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FULL PAPER

Evaluation of the RESIST-4 O.K.N.V. K-SeT for detection of carbapenemases in Gram-negative bacilli

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SUMMARY

Enterobacterales as opportunistic pathogens are commonly associated with nosocomial infections. With increasing frequency, Gram-negative bacilli, especially *Klebsiella pneumoniae* strains, are multidrug-resistant or pandrug-resistant. Carbapenems were used as the drugs of choice for the treatment of infections caused by multidrug-resistant Gram-negative bacilli. The aim of this study was to assess the usefulness of the RESIST-4 O.K.N.V. K-SeT for the rapid detection and identification of the most important carbapenemases (OXA-48, KPC, NDM, VIM) in Enterobacterales bacilli. The study involved the isolates of 97 Enterobacterales strains. The ability to produce carbapenemases was determined by the immunochromatographic RESIST-4 O.K.N.V. K-SeT test. This test detected carbapenemases OXA-48, KPC, NDM, and VIM. For the RESIST-4 O.K.N.V. K-SeT test, a positive result was obtained for 93 strains (95.9%). Four strains negative in the RESIST-4 O.K.N.V. K-SeT were positive in the Eazyplex®SuperBugCRE and PCR. These strains produce VIM enzymes. RESIST-4 O.K.N.V. K-SeT test is rapid, simple to perform and can be used for fast detection of the most important carbapenemases (OXA-48, KPC, NDM, VIM) among Gram-negative bacilli.

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INTRODUCTION

Enterobacterales are one of the frequent bacteria causing hospital and community infections. These bacteria easily acquire resistance to antibiotics, and often one strain can produce several mechanisms of resistance. The most common mechanism of antibiotic resistance in Enterobacterales is the production of extended spectrum beta-lactamases (ESBLs), but the most dangerous are carbapenemases. Strains producing carbapenemases are common in the gastrointestinal tract of healthy people. Carbapenemases efficiently hydrolyse penicillins, all cephalosporins, carbapenems, and beta-lactamase inhibitors. Enterobacterales can produce different classes of carbapenemases, i.e., class A (*Klebsiella pneumoniae* carbapenemases), class B (metallo-beta-lactamases-like VIM, NDM, IMP) and class D (OXA-48 group) (Cantón *et al*., 2012). The name KPC suggests that these carbapenemases occur only in *K. pneumoniae* strains, but they have also been detected in other species, such as *Serratia marcescens* or *Enterobacter cloacae*. Strains producing KPC enzymes are frequently isolated in the United States and Israel (Oeschlaegeret *et al*., 2010). In Poland, the predominant carbapenemases are class B (especially New-Delhi metallo-beta-lactamases, NDM) (Literacka *et al.*, 2018). In our hospital, the first metallo-beta-lactamase-producing Enterobacterales were isolated in 2006 (Sękowska *et al*., 2010). Since then, the number of Gram-negative isolates producing NDM- or VIM-beta-lactamases has increased. The OXA carbapenemases are frequently found in *Acinetobacter baumannii*. In Enterobacterales strains, the OXA-48 enzyme is prevailing, and in *A. baumannii* - OXA-23 or OXA-40 (Oeschlaeger *et al.,* 2010). Recently, strains producing more than one enzyme conditioning resistance, such as ESBLs and carbapenemase, are more frequently isolated. In such cases, therapeutic options are limited, and the detection of resistance mechanism to antibiotics is difficult. According to the data of the European Antimicrobial Resistance Surveillance System, in Poland the share of *K. pneumoniae* strains resistant to carbapenems increased from 0.5% in 2015 to 8.1% in 2018.

Therefore, the aim of this study was to assess the usefulness of the RESIST-4 O.K.N.V. K-SeT for rapid detection of the most important carbapenemases (OXA-48, KPC, NDM, VIM) in Enterobacterales bacilli.

Key words:
Carbapenemases, detection, Enterobacterales, RESIST-4 O.K.N.V. K-SeT test.

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MATERIALS AND METHODS

The study included 97 non-replicate strains. Ninety one strains were isolated from clinical samples (*K. pneumoniae* - 70, *K. oxytoca* - 4, *S. marcescens* - 8, *Raoultella ornithinolytica* - 1, *E. cloacae* - 3, *Enterobacter kobei* - 1, *Citrobacter koseri* - 1, *Citrobacter freundii* - 1, *Escherichia coli* - 2) and 6 strains were isolated from the hospital environment (*K. pneumoniae* - 3, *S. marcescens* - 3). All of the analysed strains were resistant to imipenem and/or meropenem and/or ertapenem and the phenotypic methods (CIM and discs tests with inhibitors) were performed for all of them. The isolates were identified by the mass spectrometry system (matrix-assisted laser desorption ionization-time of flight mass spectrometry, Bruker). In the mass spectrometry method, the obtained identification index for 89 of the analysed strains was over 2,300, which means reliable identification at the genus and species level. For 8 strains, the mentioned score reached value between 2,000 and 2,300, which means reliable identification at the genus and probable identification at the species level.

The Resist-4 O.K.N.V. K-SeT immunochromatographic assay (Coris BioConcept) was performed according to the manufacturer's instructions. The test detected the OXA-48, KPC, NDM and VIM enzymes in several minutes (preparation 5 minutes, incubation-reaction 15 minutes, reading 1 minute). The test was also performed with the reference strains and 10 clinical strains (5 sensitive to carbapenems and 5 resistant to carbapenems, carbapenemase-negative). The positive controls were *K. pneumoniae* NCTC 13438 (OXA-48 positive), BAA-2146 (NDM-1-positive) and NCTC 13440 (VIM-positive).

The potential of carbapenemases synthesis was also evaluated using loop mediated-method isothermal amplification (LAMP), detecting blaKPC, blaNDM, blaOXA-48, blaVIM, bla OXA-181 and blaCTX-M-1-group and blaCTX-M9 group genes (coding ESBLs enzyme). Eazyplex®SuperBugCRE test (Amplex Diagnostics) was used along with Genie II instrument (OptiGene). This test was performed according to the manufacturer's instructions (preparation 1 minute, incubation at 99°C 2 minutes, reaction/reading 15 minutes).

For selected strains (negative in the Resist-4 O.K.N.V. K-SeT and positive in the Eazyplex®SuperBugCRE test) additional PCR was performed (Sękowska *et al*., 2020).

RESULTS

The immunochromatographic test allowed the identification of the enzyme produced. The production of carbapenemases was detected in 93 (95.9%) of the strains analysed. This test confirmed the production of VIM enzyme in 47 strains, NDM enzyme in 40 strains, KPC enzyme in 4 strains, and OXA-48 enzyme in 2 strains (*Table 1*). In four *K. pneumoniae* strains, the results were impossible to interpret: a smudged streak appeared in place of the coloured line. In the Eazyplex®SuperBugCRE test, 51 strains were identified as VIM-positive, 40 as NDM-positive, 4 as KPC-positive and 2 as OXA-48-positive. Four strains negative in the RESIST-4 O.K.N.V. K-SeT and positive in the Eazyplex®SuperBugCRE were positive in PCR. These strains produce VIM enzymes.

Table 1 - *Results obtained with Resist-4 O.K.N.V. K-SeT and Eazyplex®SuperBugCRE (n=97).*

Sample type	*Species*	*Enzyme*	*Resist-4*	*Eazyplex*
Clinical samples (n=91)	*K. pneumoniae* (n=70)	NDM	39	39
		VIM	22	26
		KPC	3	3
		OXA-48	2	2
		None	4	0
	K. oxytoca (n=4)	VIM	3	3
	S. marcescens (n=8)	VIM	8	8
	R. ornithinolytica (n=1)	VIM	1	1
	E. cloacae (n=3)	VIM	4	4
	E. kobei (n=1)	VIM	1	1
	C. koseri (n=1)	KPC	1	1
	C. freundii (n=1)	VIM	1	1
	E. coli (n=2)	VIM	2	2
Samples from hospital environment (n=6)	*K. pneumoniae* (n=3)	VIM	2	2
		NDM	1	1
	S. marcescens (n=3)	VIM	3	3

DISCUSSION

Numerous phenotypic and genotyping methods have been used in the laboratory isolation, diagnosis, and confirmation of carbapenem-resistant Enterobacterales (Wareham *et al*., 2017). KPC, OXA-48-like, NDM and VIM beta-lactamases are predominant resistance mechanisms among Gram-negative bacilli in Europe. Therefore, the Resist-4 O.K.N.V. K-SeT immunochromatographic assay seems to be very useful. Glupczynski *et al.* (2019) analysed 479 isolates of Gram-negative bacilli by the Resist-4 K-SeT test. These authors reported 100% specificity for the detection of all enzymes (OXA-48, KPC, NDM, VIM), 99% sensitivity for the detection of VIM enzymes, and 100% for OXA-48-like, KPC and NDM. On the other hand, Baeza *et al.* (2019) reported 100% specificity and 84.2% sensitivity for 146 Enterobacterales strains. In the present study, sensitivity was 100% for OXA-48, KPC and NDM and 92.1% for VIM. Specificity was 100% for all of the enzymes analysed. MacDonald and Chibabhai (2019) analysed 100 Gram-negative strains and 2 strains gave false negative results on the Resist-4 test. In turn, Kolenda *et al.* (2018) analysed 69 isolates and 2 strains gave negative results in Resist-4 test. In this study, the results were impossible to interpret for four strains. Three of these strains were highly mucoid, and this probably influenced the ability to read the test. Vanstone *et al*. (2018) confirmed the difficulty of interpreting the result for mucoid strains. However, Greissl *et al*. (2019) analysed 169 strains resistant to carbapenems, reporting specificity of 100% and sensitivity from 99.2% to 100%. Differences were associated with the type of culture medium. The authors suggested obtaining the inoculum from a medium with sufficient zinc. In the present study, all of the analysed strains were cultured on agar with 5% sheep blood.

The Resist-4 O.K.N.V. K-SeT test was cheap (about $10), fast (several minutes), and simple to perform, without a need for additional specialized laboratory equipment compared to the Eazyplex® SuperBug-CRE or PCR. A limitation of the RESIST-4 O.K.N.V. K-SeT test is the need to use earlier culture of the strain on a solid medium. Although the assay does not currently extend to the detection of IMP-like MBLs, the ability to detect four of the most prevalent carbapenemases in Enterobacterales is a significant advantage.

This test can be used for fast detection and identification of the most important carbapenemases among Gram-negative bacilli, especially in small laboratories.

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