Short Communication

First Report of Extended-Spectrum β -Lactamases TEM-116 and OXA-10 in Clinical Isolates of *Alcaligenes* Species from Kuala Lumpur, Malaysia

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SUMMARY: There is an alarming increase in the prevalence of extended-spectrum β -lactamases (ESBLs) present mainly in *Enterobacteriaceae* and other nonfermenting gram-negative bacteria, such as *Alcaligenes faecalis*, which is the only species in that genus that is clinically relevant. We investigated *Alcaligenes* species from 7 cases (6 inpatients and one outpatient) at our tertiary-care hospital. Four patients had urinary tract infections, and one each had systemic lupus erythematosus, pulmonary stenosis, and diabetic ulcer. All 7 isolates were identified as *Alcaligenes* spp. based on their 16S rRNA gene sequences, and antibiotic susceptibility was determined using a Vitek 2 system with AST-GN87 cards. All the strains were resistant to cefazolin; 6 were resistant to trimethoprim/sulfamethoxazole; 5 manifested resistance to ampicillin/sulbactam, cefepime, tobramycin, ciprofloxacin, and nitrofurantoin; whereas 5 had multidrug resistance profiles. All the strains (7/7) expressed ESBL activity; PCR screening and sequencing showed evidence of genes *bla*_{TEM-116} (7/7) and *bla*_{OXA-10} (4/7), and we believe that this is the first report on the presence of TEM-116 and OXA-10 in an *Alcaligenes* spp. A combination of the 2 genes was present in 4 strains. All 7 strains were found to harbor at least one ESBL gene probably contributing to the drug resistance.

Alcaligenes faecalis, an emerging potential pathogen, is commonly found in soil, water, and hospital environments. It is usually a harmless saprophyte in the microbiota of the human gut but occasionally may give rise to infections especially in immunocompromised hosts. There are reports of *A. faecalis* infections in patients with acute or chronic otitis media (1), urinary tract infections (UTI) (1,2), wounds (3), skin and soft tissue infections (4), endophthalmitis (5), pancreatic necrosis, and abscess (6). These are usually opportunistic infections and are often linked to contamination of hospital equipment, such as nebulizers and respirators, or fluids used for lavage (2). Filipe and coworkers (7) reported in Angola the isolation of *A. faecalis* from patients with chronic suppurative otitis media due to the use of bird droppings as a traditional remedy to prevent ear discharge.

A. faecalis infection is often difficult to treat due to increased resistance to many antimicrobials, such as β -lactams, aminoglycosides, and quinolones. Previous studies have revealed the presence of extended-spectrum β -lactamase (ESBL)-producing *A. faecalis* in clinical samples. ESBLs are a group of enzymes capable of breaking down various β -lactam antibiotics. ESBL types PER-1 and TEM-21 have been detected in urinary samples from intensive care patients in Northern Italy

(2), and TEM-21 was detected in an 89-year-old woman living in a nursing home in France (8). VIM-2 and VIM-4 metallo- β -lactamases have been linked to a small outbreak in a large hospital in Gaza, Palestine, as demonstrated by next-generation sequencing analysis (3). However, information on *A. faecalis* recovered from clinical material in Malaysia is scarce. Hence, the objectives of this study were to (i) confirm the identity of *Alcaligenes* spp., (ii) examine their resistance profiles, and (iii) determine the presence and diversity of ESBLs in the *Alcaligenes* spp. from clinical samples.

A total of 7 archived isolates of *Alcaligenes* spp. from patients at the tertiary-care University Hospital, University of Malaya, Kuala Lumpur, was investigated. All the patients were females (15 to 63 years), 3 with underlying conditions such as systemic lupus erythematosus, pulmonary stenosis, and diabetic ulcer. Five samples came from urine, one from a swab, and one from blood culture. The pathogens were identified at the genus level by biochemical tests (Table 1).

DNA of the isolates was extracted and then amplified using a universal primer pair: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), as described in a previous report (9). The amplicons were purified and sent for sequencing (First Base Laboratories, Sdn.Bhd, Malaysia), and the nucleotide sequences were deposited in a public database (DNA Data Bank of Japan [DDBJ] ID numbers LC458689–LC458695). A phylogenetic tree constructed from partial 16S rRNA gene sequences indicated that all the 7 isolates shared 89% of the region of the 16S rRNA gene with that of the reference strain *A. faecalis* subsp. *faecalis* NBRC 13111 (Accession AB680368.1 published on 2011/12/11) (Fig.1).

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ESBLs in *Alcaligenes* Species

Table 1. Characteristics of 7 clinical strains of *Alcaligenes* species

Characteristic		AF28	AF55	AF86	AF99	AF113	AF128	AF129
Age/sex		20/F	37/F	25/F	15/F	17/F	63/F	30/F
Diagnosis		UTI	UTI	SLE with UTI	Pulmonary stenosis	UTI & bacteremia	Diabetic ulcer	Acute cholecystitis
Specimen		Urine	Urine	Urine	Urine	Urine	Swab	Blood
ESBL genes		<i>TEM-116, OXA-10</i>	<i>TEM-116, OXA-10</i>	<i>TEM-116, OXA-10</i>	<i>TEM-116</i>	<i>TEM-116, OXA-10</i>	<i>TEM-116</i>	<i>TEM-116</i>
Type	Antimicrobial	MIC (µg/mL) [interpretation]						
Aminopenicillin	Ampicillin/Sulbactam	≥ 32 [R]	≥ 32 [R]	≥ 32 [R]	≥ 32 [R]	≥ 32 [R]	≤ 2 [S]	≤ 2 [S]
Ureidopenicillin	Piperacillin/Tazobactam	8 [S]	≤ 4 [S]	8 [S]	64 [I]	8 [S]	≤ 4 [S]	≤ 4 [S]
Cephalosporin	Cefazolin	32 [R]	32 [R]	≥ 64 [R]	32 [R]	≥ 64 [R]	32 [R]	32 [R]
	Cefepime	16 [R]	8 [R]	16 [R]	≥ 64 [R]	16 [R]	4 [I]	2 [S]
	Ceftazidime	4 [S]	4 [S]	4 [S]	8 [S]	4 [S]	4 [S]	4 [S]
Carbapenem	Imipenem	0.5 [S]	0.5 [S]	0.5 [S]	0.5 [S]	0.5 [S]	≤ 0.25 [S]	0.5 [S]
	Meropenem	≤ 0.25 [S]	≤ 0.25 [S]	≤ 0.25 [S]	≤ 0.25 [S]	≤ 0.25 [S]	≤ 0.25 [S]	≤ 0.25 [S]
Aminoglycoside	Amikacin	8 [S]	8 [S]	8 [S]	32 [I]	8 [S]	8 [S]	16 [S]
	Tobramycin	≥ 16 [R]	≥ 16 [R]	≥ 16 [R]	≥ 16 [R]	≥ 16 [R]	2 [S]	4 [S]
Fluoroquinolone	Ciprofloxacin	≥ 4 [R]	≥ 4 [R]	≥ 4 [R]	≥ 4 [R]	≥ 4 [R]	1 [S]	2 [I]
	Levofloxacin	4 [I]	4 [I]	4 [I]	≥ 8 [R]	4 [I]	1 [S]	1 [S]
Sulfonamides	Trimethoprim/Sulfamethoxazole	≥ 320 [R]	≥ 320 [R]	≥ 320 [R]	≥ 320 [R]	≥ 320 [R]	≤ 20 [S]	160 [R]
Miscellaneous	Nitrofurantoin	128 [R]	32 [S]	64 [I]	256 [R]	128 [R]	128 [R]	128 [R]

SLE, systemic lupus erythematosus; UTI, urinary tract infections.

Fig. 1. Phylogenetic relationship of partial 16S rRNA sequences (1031 bp) between 7 *Alcaligenes* spp. and 3 reference strains using neighbor joining method. Numbers next to nodes indicate percentage bootstrap values of 1,000 replicates.

All the strains were examined for antibiotic susceptibility using a Vitek 2 system with AST-GN87 cards (BioMérieux) according to Clinical & Laboratory Standards Institute (CLSI) interpretive criteria M100-25th, and the results are presented in Table 1. All the 7 strains were susceptible to ceftazidime, imipenem, and meropenem, whereas only 6 strains were susceptible to

piperacillin/tazobactam and amikacin. All the 7 strains were resistant to cefazolin with a minimum inhibitory concentration range of 32 to ≥ 64 µg/mL. Most strains (5/7) manifested resistance to ampicillin/sulbactam, cefepime, tobramycin, ciprofloxacin, trimethoprim/sulfamethoxazole, and nitrofurantoin. Multidrug resistance (MDR) was observed in 7 strains (AF28,

Fig. 2. Representative results of ESBL production with Combination Disc Test. Standard ATCC strains of *Klebsiella pneumoniae* ATCC 700603 (ESBL producer) and *Escherichia coli* ATCC 25922 (non-ESBL producer) were included in the study as control strains. CTX, 30 µg Cefotaxime; CTL, 40 µg Cefotaxime with Clavulanic acid.

AF55, AF86, AF99, AF113, AF128, and AF129); they were recovered from patients with UTI only or with underlying medical conditions. These MDR strains were sensitive to imipenem or meropenem, and sensitivity of *A. faecalis* to both drugs has been reported in studies out of Turkey (10) and Spain (4).

All the strains were evaluated for ESBL production using a combination disk test with a cefotaxime-only (CTX; 30 µg) disk and a disk also containing clavulanic acid (CA) (10 µg; Roset degli Abruzzi, Liofilchem, Italy) in addition to CTX. The assays were carried out in triplicate, and *Klebsiella pneumoniae* ATCC 700603 (ESBL producer) and *Escherichia coli* ATCC 25922 (ESBL nonproducer) were used as positive and negative controls, respectively, according to the manufacturer's instructions. All these strains tested positive and showed a ≥ 5 mm increase in inhibition zones around the CTX-CA disk compared to the disk containing only CTX (Fig.2).

Subsequently, genomic DNA samples of all the

seven strains were screened for the genes encoding β -lactamases TEM, OXA, SHV, CTX-M, PER-1, VIM, and GES by PCR using primers described in published studies (11–13). The PCR screening also showed the presence of genes *bla*_{TEM} (7/7) and *bla*_{OXA-10} (5/7), and their identity was confirmed by subsequent sequencing. The DNA sequences of *TEM-116* and *OXA-10* were deposited in a public database (DDBJ, ID numbers LC458696–LC458706). BlastX analysis indicated that the *TEM*-type gene shared 100% identity with *TEM-116* of *Vibrio parahaemolyticus* (AEL87579.1), *K. pneumoniae* (AKE33352.1), and *E. coli* (AKE33352.1), whereas the *OXA-10* sequence, at the protein level, shared 100% identity with *OXA-10* of *Pseudomonas aeruginosa* (ADI33311.1) and *Aeromonas hydrophila* (CEP69081.1). All the strains were found to harbor at least one *ESBL* gene, which probably contributed to the observed drug resistance.

A combination of genes *TEM-116* and *OXA-10* was present in 4 strains. *TEM-116* confers resistance to

most β -lactam antibiotics, penicillins, cephalosporins, and monobactams, whereas OXA-10 is an ambler class D β -lactamase conferring resistance to amino- and ureidopenicillins. The absence of the *OXA-10* gene in the ampicillin/sulbactam-resistant strain (*Alcaligenes* sp. AF99) could be due to the presence of some other type of antibiotic-resistance determinants that were not analyzed in this study. Our study is probably the first description of ESBL-type TEM-116 and OXA-10 in an *Alcaligenes* spp. (this phenomenon has not been reported in other studies) (2,3,8). The combination of TEM-116 and OXA-10 has been detected in clinical isolates of *P. aeruginosa* in China (14), suggesting that bacteria producing the combination of TEM-116 and OXA-10 may be widespread in Southeast Asia. Further studies in a larger sample size are needed for clarification.

In India, *A. faecalis* was recovered from the bronchoalveolar lavage of a hemorrhagic patient with dengue fever and acute respiratory distress syndrome (15). This is probably the first description of bacterial coinfection with this microorganism in a patient with dengue fever. Hence, there is a risk of coinfection by *A. faecalis* in all tropical and subtropical regions of the world including Malaysia, where major outbreaks occur every 3–4 years.

In conclusion, ESBL-producing *Alcaligenes* strains were detected in our clinical material, thus highlighting their potential to behave as opportunistic pathogens. However, all the 7 strains were susceptible to 2 carbapenems, suggesting that both imipenem and meropenem may be considered the agents of choice for the treatment of infection with an ESBL-producing *Alcaligenes* sp.

Conflict of interest None to declare.

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