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Predictive role of culture-based MIC testing vs. genotyping for carbapenem-resistant Enterobacterales in a non-universal screening, highly resourced setting

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ABSTRACT

A lack of evidence of accuracy for various testing modalities for carbapenem-resistant Enterobacterales (CRE) reduces the efficiency of screening and delays the isolation of carriers. This study examined the performance of phenotypic detection of CRE in comparison to molecular testing. A cross-sectional study was conducted in an academic medical institution in Saudi Arabia on CRE-screened patients during a 36-month period (April 1, 2019, through March 31, 2022). Cases were followed up for their susceptibility status by the phenotypic gradient method and genotypes. Of 3,116 samples tested, 359 carbapenemase genes were detected in 297 strains (9.5%) belonging to 292 patients. Oxacilliniase-48 (OXA-48) was the most frequently detected genotype (n=190, 64%), followed by a combined New Delhi metallo-B-lactamase (NDM)/OXA-48 genotype (n=77, 25.9%). Variable missed isolation days were encountered for various genotypes (0-18.5 days), with an excellent clinical utility index obtained for screening the OXA-48 genotype phenotypically. The data provided some insights into the predictive role and shortcomings of the e-test alone in CRE screening. While it provided a reasonable approach in a CRE population dominated by OXA-48 genotypes, it was more likely to miss the NDM-incurred carbapenemase. Thus, local epidemiology in an institution must be considered when designing a local screening protocol in addition to consideration of cost and turnaround time.

Keywords: resistance, CRE, genotype, phenotype, screening, Carba-R, TAT, CUI, NND

INTRODUCTION

The spread of infections caused by carbapenem-resistant Enterobacterales (CRE) is an urgent health issue, raising global concerns. CRE are now widely scattered across the continents and are increasingly implicated in causing hospital- and community-acquired infections, which are challenging to treat as they may exhibit resistance to old and new drugs [1-3]. Whilst CRE are defined by phenotypic testing, the carbapenemase-producing CRE (CP-CRE) is a genotypic finding that signifies the existence of a molecular basis for the carbapenem hydrolyzing enzyme. Carbapenemase genes are encoded on plasmids, which favors the possibility of horizontal spread and escalates the problem [4-7]. Hence, timely laboratory identification is needed to prevent their dissemination through implementing effective isolation measures [8-11].

Although culture-based testing is recommended by the USA Centers for Disease Control and Prevention (CDC) for identifying CRE, this process is lengthy and may take ≥72 hours [12]. A recent meta-analysis focusing on evaluating the performance of molecular assays when applied in direct CRE testing from rectal swabs demonstrated good sensitivity (95%) and specificity (99%), which was associated with high heterogeneity among the studies [13]. The currently existing testing methods for carbapenemases produced by CRE include conventional culture-based approaches, which have high diagnostic yields but with long turnaround times (TATs), and rapid diagnostics that are sensitive and efficient but costly [14-17]. Commercial rapid kits are being increasingly introduced to address this need, which are based on nucleic acid amplification technologies, immunochromatographic tests, or syndromic assays [4]. Culture-based phenotypic methods detect a broader spectrum of enzymes, while molecular testing offers the advantage of revealing silent mutations that are not expressed *in vitro* yet can be a potential cause of therapeutic failures [18, 19]. Hybrid testing of culture- and molecular-based methods to detect all resistance in CRE can be challenging for laboratories with a high prevalence and/or low resources.

Hospitalized patients who are either infected or colonized with CP organisms should be admitted under contact precautions [20-22]. Contact precautions are recommended to be maintained for the entirety of their inpatient stay, and indefinitely during future hospital encounters, due to the extended colonization period and limited therapeutic options [22]. In addition, standard precautions that include hand hygiene and restriction of unnecessary usage of broad spectrum antimicrobial agents are crucial to preventing infection and limiting the spread of these difficult to treat

pathogens. Other effective preventive measures should focus on terminal cleaning and disinfection of patients' facility and invasive devices. Rapid detection tools for CRE can have impacts on the prompt isolation of a colonized or infected case with subsequent prevention of horizontal transmission. Simulation is used in studying infectious diseases to provide screening options and other preventive tools, which can be done retrospectively or prospectively through mathematical modeling or [23, 24]. Most previous studies have assessed the performance of molecular CRE testing in direct patients' swabs on admission versus culture-based testing [25-27]. In this study, culture-based minimal inhibitory concentrations (MIC) were determined and evaluated against genotypic testing for suspected CRE isolates using a commercial multiplex molecular platform. The aim is to address the potentially missed days of isolation in case of adopting a phenotypic modality for CRE screening in a low prevalence setting.

MATERIALS AND METHODS

Research Settings and Bacterial Identification

This is a retrospective, cross-sectional study conducted at a 550-bed academic medical institution in Alkhobar, Eastern Region of Saudi Arabia. All screening samples received from adults and children admitted to the hospital during the period between April 1, 2019, through March 31, 2022, were included. Non-replicate strains of *Escherichia coli* and *Klebsiella pneumoniae,* isolated from various anatomical sites and representing a colonization status (respiratory, rectal, and chronic wounds) were identified by the VITEK® MS (bioMe´rieux Inc., Durham, NC, USA), an automated mass spectrometry microbial identification system based on matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology. The colonies were tested for carbapenemase production phenotypically and genotypically as described. The electronic charts were reviewed to assess the isolation status of each case upon generating a laboratory report and to retrieve their clinical data.

Detection of Carbapenemases

As per the laboratory protocol, the strains were routinely tested for the production of carbapenemases using a molecular assay (Xpert® Carba-R, Cepheid Inc., Sunnyvale, CA, USA), which detects five major genes (IMP, KPC, NDM, VIM, and OXA-48). Additionally, e-test (AB-BIODISK, Sweden)-based MICs for both imipenem and meropenem were measured and interpreted following the manufacturer's instructions and the Clinical and Laboratory Standards Institute guidance (CLSI 2022) [28]. Muller Hinton agar plates (SPML, Riyadh, Saudi Arabia) were incubated overnight at ambient air (35°C) using a 0.5 McFarland inoculum to determine the MIC. The point of intersection of the inhibition ellipse on e-test strip was recorded. Phenotypic susceptibility testing was also routinely performed for all the isolates using the VITEK 2 automated system (bioMe´rieux Inc., Durham, NC, USA). Only strains with available MIC to both imipenem and meropenem were included in the cohort. Quality control strains (*Pseudomonas aeruginosa* ATCC® 27853 and *Escherichia coli* ATCC®a 25922) were run weekly as per the manufacturer's recommendations. The consumables-based cost was calculated for each testing modality.

Discrepant Analysis

The discrepant cases were resolved, as follows: for Carba-R negative but culture-positive cases, the organism was sub-cultured, and PCR was repeated from three-day incubated colonies. For culture-MIC negative but Carba-R positive cases, an additional assay was performed (mCIM test) as previously described [28]. Samples were divided into three groups based on the discrepant analysis results: the concordant group, which showed concordant Carba-R and culture-MIC results; the resolved group, which initially showed a discrepancy between the two assays that was resolved upon supplementary testing, and the discordant group, which remained as unresolved discrepancies.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.3.1 for Mac. To compare the two assays, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Differences in TATs based on the electronic reports, and times to initiate isolation actions were analyzed by the one-way analysis of variance (ANOVA) test, which compares multiple means. The null hypothesis was all population means were equal, and the alternative hypothesis was that at least one mean was different. The Ct values of the concordant, resolved, and discordant groups were analyzed using the Kruskal-Wallis test. The number needed to diagnose (NND) was calculated as NND=1/[sensitivity-(1-specificity)], where the smaller the NND, the more useful the assay. The clinical utility index (CUI) was calculated as previously described [29]. The missed isolation days were estimated based on the historical data of isolation days per confirmed CRE case in the institution that considers any CRE screening culture result representative of a true carrier state in routine clinical practice. A p-value of less than 0.05 was considered significant.

RESULTS

Of 3,116 samples tested (2,037 *E. coli* and 1,079 *Klebsiella pneumoniae*), 359 carbapenemase genes were detected by Carba-R in 297 strains (9.5%) belonging to 292 patients (median age=63.5 years±18.4, **Table 1**), where the prevalence was 2.7% among *E. coli* and 22.5% in *K. pneumoniae* isolates. 80 strains,

Table 1. Baseline characteristics of 292 patients who were colonized with CRE

Baseline chrematistics	Results
Median age (years±SD)	63.5 years±18.4
Gender (male)	151 (51.7%)
ICU admission	202 (69.2%)
Prior antimicrobial therapy (three months)	292 (100.0%)
Indwelling device	228 (78.1%)
Comorbidities	
Diabetes mellitus	203 (69.5%)
Hypertension	209 (71.6%)
End stage renal disease	96 (32.9%)
Malignancy	17 (5.8%)
Respiratory failure	134 (45.9%)
Hepatic insufficiency	14 (4.8%)
Cardiovascular disease	76 (26.0%)
Neurological illness	55 (18.8%)
Concurrent COVID-19	61 (20.9%)

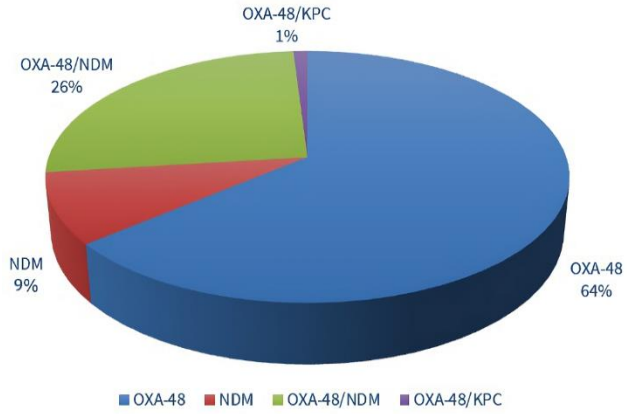

Figure 1. Frequency of CRE genotypes amongst 297 isolates (Source: Author's own elaboration)

which were identified as *K. pneumoniae,* possessed two carbapenemase genes representing 22.3% of all CRE.

OXA-48 was the most frequently detected genotype (n=190, 64%), followed by a combined OXA-48/NDM genotype (n=77, 25.9%) and NDM in 27 cases (9.1%).

Three KPC-harboring microorganisms (1%) were also carrying OXA-48, while no KPC, IMP, or VIM was detected as single genotypes in the cohort (**Figure 1**).

Based on the phenotypic MIC, 280 strains (9.0%) fulfilled the CLSI definition for carbapenem resistance (MIC≥4 µg/ml to either carbapenem tested or not), including 12 isolates (4.3%) of *K. pneumoniae* with no resistance genes detected. The MIC90 and MIC50 for both meropenem and imipenem were ≥32 µg/ml (**Figure 2**).

The overall sensitivity and specificity of the e-test were 90.6% (95% CI=86.8% to 93.6%) and 99.6% (95% CI=99.3% to 99.8%). PPV and NPV for MIC testing to detect carbapenemases were 99.0% (95% CI=98.6% to 99.3%) and 98.7% (95% CI=98.3% to 99.1%), respectively with variable performance of e-test based on the underlying genotype (**Table 2**).

In discrepant cases (n=41, 13.3%), Carba-R positive but MIC-susceptible strains were encountered in 29 cases (9.9%) with 100% concordance between imipenem and meropenem tests. These were mostly seen in case of a NDM genotype while they were less frequently encountered with OXA-48; n=15 (55.6%) vs. 14 (7.4%) respectively (p=0.08). On the other hand, 12 strains (3.9%) were resistant to both carbapenems phenotypically but no carbapenemase gene was detected. The samples were categorized into three groups: concordant, resolved, and discordant, with median Ct values of 18.5, 26.5, and 34.0 respectively (p=0.02). According to carbapenemase type, the difference in Ct value was statistically significant for NDM only (p≤0.01), but not in the case of OXA-48 or KPC (p=0.33 and 0.67, respectively). The consumable-based cost per sample was 13.3 times more in the case of molecular genotyping ($120) in comparison with combined MIC-testing for both carbapenems, inclusive of susceptibility testing media ($9). Conversely, median TAT for generating a report with

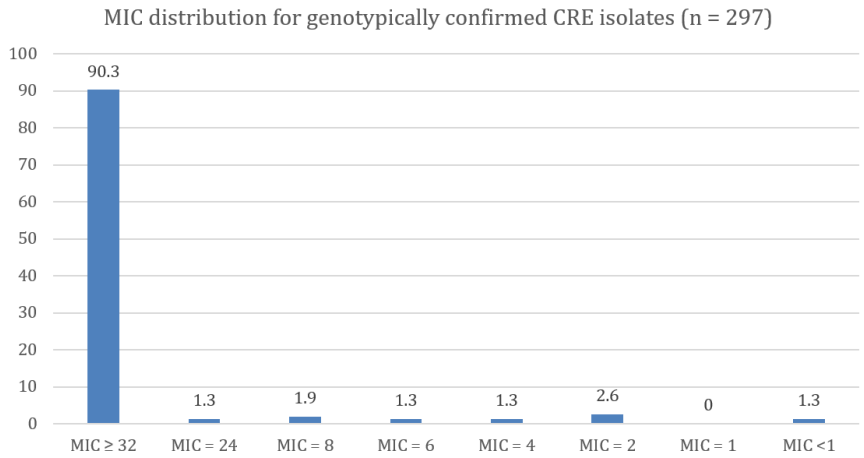

Figure 2. MIC distribution for genotypically confirmed CRE isolates (n=297) (Source: Author's own elaboration)

Table 2. Diagnostic performance of minimum inhibitory concentration-based testing for carbapenemases vs. genotyping in 297 Enterobacterales strains

Gene	*No (%)		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
All genes	297 (100)	Initial analysis	95.2% (92.1%-97.4%)	99.6% (99.3%-99.8%)	99.0% (98.6%-99.3%)	99.0% (98.6%-99.3%)
		Discrepancy resolved	90.6% (86.8%-93.6%)	99.7% (99.8%-99.8%)	99.6% (92.8%-99.9%)	98.7% (98.3%-99.1%)
OXA-48	190 (64)	Initial analysis	93.1% (88.8%-96.2%)	99.6% (99.3%-99.8%)	95.6% (92.5%-97.4%)	99.3% (98.8%-99.6%)
		Discrepancy resolved	87.2% (82.1%-91.2%)	99.9% (99.8%-100%)	99.7% (97.6%-99.9%)	98.7% (98.1%-99.1%)
NDM	27 (9.1)	Initial analysis	64.3% (48.0%-78.5%)	99.9% (99.9%-100%)	99.3% (95.2%-99.9%)	96.6% (94.9%-97.7%)
		Discrepancy resolved	48.2% (82.1%-91.2%)	99.9% (99.8%-100%)	100% (99.9%-100%)	94.5% (93.5%-96.0%)
OXA-48/NDM	77 (25.9)	Initial analysis	100% (95.3%-100%)	100% (99.9%-100%)	100% (99.9%-100%)	100% (99.9%-100%)
		Discrepancy resolved	100% (95.3%-100%)	100% (99.9%-100%)	100% (99.9%-100%)	100% (99.9%-100%)
OXA-48/KPC	3 (1)	Initial analysis	100% (29.2%-100%)	100% (99.9%-100%)	100% (99.9%-100%)	100% (99.9%-99.8%)
		Discrepancy resolved	100% (29.2%-100%)	100% (99.9%-100%)	100% (99.9%-100%)	100% (99.9%-99.8%)
Non-OXA-48	27 (9.1)	Initial analysis	64.3% (48.0%-78.5%)	99.9% (99.9%-100%)	99.3% (95.2%-99.9%)	96.6% (94.9%-97.7%)
		Discrepancy resolved	48.2% (82.1%-91.2%)	99.9% (99.8%-100%)	100% (99.9%-100%)	94.5% (93.5%-96.0%)

Note. *The number of strains with CRE genes (samples harboring multiple genes were counted as one sample in this table); **Non-OXA-48 in this cohort was equivalent to NDM alone; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; KPC: *Klebsiella pneumoniae* carbapenemase; NDM: New Delhi metallo-β-lactamase; & OXA-48: Oxacillinase-48

Table 3. Prediction of minimum inhibitory concentration-based carbapenemase testing in various CRE genotypes

Carbapenemase gene	*No (%)	NND*	CUI**	Missed isolation days (95% CI)
All genes	297 (100.0)	1.11	0.90	22.5 (18.6-29.1)
OXA-48	190 (64.0)	1.15	0.87	18.5 (12.8-23.6)
NDM	27 (9.1)	2.08	0.48	10.5 (7.7-12.9)
OXA-48/NDM	77 (25.9)	1.00	1.00	0.0
OXA-48/KPC	3 (1.0)	1.00	1.00	0.0
Non-OXA-48	27 (9.1)	2.08	0.48	10.5 (7.7-12.9)

Note. *NND: Number needed to diagnose & **CUI: Clinical utility index

Table 4. Antimicrobial susceptibility profiles of 297 genotypically confirmed CRE isolates

Drug (no tested)	Susceptible no (%)
Doxycycline (297)	212 (71.4)
Gentamicin (297)	211 (71.0)
Amikacin (297)	229 (77.1)
Ciprofloxacin (297)	127 (42.8)
Levofloxacin (297)	82 (27.6)
Nitrofurantoin (297)	71 (23.9)
Trimethoprim-sulfamethoxazole (297)	53 (17.8)
Ceftazidime-avibactam (68)	39 (56.5)
Ceftolozane-tazobactam (68)	0 (0.0)

notification to the infection control unit was 21.5 hours±10.7 and 39.0 hours±14.1 for colony-based molecular detection and MIC testing respectively, resulting in missed isolation days ranging between 0-22.5 days, as shown in **Table 3**.

The resistance rates for the CRE isolates to other antimicrobial agents are summarized in **Table 4**. In addition, MIC90 and MIC50 were also determined for two B-lactam-B-lactamase inhibitor combinations. There were ceftazidime-avibactam (MIC50=0.38 µg/ml, MIC90>256 µg/ml) and ceftolozane-tazobactam (MIC50=32 µg/ml, MIC90>256 µg/ml).

DISCUSSION

Acquired carbapenem resistance in Enterobacterales requires real-time interventions to prevent its dissemination. Therefore, institutions often seek cost-effective measures to aid in earlier detection at a reasonable cost. There is currently insufficient evidence to guide the use of various detection tools for CRE screening nor to determine the duration of isolation needed for colonized cases. In the present study, the rate of CRE (9.5%) among two commonly encountered species, *E. coli* and *Klebsiella pneumoniae*, was obtained using a combined culture-based approach of phenotypic and genotypic testing. This finding is comparable to the reported rates (7.8-12.2%) in studies conducted among similar patient populations in geographically related areas [1, 30]. However, our study assessed screening samples from cases throughout the hospital units rather than being an intensive-care-based screening. Furthermore, the study by Al Fadhil et al focused on multi-point serial testing in contrast to the single-point testing adopted in this study. It is known that the shedding of CRE among carriers is intermittent and thus a single-point test is likely to underestimate the prevalence [31, 32]. Of note, the currently found CRE rate among screening samples (9.5%) shows a significant increment from a baseline study (0.5%) conducted in the same institution seven years earlier [33]. Although this finding may suggest a rapidly increasing trend that necessitates close surveillance and prompt antimicrobial stewardship actions, variation in the testing strategy from direct rectal swab testing to culture-based screening can be an additional contributing factor. Because this study overlaps with the COVID-19 pandemic, 20.9% of CRE strains were detected in patients infected by SARS-CoV-2 (**Table 1**), leading to more challenges to the isolation facility and predisposing them to bacterial co-infection [34-38]. Bacterial coinfection and secondary infections were reported to be around 8% and 20% respectively in a systematic review of 118 COVID-19 studies [34]. *Klebsiella pneumoniae* was the most commonly isolated pathogen in coinfection in that meta-analysis, which makes CRE screening for such cases useful.

Monitoring the genetic mechanism of carbapenem resistance not only helps in selecting optimal therapeutic regimens but also identifies emergent, newly circulating clones in a region [3, 39]. Whilst new β-lactam-β-lactamase inhibitor combinations such as ceftazidime-avibactam, and meropenem-vaborbactam can be potential therapeutic agents for infections caused by the serine carbapenemases, they do not offer coverage for metallo-carbapenemases such as NDM or IMP. Earlier studies in the Arabian Gulf Peninsula have consistently shown the predominance of the OXA-48 family among CRE, which support the current findings shown in **Figure 1** [40-42]. This is in contrast to the USA, where another serine-carbapenemase “KPC” has been described as the most frequent genotype in *K. pneumoniae* [43-45].

Most of the CRE isolates in this cohort possessed MIC>32 µg/ml to both carbapenems, denoting high resistance patterns (**Figure 2**). In a retrospective Southeastern Asian report of 121 CRE isolates [46], MICs<16 were described in the majority of the strains (65%). Conversely, we did not find the OXA-48 type in the present cohort reflecting lower MIC values. With limited therapeutic options, the treatment of CRE infections is uncertain and relies mainly on *in vitro* susceptibility testing. Knowing the local epidemiology can assist in outbreak settings, where empiric therapy should be considered for patients with serious infections until laboratory results become available (**Table 4**).

A longer turn-around time of a screening assay results in additional medical expenses, overutilization of isolation facility, and on certain occasions may increase the risk of exposure to the tested pathogen by other patients if preemptive measures are not taken. By comparing the two modalities, molecular tests for CRE in suspected colonies enabled an accelerated action by the infection control team in our institution based on the shorter TAT. This has a clinical impact as it can support bed management in institutions, especially during critical periods such as an outbreak or a pandemic. Furthermore, molecular screening resulted in minimal missed isolation days in the institution because of its high sensitivity. The overall performance of Carba-R after resolving the discrepancy showed its higher concordance with the e-test MIC method for OXA-48, which is the dominating genotype (**Table 2**).

Although we conservatively considered the 17 cases out of 3,114 (0.5%) that harbored a carbapenemase gene as a colonized patient, caution needs to be exercised as false positivity has been reported for Carba-R assay, which can result in unnecessary isolation of the patients [47]. Further studies are required to clarify the clinical impact of Carba-R-positive and MIC-negative samples. On the other hand, sensitivity is an important advantage of a molecular screening method for CRE, which can be impacted by the genotype under testing. After resolving the discrepancy by the mCIM test, 12 isolates (4.0%) remained Carba-R negative but with elevated MIC to both carbapenems. This can be explained by having another rare gene not included in the multiplex cartridge or a low load of certain metallo-lactamase genes. The study [48] demonstrated up to a 100-fold higher limit of detection in cases of NDM- and VIM-harboring strains in comparison to the serine-based enzymes, which is also demonstrated in the Ct values in our study. The comparative cost of the different molecular- and culture-based screening tools should be analyzed considering other factors in each institution.

Active surveillance for patients with a high risk for CRE colonization can be an informative infection control tool [49, 50]. Reduced transmission of KPC-producing *K. pneumoniae* was evident in multiple studies that adopted comprehensive infection control measures inclusive of active screening [51-53]. In particular, screening is useful in outbreak settings, where rapid molecular testing can enable the detection of colonized cases and there has been emerging data suggesting that colonization with certain CRE clones harboring NDM can be linked to bloodstream infections although further assessment of this finding is necessary [54]. Yet, the data on optimal and cost-effective testing for CRE screening in routine infection control practice is limited. The missed isolation days for phenotypic CRE screening in this study were 22.5 days (18.6-29.1). It is important to note that the extent of missed isolation days can be influenced by the background rate of CRE carriage among the tested population, and also the predominant genotype within an institution. In addition, the patients' risk factors, and local infection control policies for suspected or confirmed CRE carriers are among other contributing factors that can justify the need to shift to rapid diagnostics. CUI and NND results suggest that phenotypic testing is accurate in detecting most genotypes, particularly the strains with mixed genes (**Table 3**).

Nevertheless, it is important to consider the difference in TAT of both testing modalities when assessing the preventive potential of a laboratory assay. Because multifaceted approaches are often followed to reduce the occurrence of outbreaks caused by multidrug-resistant organisms including CRE, the individualized role of a screening tool remains uncertain. Further studies are required in areas with variable CRE genotypes to investigate the cost-efficacy of screening yields in high and low prevalence settings. The duration of CRE asymptomatic colonization is also uncertain and can be persistent for long periods due to a lack of effective decolonization protocol [22, 55]. Thus, active surveillance is needed to continue as an important measure in order to contain the spread of these organisms in health care settings, while some institutions opt to indefinitely apply contact precautions for a patient after being identified as a CRE colonizer.

Our study simulates clinical practice in low CRE prevalence settings, where a large number of patients' samples are screened with a less than 10% positivity rate. Its main limitation is the underrepresentation of certain carbapenemase genes that are not endemic in the region. Additionally, only *E. coli* and *Klebsiella pneumoniae* were included in the study, which are together responsible for > 99% of CRE cases, since the routine local laboratory algorithm does not offer reflux testing for CRE on other genera such as *Serratia, Enterobacter*, and others [56, 57]. Furthermore, we excluded strains, where only one carbapenem MIC was performed (n=33) due to logistic issues. Other resistance genes and efflux mechanisms were not tested in the described strain panel, and although the data represent a specific institutional experience rather than being a population-based study, it contributes to the growing evidence of optimizing CRE screening considering various prevalence and resources.

CONCLUSIONS

In conclusion, the study has shed light on two culture-based testing tools for CRE, the determination of MIC and genotyping. It illustrated that either method can be used to detect CRE in a low prevalence setting that is dominated by the OXA-48 genotype with high accuracy. The superior sensitivity and shorter time to action displayed by molecular testing can be outweighed by its higher costs in less-resourced laboratories. A combined approach of CRE genotyping and MIC determination remains the most optimal in guiding clinical management as well as in detecting all CRE isolates.

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Ethical statement: Author stated that the Institutional Review Board approval was obtained from the Research Bioethics Committee at Imam Abdulrahman bin Faisal University approval (IRB-2022-1-081). The study was compliant with the Helsinki Declaration of 1964 and all its subsequent amendments.

Declaration of interest: No conflict of interest is declared by the author.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the author.

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