**Abstract:** Rapid and accurate detection can help optimize patient treatment and improve infection control against nosocomial carbapenemase-producing organisms (CPO). In this study, a total of 217 routine clinical isolates (Enterobacterales and A. baumannii), including 178 CPOs and 39 non-CPOs, were tested to evaluate the performance of six phenotypic carbapenemase detection and classification assays, i.e., BD Phoenix CPO detect panel, Rapidec Carba-NP, O.K.N detection kit, and three carbapenem inactivation methods (CIMs; mCIM, eCIM, sCIM). The overall detection sensitivity and specificity were 98.78% (95.21–99.79%) and 79.49% (63.06–90.13%), respectively, for the BD phoenix CPO P/N test; 91.93% (86.30–95.45%) and 100% (88.83–100%), respectively, for the Rapidec Carba-NP; 98.06% (94.00–99.50%) and 97.44% (84.92–99.87%), respectively, for mCIM; and 96.89% (92.52–98.85%) and 94.87% (81.37–99.11%), respectively, for sCIM. The classification sensitivity and specificity for the BD phoenix CPO Ambler test, the O.K.N detection kit, and the mCIM and eCIM were 56.71% (48.75–64.34%) and 94.87% (81.37–99.11%), 99.28% (95.43–99.96%) and 100% (88.83–100%), and 92.90% (87.35–96.23%) and 97.44% (84.92–99.87%), respectively. All detection assays were reliable in detecting carbapenemase. However, the Rapidec Carba-NP and mCIM were insufficient in detecting OXA-48-like enzymes. The BD phoenix CPO detect panel had a strong ability to detect carbapenemase but failed to classify 48/59 (81.36%) KPC, 8/52 (15.38%) NDM, 8/22 (36.36%) OXA-23-like, and 6/11 (54.55%) dual enzymes. The O.K.N detection kit accurately detected and differentiated KPC, NDM, and OXA-48-like enzymes existing alone or in combination. The results of this study will support reliable laboratory work tools and promote therapeutic and infection control decisions.

**Keywords:** BD CPO detect panel; O.K.N detection kit; Rapidec Carba-NP; mCIM; eCIM; sCIM

### 1. Introduction

Carbapenems are considered as the last resort to defend against severe multidrug-resistant Gram-negative bacterial infections owing to their broad spectrum of activity and stability to most β-lactamases [1]. Gram-negative organisms become carbapenem-resistant mainly through carbapenemase production, cephalosporinase (AmpC), and/or extended-spectrum β-lactamase (ESBL) expression in combination with porin-encoding genes (such as oprD) mutation and/or efflux pump (such as MexAB-OprM, MexXY-OprM, MexCD-OprJ) overexpression, which lead to limited cell membrane permeability [2–5]. Carbapenemase, a primary mechanism of carbapenem resistance in Gram-negative bacteria, is mainly encoded on plasmids and is highly transmissible [6]. Over the past decades, carbapenem-producing organisms (CPOs) have become increasingly prevalent worldwide, and infections caused by carbapenem-resistant organisms, especially CPOs, are associated with limited clinical treatment options, a high mortality rate, and a heavy healthcare burden.

Early screening and identification of carbapenem-producing organisms (CPO), at clinical infections or the carriage state, is efficient to prevent the development of hospital
outbreaks and to instruct clinical treatment and intervention of these severe multidrug-resistant bacteria infections. Phenotypic detection assays, including chromogenic media, the Carba-NP test [7], carbapenem inactivation method (CIM) [8], and matrix-assisted laser desorption ionization–time of flight mass spectrometry [9,10] analysis, are widely used to detect carbapenemases in clinical isolates, but cannot accurately classify the enzymes produced by the isolates. Commercial diagnostic kits based on immunochromatography principle, such as the OXA-48 K-Se T [11], O.K.N K-Se T [12], and CARBA-5 reagents [13], have high sensitivity and specificity. Genetic detection methods are considered to be the gold standard, such as traditional PCR, Xpert Carba-R, reverse transcription quantitative PCR, multiplex real-time PCR, and the microfluidic chip technology, which have high sensitivity and specificity, and can directly identify carbapenemase genes. However, the high cost and the requirement of specialized instruments and skilled staff limited their application for many laboratories worldwide without such resources [14–16]. Moreover, PCR-based methods may have false positives and false negatives, and may miss new variant types of carbapenemase.

To improve carbapenemase detection and classification, this study evaluated the performance of three commercial diagnostic kits—BD Phoenix CPO detect panel, RapidC Carba-NP, and an O.K.N detection kit—and three carbapenem inactivation methods (CIMs)—mCIM, eCIM, and the simplified version sCIM—using clinical isolates collected in China.

2. Results

2.1. General Characteristics of the Tested Strains

A total of 217 clinical isolates were included in this study (A. baumannii, n = 23; E. coli, n = 41; Enterobacter cloacae, n = 20; Klebsiella oxytoca, n = 5; Klebsiella aerogenes, n = 9; K. pneumoniae, n = 102 and 17 other Enterobacterales species). Only one isolate per species of one patient was included. By genetic characterization, there were 178 isolates carry carbapenemase genes (59 class A carbapenemase (KPC, n = 59), 69 class B carbapenemase (NDM, n = 52; IMP, n = 15; VIM, n = 1; SIM, n = 1), 39 class D carbapenemase (OXA-23-like, n = 22; OXA-58-like, n = 1; OXA-48-like, n = 16), and 11 dual carbapenemase (KPC + NDM, n = 4; KPC + IMP, n = 2; NDM + IMP, n = 6)) (Table 1).

| Ambler Class | Carbapenemase | A. baumannii | Citrobacter freundii | E. cloacae | E. coli | Klebsiella aerogenes | Klebsiella oxytoca | K. pneumoniae | Serratia marcescens | Others |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Class A | KPC | 1 | 1 | 3 | 1 | 52 | 11 | 1 | 3 |
| | NDM | 1 | 7 | 25 | 2 | 2 | 1 | 3 |
| Class B | IMP | 5 | 5 | 2 | 2 | 1 | 1 | 1 |
| | VIM | 3 | | | | | | |
| | SIM | 1 | | | | | | |
| Class D | OXA-23 | 22 | | | | | | |
| | OXA-58 | 1 | | | | | | |
| Dual enzymes | OXA-48-like | 16 | | | | | | |
| | KPC + NDM | 1 | | | | | | |
| | KPC + IMP | 2 | | | | | | |
| | NDM + IMP | | | | | | | |
| non-CPO | 2 | 2 | | | | | | |
| Total | 23 | 9 | 20 | 41 | 9 | 5 | 102 | 3 | 5 |

KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-β-lactamase; IMP, imipenemase; VIM, Verona integron-encoded metallo-β-lactamase; SIM, SIM-type metallo-β-lactamases; OXA, oxacillinase.

2.2. Overall Performance

The sensitivity and specificity of the various assays for carbapenemase detection and classification are shown in Table 2. The BD phoenix CPO detect P/N test, mCIM, and sCIM had sensitivity of over 96% in detecting the carbapenemase production. The RapidC Carba-NP had high specificity of 100%, but the sensitivity was 91.93% due to its low efficiency in detecting OXA-48-like carbapenemase. The O.K.N detection kit had high sensitivity and
specificity of over 99% and 100% in detecting and classifying the carbapenemase within its detection range.

**Table 2.** Overall sensitivity and specificity of phenotype diagnostic assays.

| Diagnostic Assays | Sensitivity | Specificity |
|-------------------|-------------|-------------|
|                   | %           | 95% CI      | %           | 95% CI      |
| BD Phoenix CPO detect panel |             |             |             |             |
| P/N test          | 98.78       | 95.21–99.79 | 79.49       | 63.06–90.13 |
| Ambler test       | 56.71       | 48.75–64.34 | 94.87       | 81.37–99.11 |
| Rapidec Carba-NP  | 91.93       | 86.30–95.45 | 100         | 88.83–100   |
| mCIM              | 98.06       | 94.00–99.50 | 97.44       | 84.92–99.87 |
| sCIM              | 96.89       | 92.52–98.85 | 94.87       | 81.37–99.11 |
| CPO classification tests |             |             |             |             |
| O.K.N Detection kit | 99.28      | 95.43–99.96 | 100         | 88.83–100   |
| mCIM + eCIM       | 92.90       | 87.35–96.23 | 97.44       | 84.92–99.87 |

2.3. Accuracy of Assays in Detecting and Classifying Specific Carbapenemase

A total of 164 CPOs and 39 non-CPOs were tested by the BD Phoenix CPO detect panel. Compared to the molecular testing, the P/N test detected 162/164 (98.78%) CPOs and incorrectly detected 8/39 (20.51%) carbapenemase-negative isolates as carbapenemase producers (specificity, 79.49%). The Ambler test provided a correct Ambler classification of 93/164 (56.71%) CPOs, as well as 63/164 (38.41%) unclassified and 8/164 (4.88%) misclassified. Five of sixty-nine (7.25%) class B carbapenemase producers were misclassified as a class D producer (NDM-carrier, n = 4; IMP-carrier, n = 1) and three OXA-23-carrying *A. baumannii* (3/22, 13.64%) were misclassified as a class B producer. Furthermore, 48/63 (76.19%) KPC, 4/63 (6.35%) NDM producer, and 5/63 (7.94%) *A. baumannii* with OXA-23-like were not classified. Eleven isolates with more than one carbapenemases-encoding gene were included in this study; however, only 5/11 (45.45%) had been classified as producing one type of carbapenemase, and 5/6 (83.33%) of the heterotypic carbapenemase (class A + B producer) cannot be distinguished (Table 3).

**Table 3.** Performance of BD Phoenix CPO detect panel.

| Ambler Class | Carbapenemase | BD Phoenix CPO P/N Test | BD Phoenix CPO Ambler Test |
|--------------|---------------|-------------------------|---------------------------|
|              |               | ClassA                  | ClassB | ClassD | Unclassified |
| ClassA       | KPC (n = 59)  | 58                      | 11     | 48     |
| ClassB       | NDM (n = 52)  | 52                      | 44     | 4      | 4           |
|              | IMP (n = 15)  | 15                      | 14     | 1      |
|              | VIM (n = 1)   | 1                       | 1      |        |
|              | SIM (n = 1)   | 1                       |        |        |
| ClassD       | OXA-23 (n = 22)| 21                     | 3      | 14     | 5           |
|              | OXA-58 (n = 1)| 1                       |        | 1      |
|              | OXA-48-like (n = 2) | 2 | 2 | | |
| Dual enzymes | Class A + B   | KPC + NDM (n = 4)       | 4      | 4      |
|              | KPC + IMP (n = 2)| 2                     | 1      | 1      |
| Class A + B  | NDM + IMP (n = 5)| 5                     | 4      | 1      |
| non-CPO      | (n = 39)      | 8                       | 1      | 2      | 5           |

The performance of the Rapidec Carba-NP test, the O.K.N detection kit, and three carbapenem inactivation methods (mCIM, sCIM, mCIM, and eCIM) was evaluated on 161 and 39 routine strains with and without CPO suspicion. The carbapenemase detection sensitivity and specificity were 91.93% and 100%, 98.06% and 97.44%, and 96.86% and 94.87% for the Rapidec Carba-NP test, mCIM, and sCIM, respectively. The Rapidec Carba-
NP had detected all 128 class A and class B enzymes but failed to identify 11/16 (68.75%) OXA-48-like enzyme and 2 strains with the KPC + IMP enzyme. The mCIM seemed insufficient in detecting OXA-48-like enzymes, and the sCIM seemed insufficient in detecting Acinetobacter baumannii producing OXA-23-like enzyme. The O.K.N detection kit could detect and classify almost all strains producing carbapenemase within its test range for its high sensitivity (99.28%) and specificity (100%). The mCIM and eCIM, with classification sensitivity and specificity of 92.90% and 97.44%, respectively, failed to classify 4/59 (6.78%) KPC enzymes, 3/15 (20.00%) IMP enzymes, 4/16 (25.00%) OXA-48-like enzymes, and could only report serine carbapenemase among 6 class A + B enzyme producers (Table 4).

Table 4. Performance of other carbapenemase detection and classification diagnostic assays.

| Ambler Class | Carbapenemase                        | CPO Detection Tests | CPO Classification Tests |
|--------------|--------------------------------------|---------------------|--------------------------|
|              |                                      | Rapidec Carba-NP | mCIM | sCIM | O.K.N Detection Kit | mCIM + eCIM |
| Class A      | KPC (n = 59)                          | 59                 | 59  | 58  | 59                 | 55          |
| Class B      | NDM (n = 52)                          | 52                 | 52  | 52  | 51                 | 52          |
|              | IMP (n = 15)                          | 15                 | 15  | 15  | 0                  | 12          |
|              | VIM (n = 1)                           | 1                  | 1   | 1   | 0                  | 1           |
|              | SIM (n = 1)                           | 1                  | 1   | 1   | 0                  | 1           |
| Class D      | OXA-23 (n = 5)                        | 5                  | /   | 1   | 0                  | /           |
|              | OXA-58 (n = 1)                        | 1                  | /   | 1   | 0                  | /           |
|              | OXA-48-like (n = 16)                  | 5                  | 13  | 16  | 16                 | 12          |
| Dual enzymes | Class A + B                           | KPC + NDM (n = 4)  | 4   | 4   | 4                  | 4           |
|              | KPC + IMP (n = 2)                     | 0                  | 2   | 2   | 2                  | 2           |
| Class B + B  | NDM + IMP (n = 5)                     | 5                  | 5   | 5   | 5                  | 5           |
|              | non-CPO (n = 39)                      | 0                  | 1   | 2   | 0                  | 1           |

3. Discussion

Rapid and accurate detection and classification of CPOs is critical in avoiding treatment failures and guiding clinical antibiotics therapy and infection control decisions, including the use of expensive antibiotics and isolation measures. However, phenotypic identification of organisms with multiple carbapenemase types, low-level carbapenemase expression, extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, growth impairment, and/or other potentially uncovered carbapenemase resistance mechanisms can be difficult [17].

The BD Phoenix CPO detect panel offers (semi-)automated antimicrobial susceptibility testing (AST) systems and phenotypic detection and classification of carbapenemase [18]. The high sensitivity of the BD Phoenix CPO detect panel P/N test observed in this study supports the direct reporting of CPO-negative strains without additional tests, and directly provides the true carbapenem MICs that can be used as a screening value for carbapenemase. However, the limitation was classification performance, especially in typing class A β-lactamase, with sensitivity of only 18.64%, which corresponds with the results of Simon et al. (5/13, 38.46%) [19] and Ong et al. (13/30, 43.33%) [20], but was much lower than that reported by Thomson et al. (91/110, 82.73%) [21]. Eleven strains producing dual carbapenemases were determined as positive by the P/N test but without providing an Ambler classification or with attributing only one of the involved carbapenemases by the Ambler test. It is also noteworthy that 8 of 39 (20.51%) non-CPOs were false positive, which indicated downstream tests, such as a colorimetric assay and/or CIM tests, were needed to confirm the positive result detect by the BD Phoenix CPO detect P/N test. The performance of the BD Phoenix CPO detect P/N test could be challenged by the non-CPOs phenotypes, with decreased efficiency and specificity in detecting strains combining ESBLs
and/or AmpCs with/without porin loss [21–23]. Among the eight false positive strains, 2/8 were AmpC producers, 3/8 were ESBLs producers, and 2/8 harbored both AmpC beta-lactamases and ESBLs.

Interpretation the result of the Rapidec Carba-NP can be challenging for an inexperienced operator, and inconclusive results can occur. Moreover, the detection of carbapenemases in mucoid isolates and the detection of OXA-48-like carbapenemase could be challenging, which was consistent with several previously published studies [24–26].

The O.K.N detection kit, a method with no special equipment and professionals required, interpretate easily and clearly, and could detect KPC, NDM, and OXA-48-like enzymes and their combination efficiently and rapidly. However, IMP (IMP-4, IMP-26), which is referred to as one of “the big five” and had prevalence in China [27], could not be identified. The recently launched Carba5 system [13], the RESIST-4 O.K.N.V assay [28], and the RESIST-5 O.K.N.V.I. assay [29] could perform a multiple lateral flow immunoassay on NDM, KPC, IMP, VIM, and OXA-48-like enzymes, and can detect multiple enzymes produced by strains.

The mCIM and eCIM, recommended by CLSI guidelines for their high sensitivity and specificity in the detection of KPC, NDM, VIM, IMP, IMI, SPM, SME, and OXA enzymes carried by Enterobacterales bacteria [30], could tell serine β-lactamase (SBL) and metallo-β-lactamase (MBL), which will affect the clinical therapy decisions, such as the use of ceftazidime-avibactam [28]. However, it seemed insufficient when classified IMP and OXA-48-like enzyme. The sCIM test was an easy, rapid, efficient, and cheap method, but the results might be influenced by the amount of bacteria on the imipenem disk, since the result was negative with a small bacterial sample, but could become “uncertain” with more abundant bacteria in the tested sample.

4. Methods

4.1. Bacterial Strains

A total of 217 routine clinical isolates was tested to evaluate the performance of six phenotypic carbapenemase detection and classification assays, i.e., BD Phoenix CPO detect panel, Rapidec Carba-NP, an O.K.N detection kit, and three carbapenem inactivation methods (CIMs) (mCIM, eCIM, and sCIM). These isolates include 178 CPOs and 39 non-CPOs, the carbapenemase activity of which was previously tested by the mCIM and eCIM test according to CLSI guidelines [30], and then genetically confirmed by traditional PCR and sequencing. The primers used to amplify specific carbapenenmase genes are shown in Table 5. Strains collected from 66 general hospitals in 23 provinces of China from 2011 to 2018 were stored at −70 °C and incubated overnight on Columbia agar plate with 5% sheep blood at 35 ± 1 °C. To ensure the purity of strains, sub-incubation was carried out. Identification was conducted by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany) using α-cyano-4-hydroxycinnamic acid matrix and standard protocol. Species identification was determined by scores > 2.

| Primer Name | Targeting Gene | Nucleotide Sequence | Product Size (bp) |
|-------------|----------------|---------------------|-------------------|
| KPC-F | bla\_KPC | 5’-TGTCACTGTATCGGCCGTC-3’ | 1010 |
| KPC-R | 5’-CTGAGCTGCTCAGAGAAAAAC-3’ | | |
| NDM-F | bla\_NDM | 5’-ATGGAATGCCCATAATTTAGC-3’ | 813 |
| NDM-R | 5’-TCGGCGAGCTTTGCAGGC-3’ | | |
| IMP-F | bla\_IMP | 5’-TGAGCAAGATTATCTGATTC-3’ | 740 |
| IMP-R | 5’-TTAGTTGCTTTTGTTTATG-3’ | | |
| VIM-F | bla\_VIM | 5’-TATGGAAGCAGCAACCGATGT-3’ | 920 |
| VIM-R | 5’-CAGGATGTCCGCTCCACAG-3’ | | |
| SIM-F | bla\_SIM | 5’-TACAGAGGATCAGGCTATCG-3’ | 571 |
| SIM-R | 5’-TAATGCGCTTCCATGTG-3’ | | |
Table 5. Cont.

| Primer Name          | Targeting Gene | Nucleotide Sequence                  | Product Size (bp) |
|----------------------|----------------|--------------------------------------|-------------------|
| OXA-48-like-F        | blaOXA-48-like | 5′-GCGTGGTAAAGGATGAACAC-3′            | 438               |
| OXA-48-like-R        |               | 5′-CATCAAGTTCAACCCACCG-3′             |                   |
| OXA-23-like-F        | blaOXA-23-like | 5′-GATCGGAATTGAGAACCAGA-3′            | 501               |
| OXA-23-like-R        |               | 5′-ATTITCTGACCCTTTACAT-3′             |                   |
| OXA-58-like-F        | blaOXA-58-like | 5′-AAATATTGGGCCTTGCTGCT-3′            | 353               |
| OXA-58-like-R        |               | 5′-CCCCTCTGCCTCTACATAC-3′             |                   |

4.2. BD Phoenix CPO Detect Panel

The BD Phoenix CPO detect panel (Becton, Dickinson and Company, Sparks, MD, USA) is a growth-based qualitative confirmatory method (ID/NMIC 503 panel, NMIC 502 panel), which could identify species and use drugs such as carbapenems and β-lactams, alone and in combination with various chelators and β-lactamase inhibitors required for CPO detection and classification to detect and confirm A, B, and D carbapenemase [17].

In the CPO detect panel, the ID/NMIC 503 panel aimed to identify species and detect carbapenemase activity (P/N test), and the NMIC 502 panel aimed to identify carbapenemase producers according to the Ambler classification (Ambler test). Not all isolates determined carbapenemase positive by the P/N test could be classified by the Ambler test. The P/N test-negative results with Ambler classification were previously demonstrated to be impossible [21]. Strains of 141 carbapenemase-producing Enterobacterales (CPE), 23 carbapenemase-producing Acinetobacter baumannii (CPAB), and 39 non-CPOs were incubated overnight using the BD M50 system, and results were interpreted by Epi-Centre software.

4.3. Rapidec Carba-NP Test

The Rapidec Carba-NP kit (bioMérieux, La Balme-les-Grottes, France) is a rapid biochemical test based on detecting the hydrolysis of the β-lactam ring of imipenem. The test was performed in accordance with the manufacturer’s recommendations [31]. A full loop (10 µL) of bacterial colonies from a culture plate was mixed into the well c so that the turbidity is similar to well b. Visual observations then occurred after 30 min incubation at 37 °C and, if necessary, after 2 h. A color change from red-to-yellow or red-to-orange was considered positive. The visual assessment and results interpretation were conducted blindly by two technicians who had no information about carbapenemase production of these strains.

4.4. O.K.N Detection Kit

Then, 200 isolates were tested by O.K.N Detection kit (Beijing Gold Mountainriver Tech Development Co., Ltd., Beijing, China). A single colony on blood agar plate was suspended in 10 drops of lysis solution, and then three drops of the diluted sample were added onto the test strip. The results were read within 15 min with naked eyes according to the synopsis.

4.5. Carbapenem Inactivation Methods

The mCIM and eCIM tests were performed following the protocol recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines [30]. In the sCIM test [32], one side of the imipenem disk was smeared with the tested strain instead of soaked in tryptic soy broth (TSB) containing the strain for 4 h, and then placed on the MHA plate inoculated with meropenem-susceptible Escherichia coli ATCC 25922.

4.6. Quality Control

K. pneumoniae BAA-1705 (producing KPC), E. coli ATCC-2452 (producing NDM), and E. coli BAA-2523 (producing OXA-48) were used as positive quality control strains, whereas
K. pneumoniae ATCC 700603 was used as a negative quality control in evaluation of the BD Phoenix CPO detection panel. In the CIM tests, E. coli ATCC 25922 and K. pneumoniae BAA 1705 were used as negative and positive quality control strains, respectively.

4.7. Statistical Analysis

The sensitivity and specificity of each method were analyzed along with their 95% confidence intervals, and compared with the results from reference standard molecular characterization using the free software VassarStats (http://vassarstats.net; 28 September 2021).

5. Conclusions

Taken together, the results of this study suggest that the BD Phoenix CPO detect P/N test, mCIM and scIM were reliable for CPOs screening and detecting. However, due to the relatively low detection specificity and low classification sensitivity, additional tests and further improvements are required to confirm positive detection by the BD Phoenix CPO detect panel P/N test and to improve the performance of the Ambler test. Moreover, when the result of Rapidec Carba-NP was interpreted negative, additional tests such as mCIM and scIM were needed to exclude the OXA-48-like enzyme. The O.K.N detection kit with high classification efficiency indicates a rapid, easy and reliable tool to classify KPC, NDM, and OXA-48-like enzymes.

Author Contributions: H.W. and X.W. designed the study. The experimental part was completed by A.Z., X.L. and J.Z. and guided by X.W. and Q.W. A.Z. processed the data, wrote and revised the manuscript, C.Z. modified the manuscript, and H.W. checked the manuscript and put forward suggestions for revision. All authors have read and agreed to the published version of the manuscript.

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References

1. Mao, W.; Xia, L.; Xie, H. Detection of Carbapenemase-Producing Organisms with a Carbapenem-Based Fluorogenic Probe. Angew. Chem. Int. Ed. Engl. 2017, 56, 4468–4472. [CrossRef]
2. Madec, J.Y.; Haenni, M.; Nordmann, P.; Poirel, L. Extended-spectrum β-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: A threat for humans? Clin. Microbiol. Infect. 2017, 23, 826–833. [CrossRef]
3. Horna, G.; López, M.; Guerra, H.; Saénz, Y.; Ruiz, J. Interplay between MexAB-OprM and MexEF-OprN in clinical isolates of Pseudomonas aeruginosa. Sci. Rep. 2018, 8, 16463. [CrossRef] [PubMed]
4. Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol. Mol. Biol. Rev. 2003, 67, 593–656. [CrossRef] [PubMed]
5. Nordmann, P.; Poirel, L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. Clin. Infect. Dis. 2019, 69 (Suppl. 7), S521–S528. [CrossRef] [PubMed]
6. Bonomo, R.A.; Burd, E.M.; Conly, J.; Limbago, B.M.; Poirel, L.; Segre, J.A.; Westblade, L.F. Carbapenemase-Producing Organisms: A Global Scourge. Clin. Infect. Dis. 2018, 66, 1290–1297. [CrossRef]
7. Mathers, A.J.; Carroll, J.; Sifri, C.D.; Hazen, K.C. Modified Hodge test versus indirect carbapenemase test: Prospective evaluation of a phenotypic assay for detection of Klebsiella pneumoniae carbapenemase (KPC) in Enterobacteriaceae. *J. Clin. Microbiol.* 2013, 51, 1291–1293. [CrossRef]

8. McMullen, A.R.; Yarbrough, M.L.; Wallace, M.A.; Shupe, A.; Burnham, C.-A.D. Evaluation of Genotypic and Phenotypic Methods to Detect Carbapenemase Production in Gram-Negative Bacilli. *Clin. Chem.* 2017, 63, 723–730. [CrossRef]

9. Choquet, M.; Guiheneuf, R.; Castelain, S.; Cattori, V.; Auzou, M.; Pluquet, E.; Decroix, V. Comparison of MALDI-ToF MS with the Rapidcide Carba NP test for the detection of carbapenemase-producing Enterobacteriaceae. *Eur. J. Clin. Microbiol. Infect. Dis.* 2018, 37, 149–155. [CrossRef]

10. Prajita, D.; Tamma, P.J.S. Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. *J. Clin. Microbiol.* 2018, 56, e01140–18.

11. Dortet, L.; Jousset, A.; Sainte-Rose, V.; Cuzon, G.; Naas, T. Prospective evaluation of the OXA-48 K-SetT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. *J. Antimicrob. Chemother.* 2016, 71, 1834–1840. [CrossRef]

12. Glupczynski, Y.; Jousset, A.; Evrard, S.; Bonnin, R.A.; Huang, T.-D.; Dortet, L.; Bogaerts, P.; Naas, T. Prospective evaluation of the OKN K-SeT assay, a new multiplex immunochromatographic test for the rapid detection of OXA-48-like, KPC and NDM carbapenemases. *J. Antimicrob. Chemother.* 2017, 72, 1955–1960. [CrossRef]

13. Boutal, H.; Vogel, A.; Bernabeu, S.; Devilliers, K.; Creton, E.; Cotellon, G.; Plaisance, M.; Oueslati, S.; Dortet, L.; Jousset, A.; et al. A multiplex lateral flow immunoa assay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. *J. Antimicrob. Chemother.* 2018, 73, 909–915. [CrossRef]

14. Baeza, L.L.; Pfennigwerth, N.; Greissl, C.; Götting, S.; Saleh, A.; Stelzer, Y.; Gatermann, S.G.; Hamprecht, A. Comparison of five methods for detection of carbapenemases in Enterobacteriales with proposal of a new algorithm. *Clin. Microbiol. Infect.* 2019, 25, 1286.e9–1286.e15. [CrossRef]

15. Cai, Y.; Chen, C.; Zhao, M.; Yu, X.; Lan, K.; Liao, K.; Guo, P.; Zhang, W.; Ma, X.; He, Y.; et al. High Prevalence of Metallo-β-Lactamase-Producing Enterobacteriaceae. *Front. Microbiol.* 2019, 10, 1823. [CrossRef] [PubMed]

16. Croxatto, A.; Coste, A.T.; Pillonel, T.; Bertelli, C.; Greub, G.; Prod’hom, G. Evaluation of the BD Phoenix CPO Detect Test for the detection of carbapenemase producers. *Clin. Microbiol. Infect.* 2020, 26, 644.e9–644.e15. [CrossRef] [PubMed]

17. Fasciana, T.; Gentile, B.; Aquilina, M.; Ciammaronci, A.; Mascarella, C.; Anselmo, A.; Fortunato, A.; Fillo, S.; Petralito, G.; Lista, F.; et al. Co-existence of virulence factors and antibiotic resistance in new Klebsiella pneumoniae clones emerging in south of Italy. *BMC Infect. Dis.* 2019, 19, 928. [CrossRef]

18. Simon, M.; Gatermann, S.; Pfeifer, Y.; Reischl, U.; Gessler, A.; Jantsch, J. Evaluation of the automated BD Phoenix CPO Detect panel in combination with the β-CARBA assay for detection and classification of carbapenemase-producing Enterobacteriales. *J. Microbiol. Methods* 2019, 156, 29–33. [CrossRef]

19. Ong, C.H.; Ratnayake, L.; Ang, M.L.T.; Lin, R.T.P.; Chan, D.S.G. Diagnostic Accuracy of BD Phoenix CPO Detect Test for Carbapenemase Production in 190 Enterobacteriaceae Isolates. *J. Clin. Microbiol.* 2018, 56, e01043–18. [CrossRef] [PubMed]

20. Thomson, G.; Turner, D.; Brasso, W.; Kircher, S.; Guillet, T.; Thomson, K. High-Stringency Evaluation of the Automated BD Phoenix CPO Detect and Rapidcide Carba NP Tests for Detection and Classification of Carbapenemases. *J. Clin. Microbiol.* 2017, 55, 3437–3443. [CrossRef]

21. Simon, M.; Koestler, J.; Reischl, U.; Gessler, A.; Jantsch, J. Detection of carbapenemase-producing Enterobacteriales and the BD Phoenix CPO Detect panel. *Expert Rev. Mol. Diagn.* 2019, 19, 659–665. [CrossRef] [PubMed]

22. Doumith, M.; Ellington, M.J.; Livermore, D.M.; Woodford, N. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. clinical isolates from the UK. *J. Antimicrob. Chemother.* 2009, 63, 659–667. [CrossRef] [PubMed]

23. Dortet, L.; Tandé, D.; De Briel, D.; Bernabeu, S.; Lasserre, C.; Gregorowicz, G.; Jousset, A.B.; Naas, T. MALDI-TOF for the rapid detection of carbapenemase-producing Enterobacterales: Comparison of the commercialized MBT STAR®-Carba IVD Kit with two in-house MALDI-TOF techniques and the RAPIDEC® CARBA NP. *J. Antimicrob. Chemother.* 2018, 73, 2352–2359. [CrossRef] [PubMed]

24. Simmer, P.J.; Johnson, J.K.; Brasso, W.B.; Anderson, K.; Lonsway, D.R.; Pierce, V.M.; Bobenchik, A.M.; Lockett, Z.C.; Charnot-Katsikas, A.; Westblade, L.F.; et al. Multicenter Evaluation of the Modified Carbapenem Inactivation Method and the Carba NP Test for Detection of Carbapenemase-Producing Pseudomonas aeruginosa and Acinetobacter baumannii. *J. Antimicrob. Chemother.* 2017, 72, 1834–1840. [CrossRef] [PubMed]

25. Noel, A.; Huang, T.D.; Berhin, C.; Hobeke, M.; Bouchahrouf, W.; Yunus, S.; Bogaerts, P.; Glupczynski, Y. Comparative Evaluation of Four Phenotypic Tests for Detection of Carbapenemase-Producing Gram-Negative Bacteria. *J. Clin. Microbiol.* 2015, 53, 510–518. [CrossRef] [PubMed]

26. Cai, Y.; Chen, C.; Zhao, M.; Yu, X.; Lan, K.; Liao, K.; Guo, P.; Zhang, W.; Ma, X.; He, Y.; et al. High Prevalence of Metallo-β-Lactamase-Producing From Three Tertiary Hospitals in China. *Front. Microbiol.* 2019, 10, 1610. [CrossRef] [PubMed]
28. Pfennigwerth, N.; Gatermann, S.G.; Korber-Irrgang, B.; Honings, R. Phenotypic Detection and Differentiation of Carbapenemase Classes Including OXA-48-Like Enzymes in Enterobacterales and Pseudomonas aeruginosa by a Highly Specialized Micronaut-S Microdilution Assay. *J. Clin. Microbiol.* 2020, 58, e00171-20. [CrossRef] [PubMed]

29. Hong, J.; Kang, D.; Kim, D. Performance Evaluation of the Newly Developed In Vitro Rapid Diagnostic Test for Detecting OXA-48-Like, KPC-, NDM-, VIM- and IMP-Type Carbapenemases: The RESIST-5 O.K.N.V.I. Multiplex Lateral Flow Assay. *Antibiotics* 2021, 10, 460. [CrossRef] [PubMed]

30. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*, 30th ed.; CLSI supplement M100; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2020.

31. Kumudunie, W.G.M.; Wijesooriya, L.I.; Wijayasinghe, Y.S. Comparison of four low-cost carbapenemase detection tests and a proposal of an algorithm for early detection of carbapenemase-producing Enterobacteriaceae in resource-limited settings. *PLoS ONE* 2021, 16, e0245290. [CrossRef] [PubMed]

32. Jing, X.; Zhou, H.; Min, X.; Zhang, X.; Yang, Q.; Du, S.; Li, Y.; Yu, F.; Jia, M.; Zhan, Y.; et al. The Simplified Carbapenem Inactivation Method (sCIM) for Simple and Accurate Detection of Carbapenemase-Producing Gram-Negative Bacilli. *Front Microbiol.* 2018, 9, 2391. [CrossRef] [PubMed]