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## Article

# Assessment of Phenotypic Tools for Detection of OXA-48, KPC and NDM in *Klebsiella pneumoniae* in Oman

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**Abstract: Background:** For judicious use of reserve drugs, timely detection of carbapenemases is essential. While molecular tools are gold standard for detection of these enzymes, many laboratories have limited access to them. This study focused on evaluating in-house and commercial phenotypic tests for detection of OXA-48 in particular, KPC and NDM in general in *K. pneumoniae*, the predominant extremely drug-resistant pathogen in Oman. **Methods:** Eighty GeneXpert/PCR confirmed (40 OXA-48, twenty KPC and NDM each) and 37 whole-genome-sequenced (25 OXA-232, 6 KPC-2 plus NDM-1 and NDM-5 each) *K. pneumoniae* were subjected to screening by temocillin (30 µg disk MAST Diagnostica, Germany) and D71C (MASTDISCS®). Isolates resistant to temocillin (<11mm) and D71C were subjected to four tests -one in-house (OXA-48 disk test) and three commercial phenotypic tests (i) The MASTDISCS® Combi (D72C) (MAST Group Ltd., UK). (ii) The MASTDISCS® Combi (D73C) (MAST Group Ltd., UK) and (iii) Immunochromatographic assay (ICT); KPC/IMP/NDM/VIM/OXA-48 Combo test kit (Medomics, China for detection of OXA-48 like, KPC and NDM carbapenemases. **Results:** Temocillin exhibited good sensitivity and specificity (100% and 97.50%) compared to D71C (70% and 100%). Among the confirmatory tests, the in-house OXA-48 disk test had 92.50% sensitivity and 100% specificity, while the commercial MAST DISC tests D72C D73C and ICT had 97.50%, 95.00% and 100% sensitivity and 100%, 91.67% and 95% specificity respectively. **Conclusion:** Temocillin disc test is a good screening tool. With high sensitivity and specificity, ease of performance, short turnaround time and low cost, we recommend ICT for routine diagnostic use. In resource constrained centres, OXA-48 disk test is an excellent alternative with high sensitivity and specificity.

**Keywords:** OXA-48 disk test; ICT; D73C; Gene Xpert; WGS; *Klebsiella pneumoniae*; OXA-48; KPC; NDM

## 1. Introduction

Carbapenemase-producing Enterobacterales (CRE) constitute a significant global public health threat in an era of spiraling antimicrobial resistance [1]. In fact their incidence is on the rise in comparison to the total Enterobacterales infections with *Klebsiella* species predominating [2–6]. Rapid detection of carbapenemases and initiation of appropriate antimicrobials improves patient outcomes, strengthens antimicrobial stewardship efforts and allows judicious use of the reserve antibiotics [7]. Epidemiological studies indicate that as with other carbapenemases, OXA-48-producing strains are associated with poor patient outcomes. However, OXA-48 detection is rather challenging, due to low-level hydrolytic activity toward carbapenems and no intrinsic activity against expanded-spectrum cephalosporins [6]. Initially reported in Turkey, OXA-48 prevalence has spread

globally, with increasing reports in Europe, Asia, and North America [8,9]. In Oman, CRE was reported for the first time in 2011 among *Klebsiella pneumoniae* harbouring NDM-1 and OXA-181 carbapenemases [10]. Other reports described the molecular epidemiology of CRE in the country, where the predominant isolates were *Klebsiella pneumoniae*, followed by *Escherichia coli*, *Enterobacter cloacae* and others that carried *bla*NDM, *bla*OXA48-like and to a lesser extent *bla*VIM carbapenemases [11]. In addition, Balkhair et al. reported in 2013 an overall prevalence rate of 10.8 (95% CI: 9.3–12.4) of multidrug resistant Gram-negative bacteria cases per 1,000 admissions [12]. In 2012, 31 (24.0%) of 129 Enterobacterales strains recovered from a hospital in Muscat were CRE [12]. In 2014, 1 (0.2%) of the 645 isolates recovered from Al-Buraimi Hospital was CRE [13]. Moreover, the *bla*OXA-48, *bla*OXA-181, *bla*OXA-232, *bla*NDM-1 and *bla*NDM-5 genes were detected in other studies [14–17]. While molecular methods are considered the gold standard for the detection of CRE, their accessibility is limited, particularly in resource constrained settings. [18] This study aimed to evaluate several phenotypic tests for the detection of carbapenemases circulating in Oman with special focus on *bla*OXA-48 in *K. pneumoniae*, with the goal of identifying a rapid and reliable tool for detection of carbapenemases in general and OXA-48 like in particular.

## 2. Material and Methods

### 2.1. Sample Collection

A collaborative study was initiated between Sultan Qaboos University Hospital (SQUH) and Central of Public Health Laboratories (CPHL), Oman with the purpose of identifying a sensitive, specific and cost effective test for detection of OXA-48, KPC and NDM in *K. pneumoniae* which may be useful in low resource settings as well. One thousand and fifty five clinical isolates of *K. pneumoniae* obtained from SQUH and CPHL were screened for OXA-48 like, KPC and NDM over a six month period. All consecutive, non-duplicate i) meropenem and imipenem carbapenem resistant isolates. ii) isolates which demonstrated intermediate MICs to carbapenems, iii) resistance to piperacillin-tazobactam; iv) susceptible, intermediate, or resistant to third-generation cephalosporins.

### 2.2. Genotypic Confirmation of Carbapenemases

For genotypic confirmation of carbapenemases in CPHL, isolates were subjected to Gene Xpert®Carba-R (Cepheid, Sunnyvale, CA) in SQUH or to PCR (Platinum multiplex PCR master mix, Thermofisher, Lithuania) In brief, at CPHL, the molecular analysis involved testing for the presence of carbapenemase genes: KPC, NDM, OXA-48 like, IMP and VIM. A pure isolate was used to extract the DNA, using QIAAsymphony DSP DNA Mini Kit as per manufacturer's instructions. Multiplex PCR targeted *bla*OXA-48, *bla*NDM, *bla*KPC, *bla*IMP and *bla*VIM. Gel electrophoresis was used for the molecular characterization of the CRE genes.

### 2.3. Whole Genome Sequencing (WGS)

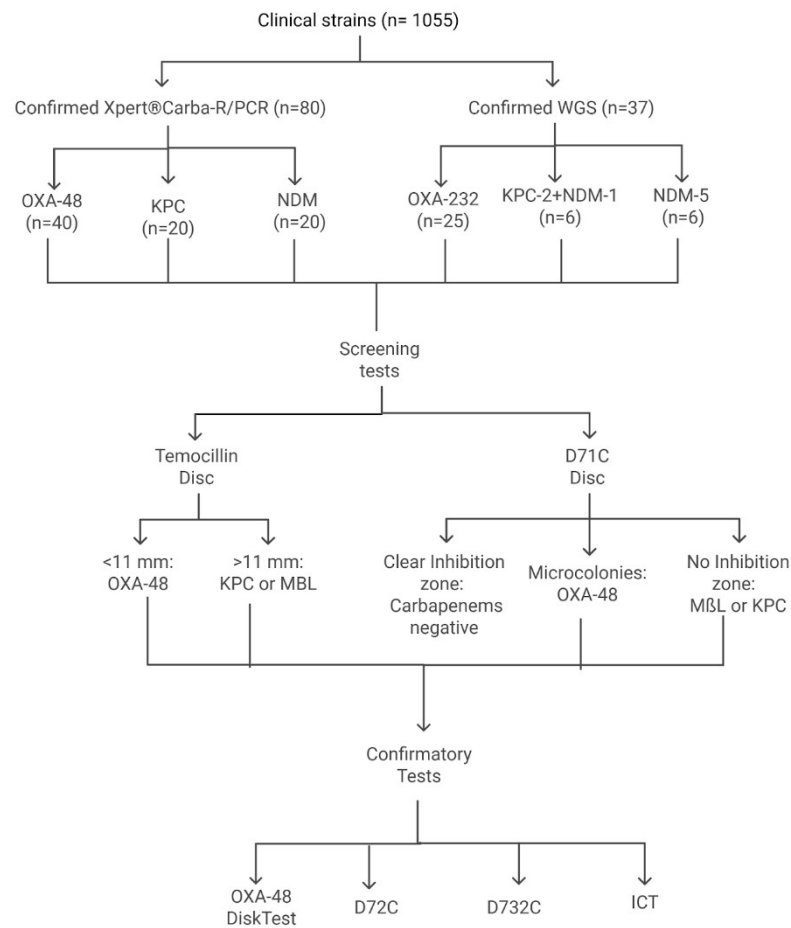
Whole-genome sequencing (WGS) was performed at MicrobesNG using Illumina next-generation sequencing for representative OXA-48, KPC and NDM in *K. pneumoniae* strains (<https://microbesng.co.uk>, Birmingham, United Kingdom). The DNA samples were prepared and sequenced according to the manufacturer's protocol. Post-sequencing, the samples were assembled using the De Novo assembly by the SPAdes program [19] (Nurk et al., 2013). The assembled contigs were annotated with Prokka [20] (Seemann, 2014) and uploaded to the website for further analysis. Detailed resistance gene profiling and bioinformatics analysis were conducted using the Center for Genomic Epidemiology (CGE) website (<https://www.genomicpidemiology.org/>) [21,22]. This included identifying multi-locus sequence typing (MLST) with the MLST tool [23]. (Larsen et al., 2012), plasmids with the PlasmidFinder tool [24]. (Carattoli et al., 2014), and antibiotic resistance genes with the ResFinder tool [25] (Bortolaia et al., 2020).

From the above mentioned pool, representative isolates from forty OXA-48-like, and twenty each of NDM and KPC carbapenemase producers and additionally 37 whole genome sequenced

isolates (25 OXA-232, 6 KPC-2 plus NDM-1 and 6 NDM-5) were selected for evaluating the tests. OXA-232 harboured CTX-M-15, SHV-11, SHV-28, SHV-67, SHV-75, SHV106,SHV-182, while the KPC-2+NDM-1 carried CTMX-65, SHV-12, SHV-12, SHV-182 and SHV-182 and NDM-5 carried CTX-M-65, TEM-1B, TEM-1C, SHV-11, SHV-12, SHV-67, SHV-75.

2.4. Phenotypic Confirmation of Carbapenemases

First, all isolates were screened by one in-house test (temocillin disc test) and one commercial test (D71C). Subsequently, they were subjected to four confirmatory phenotypic tests to detect the presence of carbapenemases. The D72C MASTDISCS® Combi (MAST Group Ltd., UK) assay detected the presence of carbapenemases, as well as extended-spectrum beta-lactamases (ESBL) and AmpC enzymes, while the OXA-48 disc test, D73C MASTDISCS® Combi (MAST Group Ltd., UK), and the immunochromatographic KPC/IMP/NDM/VIM/OXA-48 Combo test kit (Medomics, China) were used to determine the specific carbapenemase type. **Figure 1** shows the algorithm followed. Xpert®Carba-R and WGS were used as the gold standard methods.



**Figure 1.** Algorithm for screening and confirmation of carbapenemases.

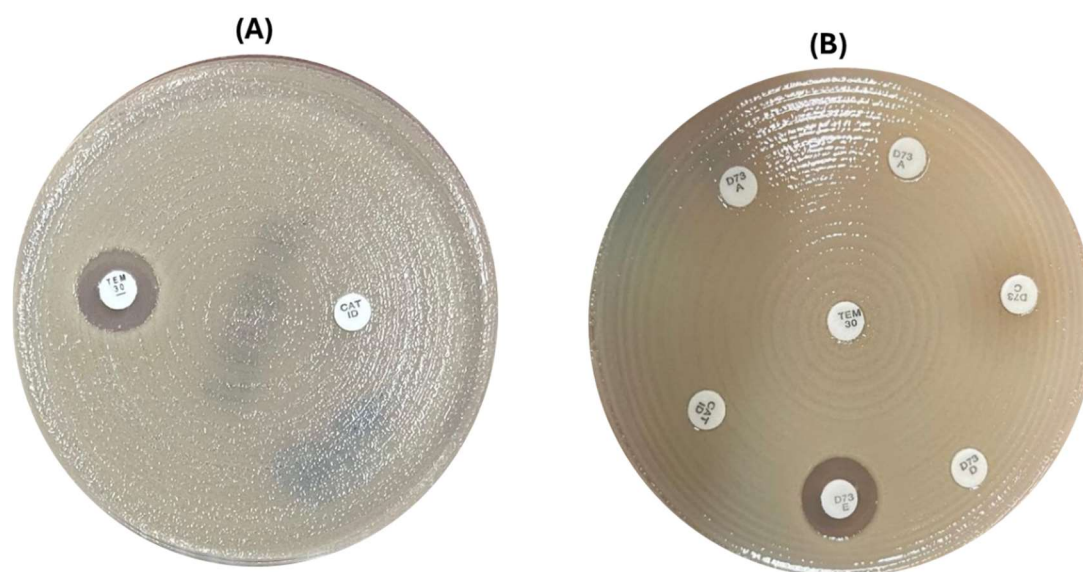
2.5. Screening Test:

Temocillin and D71C Disc Test

A 30 µg temocillin disc (MAST Group Ltd., UK) and faropenem disc from D71C (MAST Group Ltd., UK). were placed on a Mueller-Hinton plate inoculated with a fresh bacterial suspension equivalent to a 0.5 McFarland standard. Plates were incubated for 16 to 20 h under aerobic conditions at 35°C. An inhibition zone diameter of 11 mm for temocillin was considered positive for OXA-48 [26,27]. The CAT-ID (D71C) test was interpreted according to the manufacturer’s recommendation



as follows: a clearly defined zone of inhibition was indicative of no carbapenemase activity; no zone of inhibition was indicative of MBL or KPC and single colonies in the zone of inhibition/double zone of inhibition was considered OXA-48 positive [28]. (Figure 2).

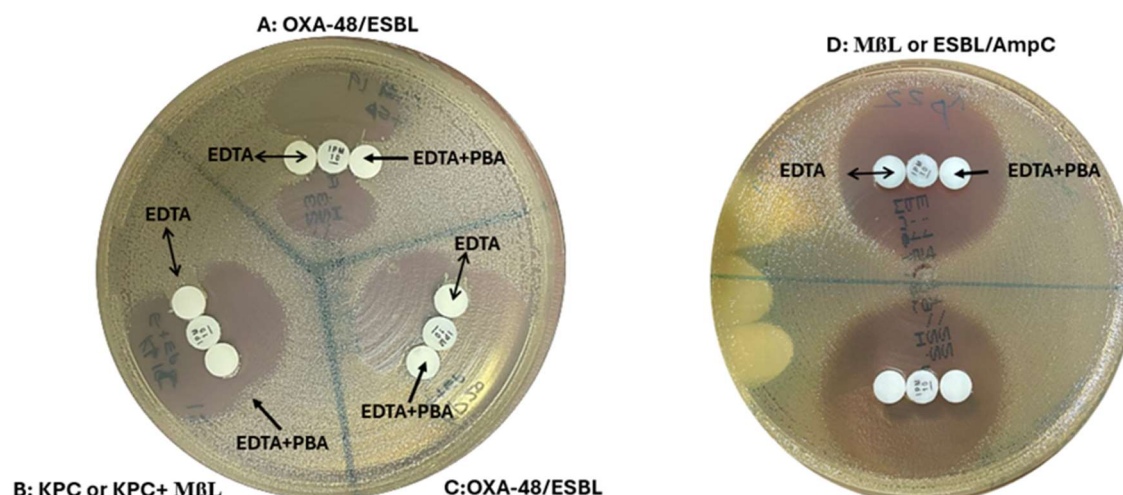


**Figure 2. Phenotypic tests used for detection of carbapenemase:** (A) The screening tests (*Temocillin* and *D71C*): where temocillin inhibition zone was > 11 mm with no zone of inhibition around *D71C* (it was an indicative of MBL or KPC). (B) Screening plus confirmatory tests for NDM detection: Temocillin < 11 mm with no zone of inhibition around *D71C* and the confirmatory *D73C* was positive for NDM.

## 2.6. Confirmatory Tests:

### OXA-48 Disk Test

OXA-48 disk test is based on utilization of EDTA to permeabilize bacterial cells, releasing  $\beta$ -lactamases into the environment. EDTA also inhibits MBL carbapenemases, while PBA inhibits KPCs. A 0.1 M EDTA stock solution was prepared by dissolving anhydrous EDTA in distilled water [29]. From this, 10  $\mu$ l (292  $\mu$ g of EDTA) was dispensed onto two blank paper disks. A PBA stock solution was made by dissolving PBA in dimethyl sulfoxide and water at 60 mg/ml [30]. From this, 10  $\mu$ l (600  $\mu$ g of PBA) was added to the EDTA disks. The disks were dried and used within 60 minutes. A Mueller-Hinton agar plate was inoculated with carbapenem-susceptible *E. coli* ATCC 25922 at 0.5 McFarland standard turbidity. A 10- $\mu$ g imipenem disk was placed on the agar surface. Four to six colonies of the test microorganism were applied to the EDTA and EDTA plus PBA disks, which were then placed inoculum-side down on the lawn adjacent to the imipenem disk. The plate was incubated overnight at 35°C in ambient air. After 18 hours, the plates were examined for an indentation or flattening of the inhibition zone. Indentation towards both EDTA and EDTA/PBA disks indicated OXA-48 carbapenemase production. Indentation towards the EDTA disk but not the EDTA/PBA disks suggested KPC or KPC plus MBL production. No growth towards both disks indicated MBL carbapenemase production or carbapenem non-susceptibility due to ESBL/AmpC production plus porin loss [31] (Figure 3).



**Figure 3. OXA-48 Disk test:** (A,C) Indentation towards both EDTA and EDTA/PBA disks indicated OXA-48 carbapenemase production. (B) Indentation towards the EDTA disk but not the EDTA/PBA disks suggested KPC or KPC plus MBL production. (D) No growth towards both disks indicated MBL carbapenemase production or carbapenem non-susceptibility due to ESBL/AmpC production plus porin loss.

#### MASTDISCS® Combi (D72C): AmpC, ESBL & Carbapenemase Detection Set

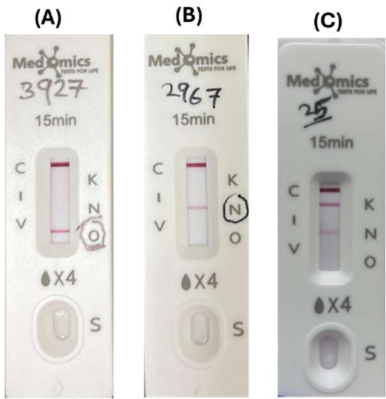
The AmpC, ESBL & Carbapenemase Detection set D72C (Mast Group Ltd., UK), is designed to detect ESBL and or AmpC (both chromosomal and plasmid-mediated), and the co-production of ESBL and AmpC enzymes, as well as carbapenem resistance status in Enterobacteriaceae. This set includes six cartridges: cartridge A (cefpodoxime 10 µg), cartridge B (cefpodoxime 10 µg + ESBL inhibitor), cartridge C (cefpodoxime 10 µg + AmpC inhibitor), cartridge D (cefpodoxime 10 µg + ESBL inhibitor + AmpC inhibitor), cartridge E (cefpodoxime 10 µg + ESBL inhibitor + AmpC inducer), cartridge F (penem antibiotic). The procedure was executed, and the results were analyzed in accordance with the manufacturer's instructions [32,33].

#### MASTDISCS® Combi Carba Plus (D73C)

The Carba plus (D73C) (Mast Group Ltd., UK), is designed to detect carbapenemase and OXA-48 enzyme production in Enterobacterales. This set includes five cartridges: cartridge A (penem), cartridge B (penem+ MBL inhibitor), cartridge C (penem+ KPC inhibitor), cartridge D (penem+ AmpC inhibitor), cartridge E (Temocillin + MBL inhibitor). The procedure was executed, and the results were analyzed in accordance with the manufacturer's instructions.

#### Immunochromatographic Assay (ICT)

The KPC/IMP/NDM/VIM/OXA-48 Combo test kit (Medomics, Nanjing, China) is a lateral flow colloidal gold immunochromatographic assay (LFIA) designed to specifically detect the five most common carbapenemases (KPC, IMP, NDM, VIM, and OXA-48) using a double antibody sandwich method [34–36]. The procedure was carried out and the results were interpreted according to the manufacturer's guidelines (Figure 4).



**Figure 4.** Immunochromatographic assay (ICT) where: (A) O line indicated positive result for OXA-48 carbapenemase; (B) N line indicated positive result for NDM carbapenemase; (C) K line and N line indicated positive results for both KPC+NDM.

2.7. Statistical Analysis

Data analysis was carried out by IBM SPSS Statistics for Windows Version 25.0. (IBM Corp, 2017). Chi-square test was used to compare categorical data between two groups. Cross-tabulation was used taking WGS, Xpert®Carba-R and ICT as a gold standard to determine the sensitivity and specificity. Positive predictive value (PPV) and negative predictive value (NPV) were calculated.

3. Results

The screening and confirmatory tests were evaluated against Xpert®Carba-R/PCR and WGS confirmed carbapenemase (OXA-48 like, KPC and NDM) producing strains.

3.1. Screening Tests

D71C identified all except one isolate as a carbapenemase-producer. Temocillin disc test identified OXA-48 in all 40 (100%) OXA-48 positive strains. KPC and NDM were identified in 18/20 (90%) each. (Table 1) All OXA-232 were also identified as OXA-48 while 4/6 isolates co-harbours KPC-2 and NDM-1 were identified as KPC. Only 1 out of 6 NDM-5 isolates were identified as NDM (Table 2). OXA-48 like producers demonstrated complete temocillin resistance. (inhibition zone: 6 mm), far below the cutoff of 11 mm. It was observed that the KPCs and NDMs too demonstrated inhibition zones below the cut off values but near 11mm [37].

**Table 1.** Assessment of phenotypic tests for detection of OXA-48, KPC and NDM in *Klebsiella pneumoniae* using Xpert®Carba-R/PCR as gold standard.

| Carbapenemase | Gold standard      | Screening test       |           | Confirmatory tests |                  |              |            |
|---------------|--------------------|----------------------|-----------|--------------------|------------------|--------------|------------|
|               | Xpert Carba-R/ PCR | Temocillin disc test | D71C      | In-house           | Commercial tests |              |            |
|               |                    |                      |           | OXA-48 disk test   | D72C             | D73C (n=32)  | ICT        |
|               |                    |                      |           | N (%)              | N (%)            | N (%)        | N (%)      |
| OXA-48 (n=40) | 40 (100%)          | 40 (100%)            | 39(97.5%) | 38(95%)            | 39(97.5%)        | 19(95%) *    | 40 (97.5%) |
| KPCs (n=20)   | 20 (100%)          | 18 (90%)             | 20 (100%) | 20 (100%)          | 20 (100%)        | 5 (83.3%) ** | 19 (95%)   |
| NDM (n=20)    | 20 (100%)          | 18 (90%)             | 20 (100%) | 20 (100%)          | 20 (100%)        | 6 (100%) *** | 19(95%)    |

\*Total number of OXA-48 tested by D73 C were 20 isolates  
\*\* Total number of KPCs tested by D73C were 6 isolates  
\*\*\* Total number of NDMs tested by D73C were 6 isolates

**Table 2.** Assessment of phenotypic tests for detection of OXA-232, KPC-2+NDM-1 and NDM-5 in *Klebsiella pneumoniae* using WGS as gold standard.

| Carbapenemase     | Gold standard | Screening test       |          | Confirmatory tests |                  |              |           |                   | Extended spectrum $\beta$ -lactam genes                   |
|-------------------|---------------|----------------------|----------|--------------------|------------------|--------------|-----------|-------------------|-----------------------------------------------------------|
|                   | WGS           | Temocillin disk test | D71C     | In-house           | Commercial tests |              |           |                   |                                                           |
|                   |               |                      |          | OXA-48 disk test   | D72C             | D73C (n=32)  | ICT       | Xpert Carba-R/PCR |                                                           |
|                   | N (%)         | N (%)                | N (%)    | N (%)              | N (%)            | N (%)        | N (%)     | N (%)             |                                                           |
| OXA-232 (n=25)    | 25 (100%)     | 25 (100%)            | 25(100%) | 22(88%)            | 25(100%)         | 19(95%) *    | 25(100%)  | 25(100%)          | CTX-M-15, SHV-11, SHV-28, SHV-67, SHV-75, SHV106, SHV-182 |
| KPC-2+NDM-1 (n=6) | 6 (100%)      | 2 (33.3%)            | 6 (100%) | 5(83.3%)           | 6 (100%)         | 5 (83.3%) ** | 5 (83.3%) | 6 (100%)          | CTMX-65, SHV-12, SHV-12 and SHV-182 and SHV-182           |
| NDM-5 (n=6)       | 6 (100%)      | 1 (20%)              | 6 (100%) | 6 (100%)           | 6 (100%)         | 6 (100%) *** | 6 (100%)  | 6 (100%)          | CTX-M-65, TEM-1B, TEM1C, SHV-11, SHV-12, SHV-67, SHV-75   |

\*Total number of OXA-48 tested by D73 C were 20 isolates

\*\* Total number of KPCs tested by D73C were 6 isolates

\*\*\* Total number of NDMs tested by D73C were 6 isolates

### 3.2. Confirmatory Tests

#### OXA-48 Disk Test

This test correctly identified 38/40 (95%) isolates as OXA-48 like, exhibiting high sensitivity (100% and 92.5%) and specificity (91.67% and 100%) with WGS and Xpert®Carba-R/PCR as gold standards, respectively as seen in **Table 3**. The negative predictive value (NPV) was 100% for WGS and 93.02% for Xpert®Carba-R/PCR. Two false-positive isolates producing OXA-48 tested negative OXA-232 were identified as OXA-48 in 22/25 (88%) cases. It correctly identified all NDMs and KPCs. Five of the six isolates co-harboured NDM-1 and KPC-2 were identified as KPC and all NDM-5s as NDM. Overall, the OXA-48 disk test showed high sensitivity across all groups: OXA-48: 38/40 (95%) and KPC and NDM: 20/20 (100%).

**Table 3.** Sensitivity and specificity of phenotypic tests for detection of OXA-48 like, KPC and NDM using WGS, Xpert®Carba-R and ICT as the gold standards.

| Gold standards            |         | Screening test (%) |        | Confirmatory tests (%) |                  |       |        |                |            |
|---------------------------|---------|--------------------|--------|------------------------|------------------|-------|--------|----------------|------------|
|                           |         | Temocillin         | D71C   | In-house               | Commercial tests |       |        |                |            |
|                           |         |                    |        | OXA-48 disk            | D72C             | D73C  | ICT    | X-pert Carba-R | WGS (n=37) |
| WGS (n=37)                | SN (%)  | 100.00             | 100.00 | 100.00                 | 100.00           | 96.15 | 100.00 | 100.00         | –          |
|                           | SP (%)  | 76.92              | 100.00 | 91.67                  | 100.00           | 91.67 | 92.31  | 100.00         | –          |
|                           | PPV (%) | 89.29              | 100.00 | 95.65                  | 100.00           | 96.15 | 96.15  | 100.00         | –          |
|                           | NPV (%) | 100.00             | 100.00 | 100.00                 | 100.00           | 91.67 | 100.00 | 100            | –          |
| X-pert Carba-R/PCR (n=80) | SN (%)  | 100.00             | 97.50  | 92.50                  | 97.50            | 95.00 | 100.00 | –              | 100.00     |
|                           | SP (%)  | 70.00              | 100.00 | 100.00                 | 100.00           | 91.67 | 95.00  | –              | 100.00     |
|                           | PPV (%) | 76.92              | 100.00 | 100.00                 | 100.00           | 95.00 | 95.24  | –              | 100.00     |
|                           | NPV (%) | 100.00             | 97.56  | 93.02                  | 97.56            | 91.67 | 100.00 | –              | 100        |
| ICT (n=80)                | SN (%)  | 100.00             | 95.12  | 90.24                  | 95.12            | 95.00 | –      | 100.00         | 100.00     |
|                           | SP (%)  | 69.23              | 100.00 | 100.00                 | 100.00           | 91.67 | –      | 95.00          | 92.31      |
|                           | PPV (%) | 77.36              | 100.00 | 100.00                 | 100.00           | 95.00 | –      | 95.24          | 96.15      |
|                           | NPV (%) | 100.00             | 95.12  | 90.70                  | 95.12            | 91.67 | –      | 100.00         | 100        |

#### D72C Test

The D72C test detected the presence of a carbapenemase in 79/80 (OXA-48 like, KPC and NDM) as well as 25 OXA-232, 6 NDM-5 and 6 isolates co-carrying NDM-1 and KPC-2, missing only one OXA-48 like *K. pneumoniae*. It had high sensitivity (100% with WGS as gold standard and 97.50% with Xpert®Carba-R/PCR), Specificity and PPV was 100% against both and NPV (100% and 97.5%).

#### D73C Test

D73C showed slightly higher sensitivity (95%) compared to the OXA-48 disk test (92.5% with Xpert®Carba-R/PCR but lower (96%) with WGS. In comparison to the OXA-48 disk test D73C had



lower specificity (91.67%) across both gold standards. PPV was 96.15%, 95% and NPV was 91.67% for both gold standards. Similar to the OXA-48 disk test, this test missed one OXA-232 and one isolate which co-harboured NDM-1 and KPC-2.

#### ICT Test

ICT detected all OXA-48 like isolates but missed one KPC and NDM each. It demonstrated 100% sensitivity and NPV against both gold standards and 92%, 95% specificity with 96.15%, 95.24% PPV. Among the sequenced strains, it missed one isolate which co-harboured NDM-1 and KPC-2 but identified all OXA-232 and NDM-5.

## 4. Discussion

Identifying the precise carbapenemase enzyme is essential for optimising patient care and promoting appropriate antimicrobial stewardship efforts. In many parts of the world and particularly the Middle East, OXA-48 like carbapenemase predominates followed by NDM and KPC [9]. Amongst these, phenotypic detection of OXA-48 like carbapenemase is particularly challenging. While Xpert®Carba-R/PCR and WGS are preferred tools for detecting them, they are expensive and not readily available in most diagnostic laboratories, particularly in the low- and medium-income countries (LMICs). In this study, we tested an array of commercial and in-house screening and confirmatory tests and assessed their utility in detecting different carbapenemases with a particular focus on OXA-48 like genes.

Temocillin disc test proved to be a good, simple and cheap screening test for carbapenemases, with a good discriminatory index in detecting OXA-48. It exhibited 100% sensitivity for OXA-48 detection, and 90% for KPCs and NDMs [28]. On the other hand D71C achieved 100% sensitivity for the latter two and 97.5% for OXA-48 like carbapenemases [37].

This study reveals that the OXA-48 disk test is a reliable and cost effective method for detecting carbapenemases in general and OXA-48 in particular. With high sensitivity (100%, 92.5%) and specificity (91.67%, 100%) with WGS and Xpert®Carba-R/PCR as gold standard, OXA-48 disk test is a valuable tool for detecting OXA-48, KPC and NDM carbapenemases, in clinical laboratories especially in resource constrained settings. Tsakris et al. reported a 96.3% sensitivity and 97.7% specificity [31]. However, Koroska et al. reported a lower sensitivity of 53.6% which improved to 98.8% when a high inoculum was used [37]. In this study too, a higher inoculum (4-6 colonies) yielded better results. The OXA-48 disk test is a cheap test, but needs to be validated in individual laboratories which is time consuming and correct interpretation requires experience. Poor detection rate of co-carriage of dual carbapenemases is a distinct limitation of this test [37].

While the D72C test is an excellent tool for detecting ESBL, AmpC and carbapenemases with good reproducibility and discrimination, it does not identify the type of carbapenemase which is a disadvantage. For detecting carbapenemases in general, it had high sensitivity (100%, 97.50%) with WGS and GeneXpert gold standards, with 100% specificity. Another study reported a lower sensitivity (90%) for carbapenemase detection [32]. In a comparative evaluation of various phenotypic tests, the D72C test achieved an accuracy of 95.4% for detecting AmpC and carbapenemase production.[33].

The D73C test supersedes D71C entirely and if used in conjunction with D72C which detects ESBL and AmpC as well, it could promote antimicrobial stewardship considerably. Although this combination does increase the cost, the benefit of identifying ESBL and AmpC and instituting appropriate antimicrobials may outweigh the incurred cost. However, ESBL and AmpC can be detected by cheap and easy in-house tests as well. Al Mamari et al. demonstrated that D73C was slightly inferior to the OXA-48-disc test with 96% sensitivity and 100% specificity with WGS as gold standard [33]. However, it has an advantage over D71C and D72C that it identifies the carbapenemase types. All (100%) OXA-48 and OXA-232 were identified as OXA-48 like. It demonstrated high sensitivity (97.5% for OXA-48, 100% for NDM) and 91.67% specificity, making it highly accurate. The negative predictive values were comparable, although lower in D73C (91.67%), indicating that while

both tests are effective, the OXA-48-disc test may offer more reliable results overall, once appropriately standardised.

ICT with 100% sensitivity and 100% specificity, surpassed both the in-house OXA-48-disc test and the D73C in performance, making it the gold standard among phenotypic tests. Compared to Xpert®Carba-R, ICT is superior in specificity but slightly weaker in sensitivity. With other commercial phenotypic tests being time-consuming and expensive ICT provides rapid results within 20 minutes at a moderate cost. Molecular tests with comparable sensitivity and specificity are expensive and not widely available in most parts of the world [7]. Of course, for maximal detection accuracy, WGS remains the ideal gold standard although Xpert®Carba-R and ICT have excellent sensitivity, specificity, reproducibility and discrimination [37].

## 5. Conclusions

Temocillin offers a degree of discrimination of carbapenemase type, especially for OXA-48 and we recommend that it may be incorporated in the laboratory as a screening tool. Excellent sensitivity and specificity, rapid results, ease of performance, and relatively low cost makes ICT an excellent tool for detection of carbapenemases in diagnostic laboratories. This test has the potential to be a game changer in optimising patient care and promoting antimicrobial stewardship at scale. OXA-48 disc test is an excellent tool too and will serve well in resource constrained centres. We believe that in-house screening and confirmatory tests are both equally sensitive and specific as the commercial tests. Hence depending on the resources, laboratories may exercise their discretion and adopt appropriate tests to identify the carbapenemase types and promote antimicrobial stewardship and optimum patient management.

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