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ORIGINAL ARTICLE

Molecular detection of *Enterobacteriaceae* isolates producing bla_{OXA-48} and $bla_{OXA-181}$ genes: A single centre study

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Abstract

Introduction: OXA-48, a carbapenem-hydrolysing class D β-lactamase, and its variant, OXA-181, are increasingly reported worldwide. This study aimed to describe the prevalence and distribution of OXA-48 and OXA-181 carbapenem-resistant *Enterobacteriaceae* (CRE) in a tertiary medical centre in Malaysia. *Materials & Methods:* A total of 13,098 *Enterobacteriaceae* isolates from various clinical samples were sent to our laboratory between January 2011 and December 2012. Of these, 90 demonstrated reduced susceptibility to at least one carbapenem and were included in this study. Only 88 isolates were successfully subcultured on blood agar (BA). Another 2 isolates failed to grow and were excluded. Of the 88, 2 isolates had the same identification number (repetitive isolates); therefore, 1 isolate was excluded from further analyses. Only 87 isolates were subjected to molecular detection of the bla_{OXA-48} and $bla_{OXA-181}$ genes by polymerase chain reaction. *Results*: Eighty-seven non-repetitive isolates grew following subculture on BA. Of these, 9 (10.34%) were positive for OXA-48 (7 *Klebsiella pneumoniae*, 2 *Escherichia coli*). Each isolate originated from different patients. All patients had a history of treatment with at least one cephalosporin and/or carbapenem prior to the isolation of OXA-48 CRE. OXA-181 was detected in one (1.15%) out of the 87 isolates; *Conclusions:* The prevalence of OXA-48 and OXA-181 CRE among all *Enterobacteriaceae* isolates in our institution is 0.069% and 0.008%, respectively. Nevertheless, our findings suggest that OXA-48 and OXA-181 carbapenemases appear to be important and possibly under-recognised causes of carbapenem resistance in Malaysia.

Keywords: carbapenemase, Enterobacteriaceae, OXA-48, OXA-181

INTRODUCTION

The rapid emergence, evolution and dissemination of carbapenem-resistant *Enterobacteriaceae* (CRE) are a source of concern globally. To date, carbapenemase production remains the predominant mechanism of resistance employed by many *Enterobacteriaceae* species to render the carbapenem group ineffective.[1] Following its first description in a *Klebsiella pneumoniae* isolate in Turkey in 2004, the carbapenem-hydrolysing class D β-lactamase OXA-48 has steadily spread to North Africa, the Middle East, Europe, and the United States.[2] Its variant of 4 amino acid substitutions, OXA-181, was first identified in an *Enterobacter cloacae* isolate in India in 2006; it has also been sporadically detected worldwide, from the Netherlands, France and the United Kingdom, to the Sultanate of Oman, New Zealand and Singapore.[1,3-8]

A primary concern of OXA-48 and OXA-181 carbapenemases is that they weakly hydrolyse both carbapenems and broad-spectrum cephalosporins; hence, elevated minimum inhibitory concentrations (MICs) to carbapenems and cephalosporins may not be noticeable in these isolates. Therefore, isolates harbouring OXA-48 and OXA-181 may go undetected in routine laboratory settings, complicating treatment options. In addition, they have a high dissemination rate due to transferable plasmids, making them an important cause of a wide range of infections, both in the community and healthcare settings.[1]

The aim of this study is to establish the

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prevalence and distribution of *bla*$_{OXA-48}$ and *bla*$_{OXA-181}$ genes among *Enterobacteriaceae* isolates with reduced susceptibility to carbapenems in our medical centre. To the best of our knowledge, this is the first study to ascertain the prevalence of OXA-48- and OXA-181-producing *Enterobacteriaceae* in a healthcare institution in Malaysia.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was conducted at the Department of Medical Microbiology and Immunology, Universiti Kebangsaan Malaysia Medical Center (UKMMC), over a period of one year. A total of 13,098 *Enterobacteriaceae* isolates were sent to our laboratory between January 2011 and December 2012. Of these, 90 *Enterobacteriaceae* isolates demonstrated reduced susceptibility (intermediate susceptibility or resistant) to at least one carbapenem. Only 88 isolates were successfully subcultured on blood agar (BA). Another 2 isolates failed to grow and were excluded. Of the 88, 2 isolates had the same identification number (repetitive isolates); therefore, 1 isolate was excluded from further analyses. Only 87 isolates were subjected to molecular detection of the bla$_{OXA-48}$ and bla$_{OXA-181}$ genes by polymerase chain reaction. They originated from various clinical samples (blood, urine, pus [including from catheter tip], tissue, swab, bile, tracheal aspirate, and sputum) from adult and paediatric subjects. All samples were previously processed and identified according to standard laboratory procedures. This included primary culture, biochemical testing, antibiotic disc susceptibility testing, confirmation of bacterial species via Analytical Profile Index 20E strips (bioMérieux, France), and modified Hodge test (MHT) as a screening for carbapenemase production. These isolates were also previously subjected to the detection of New Delhi metallo-β-lactamase-1 (NDM-1) CRE by Abidin et al.[9] All 87 isolates were maintained in cryobank vials and had been in storage in a -80°C freezer since 2012. As the bacterial isolates were already established as *Enterobacteriaceae,* the inclusion and exclusion criteria of this study were based on the success of subculturing the isolates onto blood agar (BA) plates. Bacterial isolates that successfully grew upon subculture on BA were included in this study; those that failed to grow were excluded.

Subculture of isolates onto blood agar

Bacterial isolates in cryobank vials were removed from -80°C storage. Under aseptic conditions, a sterile needle was used to remove one coloured bead from each cryovial (vials were immediately recapped and returned to the freezer to avoid thawing of contents). Subculture was achieved by directly rolling the inoculated bead onto a BA plate surface. Using a sterile loop, inoculation was performed as outlined by Cheesbrough *et al*.[10] The inoculated BA plates were incubated at 37°C in 5% carbon dioxide atmosphere for 24 hours.

DNA extraction from cultured bacterial colonies

All isolates that grew upon subculture on BA were subjected to DNA extraction for molecular detection of OXA-48 and OXA-181. DNA extraction for OXA-48 was conducted using a high-pure polymerase chain reaction (PCR) preparation kit (Roche Diagnostics, Germany), in accordance with the manufacturer's instructions. For OXA-181, DNA extraction was conducted using the boiling method as follows: four to five bacterial colonies were mixed with 200 μl of PCR-grade water in a 1.5 ml screw cap centrifuge tube. The tube was placed on a heating block at 96°C for ~30 min. Following this, the tube was centrifuged at 10,000 *g* for 5 min in order to pellet the cellular debris. The supernatant (containing the bacterial DNA) was then transferred into a labelled 1.5 ml microcentrifuge tube.

Molecular detection of bla$_{OXA-48}$

Amplification and detection of the *bla*$_{OXA-48}$ gene were undertaken on the Cobas z 480 analyzer (Roche Diagnostics, Germany) using the LightMix® Modular OXA-48 Carbapenemase assay (Roche Diagnostics, Germany). According to the manufacturer, this assay is able to detect major OXA-48-type members, in particular OXA-162, -163, -244, -245, -247, and most OXA-181, -204 and -232 (single mismatches). However, any positivity will be reported only as "OXA-48 positive". For each PCR reaction, a 10 μl reaction mixture that consisted of 5.5 μl of PCR-grade water (Roche Probes Master Kit, Roche Diagnostics), 4 μl of master (LC®480 Probes Master, Roche Diagnostics), and 0.5 μl of reagent mix containing primers and probes to OXA-48 was prepared. This 10 μl reaction mixture was pipetted into a well of a 96-well plate; 10 μl of sample or control DNA was added, to give each well a final reaction volume of 20 μl. Each run was always performed with the kit's

positive and negative control. The plate was then sealed and the run started. The protocol consisted of three steps: initial denaturation at 95°C for 5 min, followed by 45 cycles of amplification (denaturation at 95°C for 5 s, annealing at 60°C for 15 s, and elongation at 72°C for 15 s), and finally cooling at 40°C for 30 s. Each run was completed in approximately 90 min.

Analysis of a run was performed using the second derivative maximum method with colour compensation. Channel 580 was used for visualisation of the amplified bla_{OXA-48} gene. A run was considered valid when the kit's positive control was detected at a quantification cycle (Cp) of <37 cycles. All samples were considered as negative when Cp >37. The negative control must show no signal.

Molecular detection of $bla_{OXA-181}$

Two sets of primers (Integrated DNA Technologies, Inc., Singapore) were used for the detection of OXA-181 using conventional single-target PCR amplification. The primers were described by Castanheira *et al*. (2011) and Shanthi *et al*. (2013), and are as listed in Table 1. For each PCR reaction, a 20 μl reaction mixture that consisted of 10 μl of Atlas Taq 2x PCR mix (BioAtlas, Estonia), 0.75 μl of 0.3 μM forward primer, 0.75 μl of 0.3 μM reverse primer, 2.5 μl of sample or control DNA, and 6 μl of PCR-grade water was prepared. Each run was performed with an in-house OXA-181 positive control isolate. The DNA reaction mixtures were amplified using the T100™ Thermal Cycler (Bio-Rad Laboratories, USA). Amplification was carried out under the following conditions, as described by Castanheira *et al*. (2011): initial denaturation at 94°C for 5 min, 35 cycles of amplification consisting of denaturation at 94°C for 60 s, annealing at 54°C for 60 s, and elongation at 72°C for 1 min, followed by final elongation at 72°C for 5 min.

Visualisation of OXA-181 DNA fragments was achieved via gel electrophoresis. 5μl of the PCR product was mixed with 5 μl loading dye (NEB, USA). The DNA fragments were separated by electrophoresis using 90 V for 45 minutes in 1.5% agarose gel in 0.5X TBE buffer (Merck, USA.). Amplified products were visualised with UV light after staining with EZ-VISION Blue Light DNA Dye (Amresco, USA). The image was captured under an ultraviolet reader using the Gel Doc documentation system (Bio-Rad Laboratories). A run was only considered valid when the positive control was detected.

Determination of carbapenem MIC levels of OXA-48/OXA-181-positive isolates

All isolates positive for OXA-48/OXA-181 were subjected to determination of MIC towards ertapenem, imipenem, meropenem, and doripenem by E-test (bioMérieux, France). MIC levels were interpreted according to breakpoints established by the Clinical & Laboratory Standards Institute (CLSI).[11]

RESULTS

A total of 87 non-repetitive isolates was successfully grown following subculture on BA and were subjected to molecular detection of the bla_{OXA-48} and $bla_{OXA-181}$ genes by polymerase chain reaction. Of these, 9 (10.34%) isolates were positive for the bla_{OXA-48} gene (Figs. 1 and 2); 7 (77.8%) were *K. pneumoniae* and 2 (22.2%) were *E. coli*. Specimen sources consisted of urine (n=3), tissue (n=2), sputum (n=2), blood (n=1), and catheter tip for culture (n=1). Corroboration with previous data[9] revealed that none of these isolates co-produced bla_{NDM-1}. Of the 87 isolates, only one (1.15%) was positive for the $bla_{OXA-181}$ gene (Fig. 3). This *E. coli*, which originated from a sputum sample of a 16-year-male patient with APML, was among the 9 isolates positive for

TABLE 1: Primer sequences used for the detection of the $bla_{OXA-181}$ gene

Primer	Primer sequence (5'-3')	Amplicon size (bp)	References
OXA-181 forward sequence	ATGCGTGTATTAGCCTTATCG	888	Shanthi *et al*. (2013)[13]; Castanheira *et al*. (2011)[3]
OXA-181 reverse sequence	AACTACAAGCGCATCGAGCA		

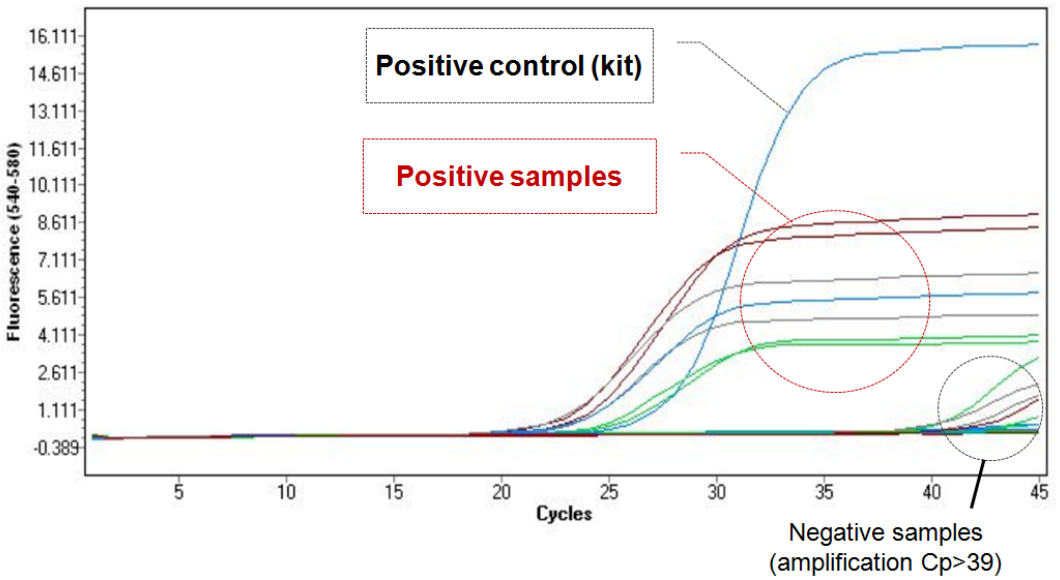

FIG. 1: Amplification curve shows detection of 7 isolates with blaOXA-48 gene among the samples tested.

OXA-48. Patient demographics are summarised in Table 2.

All 9 OXA-48-positive isolates were positive for MHT, and the rate of resistance between different classes of antibiotics ranged between 56% and 100% (Fig. 4). Table 3 depicts the carbapenem MICs of these isolates; all 9 were resistant to ertapenem (MICs 12 - >32 µg/ml), with variable susceptibility towards imipenem, meropenem, and doripenem. Only three isolates exhibited low levels of resistance towards imipenem, meropenem and doripenem (MICs 2 - 4 µg/ml); the rest demonstrated high levels of resistance (MICs 16 - >32 µg/ml). In particular, the sole isolate positive for OXA-181 had MICs >32 µg/ml towards all four carbapenems.

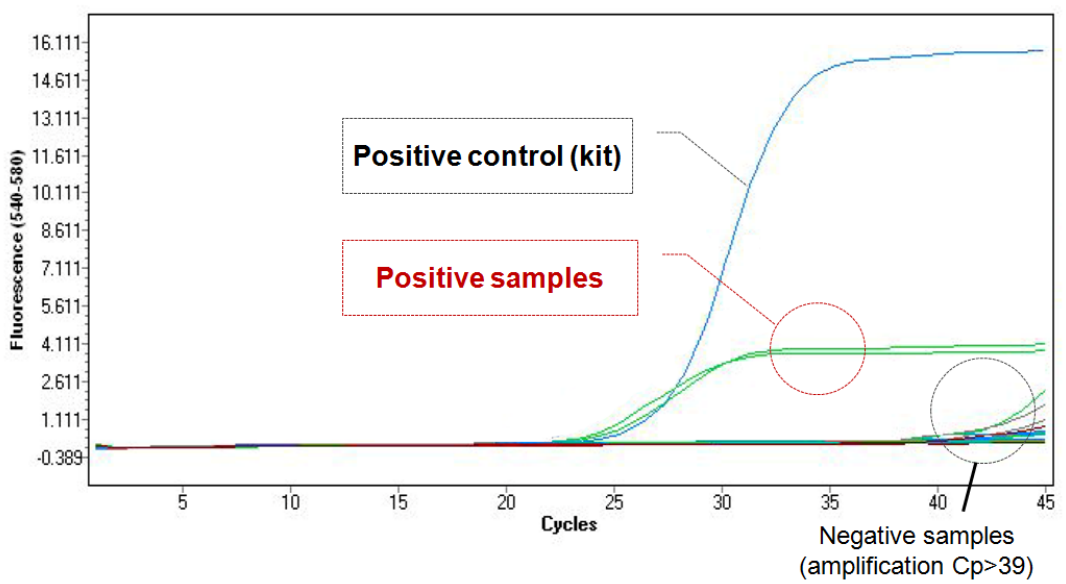

FIG. 2: Amplification curve shows detection of 2 isolates with blaOXA-48 gene among the samples tested.

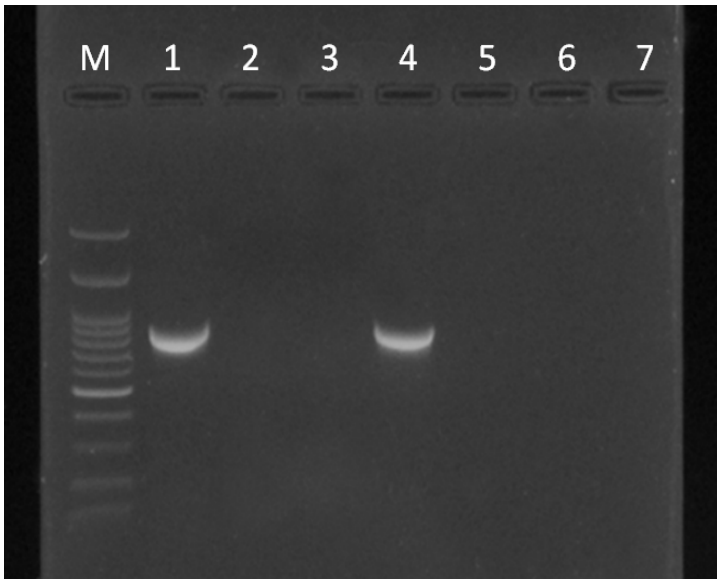

FIG. 3: PCR products from the molecular detection of OXA-181 on agarose gel, visualised under UV light. Lane M: 100 bp DNA molecular weight ladder; Lane 1: OXA-181 positive control (amplicon size: 888 bp); Lane 4: Isolate positive for OXA-181; Lanes 2, 3, 5, 6: Negative isolates; Lane 7: Negative control.

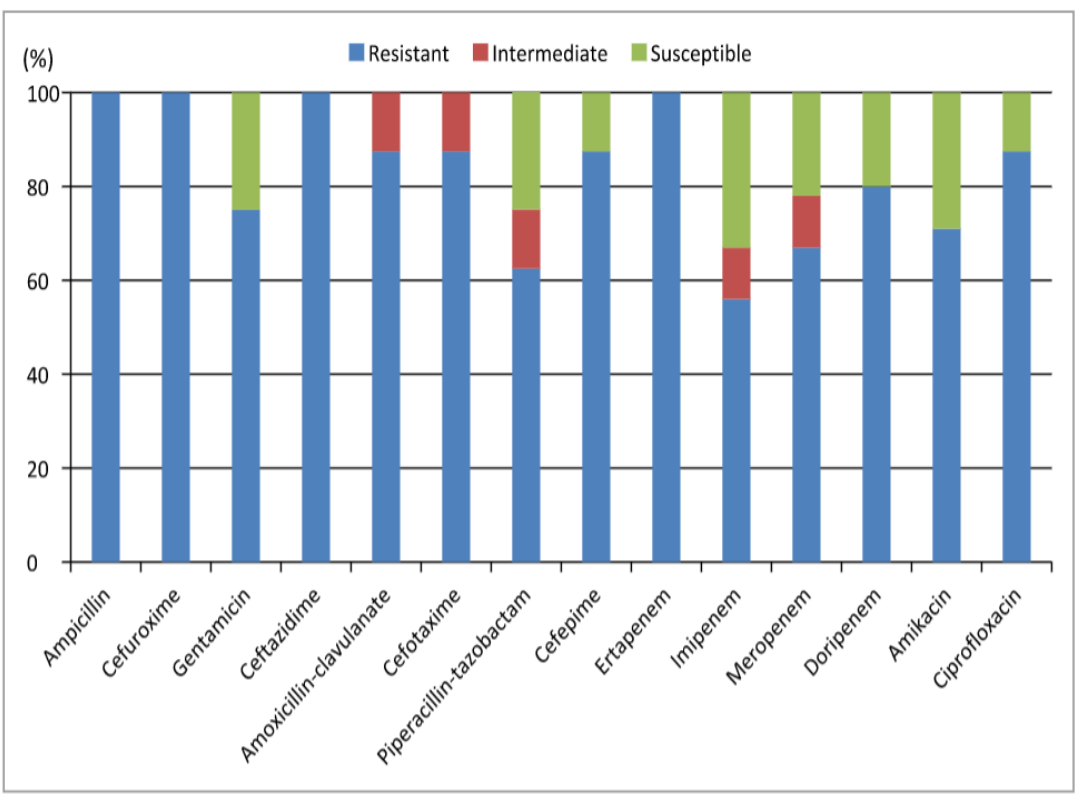

FIG. 4: Antibiogram of positive isolates based on disc diffusion.

TABLE 2: Demographics of patients with OXA-48- and OXA-181-producing *Enterobacteriaceae*

Isolate/ Case	Age (year)/ race/sex[a]	Diagnosis[b]	Comorbidity[c]	Prior hospitalization (no. of days)	Species	Source	Gene detected	Directed therapy	Outcome
1	13/C/♂	Sepsis secondary to nosocomial infection	None	34	*Klebsiella pneumoniae*	Urine	*bla*OXA-48	Meropenem	Deceased
2	59/M/♂	Urosepsis post-percutaneous nephrolithotomy	None		*Klebsiella pneumoniae*	Urine	*bla*OXA-48	Imipenem	Recovered
3	23/My/♀	Septicemic shock secondary to fungaemia and HAP	Acute myeloid leukaemia	25	*Klebsiella pneumoniae*	Blood	*bla*OXA-48	Meropenem	Deceased
4	33/M/♂	Infected right ankle wound	Juvenile dermatomyositis, history of stroke with residual dysarthria secondary to TB meningitis, HPT	0 (isolated from sample sent on day of admission); however, recent hospitalization 1 month prior	*Klebsiella pneumoniae*	Tissue	*bla*OXA-48	Piperacillin/ tazobactam, cefepime	Recovered
5	24/M/♂	Right foot abscess	DM	6	*Klebsiella pneumoniae*	Urine	*bla*OXA-48	Imipenem	Recovered
6	61/C/♂	Fluid overload secondary to non-compliance to ROF and HAP	ESRF	1	*Klebsiella pneumoniae*	Sputum	*bla*OXA-48	Piperacillin/ tazobactam, ceftriaxone, cefepime, meropenem	Recovered
7	24/C/♀	Neutropenic sepsis secondary to chemoport infection and HAP	Relapsed Hodgkin's Lymphoma (stage 4) with bone marrow infiltration	56	*Klebsiella pneumoniae*	Swab (triple lumen tip)	*bla*OXA-48	Imipenem, meropemen, colistin	Recovered (palliative care)
8	58/M/♂	Infected left DFU	CKD, DM, HPT	10	*Escherichia coli*	Tissue	*bla*OXA-48	Cefepime, tigecycline	Recovered
9	17/C/♂	Severe neutropenic sepsis	Acute promyelocytic leukaemia	54	*Escherichia coli*	Sputum	*bla*OXA-48 and *bla*OXA-181	Cefepime, meropenem, doripenem, colistin	Deceased

[a]♂- male; ♀ - female; C - Chinese; M - Malay; My - Myanmar;
[b]CKD - chronic kidney disease; DFU - diabetic foot ulcer; HAP - hospital-acquired pneumonia; ROF - restriction of fluid;
PVD - peripheral vascular disease; UTI - urinary tract infection
[c]DM - diabetes mellitus; HTN - hypertension; ESRD - end-stage renal disease; TB - tuberculosis

DISCUSSION

The emergence of CRE has been reported in many studies worldwide.[1,3,6] In general, OXA-48 carbapenemase and its variants (with the exception of OXA-163) hydrolyse carbapenems at low levels, on top of demonstrating weak activity against extended spectrum cephalosporins.[1] This leads to difficult detection of OXA-48 and OXA-181 in the routine laboratory setting, as their carbapenem MICs may remain in the susceptible range.[12] Therefore, the accurate and timely identification of these carbapenemases is important for the initiation of appropriate

TABLE 3: Carbapenem minimum inhibitory concentrations of the 9 positive isolates.

			MIC (µg/ml)			
Isolate	Gene detected	Patient[a]	ETP	IMP	MEM	DOR
1	*bla*OXA-48	13/C/♂	24	16	16	16
2	*bla*OXA-48	59/M/♂	12	2	3	2
3	*bla*OXA-48	23/My/♀	> 32	24	> 32	24
4	*bla*OXA-48	33/M/♂	12	2	2	3
5	*bla*OXA-48	24/M/♂	24	16	16	24
6	*bla*OXA-48	61/C/♂	> 32	> 32	16	24
7	*bla*OXA-48	24/C/♀	16	24	24	24
8	*bla*OXA-48	58/M/♂	12	2	2	4
9	*bla*OXA-48 and *bla*OXA-181	17/C/♂	> 32	> 32	> 32	> 32

[a]♂- male; ♀ - female; M - Malay; My - Myanmar
DOR - doripenem; ETP - ertapenem; IMP - imipenem; MEM - meropenem; MIC - minimum inhibitory concentration

antimicrobial therapy and preventive measures. The present study sought to identify the presence of bla_{OXA-48} and $bla_{OXA-181}$ genes among 87 *Enterobacteriaceae* isolates with reduced susceptibility to at least one carbapenem. Of the 9 bla_{OXA-48}-positive isolates, 1 co-harboured $bla_{OXA-181}$. This was not surprising as OXA-181 is a variant of OXA-48, differing from the latter by only 4 amino acid substitutions.[12] Our finding supported previous studies which revealed that their OXA-181-positive isolates also demonstrated positivity for OXA-48.[13-14] We postulate that the remaining 8 isolates we found may represent other OXA-48 variants, the identification of which was beyond the scope of this study.

Corroboration with previous data[9] revealed no coexistence of bla_{OXA-48}/$bla_{OXA-181}$ and bla_{NDM-1}. The coproduction of OXA-48/OXA-181 and NDM-1 have previously been described by Castanheira *et al*., Dortet *et al*., Barguigua *et al*., Khajuria *et al*., Kilic & Baysallar, and Uwaezuoke *et al*., to name a few.[3,6,15-18] Furthermore, most of these co-producing isolates demonstrated high levels of carbapenem resistance. Khajuria *et al*. found that isolates harbouring both bla_{OXA-48} and bla_{NDM-1} showed higher MICs against carbapenems compared to those harbouring bla_{NDM-1} alone.[16] Nevertheless, the occurrence of OXA48/OXA-181 CRE in isolation is not unusual.

According to CLSI, ertapenem non-susceptibility is the most sensitive indicator of carbapenemase production.[11] Our findings were in agreement with this; all of our OXA-48/OXA-181-positive isolates were resistant to ertapenem (disc diffusion and MIC). However, contrary to previous reports on OXA-48/OXA-181, the majority of our positive isolates exhibited high levels of resistance towards carbapenems. In particular, the sole isolate positive for OXA-181 demonstrated MICs above 32 µg/ml. A possible explanation for this might be the coexistence of ESBLs in combination with porin loss.[19] Additionally, as mentioned earlier, coexistence of carbapenemases usually confers a more resistant antibiotic susceptibility pattern. While our isolates did not co-produce NDM-1, the possibility that they may be harbouring other carbapenemase genes remains. Future studies may endeavour to address this.

In this study, all 9 OXA-48-positive isolates were positive for MHT. This was in line with Brink *et al*. case series in 2013 whereby all 4 OXA-48 and 5 OXA-181 isolates they encountered were MHT positive.[20] A review of other studies pertaining to OXA-48 and OXA-181 also revealed that the majority of molecularly-confirmed isolates were MHT positive.[3,7,13,21-22] These and our results suggest that MHT, using the ertapenem disc, may reliably detect OXA-48/OXA-181 CRE. Additionally, Girlich *et al*. study reported that MHT possessed a good sensitivity for detecting *Enterobacteriaceae* isolates producing OXA-48-like carbapenemases.[23] However, it is also worth noting that MHT is usually performed only when carbapenem resistance is suspected (i.e., when disc diffusion and/or MIC levels are intermediate/resistant). As previously discussed, OXA-48 and its variants may exhibit susceptible carbapenem MICs. Therefore, while current and past results indicate that MHT may be useful in the detection of OXA-48, it may be under-utilised among isolates which are carbapenem-susceptible.

Furthermore, the specificity of MHT is limited, as any positivity does not discriminate the types of carbapenemases. In fact, it may even produce false-positivity among non-carbapenemase producers[24]. The differentiation between metallo-β-lactamase (MBL)- or *Klebsiella pneumoniae* carbapenemase (KPC)-producers has been successfully demonstrated through the use of boronic acid derivatives and EDTA or dipicolinic acid as inhibitors in disc potentiation tests.[25-28] While several phenotypic tests, such as the temocillin disc test, faropenem disc test, OXA-48 disc test, and an immunochromatographic lateral flow test (ICT) have been described for the detection of OXA-48 carbapenemases, these methods have not been independently evaluated by CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[29-32] Recently, an algorithm for the reliable phenotypic detection of OXA-48 has been proposed by Koroska *et al*., following their comparison between these phenotypic tests and ICT.[33] It would be interesting to see how CLSI and/or EUCAST would respond to this in the near future.

In our study, *K. pneumoniae* accounted for 77.8% of OXA-48 positive isolates, followed by *E. coli* (22.2%). This was consistent which previous studies which found *K. pneumoniae* as the commonest *Enterobacteriaceae* to be associated with OXA-48. Interestingly, OXA-48 and its variants have only been identified in the *Enterobacteriaceae* family to date. This is in contrast with other OXA carbapenemases which have mostly been isolated from *Acinetobacter*

species.[34] Besides *K. pneumoniae* and *E. coli*, other bacteria that have been reported to harbour OXA-48 and OXA-181 include *Enterobacter cloacae, Citrobacter freundii*, and *Providentia rettgeri*.[1,13] Surprisingly, the sole isolate that was positive for OXA-181 was an *E. coli*. This contradicted most studies, which found that OXA-181 occurred mainly among *K. pneumoniae*, similar to OXA-48. In Castanheira *et al*.'s study, out of 11 OXA-181 positive isolates, only 2 (18.2%) were *E. coli*, while the remaining 9 (81.8%) were *K. pneumoniae*[3] A case series by Brink *et al*. found all 5 OXA-181 positive isolates to be *K. pneumoniae*.[20]

CRE infections pose a serious threat to hospitalised patients, especially vulnerable populations such as the elderly, the debilitated, children with oncological/haematological diseases, and burn patients.[35] Of the OXA-48-positive isolates in the present study, 33% were associated with haematological malignancies. This result corroborates the findings of Navarro-San Francisco *et al*. and Balkan *et al*., who reported that 30% and 33%, respectively, of their OXA-48-producing *Enterobacteriaceae* originated from patients with haematological malignancies.[36-37] Furthermore, haematological malignancies and prolonged neutropenia have been reported to be more common among non-survivors of CRE secondary to OXA-48-like carbapenemases.[37] Two of the three (66.7%) patients with underlying haematological malignancy in this study died following isolation of OXA-48 CRE.

With limited treatment options, infections secondary to CRE usually result in high morbidity and mortality, as evidenced by our study's mortality rate of 44%. A study on bloodstream infections secondary to OXA-48-producing *Enterobacteriaceae* reported that 18 out of 40 (45%) cases presented with severe sepsis or shock, while the crude mortality during admission and within 30 days from bacteraemia was 65% and 50%, respectively.[36] These high rates were contributed by delays in diagnosis and initiation of optimal antimicrobial therapy, as well as limited therapeutic choices. Most oxacillinases exhibit *in-vitro* susceptibility to polymyxin, fosfomycin and tigecycline. In the present study, only 3 out of 9 (33.3%) patients received directed therapy with colistin or tigecycline. While these antibiotics are frequently effective, judicious use is required, as extremely drug-resistant producers have already been documented. For instance, the OXA-181 *Citrobacter freundii* isolate reported by Poirel *et al*. was resistant to tigecycline but remained susceptible to fosfomycin.[1] One fatal *K. pneumoniae* isolate co-producing OXA-181 and NDM-5 was resistant to colistin, but susceptible to tigecycline, as reported by Cho *et al*. in South Korea.[22] In 2013, Balm *et al*. encountered two $bla_{\text{OXA-181}}$ isolates in Singapore which were resistant to both colistin and tigecycline.[21]

Limitations of the study

There are several limitations to the present study. First, the sample size was relatively small. However, as this was a prevalence study, we were limited by the number of samples that were sent for culture and sensitivity during the study period. Second, we only included isolates with reduced susceptibility (intermediate susceptibility or resistant) to at least one carbapenem. As discussed, OXA-48/OXA-181 isolates may demonstrate susceptibility towards carbapenems; therefore, the prevalence of OXA-48/OXA-181 CRE in our cohort may have been underestimated. Unfortunately, resources did not permit the inclusion of all isolates. Finally, the isolates studied were collected between 2011 and 2012, and thus, may not represent the current state of CRE in our centre. Nevertheless, as this is the first report on the prevalence and distribution of OXA-48 and OXA-181 CRE in Malaysia, we believe that our findings will be of relevance to future local studies.

CONCLUSIONS

The prevalence of OXA-48 and OXA-181 CRE in UKMMC during this study period was 0.069% and 0.008%, respectively. To our knowledge, this is the first report on the prevalence of OXA-48- and OXA-181-producing *Enterobacteriaceae* in a healthcare institution in Malaysia. The high mortality rate in the present study may or may not be attributable to OXA-48/OXA-181, as other confounding factors may have contributed to the patients' deaths. Nevertheless, our findings suggest that OXA-48/OXA-181 carbapenemases appear to be important and possibly under-recognised causes of carbapenem resistance in Malaysia. We implore the judicious use of antibiotics (especially carbapenems) in combination with strict infection control measures to prevent their dissemination.

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