



Confirmatory testing for the carbapenem-non-susceptible and carbapenemase producing *Enterobacterales* by whole genome sequencing and rapid PCR assay in the National Microbiology Reference Laboratory of Latvia

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INTRODUCTION

Referral of the carbapenem-non-susceptible and/or carbapenemases producing clinical isolates of *Enterobacterales* to the National Microbiology Reference Laboratory (NMRL) for confirmatory testing is mandatory in Latvia. Recently, the confirmatory PCR assay was complemented by the whole genome sequencing (WGS).

OBJECTIVES

We evaluated the results of the rapid PCR assay with WGS as a control.

METHODS

397 *Enterobacterales* isolates (26 *Citrobacter* spp., 8 *Enterobacter* spp., 33 *Escherichia* sp., 325 *Klebsiella* spp., 1 *Morganella* sp., 2 *Proteus* sp., 1 *Raoultella* sp., 1 *Serratia* sp.) received from central and regional hospitals (Jul–2022 to Feb–2024) were assessed by WGS and Xpert Carba-R (Cepheid, USA). Libraries prepared with DNAPrep (Illumina, USA) and sequenced either on Illumina NextSeq550 or NovaSeq6000 in 150PE or 250PE configuration (ave. 5 million paired-end reads per sample). Bioinformatic analysis performed by an in-house pipeline Ardetype^[1] v0.1.0-dev, employing Fastp v0.22.0, Shovill v1.1.0, AMRFinderPlus v3.10.42 (database v2022-10-11.2), ResFinder v4.1.11 (database v2023-03-29), RGI v5.2.1 (card_v3.1.4), 7-gene MLST v2.19.0 (PubMLST schemes), and Kleborate v2.2.0. At least two votes from three antimicrobial resistance gene identification resources were considered for the presence of a gene.

RESULTS

- WGS enabled differentiated detection of:
 - bla*_{OXA-244} in *E. coli* ST10 (2 of 3 isolates), ST167 (2/3)
 - bla*_{NDM-5} in *E. coli* ST167 (2/3), ST361 (4/4)
 - bla*_{NDM-2} in *K. pneumoniae* ST147 (1/119)
 - bla*_{NDM-1} in *C. freundii* ST540 (6/9), *K. pneumoniae* ST45 (1/2), ST147 (70/119), ST147-1LV (1/1), ST147-3LV (1/1), *P. mirabilis* (1/1), *R. ornithinolytica* (1/2)
 - bla*_{KPC-2} in *K. pneumoniae* ST37 (1/5), ST39 (2/2), ST307 (3/9), ST392 (1/1), ST395-1LV (10/10)
 - bla*_{KPC-3} in *E. coli* ST73 (1/1), *K. pneumoniae* ST512 (39/40), ST5020 (1/5), *M. morganii* (1/1)
 - bla*_{VIM-1} in *C. freundii* ST8 (1/4) (Table 1).
- Beside these double tested isolates, the systematic genomic surveillance has enabled NMRL to spot the emergence of the NDM-5 carbapenemase in *K. pneumoniae* ST395, isolated Oct–2023 (not tested by Carba-R).

REFERENCES

[1] BODRENKO J., VANGRAVS R. Ardetype: pipeline for antimicrobial resistance detection and species specific typing. Github. <https://github.com/NMRL/Ardetype>

TABLE 1 The number of the carbapenem-non-susceptible and/or carbapenemases producing clinical isolates of *Enterobacterales* with carbapenemase genes detected by the whole genome sequencing (WGS) and the rapid PCR assay. The results are grouped according to the genera of the isolates and the carbapenemase gene families detected by PCR.

GENERA	PCR RESULT	SPECIES (n)	WGS RESULT							
			<i>bla</i> _{KPC-2}	<i>bla</i> _{KPC-3}	<i>bla</i> _{NDM-1}	<i>bla</i> _{NDM-2}	<i>bla</i> _{NDM-5}	<i>bla</i> _{OXA-244}	<i>bla</i> _{OXA-48}	<i>bla</i> _{VIM-1} negative
<i>Citrobacter</i>	NDM, OXA-48	✓ <i>C. freundii</i> ST540(6)			6				6	
	OXA-48	✓ <i>C. amalonaticus</i> ST-(1)							19	
		✓ <i>C. braakii</i> ST88(1)								
		✓ <i>C. freundii</i> ST8(3), ST22(3), ST114(1), ST116(1), ST415(1), ST481(1), ST540(3)								
✓ <i>C. koseri</i> ST-(1)										
✓ <i>C. murlinae</i> ST-(1)										
✓ <i>C. werkmanii</i> ST-(1)										
		✓ <i>C. youngae</i> ST218(1)								
	VIM	✓ <i>C. freundii</i> ST8(1)							1	
<i>Enterobacter</i>	OXA-48	✓ <i>E. hormaechei</i> ST-(1), ST114(2), ST336(1), ST350(2), ST974(1), ✓ <i>E. roggenkampii</i> ST-(1)						8		
<i>Escherichia</i>	KPC	✓ <i>E. coli</i> ST73(1)		1						
	NDM	✓ <i>E. coli</i> ST167(1), ST361(4)					5			
		✓ <i>E. coli</i> ST10(2), ST167(2)						4		
	OXA-48	✓ <i>E. coli</i> ST10(1), ST38(1), ST58(1), ST69(1), ST88(1), ST155(2), ST327(1), ST349(1), ST398(3), ST405(1), ST657(1), ST1057(1), ST1125(1), ST1167(1), ST2015(4), ST2178(1), ST6969(1)						23		
<i>Klebsiella</i>		✓ <i>K. pneumoniae</i> ST512(1)								1
	KPC	✓ <i>K. pneumoniae</i> ST37(1), ST39(2), ST307(3), ST392(1), ST395-1LV(10)	17							
			✓ <i>K. pneumoniae</i> ST512(39), ST5020(1)		40					
			✓ <i>K. pneumoniae</i> ST512(39), ST5020(1)							
	NDM	✓ <i>K. pneumoniae</i> ST45(1)			1					
	NDM, OXA-48	✓ <i>K. pneumoniae</i> ST147(70), ST147-1LV(1), ST147-3LV(1)			72			72		
		✓ <i>K. pneumoniae</i> ST147(1)				1		1		
	OXA-48	✓ <i>K. aerogenes</i> ST-(2), ST93(10)							186	
		✓ <i>K. michiganensis</i> ST98(1)								
		✓ <i>K. oxytoca</i> ST37(2)								
		✓ <i>K. pneumoniae</i> ST23(1), ST36(18), ST37(4), ST45(1), ST86(4), ST147(48), ST188-1LV(1), ST215(32), ST218(2), ST219(33), ST229(1), ST268(2), ST281(1), ST307(6), ST380(1), ST395(1), ST405(1), ST420(1), ST490(1), ST873(1), ST1235-1LV(1), ST1537(1), ST1623(1), ST4327(1), ST5020(4)								
		✓ <i>K. variicola</i> ST1562(1), ST681(1), ST695(1)								
		✓ <i>K. variicola</i> ST1562(1), ST681(1), ST695(1)								
	negative	✓ <i>K. aerogenes</i> ST93(2)							7	
✓ <i>K. michiganensis</i> ST226(2)										
✓ <i>K. pneumoniae</i> ST16(1), ST396(1)										
✓ <i>K. variicola</i> ST3956(1)										
<i>Morganella</i>	KPC	✓ <i>M. morganii</i> ST-(1)		1						
<i>Proteus</i>	NDM	✓ <i>P. mirabilis</i> ST-(1)			1					
	OXA-48	✓ <i>P. mirabilis</i> ST-(1)						1		
<i>Raoultella</i>	NDM	✓ <i>R. ornithinolytica</i> ST-(1)			1					
<i>Serratia</i>	OXA-48	✓ <i>S. nevei</i> ST-(1)						1		

CONCLUSIONS

Since the whole genome sequencing provides a clear resolution of the carbapenemases in *Enterobacterales* combined with the genomic surveillance, the results question the need for the PCR assay in the era of whole genome sequencing.