $bla_{GES-1-B}$, another with HS286 and $bla_{GES-1-B}$[1,7]. The amplified products were further sequenced. Transformation

was carried out using *Escherichia coli* JM107 as recipient. Transformants were selected on Luria-Bertani (L-B) agar (Hi-Media, Mumbai, India) plates containing 0.5 mg/l cefotaxime. L-B agar with and without cefotaxime 0.5 mg/l control plate was used. Conjugation experiments were performed between clinical isolates as donors and a streptomycin resistant *E. coli* recipient strain B (Genei, Banglore). Overnight culture of the bacteria was diluted in L-B broth and was grown at 37 °C till the optical density (O.D.) of the reciepient and donor culture reached 0.8-0.9 at A_{600} absorbance. Donor and recipient cells were mixed at 1:5 donor-to-recipient ratios and transconjugants were selected on cefotaxime (0.5 mg/l) and streptomycin (800 mg/l) agar plates; 1.0 µl of each sample was used for plasmid profiling and analysed by agarose gel electrophoresis (1% agarose, Hi-Media, Mumbai, India). For the detection of incompatibility group type of plasmid in all bla_{GES-5} producing strains, PCR based replicon typing was carried out targeting 18 different replicon types, to perform five multiplex and three simplex PCRs to amplify the FIA, FIB, FIC, HI1, HI2, I1/Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA replicons as described previously[8]. Typing of bla_{OXA-2} harbouring isolates was done by Enterobacterial repetitive intergenic consensus sequences PCR[9].

When characterized genotypically, the first isolate AM 328 possessed multiple beta lactamase genes, harboured *CTX-M*, *SHV* and *GES* while the second isolate AM 438 harboured only single bla_{GES} gene. Sequencing of the PCR product with the GES primers revealed that both the isolates harboured a bla_{GES-5} variant gene. Efflux pump mediated carbapenem resistance was also noticed in both isolates. A sharp reduction in MIC was observed against ertapenem when CCCP was added at a fixed concentration of 20 µg/ml. Class 1 integron was found in both isolates (Table). Sequencing results confirmed that bla_{GES-5} was class 1 integron borne. Both isolates were carrying multiple plasmids. A plasmid of ~ 40 Kb was common in both. Incompatibility typing of the first isolate showed that there was diverse Incompatibility groups Inc groups: I1/Iγ, FIA, FIB, W, FIC, FrepB and K while in the second isolate group I1/Iγ, FIA, FIB, W, FIC and FrepB were found (Table). Transformation was successful with AM-438 where it was found that bla_{GES-5} was located within W Inc type plasmid. However, these were not conjugatively transferable to *E. coli.* The first isolate was found to be susceptible to imipenem and cefoxitin and the second isolate showed suceptibility against imipenem, meropenem and gentamicin. While the first isolate showed MIC for all tested antibiotics as >256 mg/l, the second isolate showed 64 mg/l to carbapenems and monobactam and 32 mg/l to cephalosporins. ERIC PCR result showed that both isolates belonged to diverse clonal types.

The presence of *GES-5* gene has been frequently detected in *E. coli*[10, 11], *Klesbsiella pneumoniae*[12] and *P. aeruginosa*[13,14].

In agreement with the current study, integron mediated GES-5 has been earlier reported from other parts of the Asia[11]. However, in our study, presence of bla_{GES-5} was traced from the community isolate which emphasized the need for epidemiological investigation, origin and evolution of this resistant determinant from this geographical location. Unlike the previous concept[15], the studied isolates were phenotypically susceptible to carbapenem, whereas presence of efflux pump activity was noticed with ertapenem.

In conclusion, the presence of bla_{GES-5} in *P. aeruginosa* is perhaps the first report from this part of India. Presence of this rare type ESBL in community and their presence within plasmid require further investigation for potential transmission dynamics and proper therapeutic alternatives.

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