Letter to the Editor

Emergence of VIM-6 metallo-beta-lactamase-producing *Alcaligenes faecalis* clinical isolates in a hospital in India

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Background

Alcaligenes faecalis (*A.faecalis*) is a Gram-negative, rod-shaped, motile, oxidase, catalase and citrate positive obligate aerobe that is commonly found in the environment. *A. faecalis* is the most frequently isolated member of family Alcaligenaceae in the clinical laboratory. Infections caused by *A. faecalis* are opportunistic and are acquired from moist items such as nebulizers, respirators, and lavage fluid [1]. It has been reported to cause peritonitis, urinary tract infection (UTI), bacteremia and acute otitis media [1,2]. Although presence of PER-1 and TEM-21 Extended Spectrum Beta-Lactamases (ESBL) have been reported in clinical isolates of *A. faecalis* [3,4], carbapenem resistance has not yet been reported. This study aimed to investigate the mechanism of carbapenem resistance among clinical isolates of *A. faecalis* recovered from hospitalized patients in a tertiary care centre in Pune, India.

The study

During the study period from January 2012 to December 2012, a total of 15 clinical isolates of *A. faecalis* were recovered from different clinical specimens such as urine, pus, blood and body fluids, from patients admitted to the medical and surgical intensive care units in a 1,000-bed tertiary care hospital in Pune, India. Bacterial identification was performed by routine conventional microbial culture and biochemical tests [5]. The organisms were identified up to the species level using VITEK-GNI cards (bioMérieux, Marcy l'Etoile, France). The antibiotic sensitivity test was performed by the standard Kirby Bauer disc diffusion method per the guidelines of the Clinical Laboratory Standards Institute (CLSI), using commercially available antibiotic discs (Hi Media, Mumbai, India) on Mueller Hinton agar plates [6]. Minimum inhibitory concentrations (MIC) of antibiotics were determined by VITEK-2 against imipenem, meropenem, ticarcillin, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, tigecycline, trimethoprim/sulfamethoxazole, ampicillin/sulbactam, piperacillin/tazobactam, cefoperazone/sulbactam, cefepime, tetracycline, ceftazidime, ceftriaxone and colistin. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 strains were used for quality control. Seven of these isolates were found to be carbapenem resistant according to CLSI breakpoints. All the isolates exhibiting reduced susceptibility to any of the carbapenems (meropenem and imipenem) by disc diffusion were screened for the production of carbapenemase by the Modified Hodge Test (MHT). Clover leaf type indentation by the isolates was interpreted as positive MHT. All seven isolates showed a positive MHT. Screening for metallo-beta-lactamase (MBL) production was performed on these isolates by the E-test MBL IP/IPI method and all of them were found to be positive for MBL. DNA was extracted using the spin column method (QIAGEN; GmbH, Hilden, Germany) per the manufacturer's instructions. PCR amplification for detection of Ambler class B MBLs bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{GIM}, bla_{SIM} and $bla_{\text{NDM-1}}$ and Ambler class D $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$ and bla_{OXA48} was carried out on the isolates in a Gene Amp 9700 PCR System (Applied Biosystems, Singapore). Primers used for the PCR were as described earlier [7]. These isolates were

found to be positive for bla_{VIM-6} by polymerase chain reaction. The amplicons were purified using QIAquick PCR purification kit (QIAGEN; GmbH, Hilden, Germany) and sequenced with the ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). The sequence of the bla_{VIM-6} gene showed 100% identity with previously reported genes.

MIC values of these seven isolates are shown in the Table. The clonality of *A. faecalis* was confirmed by REP-PCR and ERIC PCR; the resulting banding pattern confirmed that two clones were circulating in the hospital environment [8].

The development and spread of resistance is a complex process that is influenced by selective pressure, pre-existence of resistance genes, and the use of infection control measures. *Achromobacter xylosoxidans* (previously *Alcaligenes xylosoxidans*) is recognized as a clinically significant nosocomial pathogen with its tendency to contaminate fluids. Many outbreaks of this organism have been reported earlier. The presence of metallo-beta-lactamases, such as IMP-1, VIM-1, or VIM-2, has been reported for *A. xylosoxidans*, consequently leading to high-level resistance to carbapenems [9]. Carbapenem resistance has not yet been reported for *A. faecalis*. The importance of the microbiology laboratory cannot be overemphasized in the detection and control of the spread of carbapenemase-resistant organisms. It is therefore imperative that the laboratory should have measures in place for the detection of carbapenemases.

The nucleotide sequence data of the bla_{VIM-6} gene from the three clinical isolates of *A. faecalis* reported in the present study have been assigned GenBank nucleotide numbers KC493967, KC493968 and KC493969.

Table. Antibiotic susceptibilities of bla_{VIM-6} positive *Alcaligenes faecalis* (mg/L)

Antibiotics/ Isolate No.	AF/1860	AF/1991	AF/2260	AF/2076	AF/2077	AF/2089	AF/2173
IPM	16	16	16	32	32	16	16
MEM	16	16	16	32	32	16	16
AMK	64	64	64	64	64	64	64
GEN	16	16	16	16	16	16	16
TOB	16	16	16	16	16	16	16
CIP	4	4	4	4	4	4	4
LVX	4	4	4	8	8	4	4
TIC	128	128	128	128	128	128	128
TGC	2	2	2	4	4	2	2
SXT	320	320	320	320	320	320	320
SAM	32	32	32	32	32	32	32
TZP	128	128	128	128	128	128	128
FEP	64	64	64	64	64	64	64
SFP	64	64	64	64	64	64	64
CRO	64	64	64	64	64	64	64
CAZ	64	64	64	64	64	64	64
TET	4	4	4	16	16	4	4
CST	0.5	0.5	0.5	0.5	0.5	0.5	0.5

IPM = imipenem; MEM = meropenem; AMK = amikacin; GEN = gentamicin; TOB = tobramycin; CIP = ciprofloxacin; LVX = levofloxacin; TIC = ticarcillin; TGC = tigecycline; SXT = trimethoprim/sulfamethoxazole; SAM = ampicillin/sulbactam; TZP = piperacillin/tazobactam; SFP = cefoperazone/sulbactam; FEP = cefepime; TET = tetracycline; CAZ = ceftazidime; CRO = ceftriaxone; CST = colistin

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