

OXA-181 Beta Lactamase is not a Major Mediator of Carbapenem Resistance in *Enterobacteriaceae*

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ABSTRACT

Background: Detection of carbapenem hydrolyzing class D beta lactamase OXA-181, (a variant of OXA-48) in *Enterobacteriaceae*, is important, to institute appropriate therapy and to initiate preventive measures. This study was done to determine the presence of OXA 48 and its derivative OXA-181 in *Enterobacteriaceae* of pathogenic significance.

Material and Methods: One hundred and eleven non-repetitive *Enterobacteriaceae* isolates which were resistant to any of the cephalosporin subclasses III and which exhibited reduced susceptibility to carbapenems were included in the study. Minimum inhibitory concentrations (MICs) to imipenem and meropenem was determined by broth microdilution. Production of carbapenamase was screened by Modified Hodge test (MHT).

Polymerase Chain Reaction (PCR) was done to detect the presence of *bla* OXA-181 and *bla* OXA-48. Coexistence of other carbapenamase encoding genes, namely, NDM-1, VIM, IMP and KPC were also looked for, by PCR.

Results: Of all the isolates which were tested, only 2 (1.8%) revealed the presence of OXA-181 and OXA-48. These were *Klebsiella pneumoniae* and *Citrobacter freundii*. MICs of imipenem and meropenem for *Klebsiella pneumoniae* were 128mg/l and 64 mg/l and for *Citrobacter freundii*, they were 32mg/l and 16mg/l respectively. MHT was positive in both isolates.

Conclusion: Production of OXA-48 / OXA-181 is not a major mechanism of carbapenem resistance. PCR is the gold standard for its routine identification in clinical microbiology laboratory.

Key words: *Enterobacteriaceae*, OXA-181 carbapenamase, Polymerase chain reaction

INTRODUCTION

Carbapenems are the main stay of therapy for patients with serious and life threatening infections that are caused by *Enterobacteriaceae* which produce extended spectrum beta lactamases (ESBL) [1]. However, the widespread emergence and dissemination of carbapenem resistance among this family has resulted in a public health crisis [2]. This resistance is mediated by production of carbapenemases or by the combined mechanisms of outer membrane permeability defects and ESBL or hyperproduction of Amp C beta lactamase and upregulation of efflux pumps. Carbapenem-hydrolyzing beta lactamases which belong to Ambler classes A, B, and D have been reported worldwide among *Enterobacteriaceae*. The most clinically significant ones are KPC-type (class A), IMP, VIM and NDM-1 types (class B), and OXA-48 (class D). These genes encoding them are located on mobile genetic elements, which allow them to spread [1, 3,4].

The class D, carbapenem hydrolyzing OXA-48 was first reported in Turkey in *Klebsiella pneumoniae* [3]. Since then, outbreaks which were linked to OXA-48 have occurred worldwide [4]. Variants of OXA-48 that differ by substitution of one or more amino acids were identified subsequently, namely, OXA-163, OXA-181, OXA-204 and OXA-232. Of these, so far, only OXA-181 variant has been found to possess significant carbapenamase activity. It has been found to be associated with other carbapenamase encoding genes such as *bla*_{NDM-1} and *bla*_{VIM}. OXA-181 displays 45 nucleotide substitutions which lead to 4 amino acid differences as compared to OXA-48 [4,5]. Globally OXA-48 and its variant OXA-181 have been reported in several species of *Enterobacteriaceae*, particularly in *Klebsiella pneumoniae*. Case reports which referred to infections caused by OXA-181 producers have been described from The Netherlands, Singapore, France and Sultanate of Oman. The origin of most of these strains has been traced to the Indian subcontinent [1,6-8]. More recently, a Canadian publication reported the occurrence of OXA-181 in patients from Dubai and Lebanon, who had no contact with India [9]. There are only two publications from India which have indicated the presence of *bla* OXA 48/ *bla* OXA-181 in strains

from India [2,5]. So, the magnitude of infections which are caused by OXA-48 and OXA-181 producing *Enterobacteriaceae* in India is largely unknown. Therefore, this study was done to determine the prevalence of OXA-48 and its variant OXA-181 in clinically significant isolates of *Enterobacteriaceae* which have a pathogenic significance.

MATERIAL AND METHODS

Approval of the institutional ethics committee was obtained for the conduct of this study.

Bacterial Isolates: This study was conducted for a period of 7 months, from April- October 2010, in a 1600 bedded tertiary care university teaching hospital. It included 111 non repetitive *Enterobacteriaceae* isolates which were resistant to any of the cephalosporin subclasses III and were either resistant or had reduced susceptibilities (zones of inhibition of 20-22 mm) to imipenem and or meropenem by disc diffusion. The source of the isolates was blood (23), respiratory secretions (24), exudative specimens (19) and urine (45). Species included were *Klebsiella pneumoniae* (52), *Escherichia coli* (25), *Enterobacter cloacae* (16), *Citrobacter freundii* (16) and *Providencia rettgeri* (2).

METHODS

Susceptibility of these organisms to aztreonam (30µg), cefotaxime (30 µg), ceftazidime (30µg), cefepime (30 µg), piperacillin-tazobactam (100/10 µg), ciprofloxacin (5 µg), amikacin (30 µg), ertapenem (10 µg), imipenem (10 µg) and meropenem (10 µg) (Hi-Media Laboratories, India) was tested by the disc diffusion method and results were interpreted as per CLSI 2011 guidelines [10]. Susceptibility of these organisms to tigecycline was determined using 15µg tigecycline disc (BBL TM BD, USA). The criteria of the United States, Food and Drug Administration, was used for interpretation [11].

Minimal Inhibitory Concentrations (MICs) to imipenem and meropenem were determined by broth microdilution method and results were interpreted according to CLSI document M100-S21. MIC to colistin was determined by E test (Biomerieux SA, France) [10].

Production of Carbapenemase was screened by the Modified Hodge test (MHT) [10].

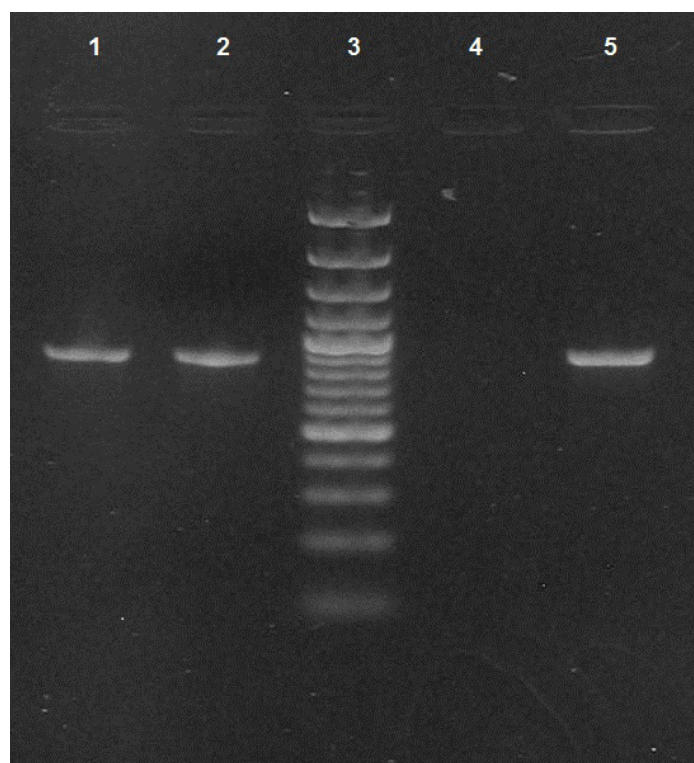
Polymerase chain reaction (PCR): All study isolates were subjected to PCR by using primers which targeted *bla* OXA-181 and *bla* OXA-48.

The primers which were used were: OXA- 181 forward seq: ATGCGTGTATTAGCCTTATCG; OXA-181 reverse seq: AACTACAAGCGCATCGAGCA; [5] and OXA-48 like forward seq: GCTTGATCGCCCTCGATT; OXA-48 like reverse seq: GATTTGCTCCGTGGCCGAAA [12].

DNA sequencing: PCR products of the isolates that carried the *bla* OXA-181 gene were purified by using DNA purification kit (QIA quick Gel Extraction Kit , Qiagen ,Valencia, CA, USA) and they were subjected to automated DNA sequencing (ABI 3100, Genetic Analyser, Applied Biosystems, Foster city, CA, USA). The aligned sequences were analysed using the Bioedit sequence program and similarity searches for the nucleotide sequences were performed using the BLAST program (<http://www.ncbi.nlm.nih.gov>).

RESULTS

Two isolates were found to be positive for OXA-48 and OXA-181 by PCR. The PCR screening results were validated by sequencing and the sequences of the *bla* oxa-181 genes showed 100% identity with previously reported genes [Table/Fig-1]. Shows the results of PCR for detection of *bla* OXA-181. These were *Klebsiella pneumoniae* and *Citrobacter freundii*, which were isolated from patients who were admitted to the Intensive Care Units (ICU). While *Klebsiella pneumoniae* was isolated from a patient with septicaemia, *Citrobacter freundii* was isolated from the urine of a catheterised patient with multiple injuries and had undergone a neurosurgical procedure. MICs to imipenem and meropenem for *Klebsiella pneumoniae* were 128mg/l and 64 mg/l and for *Citrobacter freundii*, were was 32mg/l and 16mg/l respectively. The Modified Hodge test revealed that both were strong carbapenemase producers. The OXA-181 producers were susceptible only to tigecycline and colistin (MIC- 0.38mg/L) and were resistant to cefotaxime, ceftazidime, aztreonam, amikacin, ciprofloxacin, piperacillin-tazobactam and ertapenem. [Table/Fig-2]



[Table/Fig-1]: PCR for detection of *bla* OXA-181

Lanes 1 and 2: *bla* OXA-181 positive (amplicon size- 888bp) ; Lane 3: Molecular mass marker (100bp DNA ladder); Lane 4: negative control ; Lane 5: positive control

Characteristics	Patient -1	Patient -2
Isolate Number	MS 2218	MS2287
Isolate	<i>Klebsiella pneumoniae</i>	<i>Citrobacter freundii</i>
Age /sex	73 years, female	56 years ,male
Hospital location	ICU	ICU
Admitting unit	Cardiology	Neurosurgery
Underlying disease	Coronary heart disease	Road traffic accident
Co-morbid conditions	Diabetes mellitus	none
Days in hospital	35 days	45days
Surgical procedures ,if any	Coronary artery bypass grafting	Craniotomy
Anti-microbials used prior to detection of OXA-181	Cefoperazone-sulbactam, vancomycin	Ceftriaxone, cefoperazone-sulbactam, amikacin, imipenem, metronidazole
Indwelling devices	Internal jugular venous catheter, peripheral venous line, urinary catheter	Internal jugular venous catheter, peripheral venous line, urinary catheter
Mechanical ventilation	Intubated	Intubated
Outcome	Recovered	Expired
Source specimen	Blood	Urine
MIC –IMP mg/L	128	32
MIC –MEM mg/L	64	16
MHT	Positive	Positive
ESBL screen test	Positive	Positive
Carbapenemase	OXA-48/OXA-181	OXA-48/OXA-181
Anti-microbial susceptibility profile Susceptible to	Colistin and tigecycline	Colistin and tigecycline
Anti-microbial susceptibility profile Resistant to	amikacin, ciprofloxacin,aztreonam, piperacillin-tazobactam and ceftazidime	amikacin, ciprofloxacin, aztreonam, piperacillin-tazobactam and ceftazidime

[Table/Fig-2]: Clinical Characteristics of the patients infected with OXA -48 producing isolates

shows the clinical characteristics of OXA-48/OXA-181 producing *Klebsiella pneumoniae* and *Citrobacter freundii*.

DISCUSSION

Generally, OXA-48 carbapenemase and its variants hydrolyse penicillins at a high level, but hydrolyse carbapenems at a low level only. They show very weak activity against extended spectrum cephalosporins. Hence, the *bla* OXA-48 producers exhibit reduced susceptibility to carbapenems .Their MIC to carbapenems may remain in the susceptible range, thus making their detection problematic [4]. In such infections, treatment with carbapenems results in adverse outcomes. Their detection is therefore crucial for institution of appropriate therapy and to initiate preventive measures [13].

In this study, *bla* OXA-48 and *bla* OXA-181 were detected in two of the 111 isolates. These were *Klebsiella pneumoniae* and *Citrobacter freundii*, isolated from patients admitted to the Intensive Care Units (ICU). The Modified Hodge test was positive in both. Since it has been reported that *bla* OXA-181 coexists with other carbapenemase genes [2, 5], we have screened these isolates for the concurrent presence of *bla*_{NDM-1}, *bla*_{VIM-1}, *bla*_{IMP} and *bla*_{KPC}. However, none of these genes were detected in them. In the SENTRY study which was conducted between 2006-07, out of a total of 1443 isolates, 39 were carbapenem resistant. Among these 39, 10 were found to harbour OXA-181 alone and one *Klebsiella pneumoniae* isolate carried both *bla* OXA-181 and *bla* VIM-5 [5]. Data from the SMART study of 2009, which was done on 235 Indian isolates that were non susceptible to ertapenem, indicated that *bla* OXA-48 existed alone (n= 3) or in combination with *bla* NDM-1 in *Enterobacter cloacae* (n=2) [2].

The indication for the presence of *bla*_{OXA-48} is often very ambiguous,

because some of the isolates may be carbapenem resistant but may remain susceptible to cephalosporin subclass III, which may be regarded as the potential treatment of choice [14]. However, in the Indian scenario, this phenomenon may be encountered very rarely, because of the high prevalence of ESBLs among *Enterobacteriaceae*. Due to this the resistance pattern becomes broader, leaving behind only limited therapeutic options such as polymyxins and tigecycline. [14]. The OXA -181 producers in this study were ESBL screen test positive.

Unlike the observation that OXA-48/ OXA -181 had only a low level resistance to carbapenems, our isolates had MICs which were in the range of 16-128 mg/L, way above the resistance breakpoints. This may be attributed to the coexistence of ESBLs in combination with porin loss [9].

The OXA-181 producers remained susceptible only to colistin and tigecycline in-vitro. Both patients were treated with colistin. While the patient with *Klebsiella pneumoniae* septicaemia recovered, the other patient expired. Many patients with infections caused by OXA producers have significant comorbidity and a prolonged hospital stay, as has been observed in this study [13].

Therefore it can be reasonably assumed that production of OXA-48 /OXA-181 is not a major mechanism of carbapenem resistance. Out of 111 *Enterobacteriaceae* which were screened, only 2 (1.8%) were found to be OXA-181 producers. This was in sharp contrast to the findings of study of Castanheira et al., where of 39 *Enterobacteriaceae*, 10 were OXA-181 producers [5] and in the SMART study, among 66 isolates with a carbapenemase encoding gene, 5 were found to be *bla*_{OXA-48} positive [2]. Additionally, *bla* OXA-48 / *bla*_{OXA-181} in our study did not coexist with other carbapenemase encoding genes, as was reported in the earlier studies [2,5]. Notably, the carbapenem MICs were high. Since there are no specific phenotypic tests for the detection of this enzyme, PCR is the gold standard for their routine identification in clinical microbiology laboratory.

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