Assessing O.K.N.V.I. RESIST-5 performance for post-mortem biological samples: A prospective pilot study

IULIANA DIAC[1,2], LAVINIA NECULAI-CÂNDEA[3], MIHAELA HORUMBĂ[4], CĂTĂLIN DOGĂROIU[5], MIHNEA COSTESCU[2,6] and ARTHUR-ATILLA KERESZTESI[7,8]

[1]PhD School, 'Carol Davila' University of Medicine and Pharmacy, 020021 Bucharest; [2]Department of Clinical Legal Medicine and Forensic Pathology, Mina Minovici National Institute of Legal Medicine, 042122 Bucharest; [3]Department of Legal Medicine and Forensic Pathology, Forensic Clinical County Service Constanța, Faculty of Medicine, 'Ovidius' University of Constanța, 900439 Constanța; [4]Department of Cardiology, County Clinical Emergency Hospital Constanta, 900591 Constanta; [5]Department of Morphological Sciences, Discipline of Forensic Medicine and Bioethics, Faculty of Medicine, 'Carol Davila' University of Medicine and Pharmacy, 020021 Bucharest; [6]Department of Functional Sciences, Discipline of Pharmacology and Pharmacotherapy, Faculty of Medicine, 'Carol Davila' University of Medicine and Pharmacy, 020021 Bucharest; [7]Department of Legal Medicine and Forensic Pathology, Covasna County Institution of Forensic Medicine, 520068 Covasna; [8]Faculty of Medicine, Transilvania University of Brasov, 500036 Brasov, Romania

Received February 2, 2023; Accepted September 13, 2023

DOI: 10.3892/etm.2023.12302

Abstract. In recent years, the emergence of carbapenem-resistant strains has been increasing worldwide, including in Romania. Rapid tests for post-mortem examinations have been researched and currently have several applications. In the present study, we aimed to test the performance of O.K.N.V.I. RESIST-5 tests on impure post-mortem biological samples compared with a standard of pure cultures. When a death occurs during hospitalization and the issue of malpractice arises, the medico-legal practice would benefit from rapid tests applicable to post-mortem samples. Thus, detection and differentiation of the five targeted carbapenemases, namely oxacilinase-48, *Klebsiella pneumoniae* carbapenemase, New Delhi metallo-β-lactamase, Verona integron-encoded metallo-β-lactamase and imipenemase, could be useful in guiding sampling for third-party microbiological assessment and could also be an asset from an epidemiological standpoint. The present prospective and observational pilot study included medico-legal autopsy cases performed at Mina Minovici National Institute of Legal Medicine (Romania) between June and July 2022. A total of two sets of O.K.N.V.I. RESIST-5 tests were performed: Test I, which was performed on-site from biological samples obtained during autopsy; and Test II, which was performed on pure cultures after sample inoculation and incubation. Total of 39 O.K.N.V.I. RESIST-5 rapid tests were performed on 19 biological samples, at least one sample per case. The O.K.N.V.I. RESIST-5 tests performed on-site showed an overall sensitivity of 92.3% with a 100% specificity. The results obtained through rapid tests using post-mortem impure samples were comparable to the results obtained from sample cultures with good sensitivity and specificity. Through post-mortem screening for carbapenem resistance, it would be possible to narrow down the number of cases that require further bacteriological assessment.

Correspondence to: Dr Mihnea Costescu, Department of Functional Sciences, Discipline of Pharmacology and Pharmacotherapy, Faculty of Medicine, 'Carol Davila' University of Medicine and Pharmacy, 8 Eroii Sanitari Bulevardul, 020021 Bucharest, Romania
E-mail: mihnea.costescu@umfcd.ro

Dr Lavinia Neculai-Cândea, Department of Legal Medicine and Forensic Pathology, Forensic Clinical County Service Constanța, Faculty of Medicine, 'Ovidius' University of Constanța, 2 Aleea Zmeurei, 900439 Constanța, Romania
E-mail: lavinia_candea@ymail.com

Key words: rapid test, O.K.N.V.I. RESIST-5, post-mortem sampling, legal-medicine, carbapenem-resistance

Introduction

Carbapenems used to be antibiotics reserved for the treatment of patients with infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. In recent years, the emergence of carbapenem-resistant strains has been increasing worldwide and as such also in Romania (1,2). Based on the Ambler classification of β-lactamases, according to their molecular structure, these enzymes with versatile hydrolytic capacities, known as carbapenemases (3), are divided into class A (clavulanic acid inhibitory enzymes), B (metallo-β-lactamases) and D (oxacillinases) (4).

Class A β-lactamases include the *Klebsiella pneumoniae* carbapenemase (KPC), the first reported carbapenem-hydrolysing β-lactamase. KPC was initially extracted from a carbapenem-resistant (CR) strain of *Klebsiella pneumoniae* in a North Carolina hospital in 2001 (5). KPC-producing

organisms are usually resistant to penicillin, cephalosporins and carbapenems. Moreover, their growth is not inhibited by clavulanic acid or other common β-lactamase inhibitors such as sulbactam and tazobactam. Thus, only a few therapeutic options remain, namely colistin, polymyxin B and tigecycline (6).

The New Delhi metallo-β-lactamase (NDM) emerged from the Indian subcontinent as a major cause of carbapenem resistance and has since been identified all over the world (7). NDM strains are usually resistant to most antibiotics except for tigecycline and colistin. Regarding NDM resistance, of great concern is its frequent association with *Escherichia coli*, a ubiquitous and non-nosocomial microbe, making it extremely difficult to contain community transmission (2). Other MBLs are the Verona integron-encoded metallo-β-lactamase (VIM) and the imipenemase (IMP). Both were reported worldwide, with a higher prevalence in southern Europe and Asia (8).

Class D β-lactamases include oxacilinase-48 (OXA-48), a carbapenem-hydrolysing β-lactamase, initially identified in Europe, which has been increasingly reported in various species of *Enterobacteriaceae*. Though colistin and tigecycline are most likely to be active against OXA-48 producer strains, some resistance has been reported (9). Fosfomycin as part of combination therapy could be used as a last resort, though the emergence of resistance is highly likely (10).

Point-of-care (POC) tests for the identification of carbapenemases were developed during the second half of the last century and gradually gained a foothold as an aid in the fast and accurate diagnosis of CR strains. They are easy to perform, fast, inexpensive and allow for on-site application. The technologies used in POC tests include particle agglutination assays, monoclonal antibody-based immunodot tests, immunochromatography and immunofiltration (11). Most immunochromatographic tests (ICT) used in microbiological laboratories are lateral flow double-antibody sandwich assays. Antigens from the biological sample migrate along a nitrocellulose membrane by capillarity. In doing so, they encounter the conjugate pad which contains dye-labelled antibodies, forming an antigen-antibody complex. A second wave of antibodies is immobilized at the level of the test line; when the antigen-antibody complex encounters the test line antibodies a colour reaction occurs and produces a visible band (positive result). The control line contains immobilized anti-IgG antibodies aimed at the excess dye-labelled antibodies (with or without the antigen) and is used to confirm that the sample migrated along the nitrocellulose membrane (Fig. 1) (4).

In terms of post-mortem use, POC tests are already in use for the diagnosis of acute coronary syndrome (rapid troponin T test) (12), sepsis (a semi-quantitative procalcitonin test) (13) as well as several antibody-based tests for the diagnosis of hepatitis virus C and HIV (14,15). More recently, the post-mortem application of a SARS-COV-2 rapid antigen test was assessed on nasopharyngeal exudate samples collected from 30 autopsy cases in a pilot study with promising results; this showed an overall lower sensitivity compared with reverse transcription-quantitative PCR considered to be due to viral loads below the threshold of cultivability in cases with false-negative results (16).

Rapid diagnostic tests are currently commercially available for the detection of carbapenem resistance. The O.K.N.V.I. RESIST-5 (Coris BioConcept) targets KPC, NDM, VIM, IMP and OXA-48 types of carbapenemases and is performed on pure microbial cultures, in laboratories, without the use of specialized equipment (17). The detection limit, as provided by the manufacturer, varies according to carbapenemase and was determined using purified recombinant proteins obtained from KPC (0.5 ng/ml), NDM (0.0625 ng/ml), VIM (0.23 ng/ml), IMP (0.781 ng/ml) and OXA-48-like carbapenemases (0.25 ng/ml) (18). To the best of our knowledge, currently, there are no ICT or POC tests available for the identification of CR strains directly from clinical samples (without prior isolation), as the sensitivity of these assays is not high enough (19). However, simplified procedures are under research in both laboratory and clinical settings, aiming to concentrate sample antigens and eliminate any potential interference with impurities. Simplified procedures have been tested on blood cultures (NG-Test Carba 5 assay) (20), rectal swabs (RESIST-4 O.K.N.V. K-SeT test) (21,22) and urine samples using a dedicated device (19).

Not yet available for routine clinical practice, rapid tests for the detection of antimicrobial resistance using immunochromatographic analysis would guide the diagnosis and management of infections with CR strains. Some of its potential applications lie in the field of legal medicine/forensic pathology, where other rapid tests have already proven useful [troponin (12), C reactive protein (23), HIV (15) etc.]. Post-mortem sampling offers the advantage of targeted access to biological samples, irrespective of the area of interest, by allowing sampling of fluid collections, including pus, as well as profound structures, such as the distal respiratory tract. Moreover, the post-mortem dehydration results in a concentrated sample of antigens.

In Romania, autopsy-performing facilities are dependent on external laboratories for microbiological assessment; thus, sampling is strictly case-oriented and not routinely performed. A cheaper, faster and easier method of assessing antibiotic resistance would potentially redefine the incidence and prevalence of ESBL-producing microorganisms. For patients who die during hospitalization where the issue of malpractice arises, medico-legal practice would benefit from rapid tests validated for post-mortem samples. Thus, detection and differentiation of the five targeted carbapenemases, KPC, NDM, VIM, IMP and OXA-48, is useful in guiding sampling for external microbiological assessment and could also be an asset from an epidemiological standpoint.

The current study aimed to test the performance of the O.K.N.V.I. RESIST-5 test on impure post-mortem biological samples compared with a standard consisting of pure microbial cultures obtained from the initial sample through inoculation and incubation.

Materials and methods

The present study was a prospective, single-centre pilot study conducted at Mina Minovici National Institute of Legal Medicine (Bucharest, Romania) aimed to compare CR identifying rapid test results obtained from post-mortem samples either on-site or as pure microbial cultures after inoculation, isolation and identification of the causative agents.

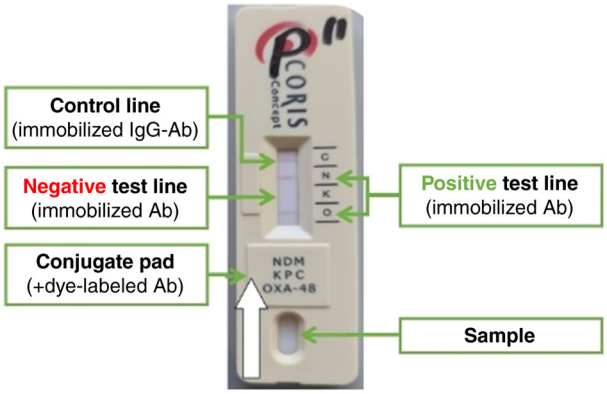

Figure 1. Lateral flow double-antibody sandwich assay is a common immunochromatographic technique used in microbiological laboratories. The white arrow depicts the direction in which sample antigens migrate across the nitrocellulose membrane through capillarity. The dye-labelled antibodies in the conjugate pad form immune complexes with the antigen. The immune complex then reacts with the immobilized antibodies at the test line, producing a positive result. If there is no corresponding antigen in the sample, the antibodies in the test line have no substrate with which to interact so no line becomes visible (negative test). The control line is proof that sample particles migrated through the nitrocellulose membrane. Ab, antibody.

Sample collection. Bronchial swabs were preferred to tracheal swabs due to agonal aspiration of gastric content. The trachea was dissected just above the bronchial bifurcation and four sterile swabs were inserted distally into the bronchi. For other biological samples, four swabs per site were collected through a sterile technique (using sterile gloves and dissection instruments, immediately after removing the wound dressings or opening the anatomical cavities, prior to other extensive manipulation of body or organs, wound swabbing, collection of meningeal fluid etc.). Following sample collection, two swabs were used for O.K.N.V.I. RESIST-5 testing and the other two were sent to a third party laboratory for further testing the results obtained from the latter were outside the scope of the study.

Patient inclusion. A total of 200 autopsies were performed at The Mina Minovici National Institute of Legal Medicine between June and July 2022. Patient inclusion criteria were: Hospitalization over 3 days and a carbapenem-resistant (CR) strain identified during hospitalization.

Autopsies that did not have post-mortem bacteriology results available for review were excluded from the present study. A total of 21 medico-legal autopsies met the inclusion criteria and rapid on-site tests were performed in 14 cases out of the 21. The location of post-mortem microbiological sampling was selected in a case-oriented manner, guided by the anatomical area or biological sample from which a positive culture for CR strains was identified during hospitalization, thereby suspected as a possible cause of death.

Examination technique and O.K.N.V.I. RESIST-5 *testing*. A total of two sets of O.K.N.V.I. RESIST-5 tests (Test I and II) were performed for each collected specimen. Test I was performed on-site from post-mortem samples, while Test II was performed on pure cultures obtained after isolation and served as standard, as described below:

The swab for Test I was tested on-site, immediately after post-mortem sampling. The swab was immersed into a semi-rigid tube containing 12 drops of buffer (LY-D) provided by the manufacturer of the rapid diagnostic kits (Coris BioConcept), mixed thoroughly, and the diluted sample transferred using a pipette onto each of the two cassettes of O.K.N.V.I. RESIST-5. The results were read within 15 min of sampling (Fig. 2; bottom green arrow, Test I).

The swab for Test II was tested locally. The swab was used to inoculate Brilliance™ ESBL Agar (Oxoid, Ltd.; Thermo Fisher Scientific, Inc. Fisher Scientific), a chromogenic screening plate with a selective medium for the detection of extended-spectrum β-lactamase-producing strains. The Brilliance™ ESBL Agar plates were examined after incubation at 36±2˚C, for 18-24 h. If bacterial growth showed mixed populations, an isolation plate was performed for further differentiation, by aseptically transferring a small amount of an isolated colony using a smear loop onto CLED Agar plate (Oxoid, Ltd.; Thermo Fisher Scientific, Inc.), followed by incubation at 36±2˚C for 18-24 h. Once pure cultures were obtained, O.K.N.V.I. RESIST-5 tests were performed according to manufacturer instructions (Fig. 2; top green arrow, Test II). Results were obtained within 1-2 days of sampling depending on culture growth rate and incubation facilities.

Statistical analysis. Categorical variables were reported as counts or frequencies (%). Continuous data are presented as mean ± standard deviation for normally distributed data, while non-normally distributed data are presented as median and interquartile range (IR) or range (R). $P<0.05$ was considered to indicate a statistically significant difference. The statistical analysis was performed using IBM® SPSS® Statistics software for Windows, version 24.0 (IBM Corp.). Sensitivity, specificity, and positive and negative predictive values were assessed using crosstabs column and row percentages with syntax for 95% CIs. The effect of postmortem interval upon concordance of the two sets of test for each type of carbapenemase was analyzed by dividing the samples into two groups and since >20% of the cells in the contingency table contained an expected count of five or less, Fisher's exact test was used (Table SII).

Ethics. This study was approved by the Institutional Research Ethics Committee from Mina Minovici National Institute of Legal Medicine, Bucharest (approval no. 5152/2022).

Results

Descriptives of included cases, sampling and rapid testing. The present study included four female (28.8%) and 10 male (71.4%) patients with a median age of 63 years (IR, 48.7-77.0 years). Patients had a hospital stay of 43.5 days (IR, 23.5-58.2 days) with a maximum of 107 days. The post-mortem interval (PMI), i.e. time to autopsy and sample collection, was 3 days (IR, 1.7-4.0 days), the longest being 6 days. A total of 10 patients (71.4%) were hospitalized due to violent circumstances such as traumatic brain injury, polytrauma (motor vehicle collision) or thermal burn injuries, of whom six (60.0%) had pre-existing conditions (Table I). Patient characteristics

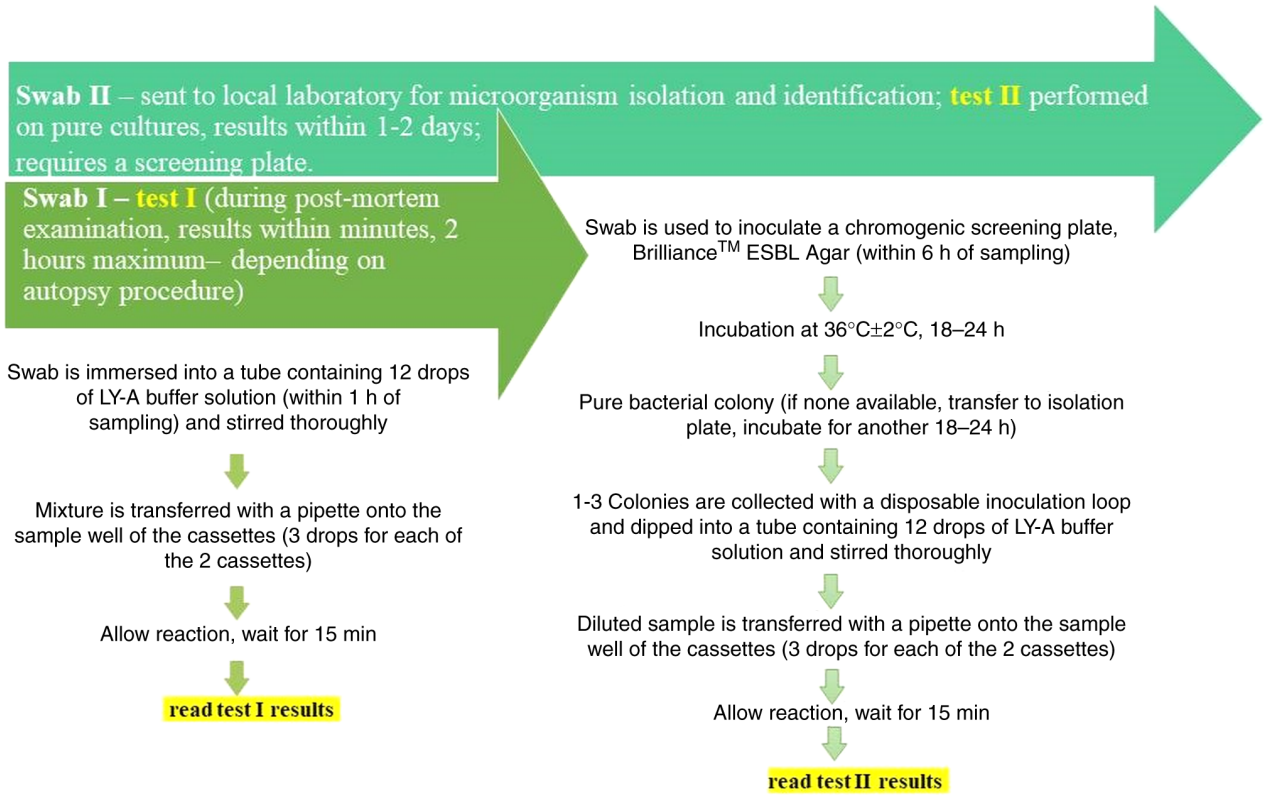

Figure 2. Testing sequence to determine carbapenemase-resistant strains present in post-mortem samples. O.K.N.V.I. RESIST-5 tests were performed from the post-mortem samples either on-site (in the autopsy room; Test I) or in a specialized laboratory after isolation of pure cultures (Test II).

and additional data extracted from medical records obtained before death are available in Table I.

A total of 39 O.K.N.V.I. RESIST-5 rapid tests were performed on 19 biological samples collected from 14 patients; one sample per case and, in five cases, two samples. For two of the samples, Test I was negative and Test II was not performed since the 24 h incubation showed no microbial growth on the Oxoid Brilliance ESBL Agar screening plate. The first negative sample (Table I, patient no. 6) was a tracheal swab collected from a patient who tested positive for CR *Klebsiella pneumoniae* during hospitalization (wound swab). They had been treated empirically with ceftriaxone for 1 day, followed by combination therapy with colistin and tigecycline for 10 days. The second negative sample (Table I, patient no. 11) was a meningeal swab collected from a patient with meningoencephalitis of unknown origin. The patient had been treated empirically with meropenem for 3 days followed by combination therapy with meropenem, vancomycin and levofloxacin.

Moreover, fourteen of the inoculated samples generated monocultures, while three samples required transfer to a second plate for microbial pure culture. An invalid result was obtained from one rapid test performed on a microbial culture (Test II); therefore a third test was performed. The following Enterobacteriaceae species were identified in pure culture: *Klebsiella pneumoniae* (16 cultures), *Pseudomonas aeruginosa* (two cultures) and *Escherichia coli* (one culture). For 10 of the pure cultures, the same CR strain showed positive results for two different types of carbapenemases (Fig. 3).

Antimicrobial therapy. All patients included in the present study had positive microbiology results for carbapenem-resistant microorganisms while in-hospital (Table I). All but one (13 patients) received empirical antibacterial therapy with broad-spectrum antibiotics. Of the 13 patients, 10 received single-agent antimicrobial therapy as empirical treatment. Ceftriaxone was the most popular option for empirical antibacterial therapy being used in 10 of the 13 patients, with a median duration of treatment of 7 days (range, 1-14 days).

All 14 patients received targeted antibacterial therapy for a variable number of days after CR strain identification. Combination therapy regiments most often included colistin (9/14 patients) for a mean duration of 10 days (range, 3-14 days), tigecycline (seven patients) for a mean duration of 10 days (range, 2-14 days), piperacillin/tazobactam (six patients) for a mean duration of 14 days (range, 8-14 days), vancomycin (three patients) for a mean duration of 10 days (range, 2-10 days) and Recarbrio (imipenem/cilastatin/relebactam; Merck Sharp & Dohme-Hoddesdon) in two patients for a mean duration of 13 days (range, 5-21 days).

In the context of the present study, for any type of carbapenemase, the results were interpreted as follows: A negative result during Test I with a positive result during Test II as a false negative; a positive result during both sets of tests as a true positive; a negative result during both sets of tests as a true negative; and a positive result during Test I with a negative result during Test II as a false positive. The Test I results for the two samples that showed no microbial growth were excluded from the interpretation.

Table I. Study patient characteristics and additional data extracted from medical records obtained before death.

Patient no.	Age, years/sex	Cause of hospitalization	Pre-existing conditions	Hospital stay, days	Post-mortem interval, days	Empirical antimicrobial treatment (duration, days)	Positive microbiology during hospitalization	Targeted antimicrobial treatment (duration, (days)	Superimposed infection during hospitalization	CR positive sample location
1	50/F	Spontaneous pneumothorax	Chronic obstructive pulmonary disease; Obesity	59	4	Ceftriaxone (5); Moxifloxacin (5); Meropenem (7)	*Acinetobacter baumannii* (MDR); *Klebsiella pneumoniae*	Colistin (14); Tigecycline (10); TZP (13)	*Providencia stuartii*	Respiratory tract
2	83/M	Traumatic brain injury	Epilepsy; HTN	31	2	Ceftriaxone (12)	*Klebsiella pneumoniae* (CR)	Colistin (14); Tigecycline (10)	*Pseudomonas aeruginosa*; *Providencia stuartii*	Respiratory tract
3	45/M	Perforated colonic diverticulitis		38	3	Colistin (7)	*Klebsiella pneumoniae* (CR)	Recarbrio (21); Linezolid (14)	*Acinetobacter baumannii*	Respiratory tract
4	88/F	Thermal burns	T2DM; Heart failure	4	1		*Klebsiella* pneumoniae (CR)	Colistin (3); Tigecycline (2)	-	Wound swab
5	57/M	Traumatic brain injury		27	1	Ceftriaxone (14)	*Klebsiella pneumoniae* (CR)	Recarbrio (5); Vancomycin (2)	-	Respiratory tract
6	40/M	Septic arthritis of the hip	Heroin addiction; Hepatitis C virus infection	11	3	Ceftriaxone (1)	*Escherichia coli* ESBL; *Klebsiella pneumoniae* (CR)	Colistin (10); tigecycline (10)	-	Respiratory tract, wound swab
7	74/M	Traumatic brain injury	Alcoholism; HTN	53	2	Ceftriaxone (7)	*Klebsiella pneumoniae* (CR)	Colistin (14) tigecycline (14)	*Burkholderia cepacia complex*	Respiratory tract
8	69/M	Polytrauma		31	4	Ceftriaxone (6)	*Acinetobacter baumannii* (MDR); *Klebsiella pneumoniae*	Colistin (10); tigecycline (10); TZP (14)	*Pseudomonas aeruginosa*	Respiratory tract

Table I. Continued.

Patient no.	Age, years/sex	Cause of hospitalization	Pre-existing conditions	Hospital stay, days	Post-mortem interval, days	Empirical antimicrobial treatment (duration, days)	Positive microbiology during hospitalization	Targeted antimicrobial treatment (duration, (days)	Superimposed infection during hospitalization	CR positive sample location
9	37/F	Thermal burns		58	6	Ceftriaxone (6)	*Escherichia coli* (ESBL); *Acinetobacter baumannii* (MDR)	Colistin (10); tigecycline (14); TZP (8)	*Pseudomonas aeruginosa* (MDR)	Wound swab
10	89/M	Polytrauma		49	3	Ceftriaxone (10); Moxifloxacin (14)	*Pseudomonas aeruginosa* (MDR); *Acinetobacter baumannii*	Colistin (10); TZP (14)	*Klebsiella pneumoniae* (CR)	Respiratory tract
11	54/M	Meningoence-phalitis	T2DM; HTN	13	5	Meropenem (3)	*Salmonella spp.*	Meropenem (10); vancomycin (10); levofloxacin (7)	*Acinetobacter baumannii*	Respiratory tract
12	59/M	Thermal burns	T2DM; Obesity	79	2	Ceftriaxone (14)	*Pseudomonas aeruginosa* (MDR); *Acinetobacter baumannii*	TZP (14); Vancomycin (10)	*Klebsiella pneumoniae* (CR) A. baumannii	Respiratory tract
13	75/M	Traumatic brain injury	Cirrhosis	55	1	Ciprofloxacin (7)	*Proteus mirabilis* (extended spectrum beta-lactamase)	Amoxicillin clavulanate (14)	*Providencia stuartii* (CR)	Respiratory tract
14	67/F	Smoke inhalation	HTN	107	3	Doxycycline (10); Levofloxacin (7); TZP (10)	*Acinetobacter baumannii* *Pseudomonas aeruginosa*	TZP (14); Colistin (14)	*Klebsiella pneumoniae* (CR)	Respiratory tract

CR, carbapenem-resistant; HTN, arterial hypertension; COPD, chronic obstructive pulmonary disease; T2DM, Type 2 diabetes mellitus; MDR, multidrug-resistant; TZP, piperacillin/tazobactam; N/A, not applicable.

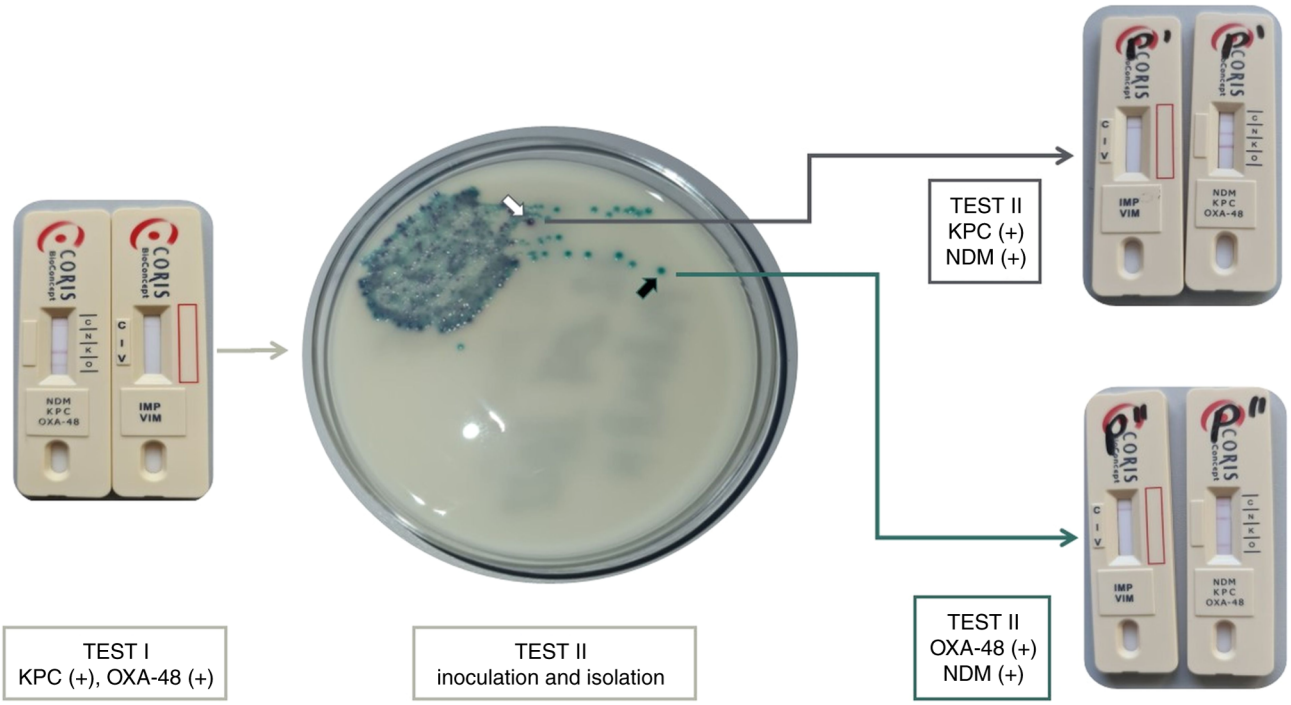

Figure 3. Some of the samples require inoculation and incubation on an additional agar plate to obtain pure cultures for microorganism identification. Rapid tests were performed separately for each of the pure cultures obtained. Test I: O.K.N.V.I. RESIST-5 from a bronchial swab was positive for KPC and OXA-48. After 24 h of incubation, two types of colonies grew on the agar plate: *Klebsiella pneumoniae* (white arrow) and *Escherichia coli* (black arrow). Test II: O.K.N.V.I. RESIST-5 performed on the *Klebsiella pneumoniae* pure culture was positive for NDM and KPC. The O.K.N.V.I. RESIST-5 for E. coli was positive for NDM and OXA-48. NDM was missed by the first rapid test and classified as a false negative. KPC and OXA-48 were both correctly identified (true positives) on Test I despite the carbapenem resistance coming from two different CR strains. KPC, *Klebsiella pneumoniae* carbapenemase; OXA-48, oxacilinase-48; NDM, New Delhi metallo-β-lactamase.

For tests performed on-site from post-mortem biological samples (Test I results-interpretation), eight false negative results were obtained: Five for NDM; two for OXA-48; and one for VIM. Five false negative results were found in cases that received CO for at least 10 days (two NDM false negative results from cases with a combination of antibacterial therapy with TZP for 14 days; one NDM and one OXA-48 false negative result from cases with a combination of antibacterial therapy with TZP for 13 days and TG for 10 days). Two false negative results (one VIM and one NDM) were encountered in a case that received RECARBRIO for 5 days and Vanco for 2 days before death, and an OXA-48 false negative in a case with the following antibacterial therapy: TZP for 14 days and Vanco for 10 days. True negative results for all five carbapenemases were found in four samples, three samples from cases that received a combination of antibacterial therapy with CO and TG for at least 10 days, with or without TZP, and one sample from a case with RECARBRIO for 21 days (Table SI).

Time to sampling (PMI). The present study aimed also to evaluate whether the PMI had any influence on the results of the O.K.N.V.I. RESIST-5 performed on-site (Test I) or from a pure culture (Test II). Given the small sample size consistent results were computed without statistical tests for each carbapenemase type, the true negative and true positive results were both considered as concordance of tests. Due to the small sample size, we took into account the length of time between death and biological sample collection (postmortem interval-PMI) as a binary variable dividing the samples into two groups (PMI under/over 24 h) (Table II) and used 2x5 contingency table with Fisher's exact test (Table SII) to determine if there was a significant association with concordance (C) for each type of carbapenemase. The O.K.N.V.I. RESIST-5 test concordance for each carbapenemase type does not seem to differ with PMI groups, respectively with time elapsed from death to biospecimen collection (P=0.989).

Sensitivity and Specificity of O.K.N.V.I. RESIST-5 *tests.* The number of positive and negative results for the O.K.N.V.I. RESIST-5 tests performed during both Test I and II are shown in Table II.

The sensitivity, specificity, positive predictive value and negative predictive value of the O.K.N.V.I. RESIST-5 tests performed on-site from a biological sample were computed for each of the types of carbapenemase (IMP, VIM, NDM, KPC and OXA-48) and obtained via the positive and negative results of both sets of O.K.N.V.I. RESIST-5 tests, Test II values serving as standard (Table III). The O.K.N.V.I. RESIST-5 tests performed on-site from biological samples (Test I) showed an overall sensitivity of 92.3% with 100% specificity, 100% positive predictive value and an 80.0% negative predictive value.

Discussion

Carbapenem-resistant strains are increasingly described in both hospital and community-acquired infections (24) as well as in animal reservoirs (25). Consequently, medical negligence accusations focusing on healthcare-associated infections that

Table II. Carbapenem-resistance is identified through RESIST-5 O.K.N.V.I. performed on-site (Test I) and from a pure culture (Test II).

CP	Imipenemase			Verona integron-encoded metallo-β-lactamase			New Delhi metallo-β-lactamase			*Klebsiella pneumoniae* CP			Oxacilinase-48		
PMI groups	Test I	Test II	C	Test I	Test II	C	Test I	Test II	C	Test I	Test II	C	Test I	Test II	C
<24 h															
1	-	-	Yes	+	+	Yes	-	-	Yes	-	-	Yes	-	-	Yes
2	-	-	Yes	-	+	No	-	-	Yes	+	+	Yes	-	-	Yes
3	-	-	Yes	-	-	Yes	-	+	No	-	-	Yes	+	+	Yes
>24 h															
1	-	-	Yes	-	-	Yes	+	+	Yes	-	-	Yes	+	+	Yes
2	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes
3	-	-	Yes	-	-	Yes	+	+	Yes	-	-	Yes	+	+	Yes
4	-	-	Yes	-	-	Yes	+	+	Yes	-	-	Yes	-	+	No
5	-	-	Yes	-	-	Yes	-	+	No	-	-	Yes	-	+	No
6	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes
7	-	IC	N/A	-	IC	N/A	-	IC	N/A	-	IC	N/A	-	IC	N/A
8	-	-	Yes	-	-	Yes	-	+	No	-	-	Yes	+	+	Yes
9	-	-	Yes	-	-	Yes	+	+	Yes	-	-	Yes	+	-	No
10	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes
11	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes	-	-	Yes
12	-	-	Yes	-	-	Yes	-	+	No	+	+	Yes	+	-	No
13	-	-	Yes	-	-	Yes	-	+	No	+	-	No	+	+	Yes
14	-	-	Yes	-	-	Yes	-	-	Yes	+	+	Yes	-	-	Yes
15	-	IC	N/A	-	IC	N/A	-	IC	N/A	-	IC	N/A	-	IC	N/A
16	-	-	Yes	-	-	Yes	+	+	Yes	-	-	Yes	+	+	Yes
Test I/Test II C rates for CP		17/17(100%)			16/17 (94.1%)			12/17 (70.6%)			16/17 (94.1%)			13/17 (76.5%)	

Test I was performed on 19 biological samples and Test II on 17 pure cultures due to 2 (10.53%) samples having no bacterial growth for 48 h. Detailed results of tests for samples divided by postmortem interval (under/over 24 h) with C rates of each CP evaluated for 17 sets of tests (89.47% of collected samples). CP, carbapenemase; PMI, postmortem interval. IC, incomplete set of tests; N/A, not applicable; C, concordance; +, positive result; -, negative result.

Table III. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the O.K.N.V.I.

Carbapenemase	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value, % (95% CI)	Negative predictive value, % (95% CI)	Accuracy % (95% CI)
IMP	N/A	N/A	-	-	-
VIM	50.0 (1.3-98.7)	100 (78.2-100)	100 (2.5-100)	93.8 (69.8-99.8)	94.1 (71.3-99.9)
NDM	50.0 (18.7-81.3)	100 (59.0-100)	100 (47.8-100)	58.3 (34.8-93.3)	70.6 (44.0-89.7)
KPC	100 (39.8-100)	92.9 (66.1-99.8)	75.0 (29.0-100)	100 (76.8-100)	94.1 (71.3-99.9)
OXA-48	75.0 (44.0-97.5)	77.8 (30.8-89.1)	75.0 (54.1-100)	77.8 (66.4-100)	76.5 (50.1-93.2)
O.K.N.V.I. RESIST-5	92.3 (63.9-99.81)	100 (39.8-100)	100 (73.5-100)	80.0 (37.9-96.3)	94.12 (71.3-99.9)

RESIST-5 tests performed on-site from a biological sample (Test I) were computed for each of the carbapenem-resistant strains (IMP, VIM, NDM, KPC and OXA-48) and overall for the test kit. N/A, not applicable. IMP, imipenemase; VIM, Verona integron-encoded metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; OXA-48, oxacilinase-48.

go unnoticed or untreated are becoming prominent issues in forensic medicine. Thus, post-mortem bacteriology analysis is of paramount importance both in establishing the cause of death and, in a wider sense, identifying CR strain outbreaks in hospitals.

Post-mortem bacteriology is rarely requested during medico-legal autopsies at The Mina Minovici National Institute of Legal Medicine where the present study was performed (26) due to its costly and time-consuming nature. However, rapid diagnostic tests are emerging, some with applications in forensic medicine (12-15,23). The present study aimed to assess their performance in the detection of carbapenem-resistant strains from post-mortem samples (bronchial swabs, wound swabs and meningeal fluid) without the need for incubation, isolation and identification. Blood cultures were also collected for each case without being included in the O.K.N.V.I. RESIST-5 testing sequence, due to the potentially lower concentration of microorganisms. The overall sensibility and sensitivity of the O.K.N.V.I. RESIST-5 tests performed on-site were satisfactory.

The O.K.N.V.I. RESIST-5 tests we used in the current study could identify up to five CR strains. However, as no IMP-producing strains were recovered and only two VIM strains among the 19 samples were collected, we hypothesise that less specialized versions of multiplex tests like RESIST-4 OKNV (27) or RESIST-3 OKN (28) may be sufficient for post-mortem screening of CR strains.

Traditionally, POC tests guide the course of treatment. The present study proposes a legal-medicine-oriented application by guiding the course of complementary examinations for determining probable cause of death. For example, in medico-legal cases with macroscopic signs of infection (with or without a previously identified causative agent), positive screening for carbapenem resistance could guide the pathologist towards requesting a complete post-mortem microbiology assessment.

Although limited by the small sample size, the present results showed a good correlation between the tests performed on-site from biological samples ('point of autopsy') and results from pure cultures after 24-48 h of incubation.

In medico-legal autopsies, the pathologist often needs to answer questions regarding the contribution of healthcare-associated infections to thanatogenesis. On the one hand, a legislative framework provides specific case definitions based on clinical and laboratory findings recorded during hospitalization. On the other, post-mortem analysis is of tantamount importance in determining if correct in-hospital management was achieved regarding the diagnosis and treatment of healthcare-associated infections.

Performing O.K.N.V.I. RESIST-5 tests on biological samples collected on-site during medico-legal autopsy are easy, fast and inexpensive. The results obtained through rapid tests using post-mortem impure samples are comparable to results from sample cultures with good sensitivity and specificity. Through post-mortem screening for carbapenem resistance, it is possible to improve the screening of cases that require further bacteriological assessment. Nonetheless, larger studies are required to confirm the present findings.

Acknowledgements

Publication of this paper was supported by the University of Medicine and Pharmacy Carol Davila, through the institutional program Publish not Perish.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

ID conceptualized the study. ID, MH and AAK discussed and designed the methodology. ID, MC and CD performed the investigation. ID and MC confirm the authenticity of all the raw data. ID provided the resources for the study. ID, MC, MH and LNC performed the data validation. ID and LNC provided the data curation. ID, MH and LNC performed the statistical analysis, figure design and interpretation of the results. ID and MH prepared the original draft. ID, MH and AAK worked on writing, reviewing and English editing. MC and CD supervised

the study. ID insured project administration. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate.

Relevant ethical issues were identified and discussed with the institutional ethical committee. All medico-legal autopsies in Romania are mandated by the police or the state prosecutor. Institutional access to data was obtained. Informed consent of relatives for data and sample collection was waived by the Institutional Research Ethics Committee of the Mina Minovici National Institute of Legal Medicine, Bucharest, considering that all microbiological samples were requested to ascertain probable cause of death. The study was conducted in accordance with the Declaration of Helsinki.

Patient consent to publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Nordmann P and Cornaglia G: Carbapenemase-producing *Enterobacteriaceae*: A call for action! Clin Microbiol Infect 18: 411-412, 2012.
2. Nordmann P: Carbapenemase-producing *Enterobacteriaceae*: Overview of a major public health challenge. Med Mal Infect 44: 51-56, 2014.
3. Queenan AM and Bush K: Carbapenemases: The versatile beta-lactamases. Clin Microbiol Rev 20: 440-458, table of contents, 2007.
4. Noster J, Thelen P and Hamprecht A: Detection of multi-drug-resistant enterobacterales-from ESBLs to carbapenemases. Antibiotics (Basel) 10: 1140, 2021.
5. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K and Tenover FC: Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 45: 1151-1161, 2001.
6. Paterson DL: 313-infections due to other members of the *Enterobacteriaceae*, including management of multidrug-resistant strains, Goldman L and Schafer AI (eds). Goldman's Cecil Medicine (Twenty Fourth Edition), W.B. Saunders, pp1874-1877, 2012. https://doi.org/10.1016/B978-1-4377-1604-7.00313-4.
7. Poirel L, Hombrouck-Alet C, Freneaux C, Bernabeu S and Nordmann P: Global spread of New Delhi metallo-β-lactamase 1. Lancet Infect Dis 10: 832, 2010.
8. Nordmann P, Naas T and Poirel L: Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 17: 1791-1798, 2011.
9. Stewart A, Harris P, Henderson A and Paterson D: Treatment of infections by OXA-48-producing *Enterobacteriaceae*. Antimicrob Agents Chemother 62: e01195-18, 2018.
10. Poirel L, Potron A and Nordmann P: OXA-48-like carbapenemases: The phantom menace. J Antimicrob Chemother 67: 1597-1606, 2012.
11. Anfossi L, Giovannoli C and Baggiani C: Introductory chapter: Rapid test-advances in design, formats, and detection strategies [Internet]. Rapid Test-Advances in Design, Format and Diagnostic Applications. InTech, 2018. Available from: http://dx.doi.org/10.5772/intechopen.79805.
12. Cina SJ, Brown DK, Smialek JE and Collins KA: A rapid post-mortem cardiac troponin T assay: Laboratory evidence of sudden cardiac death. Am J Forensic Med Pathol 22: 173-136, 2001.
13. Ramsthaler F, Kettner M, Mall G and Bratzke H: The use of rapid diagnostic test of procalcitonin serum levels for the postmortem diagnosis of sepsis. Forensic Sci Int 178: 139-145, 2008.
14. Rose CE, Duncan L and Hawes AM: Validation of OraQuick HCV rapid antibody test in postmortem specimens. Acad Forensic Pathol 10: 81-86, 2020.
15. Karhunen PJ, Brummer-Korvenkontio H, Leinikki P and Nyberg M: Stability of human immunodeficiency virus (HIV) antibodies in post-mortem samples. J Forensic Sci 39: 129-135, 1994.
16. Zacharias M, Stangl V, Thüringer A, Loibner M, Wurm P, Wolfgruber S, Zatloukal K, Kashofer K and Gorkiewicz G: Rapid antigen test for postmortem evaluation of SARS-CoV-2 carriage. Emerg Infect Dis 27: 1734-1737, 2021.
17. Hong J, Kang D and 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 (Basel) 10: 460, 2021.
18. OKNVI RESIST-5, Coris BioConcept. https://ifu.corisbio.com/corisbio/all?keycode=COR58R1104 (Visited on 02.06.2023).
19. Boutal H, Moguet C, Pommiès L, Simon S, Naas T and Volland H: The revolution of lateral flow assay in the field of AMR detection. Diagnostics (Basel) 12: 1744, 2022.
20. Giordano L, Fiori B, D'Inzeo T, Parisi G, Liotti FM, Menchinelli G, De Angelis G, De Maio F, Luzzaro F, Sanguinetti M, *et al*: Simplified testing method for direct detection of carbapenemase-producing organisms from positive blood cultures using the NG-test carba 5 assay. Antimicrob Agents Chemother 63: e00550-19, 2019.
21. Gallah S, Villageois-Tran K, Godmer A, Arlet G, Rottman M, Benzerara Y and Garnier M: Four-hour immunochromatographic detection of intestinal carriage of carbapenemase-producing *Enterobacteriaceae*: A validation study. J Clin Microbiol 59: e02973-20, 2021.
22. Rescape & Resist-Bc, Coris BioConcept. https://www.corisbio.com/pdf/Products/Coris-D-CUS-10-RES04-2023-02-V1.pdf (Visited on 02.06.2023).
23. Soejima M and Koda Y: Evaluation of point-of-care testing of C-reactive protein in forensic autopsy cases. Forensic Sci Int 237: 27-29, 2014.
24. Tilahun M, Kassa Y, Gedefie A and Ashagire M: Emerging carbapenem-resistant *Enterobacteriaceae* infection, its epidemiology and novel treatment options: A review. Infect Drug Resist 14: 4363-4374, 2021.
25. Silva JMD, Menezes J, Marques C and Pomba CF: Companion animals-an overlooked and misdiagnosed reservoir of carbapenem resistance. Antibiotics (Basel) 11: 533, 2022.
26. Diac I, Keresztesi AA, Cerghizan AM, Negrea M and Dogăroiu C: Postmortem bacteriology in forensic autopsies-a single center retrospective study in Romania. Diagnostics (Basel) 12: 2024, 2022.
27. Cointe A, Bonacorsi S, Truong J, Hobson C, Doit C, Monjault A, Bidet P and Birgy A: Detection of carbapenemase-producing *Enterobacteriaceae* in positive blood culture using an immunochromatographic RESIST-4 O.K.N.V. Assay. Antimicrob Agents Chemother 62: e01828-18, 2018.
28. Wareham DW and Abdul Momin MH: Rapid detection of carbapenemases in *Enterobacteriaceae*: Evaluation of the resist-3 O.K.N. (OXA-48, KPC, NDM) lateral flow multiplexed assay. J Clin Microbiol 55: 1223-1225, 2017.