

High Burden of Carbapenem-Resistant Organisms Screened by Xpert Carba-R Assay Versus Culture from Intensive Care Patients in Indonesia

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Abstract

The World Health Organization (WHO) considers carbapenem-resistant organisms (CROs) to be critical-level pathogens. Regular screening for high-risk CRO colonization is essential, especially in the ICU. Direct detection of carbapenem-resistant genes is possible using the FDA-approved Xpert Carba-R assay. This study evaluated its reliability compared with the culture technique at a tertiary hospital in Indonesia. A high number of CRO colonization was found using the culture technique and the Xpert Carba-R assay with about 31 and 26 positive results out of 100 total samples, respectively. Both methods detected *bla*_{NDM} in 11 samples, and the Xpert Carba-R assay detected one sample co-presenting with *bla*_{VIM} that was not detected by PCR. The Xpert Carba-R assay did not detect the gene in 73 samples following negative results with the culture technique. Fifteen samples were *detected gene* by the Xpert Carba-R assay though there was no gene by the culture method, showing that the Xpert Carba-R assay demonstrated a high degree of sensitivity in identifying carbapenem-resistance genes. Carbapenem-resistance genes common in Indonesia other than those examined by Xpert Carba-R assay in this study (i.e., *bla*_{OXA-23} and *bla*_{OXA-24}) or non-enzymatic mechanisms may also produce resistance in many colonies without the examined genes. Finally, the Xpert Carba-R assay produced faster findings than the culture technique.

Keywords: Carbapenem-resistance, Xpert Carba-R Assay, Bacterial Colonization, ICU, Infectious Disease, Indonesia

INTRODUCTION

In recent years, a worldwide outbreak of ESBL-producing bacteria led to an increase in the inappropriate use of carbapenem antibiotics and the emergence of carbapenem-resistant organisms (CRO) producing carbapenemase enzymes.¹ Bacteria that produce carbapenemase enzymes are resistant to carbapenem and also to broad-spectrum beta-lactam drugs, leaving limited antibiotic therapy options available for them.² Carbapenemases are generally divided into 3 molecular classes: penicillinases (class A carbapenemases) such as *Klebsiella pneumoniae carbapenemase* (KPC); metallo-beta-lactamases (class B carbapenemases) such as *Imipenemase* (IMP), *Verona Integron-encoded Metallo-β-lactamase* (VIM), and *New-Delhi metallo β-lactamase* (NDM); and oxacillinase (class D carbapenemases) such as *Oxacillinase-48* (OXA-48).²

The increasing prevalence of CRO infection in several countries has become very troubling for patient care, especially in high care or intensive care units (ICU), because it is difficult to treat, extends the length of hospitalization, increases hospitalization costs, and involves high mortality rates.³ CRO infection is associated with CRO colonization as a risk factor, especially in critical patients. Thus it is important to carry out

active and regular screening, especially in high-risk patients infected with CRO bacteria in the ICU, to prevent CRO infection.⁴⁻⁸

The World Health Organization (WHO) recommends continuous surveillance of patients with active or suspected CRO infections and individuals with elevated risk factors, including those undergoing multiple antibiotic medications, hemodialysis patients, transplant patients, and patients confined to intensive care.⁹ Culture surveillance for CRO-colonized asymptomatic patients should also be conducted based on epidemiology and local risk factor analysis.⁹

The limitations of culture-based surveillance include the requirement for molecular and/or phenotypic means of confirmation, the requirement for 24 to 48 hours for culture growth, issues with specificity and sensitivity based on growth media composition, and the specific enzymes of carbapenemase.¹⁰ Recently, molecular testing method, such as the Xpert Carba-R assay made by Cepheid (USA) have been developed to lessen these restrictions. This assay was an FDA-approved automated *in vitro* test using a multiplex real-time PCR technique to identify the *bla*_{NDM}, *bla*_{OXA-48}, *bla*_{KPC}, *bla*_{IMP} and *bla*_{VIM} genes related to its resistance.¹⁰⁻¹² Data on the application of this method in clinical settings is still sparse, especially in Indonesia. In a tertiary hospital in Indonesia, This study evaluated the reliability of the Xpert Carba-R

assay in comparison to the culture approach for screening CRO colonization in intensive care patients.

MATERIALS AND METHODS

Patients had rectal swab samples obtained using a double sterile swab and then inserted into Stuart's transport media. Samples that were not processed immediately were stored at 2-8°C with a maximum storage time of 7 days. After the double swab was gently rolled to minimize sampling bias probability, as directed by the manufacturer, one rectal swab was processed onto the test cartridge. The second rectal swab was cultured on MacConkey agar media supplemented with meropenem 2 µg/ml. Bacterial colonies that grew were suspected to be carbapenem-resistant. Identification of bacterial species used the VITEK® 2 Compact system with GN card. The molecular approach verified the presence of carbapenem-resistant genes. Next, using primers to identify *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, and *bla*_{IMP-1} genes, DNA from CRO isolates was extracted for conventional PCR based on a previous protocol (Table 1).

RESULTS

This study was conducted between January to June 2023. A total of 100 rectal swab

samples meeting the inclusion criteria were consecutively obtained from hospitalized patients in the critical care unit who were older than eighteen. The Xpert Carba-R assay detected 26 rectal swab samples as positive, with 26 *bla*_{NDM} genes and one *bla*_{VIM} gene detected. The culture method detected 31 of 100 samples as positive for CRO colonization. Of the 31 patient samples with CRO colonization, 23 samples were isolated with one CRO species, six samples with two CRO species, and 2 samples with three CRO species. A total of 41 CRO isolates were collected (Figure).

Eleven rectal swab samples that agreed with the results of both the Xpert Carba-R assay and the conventional PCR from isolated colonies on

Table 1. Primer sequences and product length^{13,14}

Primer	Primer Sequence (5'-3')	Product length
<i>bla</i> _{KPC}	F: TGTGCTGAAGGAGTTGGGC R: ACGACGGCATAGTCATTTC	340
<i>bla</i> _{NDM-1}	F: CTGAGCACCGCATTAGCC R: GGGCCGTATGAGTGATTGC	754
<i>bla</i> _{IMP-1}	F: CTACCGCAGCAGAGTCTTTG R: AACCACTTTTGCCTTACCAT	587
<i>bla</i> _{OXA-48-like}	R: GAGCACTTCTTTGTGATGGC R: CCCCTCTGCGCTCTACATAC	744
<i>bla</i> _{VIM}	F: TGGGCCATTACGCCAGAT C R: ATGGTGTGTCGCATATC	510

F: forward primer; R: reverse primer

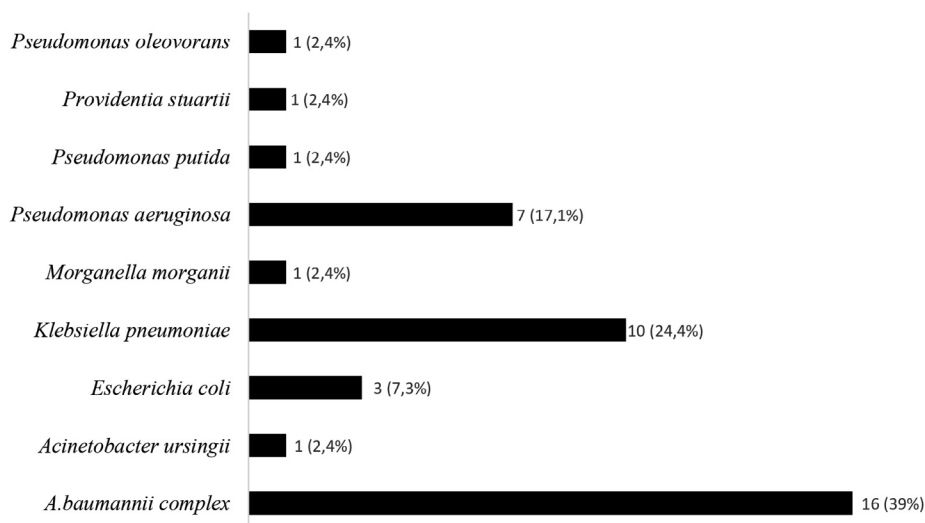


Figure. The number of CRO colonizing isolates by species (n=41)

Table 2. Xpert Carba-R assay gene identification in comparison to the culture approach

		Culture Method (gene detection by PCR)		
		Samples with isolate(s) growth		Total
		<i>bla</i> _{NDM} (+)	<i>bla</i> _{NDM} (-)	
Xpert Carba-R assay	<i>bla</i> _{NDM} (+)	11	6	9
	<i>bla</i> _{NDM} (-)	1	13	60
	Total	12	19	69
				100

selective media containing meropenem detected the *bla*_{NDM} gene (Table 2). Carbapenem-resistant bacterial isolates from the concordance samples consisted of two *Acinetobacter baumannii* complex isolates, seven *Klebsiella pneumoniae* isolates, one *Providencia stuartii* isolate, and one *Acinetobacter ursingii* isolate. One sample obtained two isolates of carbapenem-resistant bacteria and detected *bla*_{NDM} from rectal swab samples, concordant with PCR results from a *Klebsiella pneumoniae* isolate with the *bla*_{NDM} gene. The same rectal swab sample was also detected for the *bla*_{VIM} gene although PCR results for the *Acinetobacter baumannii* complex and *Klebsiella pneumoniae* isolates grown from the culture technique did not detect the *bla*_{VIM} gene.

The *bla*_{NDM} gene was identified via the Xpert Carba-R assay in 6 samples with colony growth where the same gene could not be detected by PCR from its growth isolates, including nine different isolates of the *Acinetobacter baumannii* complex, three *Pseudomonas aeruginosa*, two *Klebsiella pneumoniae*, and one *Pseudomonas putida* isolate. However, one sample with *Pseudomonas aeruginosa* colony growth was detected for the *bla*_{NDM} gene by PCR but not by Xpert Carba-R assay from its swab sample. Nine samples tested positive for *bla*_{NDM} by the Xpert Carba-R assay that yielded no colony growth on selective meropenem-containing MacConkey agar media. The gene was not found using the Xpert Carba-R assay in a total of 60 samples without colony growth. In addition, there were 13 samples with colony growth in which no resistance gene was found by either the PCR examination or the Xpert Carba-R assay (Table 1).

DISCUSSION

True positive carbapenem-resistance genes, especially *bla*_{NDM}, were detected by the Xpert Carba-R assay examination using rectal swab samples and found to be in accordance with conventional PCR examination on isolates grown from carbapenem-resistant selective media in 11 samples. One sample co-presenting the *bla*_{VIM} gene was not detected by the culture method. True negative results for carbapenem-resistance genes based on both methods were found in 73 samples.

We also identified 16 discordant results between these two methods. Of the 16 samples with discordant results, carbapenem-resistance genes were identified using the Xpert Carba-R assay method in 15 samples from the direct specimens, but not detected based on the culture method, where 9 samples showed no colony growth and 6 samples grew colonies of bacteria. One sample showed growth of two types of colonies of *Acinetobacter baumannii* complex and *Pseudomonas oleovorans*, 2 samples showed growth of *Acinetobacter baumannii* complex, 1 sample showed growth of *Klebsiella pneumoniae*, 1 sample had *Pseudomonas aeruginosa* growth, and 1 sample had *Pseudomonas putida* growth. One sample detected the *bla*_{NDM} carbapenem-resistance gene in *Pseudomonas aeruginosa* colony growth through the culture method but the direct detection of the gene from the sample did not yield the same result in the Xpert Carba-R assay method.

In the 9 samples with no colony growth and the 6 samples with colony growth negative

for carbapenem-resistant genes based on conventional PCR, the *bla*_{NDM} detected by the Xpert Carba-R assay was probably carried by too small number of bacteria to be revealed by the culture method.¹⁵ The Carba Xpert-R assay was more sensitive for detecting carbapenem-resistance genes than conventional culture methods. Resistant bacterial colonies that did not reveal the targeted genes could result from non-enzymatic resistance mechanisms (i.e., non-carbapenemase producers), such as porin alterations that often occur in *Acinetobacter baumannii* complex and *Pseudomonas* species.¹⁶⁻¹⁸ Carbapenem-resistance genes other than the genes examined with the Xpert Carba-R assay (i.e. *bla*_{OXA-23} and *bla*_{OXA-24} genes), especially in *Acinetobacter baumannii* complex species, also may produce resistant colonies with no detected gene in this setting.^{14,19} One sample produced a false negative for the *bla*_{NDM} gene from *Pseudomonas aeruginosa* that was not detected based on an Xpert Carba-R assay from the direct specimen. This may be due to a small number of bacteria, making it difficult to amplify the target gene to reach the limit of detection of the test.¹¹

In terms of time efficiency, there were wide differences in turn-around time. The Xpert Carba-R assay only took around an hour, including a 48-minute running period, whereas the standard culture method needed 48-72 hours. On the diagnostic side, faster results help in making treatment decisions. This study used rectal swabs to screen CRO carriers so contact precautions could be taken and transmission to other patients could be avoided.

There were limitations to this study. There is no standard procedure for screening cultivation of carbapenem-resistant organisms, so organisms with decreased susceptibility to carbapenem drugs other than meropenem (i.e., ertapenem and imipenem) would be unculturable with meropenem-containing selective media. Thus, growth-isolate-based procedures for carbapenem-resistant organisms are more complex than the molecular-based procedure with the direct specimen. Bacterial colonies could not grow even containing a carbapenem-resistance gene. Moreover, in this study, it was challenging to establish a false positive conclusion in the sample

with gene(s) detected by molecular methods from the direct specimens using the Xpert Carba-R assay without applying another molecular method also from the same direct specimens.

CONCLUSION

This study was the first in Indonesia to evaluate the FDA-approved Xpert Carba-R assay, which may be used as a screening tool for carbapenem-resistant organisms, in the ICU. The results revealed that the assay is more sensitive than the culture method on selective meropenem-containing media and has a faster processing time, making it useful in infection prevention and control strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

MAM, RS, BPS, KK, and EBK conceptualized the study. MAM, AMW, SROS, WS, BPS, PSA, KK, and EBK collected resources. MAM, SROS, and WS performed administration work. MAM, RS, KK, and EBK funding acquisition. AMW, BPS, KK, and EBK supervised the study. MAM, RS, BPS, KK, and EBK applied methodology. MAM, FSW, RS, BPS, KK, TS, and EBK performed analysis. MAM, and FSW performed visualization. MAM, FSW, RS, WS, AMW, SROS, BPS, PSA, KK, TS, and EBK performed data validation. MAM, FSW, RS, KK, and EBK wrote the original draft. MAM, FSW, RS, AMW, SROS, BPS, PSA, KK, TS, and EBK wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The study was approved by the Health Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (approval number: 0558/KEPK/I/2023).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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