First report of TEM-116 and OXA-10 extended-spectrum β-lactamase in clinical isolates of Alcaligenes species from Kuala Lumpur, Malaysia

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First report of TEM-116 and OXA-10 extended-spectrum β-lactamase in clinical isolates of 

*Alcaligenes* species from Kuala Lumpur, Malaysia

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**Key Words:** *Alcaligenes* species, Drug resistance, Extended-β beta-lactamase, Opportunistic infections

**Running title:** ESBLs in *Alcaligenes* species
Summary

There is an alarming increase of extended-spectrum β-lactamases (ESBL) being present mainly in Enterobacteriaceae and other non-fermenting gram-negative bacteria, such as Alcaligenes faecalis which is the only species present in that genus that is clinically relevant. We investigated Alcaligenes species from 7 cases (6 inpatients and 1 outpatient) at our tertiary hospital. Four patients had urinary tract infections and one each with systemic lupus erythematosus, pulmonary stenosis and diabetic ulcer. All 7 isolates were identified as Alcaligenes sp. using 16S rRNA gene sequences and antimicrobial susceptibility was determined using the Vitek 2 system with the AST-GN87 cards. All strains were resistant to cefazolin; 6 were resistant to trimethoprim/sulfamethoxazole; 5 exhibited resistance to ampicillin/sulbactam, cefepime, tobramycin, ciprofloxacin, and nitrofurantoin while 5 strains had multidrug resistance profiles. All strains (7/7) expressed ESBL activity, PCR screening and sequencing showed evidence of blaTEM-116 (7/7) and blaOXA-10 (4/7) genes and we believe this is the first report on the presence of TEM-116 and OXA-10 in Alcaligenes sp.. A combination of both genes was present in 4 strains. All 7 strains were found to have at least one ESBL gene probably contributing to drug resistance.

Alcaligenes faecalis, an emerging potential pathogen is commonly found in soil, water and in hospital environments. It is usually a harmless saprophyte in the microbiome of the human gut but occasionally may give rise to infections especially in immunocomprised hosts. A. faecalis infections have been reported in patients with acute or chronic otitis media (1), urinary tract infections (1, 2), wounds (3), skin and soft tissue infections (4), endophthalmitis (5), pancreatic necrosis and abscess (6). These are usually opportunistic infections and often linked to contamination of hospital equipment such as nebulisers, respirators and fluids used for lavage (2).
Filipe and co-workers (7) reported the isolation of *A. faecalis* from the patients with chronic suppurative otitis media in Angola 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 reported the presence of ESBL-producing *A. faecalis* in clinical specimens. 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 the latter from a 89-year-old woman living in a nursing home in France (8). VIM-2 and VIM-4 metallo-β-lactamase was associated with a small outbreak in a large hospital in GAZA, Palestine, demonstrated by next generation sequencing analysis (3). Information on *A. faecalis* recovered from clinical material in Malaysia is scanty. Hence, the objective of this study was to a) confirm the identity of *Alcaligenes* sp. b) examine their resistance profiles and c) determine the presence and diversity of ESBLs in *Alcaligenes* sp. from clinical specimens.

A total of 7 archived isolates of *Alcaligenes* sp. from patients at the tertiary University Hospital, University of Malaya, Kuala Lumpur, was investigated. All patients were females (15 to 63 years), 3 with underlying conditions such as systemic lupus erythematosus, pulmonary stenosis and diabetic ulcer; 5 from urine, 1 from a swab and 1 from blood culture, were identified to genus level by biochemical tests (Table 1).
DNA of the isolates was extracted and further amplified using 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 (1st Base Laboratories, Malaysia) and the nucleotide sequences were deposited in a public database of DDBJ (LC458689 - LC458695). A phylogenetic tree constructed with partial 16S rRNA gene sequences demonstrated that all 7 isolates shared 89% region of the 16S rRNA gene with references Alcaligenes faecalis subsp. faecalis NBRC 13111 (Accession AB680368.1 published in 2011/12/11) (Figure 1).

All strains were examined for antibiotic susceptibility using the Vitek 2 system with the AST-GN87 cards (BioMérieux) with CLSI interpretive criteria M100-25th and results are presented in Table 1. All 7 strains were susceptible to ceftazidime, imipenem, meropenem whereas only 6 strains were susceptible to piperacillin/tazobactam and amikacin. All 7 strains were resistant to cefazolin with MIC range of 32 to ≥ 64 µg/mL. Most (5/7) exhibited resistance to ampicillin/sulbactam, cefepime, tobramycin, ciprofloxacin, trimethoprim/sulfamethoxazole and nitrofurantoin. Multi-drug resistance (MDR) was observed in 7 strains (AF28, AF55, AF86, AF99, AF113, AF128 and AF129) and they were recovered from patients with urinary tract infections only or with underlying medical conditions. These MDR strains were sensitive to imipenem or meropenem and sensitivity of both drugs to A. faecalis was reported by studies in Turkey (10) and Spain (4).

All strains were evaluated for ESBL production using a Combination Disc Test with cefotaxime CTX (30µg) disk alone and in combination with CA (10µg) (Liofilchem, Italy). The assays were
carried out in triplicate, *Klebsiella pneumoniae* ATCC 700603 (ESBL producer) and *Escherichia coli* ATCC 25922 (non-ESBL producer) were used as positive and negative controls, respectively according to the manufacturer’s instructions. All these strains were positive and showed ≥ 5mm increase in inhibition zones around the CTX-CA (CTL) disk compared to the disk containing only CTX (Figure 2).

Subsequently, genomic DNA of all 7 strains was screened for gene encoding β-lactamases TEM, OXA, SHV, CTX-M, PER-1, VIM and GES by PCR using primers as described in published studies (11,12,13). PCR screening also showed presence of bla\textsubscript{TEM} (7/7) and bla\textsubscript{OXA-10} (5/7) genes and their identity was confirmed by subsequent sequencing. The DNA sequences of TEM-116, OXA-10 were deposited in a public database of DDBJ (LC458696 - LC458706). BlastX analysis indicated that the TEM-type showed 100% identity to TEM-116 of *Vibrio parahaemolyticus* (AEL87579.1), *Klebsiella pneumoniae* (AKE33352.1) and *Escherichia coli* (AKE33352.1) whereas the OXA-10 sequence, at protein level, shared 100% identity with OXA-10 of *Pseudomonas aeruginosa* (ADI33311.1) and *Aeromonas hydrophila* (CEP69081.1). All strains were found to have at least one ESBL gene, which probably contributed to the observed drug resistance.

A combination of TEM-116 and OXA-10 genes was present in 4 strains. TEM-116 confers resistance to most β-lactam antibiotics, penicillins, cephalosporins and monobactams while OXA-10 is an ambler class D β-lactamase conferring resistance to amino- and ureido-penicillins. The absence of OXA-10 gene in the ampicilin/sulbactam resistant strain (*Alcaligenes* sp. AF99) could be due to the presence of some other type of antibiotic resistance determinants that were
not targeted in this study. Our study is probably the first description of ESBL type TEM-116 and OXA-10 in *Alcaligenes* sp. and 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 *Pseudomonas aeruginosa* in China (14), suggesting that bacteria producing the combination of TEM-116 and OXA-10 may be spread in Southeast-Asia. Further studies involving larger sample size are needed for clarification.

In India *A. faecalis* was recovered from the bronchoalveolar lavage of a dengue hemorrhagic patient with acute respiratory distress syndrome (15). This is probably the first description of bacterial co-infection of this organism in a dengue patient. Hence, a potential threat exists of co-infection 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* sp. was detected in our clinical material highlighting their potential to behave as opportunistic pathogens. However, all 7 strains were susceptible to two carbapenems, suggesting both imipenem and meropenem may be considered as agents of choice for the treatment of ESBL-producing *Alcaligenes* sp. infections.

**Authors’ Disclosures of Potential Conflicts of Interest**

No potential conflict of interest relevant to this article were reported.
References


Figure legends:

Figure 1  Phylogenetic relationship of partial 16S rRNA sequences (1031 bp) between 7 *Alcaligenes* sp. and 3 reference strains using neighbor joining method. Numbers next to nodes indicate percentage bootstrap values of 1000 replicates.

(Print quality: black and white)

Figure 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: 30µg Cefotaxime; CTL: 40 µg Cefotaxime with Clavulanic acid.
Table 1 Characteristics of 7 clinical strains of *Alcaligenes* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AF28</th>
<th>AF55</th>
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<th>AF99</th>
<th>AF113</th>
<th>AF128</th>
<th>AF129</th>
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<tr>
<td>Age/sex</td>
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<td>37/F</td>
<td>25/F</td>
<td>15/F</td>
<td>17/F</td>
<td>63/F</td>
<td>30/F</td>
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<td>UTI</td>
<td>SLE with UTI</td>
<td>Pulmonary stenosis</td>
<td>UTI &amp; bacteremia</td>
<td>Diabetic ulcer</td>
<td>Acute cholecystitis</td>
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<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
<td>Swab</td>
<td>Blood</td>
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<tr>
<td>ESBL genes</td>
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<td>TEM-116, OXA-10</td>
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<td>32 [R]</td>
<td>≥64 [R]</td>
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<td>Cefepime</td>
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<td>8 [R]</td>
<td>16 [R]</td>
<td>≥64 [R]</td>
<td>16 [R]</td>
<td>4 [I]</td>
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<td>Imipenem</td>
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<td>0.5 [S]</td>
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<td>Levofloxacin</td>
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<td>4 [I]</td>
<td>≥8 [R]</td>
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</tr>
</tbody>
</table>
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