Laboratory Detection and Clinical Implication of Oxacillinase-48 like Carbapenemase: The Hidden Threat

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ABSTRACT

Carbapenemase producing Gram-negative pathogen is of great concern for physician. The challenging aspects are treatment option and infection control. Monitoring of respective carbapenemase resistance mechanism is necessary to prevent the outbreaks. Currently, the rapid emergence of oxacillinase (OXA-48) like is alarming. Increasing frequency of OXA-48 is seen than the classical carbapenemase (KPC, NDM, IMP, and VIM) across the world. The $bla_{\text{OXA-48}}$ gene is commonly identified in *Escherichia coli* and *Klebsiella pneumoniae*. The transferrable plasmid of OXA-48 is associated with rapid spread and inter-species dissemination. In general, OXA-48-like enzymes weakly hydrolyzes both carbapenem and broad spectrum cephalosporins. Except OXA-163, which effectively hydrolyze cephalosporin. This poor hydrolytic profile obscures the detection of OXA-48-like. It may go undetected in routine diagnosis and complicates the treatment option. Co-production of OXA-48-like with CTX-M-15 and other carbapenemase (NDM, VIM) leads to the emergence of multidrug resistant strains.

Key words: Carbapenemase, noscomial, OXA-48, OXA-48-like

INTRODUCTION

The global spread of carbapenemase-producing Gram-negative pathogens is of special concern in healthcare and community settings. Invasive infection causing carbapenemase producers associated with significant mortality and morbidity. Carbapenemase confer resistance to most β-lactam antibiotics including carbapenem.[1] Moreover, frequent co-existence of other antibiotic resistant genes complicates the therapy and limits the treatment option.[2] The optimal treatment remains undefined. Accurate detection of carbapenemase producers is essential for infection control and management of antibiotic therapy.[3] The most representative carbapenemase is classified in three classes: Class A (KPC), Class B metallo β-lactamase (IMP, VIM, and NDM), and Class D oxacillinase (OXA-48).[4]

Certainly, OXA-48 a Class D β-lactamase are being increasingly reported with outbreaks and case reports across the world.[5] Horizontal transfer of mobile genetic elements carrying OXA-48-like gene results in rapid spread.[6] OXA-48-like enzyme weakly hydrolyzes both carbapenem and cephalosporin. Hence, elevated minimum inhibitory concentrations (MIC) to carbapenem and cephalosporin is not noticeable with OXA-48 like.[7] It may go undetected with routine diagnosis. Molecular characterization of OXA-48-like is warranted. Recently, many studies have reported OXA-48-like in *Enterobacteriaceae*, especially in *Klebsiella* spp. Identification of OXA-48 and its variant with short turnaround time promotes the time to active treatment.

The aim of this review is to summarize the characteristics of OXA-48, including hydrolytic activity, distribution of

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enzyme variants, plasmid-mediated rapid spread with a special reference to challenges in laboratory diagnosis and clinical implication.

Classification of carbapenemase

Carbapenemase identified in *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* are placed in three classes of β-lactamase as Ambler Class A, Class B metallo β-lactamase, and carbapenem hydrolyzing Class D oxacillinase.[8] Carbapenemase are classified into two major types based on the active site of the enzyme as serine carbapenemase (KPC and OXA type β-lactamase) and metallo-β-lactamase (IMP, VIM, and NDM). The spectrum of substrate for carbapenemase activity is varied [Table 1].[9]

In the early 1990s, most of the identified carbapenemase are chromosomally encoded. Plasmid-mediated carbapenemase begins to emerge and creates the threat for rapid spread among Gram-negative pathogens.[10] Class A and Class B metallo-β-lactamase are described with well-defined set of characteristics. Notably, synergy test using the inhibitors of boronic acid derivatives (BA) and dipicolinic acid (DPA)/ ethylene diamine tetra acetic acid (EDTA) were available to detect Class A and Class B carbapenemase.[11] However, OXA-48 is difficult to identify due to poor hydrolysis of substrates (carbapenem and cephalosporin). In contrast to other carbapenemase, elevated carbapenem MIC is not noticeable with OXA-48 unless co-produced with other β-lactamases.[12] Additionally, phenotypic method using specific inhibitor for OXA-48 is not currently available.[13] It is of great concern for clonal spread and inter-species dissemination. Hence, a phenotypic method for earlier detection of OXA-48 is essential.

In *Enterobacteriaceae*, the most common carbapenemase producers are KPC, IMP, VIM and NDM. Comparatively OXA-48 and its variants are becoming widely distributed of late and commonly reported in *Enterobacteriaceae*. In contrast, so far only two studies have reported OXA-48 in *A. baumannii* and no single study with *P. aeruginosa*.[14]

Characteristics of oxacillinase-48

Carbapenem-hydrolyzing Class D β-lactamases are not inhibited by the inhibitor available in clinical use. This includes clavulanic acid, tazobactam and sulbactam.[15] Of the Class D carbapenemase, OXA-48 is of major concern due to its:

1. Difficulty in detection,
2. Association with treatment failure and
3. High dissemination rate due to transferable plasmid.

Due to this property of OXA-48, susceptible or low-level resistance to cephalosporin and/or carbapenem is seen. Remarkably, enzyme kinetic analysis shows greater catalytic activity of OXA-48 to imipenem than meropenem.[16] OXA-48-like enzyme variants is plasmid coded and is associated with rapid spread in community settings.[17] Further, OXA-48 is frequently reported in clinically important noscomial pathogens *E. coli* and *Klebsiella pneumoniae*. The high level of OXA-48 resistance is associated with co-production of extended spectrum β-lactamase(ESBL).[18]

Variants of oxacillinase-48 like enzymes

A multi-drug resistant *K. pneumoniae* was isolated from a patient in Istanbul, Turkey and found a new OXA-type β-lactamase.[19] It was identified and named as OXA-48. Since 2001 with the appearance of first report on OXA-48, 11 enzyme variants were identified and reported across the world. This includes OXA-48, OXA-48b, OXA-54, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-242, and OXA-247. These enzyme variants differ by few amino acid substitution or deletion [Table 2]. OXA-48 and its variants were named and placed under the group as OXA-48-like variants. Most common host

Table 1: Substrate, hydrolysis and inhibitory profile of carbapenemase

Ambler class	Representative carbapenemase	Hydrolysis profile				Inhibitory profile		*Enterobacteriaceae*	Nonfermenters
		Second generation cephalosporin	Third generation cephalosporin	Aztreonem	Carbapenem	Inhibitor in clinical use	*In-vitro* inhibitors		
Class A	KPC	—	++	+	++	Clavulanic acid, tazobactam	Boronic acid	+++	+
Class B	IMP, VIM*	++	++	++	—	Not affected by inhibitors in clinical use	EDTA	+++	+++
	NDM	++	++	—	+			+++	++
Class D	OXA-48-like	±	±	—	+	Not affected by inhibitors in clinical use	NaCl	+++	±

In particular, OXA-48 hydrolysis cephalosporins and carbapenem poorly and make it difficult for detection under routine laboratory diagnosis. Additionally OXA-48-like variant was not inhibited specifically by the inhibitors in clinical use. *IMP and VIM are integron-associated elements and facilitates its insertion into plasmid. In contrast, KPC, NDM and OXA-48-like are plasmid encoded. EDTA: Ethylenediaminetetraacetic acid, OXA-48: Oxacillinase-48, KPC: *Klebsiella pneumoniae* carbapenemase for production of OXA-48-like enzymes is *Escherichia coli*, *K. pneumonia, Enterobacter cloacae, Serratia marcescens, Shewanella xiamenensis, Citrobacter freundii, Providencia rettgeri, Klebsiella oxytoca, Enterobacter sakazakii*, and *A. baumannii*.[20]

The kinetic properties of OXA-181, OXA-162, and OXA-204 appear broadly similar to OXA-48 in their hydrolytic profile.[21] OXA-181 is one of the most commonly encountered OXA-48-like variants in different geographic regions. OXA-181 weakly hydrolyses both carbapenem and cephalosporin and differs from OXA-48 at four amino acid substitution. In contrast, OXA-163 effectively hydrolyses cephalosporins (ceftazidime, cefotaxime, and cefepime) and aztreonam.[22] Interestingly, this property of OXA-163 is not detectable with OXA-48. Ideally OXA-163 appears similar to ESBL than carbapenemase in substrate profile. Unlike OXA-48, OXA-232 shows relatively lower hydrolytic activity against carbapenem.[23]

Plasmid as vehicle for spread of oxacillinase-48 like determinants

Initially, $bla_{\text{OXA-48}}$ gene was reported in association with insertion sequence (IS)1999 in the upstream region. Later, $bla_{\text{OXA-48}}$ gene is identified on self-transferrable IncL/M-type plasmid.[24] The transposon Tn1999 is located at the downstream of OXA-48 gene. The $bla_{\text{OXA-48}}$ gene is a part of the transposon Tn1999, made of two copies of IS1999 as shown in Figure 1.[25] The most alarming finding is the transferable operon present in IncL/M-type. This plasmid is associated with high conjugation rate and accounts for rapid transfer and spread across the Gram-negative pathogens.[26]

Further variants of Tn1999 were identified with additional IS.Tn1999.2 contains IS*1*R element inserted into the upstream region of IS1999 seen in Figure 1. Similarly, an

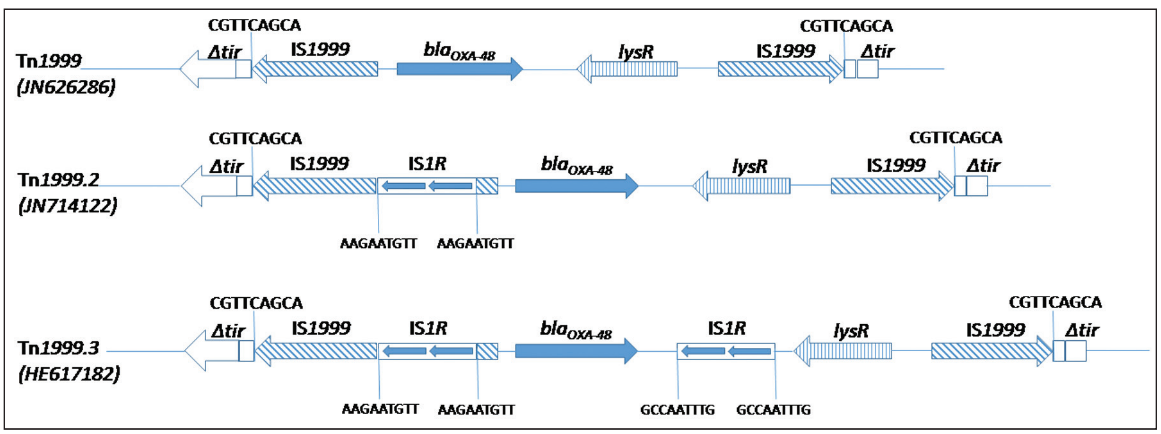

Figure 1: Schematic representation of oxacillinase-48 and its transposons, (a) schematic representation of Tn*1999* identified with oxacillinase-48 gene, (b) schematic representation of Tn1999.2 identified with oxacillinase-48 gene, (c) schematic representation of Tn*1999.3* identified with oxacillinase-48 gene. Horizontal arrowsrepresent the orientation of gene and their transcription. Δ Indicate the interruption of an element/gene by insertion sequence

Table 2: Variants of OXA-48-like enzyme and its deviation

OXA-48-like variants	NCBI reference sequence: Accession number	Deviation from OXA-48
OXA-54*	WP_011071128	20 substitution at Phe10Leu, Leu11Val, Ile16Val, Val21Met, Lys23asn, Asn28Lys, Lys29Pro, Ala33Thr, Thr36Ser, Ser40Ala, Val44Ile, Thr104Ala, Asn110Asp, Glu132Gln, Val153Leu, Ile170Val, Ser171Ala, Gly201Ser, Lys218Gln, and Ser244Ala
OXA-162	ADG27454	Single substitution at Thr213Ala
OXA-163	ADY06444	Single substitution at Ser212Asp and four deletions at Arg214, Ile215, Glu216, and Pro217
OXA-181	BAP94533	Four substitutions at Thr104Ala, Asn110Asp, Glu168Gln, and Ser171Ala
OXA-199	AFC95894	Three substitutions at His38Tyr, Val44Ala, and Asp154Gly
OXA-204	AJF39128	Two substitutions at Gln98His and Thr99Arg
OXA-232†	AGD91915	Single substitution at Arg214Ser
OXA-244	YP_009090533	Single substitution at Arg214Gly
OXA-245	YP_009090534	Single substitution at Glu125Tyr
OXA-247‡	YP_009090726	Two substitutions at Tyr211Ser and Asp212Asn

Analysis of amino acid substitution/deletion was done using the reference amino acid sequences of the respective OXA-48 variants deposited in NCBI website (http://www.ncbi.nlm.nih.gov/pubmed/). *OXA-54: It differs from OXA-48 by 20 amino acids and constitutes a subgroup of OXA-48-like enzymes, †OXA-232: A mutant derivative of OXA-181 and not derived from OXA-48, OXA-247: Two amino acid derivative of OXA-163 and was not originated from OXA-48. OXA: Oxacillinase, NCBI: National Center for Biotechnology Information

IS*1*R element located at the downstream of *bla*$_{OXA-48}$ gene is called as Tn1999.3 as shown in Figure 1.[27] The hybrid promoter of combined IS*1*R/IS1999 element results in 2-fold greater hydrolysis of imipenem. Collectively, the presence of both increases the level of carbapenem resistance than possessing either one of the IS.[28]

Each OXA-48-like variants was identified on the plasmid with different ISs and arrangements. Modification in the genetic arrangements is described for the following enzyme variants such as OXA-181, OXA-204 and OXA-232 respectively. Unlike OXA-48, *bla*$_{OXA-181}$ gene is inserted at the downstream region of ISE*cp1*and form the transposon Tn*2013*.[29] The presence of ISE*cp*1 facilitates the acquisition of ESBL genes such as CTX-M-15.[30] Co-production of OXA-181 with ESBLs makes the resistance profile broader with reliable detection.[31] However limits the therapeutic option; the *bla*$_{OXA-204}$ gene is found on the IncA/C plasmid contains the transposon Tn2016 with the ISE*cp*1 was reported in *K. pneumoniae* from Tunisia. An additional element IS*kpn*15 inserted at the upstream of ISE*cp*1. Hybrid promoter was not identified in IS*kpn*15/ISE*cp*1 chimera. The promoter of ISE*cp*1 regulates the expression of *bla*$_{OXA-204}$ gene.[32] IncA/C plasmid possess a wide host range and responsible for acquisition and spread of various β-lactamase genes. The notable among them include *bla*$_{CMY}$ and *bla*$_{CTX-M}$ genes.[33] In addition, *bla*$_{NDM-1}$ and *bla*$_{VIM-4}$ were also found on the plasmid scaffold.[34,35] Further OXA-232 associates with ISE*cp*1 and found on non-conjugative plasmids, resulting in failure of transposase to mobilize *bla*$_{OXA-232}$ gene either due to deletion or duplication in the upstream region of ISE*cp*1.[36]

Epidemiology

The true prevalence of OXA-48 is relatively unknown due to the varying level of carbapenemase activity and difficult to detect with phenotypic methods. Outbreak of OXA-48 was reported initially from Turkey, United Kingdom and France.[37-39] Clinical cases of OXA-48 producing *Enterobacteriaceae* was reported widely from Lebanon, Belgium, United Kingdom, Tunisia, Morocco,Oman and Netherland.[40-46] Currently, Turkey, Middle East countries, and North African countries were considered a major reservoir of OXA-48.[47] Many outbreak and sporadic cases were reported in Turkey.[48,49] The emergence of OXA-48 in hospital and community settings was reported sporadically in Senegal.[50] In Spain, noscomial outbreak of OXA-48 was reported with co-production of CTX-M-15.[51] Recently, an outbreak of OXA-48-like, being predominantly identified with OXA-48 from Netherland, Belgium and Germany.[52-54]

OXA-181 is the most common OXA-48-like variant reported across India. This includes two multicenter and two single center study reporting OXA-48-like. We reviewed 14 studies on OXA-48-like enzymes which include case reports, noscomial outbreaks and retrospective studies from France, Italy, Spain, the United States, North America, Argentina, Europe and America, New Zealand, Singapore, Japan, and India [Table 3].

Interestingly, most of the OXA-48-like are reported in *K. pneumoniae*. The reviewed literature on OXA-48-like reveals ESBLs were the most common co-producing β-lactamase. Among ESBL, CTX-M-15 seems to be predominantly distributed CTX-M-1 group followed by CTX-M-14 belongs to CTX-M-9 cluster as shown in Table 3. Co-production of OXA-48 was also seen with other carbapenemase including NDM-1and VIM as shown in Table 3.[69] Next to NDM, OXA-48-like is the frequently reporting carbapenemase across India. Further co-production of OXA-48-like with NDM in countries with poor sanitation is worrisome.

Clinical implication

OXA-48 is of great clinical concern due to the difficulty in detection with reduced susceptibility to both carbapenem and broad-spectrum cephalosporin. The mortality remains high in patients infected with OXA-48-like producers. The challenging aspect of OXA-48 is that it may go undetected and classified as susceptible with imipenem and meropenem MIC of (≤1 μg/ml) as per Clinical and Laboratory Standards Institute (CLSI) and (≤2 μg/ml) as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines respectively. Accurate screening for the presence of OXA-48 is not possible with MIC determination alone. This OXA-48 remains as a hidden threat that obscures the laboratory diagnosis and complicates the treatment option. The plasmid that carries OXA-48 serves as a potential vehicle for dissemination of genes among clinical isolates and to the intestinal flora.[70] Currently, the plasmid that carries OXA-48 not often carries other antibiotic resistant genes. However recently co-production of OXA-48 with ESBL are being reported which is the cause for great concern.

In case of ESBL negative OXA-48 positive producers, broad spectrum cephalosporin is considered as potential treatment option except those with OXA-163 a potent hydrolyser of cephalosporinase. A neonate infected with OXA-48 producing *K. pneumoniae* was successfully treated with cefotaxime and amikacin from France.[71] A patient with central line infection of OXA-48 producing *E. coli*

Table 3: Outbreaks, surveillance and clinical case reports of OXA-48 across the world

Organism	Number of isolate included	Source of the isolate	Study type	Study conducted period	OXA-48 and its variants reported (*n*)	Co-producers	Geographic region
K. pneumoniae	53	Clinical isolates	Surveillance	2011-2013	OXA-48 (53)	CTX-M-15	France[55]
K. pneumoniae	1	Clinical isolate	Clinical case	2011	OXA-48 (1)	CTX-M-15	Italy[56]
K. pneumoniae	71	Clinical isolate	Outbreak	2011	OXA-48 (71)	CTX-M-15	Spain[57]
K. pneumoniae	1	Clinical isolates	Clinical case	2013	OXA-232 (1)	NDM-1	United States[58]
K. pneumoniae	110	Inta-abdominal infections	Surveillance	2008-2009	OXA-48 (6) OXA-163 (2) OXA-181 (5)	CTX-M-15 CTX-M-3 VIM-5	North America[59]
K. pneumoniae	2	Clinical isolates	Clinical case	—	OXA-163 (1) OXA-247 (1)	—	Argentina[60]
E. coli, K. pneumoniae	15,948	Clinical isolates	Surveillance	2007-2009	OXA-48 (38)	—	Europe and America[61]
K. pneumoniae	1	Clinical isolate	Clinical case	2010	OXA-181 (1)	CTX-M-15	Newzealand[62]
Enterobacteriaceae	96	Clinical isolates	Surveillance	2010-2012	OXA-181 (8)	CTX-M-15	Singapore[63]
K. pneumoniae	1	Clinical isolate	Clinical case	2010	OXA-181 (1)	—	Japan[64]
Enterobacteriaceae	1443 (26-CRE)	Clinical isolate	Multicenter SENTRY surveillance program	2006-2007	OXA-181 (10)	CTX-M-15 (10) VIM-5 (1)	India[65]
Enterobacteriaceae	235 (66-CRE)	Clinical isolate	SMART study	2009	OXA-48 (3)	CTX-M-15 (3)	Only 3 isolates with OXA-48 are reported from India[66]
Enterobacteriaceae	111	Clinical isolate	Single center	2010	OXA-181 (2)	—	Reported in *K. pneumoniae*and *C. freundii*[67]
E. coli	300	Clinical isolate	Single center	2012	OXA-48 (25)	NDM-1 (25)	Only *E. coli*is included in the study[68]

E. coli: Escherichia coli, K. pneumonia: *Klebsiella pneumoniae*, *C. freundii*: *Citrobacter freundii*, OXA: Oxacillinase

was recovered on ceftazidime and non-carbapenem combinational therapy.[72] However, the studies supporting the recommendation of broad spectrum cephalosporin in treating ESBL negative OXA-48 positive producers are limited.

Infection with ESBL positive and OXA-48 positive co-producers provide reliable identification but associates with wider range of resistance and limit the treatment option. Co-production of OXA-48 with CTX-M-15 and NDM serves as a vehicle for noscomial outbreaks as multidrug resistant pathogen as shown in Table 3. A better outcome was observed with triple combination of colistin, aminoglycoside with ceftazidime/cefepime in treating co-resistance of OXA-48 and ESBL.[73] Tigecycline and fosfomycin are also served as a treatment option but carries the risk for emergence of resistance.[74] The optimal treatment option for this multi-drug resistance pattern is unclear and there is a lack of evidence-based studies.

Avibacatam, a non-β lactam β lactamase inhibitor selectively inhibits Class D OXA-48, Class A and Class C β-lactamase. It forms a stable complex with OXA-48 by establishing covalent bond.[75] The combination of avibactam with imipenem and cephalosporin (cefepime, ceftazidime, and cefotaxime) significantly reduces the MIC value of OXA-48 producers.[76] Interestingly, avibactam was identified with the broad coverage of OXA and other β-lactamase. Thus serves as an attractive treatment option.

In an *in-vivo* study, an infection is induced in murine model with OXA-48 producing *K. pneumoniae*.[77] The strains were susceptible to ceftazidime and imipenem as per MIC. However, the outcome was better with ceftazidime than with carbapenem treated group. A similar result was also revealed in experimental mice, in which peritonitis was induced with ESBL negative and OXA-48 producing *K. pneumoniae*.[78] Further clinical profile of OXA-48 producers does not concur with carbapenem MIC breakpoints ≤1 μg/ml (i.e., ≤1 μg/ml can be OXA-48 producers). Irrespective of carbapenem susceptibility, carbapenem is not reliable in treating patients infected with OXA-48 producers.

The optimal treatment remains unclear. *In vitro* susceptibility to colistin, tigecycline and aminoglycosides were promising.[79,80] However, there is a limited proven clinical efficacy. Notably, emerging resistance to this antibiotic is already reported.[81,82] Some studies have reported the better outcome with combinational therapy rather than with monotherapy.[83]

Appropriate therapy for OXA-48 is unpredictable and presents with multidrug resistant profile. Carbapenem MIC alone is not enough for treating patients with OXA-48 producers. Molecular characterization is essential to rule out the presence of OXA-48 producers that shows heterogeneous hydrolytic profile in phenotypic detection (MIC). Screening for OXA-48 in patient is necessary to prevent the noscomial outbreak prior to hospital admission. Rectal screening of the patient offers detection at the earliest and initiation of appropriate therapy.[84]

Laboratory detection of oxacillinase-48

Carbapenemase production is identified with the reduction in susceptibility to carbapenem. However, OXA-48-like exhibits low carbapenemase activity and makes it difficult to detect. At present, an OXA-48-like producer is not detectable with the well-defined phenotypic methods using β-lactam and β-lactamase inhibitor combination used to detect Class A and Class B carbapenemase. Further, a good inhibitor is not available for specific analysis of OXA-48-like enzyme. The most challenging part of OXA-48 detection is that it differs from other carbapenemase by exhibiting susceptibility to broad spectrum cephalosporins but remains resistant to carbapenem. However, the marker is not consistent to selectively identify OXA-48 as some may show resistance to both broad-spectrum cephalosporins and carbapenem equally. Most of the studies on carbapenemase detection are focused on Class A and Class B-metallo-β-lactamase. Very few studies are carried out for OXA-48-like detection.

Carbapenem minimum inhibitory concentrations and interpretative criteria

Resistance to carbapenem is reported on the basis of susceptibility testing. According to the CLSI guidelines (M100-S25), MIC breakpoints for imipenem and meropenem are susceptible (≤1 μg/ml) and resistant (>4 μg/ml). For ertapenem, the breakpoints are susceptible (≤0.5 μg/ml) and resistant (>2 μg/ml). The role of ertapenem with low MIC breakpoint in detecting OXA-48 remains to be assessed.

Modified Hodge test

Modified Hodge Test (MHT) or cloverleaf technique recommended by CLSI guidelines (M100-S25) is used extensively as a nonspecific screening test for routine diagnosis of carbapenemase producers. Carbapenemase producers inactivate the carbapenem and promote the growth of carbapenem susceptible indicator strain along the streak line toward carbapenem disk and results in a characteristic cloverleaf-like indentation. Comparable sensitivity but poor specificity was observed with MHT of 98% and 80% respectively with the longer turnaround time.[85] For OXA-48, MHT is found to be better with sensitivity and specificity of 96% and 84% respectively (unpublished data) contrast to the percentage described in CLSI. This may be due to the variation in the prevalence of OXA-48-like enzymes in different geographic regions. False negative results account for lack of specificity and low sensitivity. Unlike NDM, detection of OXA-48 seems to be better with MHT.[86]

Temocillin and minimum inhibitory concentrations

Temocillin, a semi synthetic β-lactam is identified as a suggestive marker to screen OXA-48-like determinants that confers high-level resistance.[87] Temocillin is stable against the hydrolysis of ESBL and AmpC enzymes.[88] However, high level of temocillin resistance is not only the characteristics of OXA-48-like producers but similar level of resistance is also observed with KPC and metallo-β-lactamase. Hence temocillin alone is not a diagnostic marker for OXA-48-like detection.[89] Temocillin with high-level MIC and nonsusceptibility to ceftazidime and other third generation cephalosporin gives the clue for the presence of OXA-48-like enzymes. Nonavailability of temocillin in many countries makes it impossible for screening. In CLSI or EUCAST guidelines breakpoint for temocillin is not available. Temocillin breakpoints are established by the British Society for Antimicrobial Therapy (BSAC), and it is the only available guideline to define temocillin MIC breakpoints as well zone diameter for interpretation of strains. According to BSAC, the breakpoints for temocillin are susceptible (≤32 μg/ml) and resistant (>32 μg/ml), and the zone diameter of ≥12 mm and ≤11 mm are defined as susceptible and resistant, respectively.[90]

Disc diffusion assay

Disc diffusion assay using temocillin, meropenem, BA, and/or DPA which is commercially available as KPC/MBL and OXA-48 Confirm Kit (Rosco Diagnostics) is used to discriminate OXA-48-like enzymes from other carbapenemase. If the synergy is not observed with meropenem and phenyl BA and/or DPA and temocillin with the zone diameter of <10 mm is identified as OXA-48 producers with the sensitivity and specificity of 100%.[91] Mastdiscs ID inhibitor combination disks are also useful for presumptive detection of OXA-48.

Chromogenic medium

Chromogenic medium enables rapid identification of carbapenem resistant pathogens either from clinical samples or from isolates. Although chromogenic media are a fairly rapid means of detecting CRO, its use is confined to rectal swab. Chrom ID OXA-48 is the only available chromogenic medium for selective identification of OXA-48 [Table 4]. Its exact composition is undisclosed; however,the media contains antibiotic for the inhibition of other microorganism and biochemical

Table 4: Comparison of chromogenic medium for the detection of carbapenem resistant organism

Chromogenic medium	Available	Number of isolates screened	Sensitivity (%)	Specificity (%)	Remarks
Brilliant CRE agar*[92]	Oxoid, Thermofisher Scientific	255	94	71	Sensitivity is lower with OXA-48 (84%) than with KPC, NDM (100%)
Chrom ID CARBA[93]	Biomerieux	133	92.4	96.9	OXA-48 detected at high inoculum of 10^7 CFU/ml
Chrom ID OXA-48 and SUPERCARBA[94]	Biomerieux	117	91 93	100 53	Validated only for OXA-48 detection

*Brilliant CRE agar was found to be less optimal in detecting OXA-48-like. The specificity was low due to the growth of AmpC- and/or ESBL-producing isolates. OXA-48: Oxacillinase-48

markers to differentiate species or groups of species using either chromogenic substrates or fermentable carbohydrates with a pH indicator. The sensitivity of chromogenic medium can be increased by incubating the plates for complete 24 h. In contrast to manufacturer's claims, false positives can occur. Confirmation of all positives with either disc diffusion assay or by molecular characterization is recommended.

Rapid diagnosis

Accurate and earlier detection of OXA-48-like determinants is necessary for better management of patient and infection control. The following rapid diagnostic methods tests are available for detection of carbapenemase producers.

Carba NP

Carba NP is a hydrolytic assay recommended by CLSI (M100-S25) guidelines as a confirmatory test for detection of carbapenemase that is simple and easy to perform. Hydrolysis of imipenem is indicated by the change in the pH of indicator phenol red and the color changes from red to yellow with the turnaround time <2 h. It has a sensitivity and specificity of 100% in detecting Class A and Class B metallo-β-lactamase.[95] However, the sensitivity of calorimetric microtube assay/Carba NP is shown to be 11% with OXA-48 according to CLSI guidelines (CLSI-2015). While in our experience we observed, Carba NP for OXA-48 detection shows sensitivity and specificity of 77% and 84% respectively (unpublished data). Indeed, suspected cause for low sensitivity with OXA-48-like enzymes is due to the presence of mucoid strains and/or weak carbapenemase activity. Further sensitivity of Carba NP with mucoid phenotypes may be improved by extending the incubation period from ½ to 1 h and reduction of inoculum size was preferred.[96] For weak carbapenemase producers, concentrated extract of cell suspension was recommended.[97]

Blue Carba is a variant of Carba NP in which bromothymol blue is used as an indicator instead of phenol red as in Carba NP assay. The main advantage of Blue Carba over Carba NP is the direct colony suspension approach without the need for lysis buffer.[98] The sensitivity and specificity of Blue Carba in detecting OXA-48 is yet to be established.

Molecular based techniques

Phenotypic test identifies the carbapenemase producers in general without any specification over the class of carbapenemase. Molecular characterization is the only available tool for discriminating different carbapenemase encoding genes. Polymerase chain reaction (PCR)is considered as the gold standard for identification of OXA-48 and it should be followed by sequencing for the precise identification of enzyme variants of OXA-48-like. In addition to the conventional PCR, real-time PCR and microarray designed panels are also available for rapid detection of OXA-48-like [Table 5].[99-102] Matrix-assisted laser desorption ionization time-of-flight mass spectrometry is evaluated and available for detection of OXA-48, although its use in routine diagnosis is restricted due to the cost.

Xpert®Carba-R

Xpert®Carba-R is developed on the basis of real time PCR and the results are available with the turnaround time of 2 h. The panel of Xpert®Carba-R contains the sequences for targeting bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, and $bla_{\text{OXA-48}}$ genes. Of the 11 known variants of OXA-48-like, only four variants ($bla_{\text{OXA-48}}$, $bla_{\text{OXA-162}}$, $bla_{\text{OXA-163}}$,and $bla_{\text{OXA-204}}$) were designed to detect.[103] Incorporation of geographic-specific OXA-48-like variants in the proprietary panel may improve its sensitivity and broadens the detection of OXA-48-like enzymes across the world.

The Class A (KPC) and Class B (IMP, VIM, NDM) is considered important carbapenemases with focus of high priority. However, recently Class D carbapenemase OXA-48 is rapidly emerging and disseminating in Gram-negative pathogens. Interestingly, the self-conjugative plasmids that carry bla_{OXA}-48 gene in composite transposons

Table 5: Various commercially available molecular detection for OXA-48-like enzyme variants

Features of evaluated studies	Hyplex-SuperBug ID kit	Check direct CPE assay	Check-MDR CT102 DNA microarray	MALDI-TOF MS
Number of isolates	132	83	95	372
Organism included for validation	*Enterobacteriaceae*	Gram-negative organism and noncarbapenemase producers	*Enterobacteriaceae*	*Enterobacteriaceae*
Carbapenemase coverage	KPC, OXA-48, VIM, NDM	KPC, OXA-48, VIM, NDM	KPC, OXA-48, VIM, NDM	OXA-48
Other carbapenemase				
Sensitivity (%)	100	100	97	None
Specificity (%)	100	100	100	
OXA-48				
Sensitivity (%)	100	Not mentioned	Not mentioned	98.9
Specificity (%)	99			97.1
PCR	Multiplex real-time PCR	Multiplex real-time PCR	Microarray	Ionization
Time	5 h	3 h	6.5 h	90 min
Approval	Not available	FDA	FDA	FDA

PCR: Polymerase chain reaction, FDA: Food and Drug Administration, OXA-48: Oxacillinase-48, MALDI-TOF MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

Tn*1999* account for rapid spread as a single clone. As a consequence of low carbapenemase activity and poor hydrolyzes of broad spectrum cephalosporins complicates the identification. As a result, silent spread and outbreak occurs. Further surveillance studies are necessary to understand the dynamic of transmission, risk factors and reservoir of OXA-48 producers.

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Conflicts of interest

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