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REVIEW ARTICLE

Clinical Laboratory Aspect of Carbapenem-Resistant *Enterobacteriaceae*

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카바페넴내성장내세균속균종의 임상검사 측면

박창은

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ABSTRACT

The correct distinction of carbapenem-resistant *Enterobacteriaceae* (CRE) and ccarbapenemase producing *Enterobacteriaceae* (CPE) and the rapid detection of CPE are important for instituting the correct treatment and management of clinical infections. Screening protocols are mainly based on cultures of rectal swab specimens on selective media followed by phenotypic tests to confirm a carbapenem-hydrolyzing activity, the rapid carbapenem inactivation method, lateral flow immunoassay, the matrix-assisted laser desorption ionization-time-of-flight test and molecular methods. The CPE is accurate for detection, and is essential for the clinical treatment and prevention of infections. A variety of phenotypic methods and gene-based methods are available for the rapid detection of carbapenemases, and these are expected to be routinely used in clinical microbiology laboratories. Therefore, to control the spread of carbapenemase, many laboratories around the world will need to use reliable, fast, high efficiency, simple and low cost methods. Optimal effects in patient applications would require rapid testing of CRE to provide reproducible support for antimicrobial management interventions or the treatment by various types of clinicians. For the optimal test method, it is necessary to combine complementary test methods to discriminate between various resistant bacterial species and to discover the genetic diversity of various types of carbapenemase for arriving at the best infection control strategy.

INTRODUCTION

Carbapenems are a commonly used primary therapeutic option for serious infections caused by Gram-negative bacilli such as *Enterobacteriaceae* and are often considered agents. Although current studies on the molecular epidemiology of some carbapenem-resistant *Enterobacteriaceae* (CRE) suggest that there are globally predominant clonal lineages among isolates from humans (e.g. sequence type (ST258) among KPC-producing *K. pneumoniae*), carbapenemases are not exclusively associated with defined clones [1]. Rapid identification of patients colonized with carbapenemase-producing organisms (CPOs) is mandatory to implement proper infection control measures to avoid further spread of these deadly bacteria to other hospitalized patients. Ceftazidime-avibactam (CZA) is a combination antimicrobial consisting of an established antipseudomonal cephalosporin and

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a novel non-β-lactam (diazabicyclooctane) β-lactamase inhibitor. Avibactam protects ceftazidime from hydrolysis by Ambler class A and some class D carbapenemases [2].

Overall, isolates associated with OXA-48, VIM-1 and IMP-27 carbapenemases often exhibited minimum inhibitory concentrations (MICs) below the clinical breakpoints, but were detected using the European Committee on antimicrobial susceptibility testing (EUCAST) screening cut-off values or epidemiological cut-off values (ECOFFs). CRE occur globally in livestock, pets, wildlife, and seafood. Studies on the prevalence of CRE have rarely been performed and are challenging because of the variety of bacterial species and the different genetic elements involved [3]. The β-lactam antibiotics such as penicillins, cephalosporins, and carbapenems represent of the available treatment options for antibiotic-resistant Gram-negative bacteria. These antibiotics can be hydrolyzed by the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases.

In prevalence of CRE, some recent studies indicated a higher prevalence in Asia (China: 15% in milk samples, India: 1~3% in piglets, Lebanon: 2.5% in fowl) and Algeria (6% in milk samples, 26% in chickens). Seafood was contaminated with CRE in <1% in Canada. Among wildlife, two studies found a high prevalence of CRE among silver gulls and yellow-legged gulls in France (19.4%) [4]. The occurrence of CRE in livestock, seafood, wildlife, pets, and directly exposed humans poses a risk for public health. Prospective prevalence studies using molecular and cultural microbiological methods are needed to better define the scope and transmission of CRE [3].

The major innovation is the combined use of faropenem and temocillin for reliable detection (excellent performance with 100% sensitivity and specificity) of OXA-48. its development of a new algorithm to detect the different classes of carbapenemases, for first-line diagnosis, by combining this modified MASTDISCS® Combi Carbapenemase Detection set or ID carbapenemase activity test (CAT) discs (Mast Diagnostica GmbH, Reinfeld, Germany) with immunochromatographic methods and molecular biology techniques [5].

In laboratory aspect, the first essential step is early, simple and reliable detection. The methods must be standardized and accessible to all microbiology laboratories. Therefore, it is a capital issue for worldwide public health to detects carbapenemase to issue alerts and ensure immediate implementation of strict and efficient hospital hygiene measures, in order to prevent and contain outbreaks and to stop the spread of these resistance enzymes. The purpose of this study is to review recently reported papers on the clinically used CRE test method and provide information on the selection of appropriate test method for infection control site.

MAIN ISSUE

1. Distributions of CRE

In 2001, the United States first reported a *Klebsiella pneumoniae* (KPN) strain carrying a plasmid-mediated carbapenemase gene encoding a protein later designated *K. pneumoniae* carbapenemase (KPC) [6]. In 2009, *bla*$_{NDM}$-associated carbapenem-resistant *K. pneumoniae* (KPN) was first reported in India [7]. In the past few years, cases of multiple carbapenemases in the same *Enterobacteriaceae* isolate have been reported. For example, bla$_{NDM-1}$ and blaI$_{MP-4}$ coexisted in KPN [8]. *Enterobacter cloacae* complex (ECC) were recovered from *Enterobacter cloacae* or *Citrobacter freundii* carried both bla$_{NDM-1}$ and bla$_{KPC-2}$ in China [9]. Also, *Klebsiella oxytoca* isolate coexpressing three carbapenemases (KPC-2, NDM-1, IMP-4) was identified in 2017 [10].

Most CRE were *K pneumoniae* (63.2%), followed by *Escherichia coli* (14.5%) and *Enterobacter* spp. (12.8%) at academic medical centers in the United States between 2015 and 2019 [11]. More than half of patients were residents of the intensive care unit (ICU) at

infection onset, the median SOFA (sequential organ failure assessment) score was 5. The most common infection sources were respiratory tract (37.4%), followed by urinary tract (19.7%), intra-abdominal (18.7%), skin and soft tissue (8.9%), and osteoarticular (6.9%) [11]. Primary bacteremia or respiratory tract infection and SOFA score were independently associated with higher clinical failure. GES-6 was first identified in a *Klebsiella pneumoniae* isolate from Greece [12].

2. Detection of carbapenemases by chromogenic media for CRE screening

Chromogenic plate methods was acceptable specificity, but sensitivities ranging broads. these methods were finally reporting is extended to 48 hr. False positive results have been reported in strains harbouring extended-spectrum beta-lactamases (ESBL) or overexpressing AmpC with porin deficiencies. For improvement of chromogenic media with low hydrolytic profiles detection of OXA-48 and GES-6 enzymes was not supported in carbapenemase specific media.

The OXA-48 enzyme is an Ambler class D β-lactamase that hydrolyzes carbapenems but shows very weak activity against extended-spectrum cephalosporins such as cefepime and ceftazidime. OXA-48 is known to hydrolyze penicillins at a high level and carbapenems at a low level, sparing expanded-spectrum cephalosporins. Identification of OXA-48-producing *Enterobacteriaceae* was from an isolate [13]. Since its recognition, there have been increasing numbers of reports of OXA-48-producing organisms worldwide, particularly in Europe [14]. OXA-48 carbapenemases appear to be uncommon in the United States, with the first reported ceftazidime-avibactam resistance in a KPC-3-expressing *Klebsiella pneumoniae* isolate identified [15].

Zhong et al (2019) was reported that meta-analysis and systematic review to assess the accuracy and applicability of modified Hodge's test (MHT), carbapenemase Nordmann-Poirel test (Carba NP), modified carbapenem inactivation method (mCIM), and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for carbapenemase-producing *Enterobacteriaceae* (CPE) detection. The Carba NP, mCIM and MALDI-TOF MS all demonstrated high accuracies in CPE detection, while MHT not recommended and costs and the equipments and skills involved [16]. Moubareck et al (2019) was reported that CHROMagar KPC (CHROMagar, Paris, France; suspiciously colored colonies: dark pink, metallic blue, cream to blue or opaque) and Xpert Carba-R (Cepheid, Sunnyvale, CA, USA; target carbapenemase gene: blaKPC, blaNDM, blaOXA-48-type, blaIMP-1, and blaVIM) were used to rectal surveillance swabs. The Xpert Carba-R had higher sensitivity than CHROMagar KPC. while Xpert Carba-R was more accurate and faster [17]. In detection of KPC-2 and VIM-1-producing *K. pneumoniae* isolates, the positive predictive values (PPV) and negative predictive values (NPV) of CHROMagar KPC for the detection of CRE was 100% and 98.8%, respectively [18]. To detect OXA-48-like producers, reported that the sensitivity and specificity of CHROMagar for detection of carbapenemase-producing isolates was 88.5% and 86.1% [19]. The sensitivity and specificity of commercialized chromogenic media (Table 1).

3. Phenotypic detection assays

The carbapenem inactivation method (CIM) is another effective phenotypic test, which method determines the carbapenemase activity of the tested bacteria by measuring the diameter of the inhibition zone of *E. coli* ATCC 25922 after the carbapenem disk is inactivated by the test bacterium. A study indicated that both the sensitivity and specificity of mCIM were 100% [28]. Because of its simplicity, clear criteria, cost-effectiveness, and availability in any laboratory, the mCIM has become a useful tool in microbiology laboratories. A bioluminescence-based carbapenem susceptibility detection assay was reported in 2018 that allows carbapenemase-producing CRE and non-

Table 1. Comparison of chromogenic media

Chromogenic media	Company	Sensitivity (%)	Specificity (%)	References
BBL™ CHROMagar™ CPE	Becton Dickinson GmbH, Heidelberg, Germany	88.5	86.1	[19]
Brilliance™ CRE	Thermo Fisher Scientific, Perth, Australia	75.9~81.3	58.9~91.3	[19]
CHROMagar KPC	CHROMagar Company, Paris, France	40.3~100	67.8~98.4	[24]
ChromArt CRE	Biolife Italiana, Milano, Italy	100	55.8	[19]
ChromID® CARBA	bioMérieux, Mercy-l'Etoile, France	90.7	88.5~89.1	[19]
ChromID® OXA-48 CARBA	bioMérieux, Mercy-l'Etoile, France	70.1~75.8	99.3~100	[20]
Colorex™ KPC	E&O Laboratories, Bonnybridge, UK	97	96	[22]
HardyCHROM CRE	Hardy Diagnostics, Santa Maria, USA	92~100	91.2	[27]
RambaCHROM KPC	Gibson Bioscience, Lexington, KY, USA	95.0	77.1	[26]
Rapid Carb screen test	Rosco Diagnostica A/S, Taastrup, Denmark	98	100	[23]
Rapid Polymyxin™ NP test	ELITech MICROBIO, France	82~94	88	[21]
Remel Spectra™ CRE	Remel, Lenexa, KS, USA	97.8	86.4~99.1	[25]

Table 2. Commercial tests for detection of carbapenem resistance

Test	Specimen type	Target gene(s)	References
Bogaerts-Yunus-Glupcynski (BYG) Carba (electrochemical technique)	Bacteria from pure culture	Electrochemical indicator of imipenem hydrolysis, false negative results for OXA-48-like carbapenemase	[30]
FilmArray® Blood Culture Identification Panel (BCID, BioFire Diagnostics LLC, UT, USA)	Positive blood culture broth	blaKPC	[31]
GeneXpert Carba-R (Cepheid, Sunnyvale, CA, USA) Check-Direct CPE (Check-Points, Wageningen, Netherlands), RenDx Carbaplex assay (Renshaw, UK) Amplidiag CarbaRCVRE (Mobidiag, Espoo, Finland)	Rectal swabs	blaKPC, blaIMP, blaVIM, blaNDM, blaOXA-48	[32]
MALDI-TOF MBT STAR (Selective Testing of Antibiotic Resistance)-Carba IVD kit (Bruker Daltonik GmbH, Bremen, Germany)	Bacteria from pure culture	Mass-based detection of carbapenem degradation products	[33, 34, 35]
RAPIDEC® CARBA NP (bioMerieux, Marcy L'Etoile, France) Rapid CARB Screen® (Rosco Diagnostica, Taastrup, Denmark)	Bacteria from pure culture	pH shift, Color indicator of imipenem hydrolysis, KPC and MBL producers, low imipenem hydrolysis activity, such as the OXA-48-like Both kits specificity 100%, but sensitivity differences (RAPIDEC® CARBA NP-99%; Rapid CARB Screen®-89.5%)	[23, 36]
Unyvero® P55 (Curetis AG, Holzgerlingen, Germany)	Respiratory secretions	blaKPC, blaIMP, blaVIM, blaNDM, blaOXA-48	[37]
Verigene® Gram-negative blood culture test (Nanosphere, Northbrook, IL, USA)	Positive blood culture broth	blaKPC, blaIMP, blaVIM, blaNDM, blaOXA-48	[38]

carbapenemase-producing CRE to be distinguished with a sensitivity of 99% and a specificity of 98% [29]. Modified carbapenem inactivation method (mCIM) is the best CPE inspection method at the present time. the high sensitivity and specificity of mCIM compared to the other tested methods of CPE detection. List of commercially available test methods, sample type, principles, and detection targets (Table 2).

The colormetric-Carba presented the highest sensitivity and, therefore, can be considered the best test to be used as a screening phenotypic methodology. The carbapenemase inhibition method (CIM) might be the easiest test to perform, as it does not require any special reagent and it is easier to be interpreted. The early detection of carbapenemases helps to establish infection control measures.

In case of carbapenemase activity in cultured bacteria, screening for carbapenemase producers.

Enterobacterial isolate with decreased susceptibility to carbapenems (excluding perhaps low-level imipenem resistant *Proteus* spp. and related organisms). Low-level carbapenem resistance (especially for imipenem) is observed among *Proteus* spp., *Providencia* spp., *Morganella* spp., *Enterobacter* species producing AmpC β-lactamase at a high-level and high ertapenem MICs results.

Inhibitor-based tests rely on the ability of certain compounds [i.e., ethylenediaminetetraacetic acid (EDTA), dipicolinic acid (DPA) or phenylboronic acid (PBA)] to inhibit specifically carbapenemase activity. The DPA+EDTA were synergism with meropenem in MBL. The DPA+PBA were synergism with meropenem in KPC+MBL. The KPC+MBL confirmation ID kit (Rosco Diagnostica, Denmark). Also, The MAST ISOPLEX® CRE-ART (Mast Diagnostica GmbH, Reinfeld, Germany) is a loop mediated isothermal amplification (LAMP) kit for the detection and characterisation of the seven most prevalent carbapenemase families: OXA-48, OXA-23, OXA-24/40, KPC, VIM, NDM and IMP was used.

4. Molecular-based detection methods

Tests based on molecular techniques (nested PCR, real-time PCR, and microfluidics chip technology) are considered the gold standards for the identification of carbapenemase genes. the microfluidic chip technology which allows the rapid detection of pathogens and their resistance genes was used to detect carbapenem-resistance genes for high sensitivity and specificity, and the requirements for rapidly clinical diagnoses.

The recently established multiple cross displacement amplification (MCDA) assay was a powerful innovative nucleic acid amplification technique [39]. MCDA was based on strand displacement nucleic acid synthesis in the presence of *Bst* polymerase under isothermal conditions. A total of ten primers were employed to recognize ten distinct regions on the target gene. The duplex MCDA-LFB (lateral flow biosensor, LFB) assay for simultaneous identification of *A. baumannii* strains and carbapenem-resistance based on *pagD* gene and blaOXA-23-like gene was successfully established. The duplex MCDA-LFB method established in this study displays high selectivity for target gene detection, and the limit of the method is 100 fg per reaction with pure culture [40].

The whole-genome sequencing (WGS) also provides information on the type of plasmid carrying resistance genes, the evolutionary lineage of the bacterium, and the relatedness of isolates, all of which can help to elucidate the source of the isolate or inform outbreak investigations [41].

The rapid detection of carbapenem resistance. These can be divided into 2 categories: molecular detect the resistance mechanism (presence of a carbapenemase gene) and novel, phenotypic detect the in vitro activity of carbapenemase enzymes (hydrolysis of carbapenem). A number of nucleic acid- and non-nucleic-acid-based methods for rapid detection of CRE are currently available or in development. To optimally impact patient outcomes, rapid testing for CRE should be implemented together with antimicrobial stewardship interventions or other forms of clinical decision support. The clinical and economic impact of rapid diagnostics for CRE identification will likely depend on local CRE prevalence and are areas for future research. There are compares with the commercialized carbapenem-resistant *Klebsiella pneumoniae* detection methods (Table 3).

5. Recent reported methods

A novel real-time multiplex PCR assay, BD MAX Check-Points CPO, was evaluated to detect carbapenemase-producing organisms in clinical settings on the BD MAX system. The BD MAX Check-Points CPO assay (Check-Points, Wageningen, Netherlands) detected carbapenemases KPC, VIM/IMP, NDM, and OXA-48-like producers with a high sensitivity and specificity of 97.1% and 98.8% [47]. The performance of the automated BD Phoenix CPO Detect test (BD-CPO test) for detection and Ambler classification of carbapenemases in *Enterobacteriaceae*, *P. aeruginosa* and *A.*

Table 3. Comparison of different carbapenem-resistant *Klebsiella pneumoniae* detection methods

Method	Accuracy	Sample preparation (hr)	Analysis time (hr)	Costs	References
Loop-mediated isothermal amplification PCR (blaNDM, blaKPC, blaIMP, blaVIM genes)	Middle	3~4	<4	Middle	[43]
Multiplex real-time PCR (carbapenemase genes blaVIM, blaOXA-48, blaNDM, blaKPC)	High	3~4	4~6	High	[44]
Nanopore assay (probe-target 16S rRNA complex)	Middle	6.5	1	Low	[46]
Phenotypic analysis (Verigene assays; BC-GP and BC-GN assays)	Middle	18~24	0.8	Low	[42]
TaqMan PCR genes encoding IMP, VIM, NDM, SPM, SIM, GIM MBLs)	High	3~4	<2	High	[45]

baumannii complex. The BD-CPO test showed an overall sensitivity of 89.7% and specificity of 83.5% for carbapenemase detection [48].

An agar plate-based modified carbapenem inactivation (p-mCIM) method for detection of CPE. Performance of the p-mCIM, which uses a lawn of bacterial colonies on MHA plate instead of a bacteria-suspended tryptic soy broth tube in the carbapenem inactivation (CI) step. CLSI-recommended mCIM in the detection of clinical isolates of *Enterobacteriaceae* producing carbapenemases including difficult to detect blaOXA-48-like enzymes [49]. The rapid carbapenemase detection method (rCDM) has a wide range of detection, and can be used to routinely detect carbapenemase-producing *Enterobacteriaceae* and *P. aeruginosa* in clinical microbiology laboratories if thin Mueller-Hinton agar (tMHA) could achieve commercial supply [50].

The Cica Geneus® Carbapenemase Genotype Detection kit 2 (Kanto Chemical Co., Inc.) is a commercially available product that not only targets the six major carbapenemase genes (blaKPC, blaIMP, blaNDM, blaVIM, blaOXA-48-like, and blaGES) detected in Japan, but which can also detect carbapenemase-type GES genes [51]. Carbapenemase- producing organisms (CPOs) Complete test detected all carbapenemases rapidly. The speed and accuracy of the test, coupled with its potential to classify carbapenemases, and its applicability not only to *Enterobacteriaceae* and *P. aeruginosa* but also to *A. baumannii*, can be applied successfully to meeting what has become one of the world's most urgent infectious disease challenges. The test exhibited 100% sensitivity 98.5% specificity for carbapenemase detection within 90 minutes and detected 74.1% of carbapenemases within 10 minutes [52].

The Xpert Carba-R v.2 (Cepheid, CA, USA) is a rapid and sensitive method for detecting carbapenemase-encoding genes compared with culture-based conventional PCR. The sensitivity and specificity of the Carba R were 95.0% and 98.1%, and ROC curve analysis yielded a C_t value of 28.8 as the optimal cut-off for KPC with 72.5% sensitivity and 87.5% specificity [53]. Allplex™ Entero-DR Assay (Seegene Inc., Seoul, Korea), Amplidiag® CARBAR+MCR (Mobidiag, Espoo, Finland), AusDiagnostics MT CRE EU Assay (Aus-Diagnostics, Mascot NSW, Australia), and EasyScreen™ ESBL/CPO Detection Kit (Genetic Signatures, Newtown NSW, Australia). The AusDiagnostics MT CRE EU assay displayed the highest rate of concordance with the routine cultural screening assay. Allplex and EasyScreen methods provided the highest number of positive results in conflict with both routine screening and all other molecular assays.

Three assays have shown the presence of the blaOXA48 gene in some rectal swabs, which were negative for routine methods [54]. The polymyxin B nonapeptide (PMBN) associated with a carbapenem allowed us to detect porin-deficient isolates with a sensitivity ranging from 89 to 93% and a specificity

ranging from 86 to 100%. For a diagnostic assay allowing the detection of this membrane-associated mechanism of resistance in *Enterobacteriaceae* [55]. The Revogene® Carba C assay (Meridian Bioscience, Cincinnati, OH, USA) performances were high as it was able to detect the five major carbapenemases (NDM, VIM, IMP, KPC, and OXA-48). The Revogene® Carba C assay showed excellent sensitivity and specificity for the five most common carbapenemases. It is well adapted to the CPE and CP-*Pseudomonas aeruginosa* epidemiology of many countries worldwide, which makes it suitable for use in the routine microbiology laboratory [56]. Recently reported assays use molecular-based carbapenemase detection to improve the detection rate based on nano-level particles or membranes, as well as to detect various targets at once.

CONCLUSION

The various CRE and CP-CRE detection methods discussed here address different needs and purposes; there is no one test that is ideal in all situations. When choosing a detection strategy, cost, time to results, test performance (accuracy), and the information provided by the test are all factors that need to be considered.

Furthermore, culture-based methods are still essential for the initial detection of CRE. Several culture-based techniques for screening have been tested and designed for CRE detection. Chromogenic media are the new generation of media and can be considered as a truly rapid culture-based method for carbapenemase detection. This screening media offers a sensitive, convenient, and relatively low-cost method for identifying CRE species, and is able to detect even CRE species with relatively low carbapenem MICs. Transmission between animals and humans in either direction has so far been poorly investigated, but is required for an evidence-based public health risk assessment. Combining different complementary tests appears to be the strategy to adopt to correctly cover the various bacterial species and the great genetic diversity of the different classes of carbapenemases. Domestic laboratories should select the appropriate test method and apply it to the infection control environment.

요 약

카바페넴내성장내세균속균종(carbapenem-resistant *Enterobacteriaceae*, CRE)과 카바페넴분해효소 생성 장내세균과(carbapenemase-producing *Enterobacteriaceae*, CPE)의 정확한 구분과 CPE의 빠른 탐지는 임상 감염의 치료 및 관리에 중요하다. 선별방법은 주로 선택적 배지에서의 직장 면봉 표본 배양 후 카바페넴분해 효소의 활성도, 신속한 카바페넴의 불활성화 방법, 측방유동면역분석(lateral flow immunoassay, LFI), 메트릭스보조레이저 탈착/이온화이온사이클론 공명 질량분석법(matrix assisted laser desorption/ionisation time of flight mass spectrometry, MALDI-TOF MS)을 통해 표현형을 측정하는 분자기반 방법들이다. CRE, 특히 CPE의 적절한 시기에 정확한 탐지는 감염의 임상 치료 및 예방에 필수적이다. 다양한 표현형 검출방법 및 유전자-기반 검출방법이 카바페넴의 신속한 검출을 위해 이용 가능하며, 이들은 임상 미생물학 실험실에서 일상적으로 사용된다. 신속한 처리 시간으로 현장에서 치료를 위한 검사 방법을 사용하는 CRE에 대한 능동적인 감시활동에서 카바페넴분해효소를 생성하는 CRE의 탐지는 중요한 가치를 갖는다. 따라서 카바페넴분해효소의 확산을 통제하기 위해서는 전세계의 많은 검사실에서 신뢰할 수 있고 신속하고 고효율적이며, 간편하고 저비용의 검사법을 사용해야 할 것이다. 환자의 적용에서도 최적의 효과를 가지려면 CRE에 대한 신속한 검사를 통해 항균제의 관리 개입이나 다양한 형태의 임상 의사의 치료에 결정적인 지원을 재현성있게 나타나야 할 것이다. 최적의 검사법을 위해서는 보완되는 검사법을 결합하여 다양한 내성 박테리아 종을 감별하고 다양한 종류의 카바페넴분해효소의 유전적 다양성을 발굴하여 최상의 감염관리 전략을 포괄하는 시스템이 마련되어야 할 것으로 사료된다.

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