The Antibiotic Resistance Status of Gram-negative Bacilli causing Hospitalized Pneumonia Directly Detected by the MPL real-time PCR to Detect the Antibiotic Resistance Genes

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Background

The causative agents of pneumonia requiring hospitalization are often Gram-negative bacilli, and this presents a challenge in treatment because Gram-negative bacilli, especially *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli*, have a relatively high rate of antibiotic resistance, including to potent and broad-spectrum antibiotics such as third and fourth generation cephalosporins and carbapenems.

Aims of the study

To investigate the antibiotic resistance of Gram-negative bacilli causing hospital-acquired pneumonia, utilizing the multiplex realtime PCR (MPL-rPCR) assay developed by the Vietnam Institute of Clinical Microbiology Research and Development (VCM)

Materials and methods

This retrospective cross-sectional study analyzes results from the MPL-rPCR assay, which simultaneously detects multiple pathogens and antibiotic resistance genes of Gram-negative bacilli in lower respiratory tract specimens from hospitalized patients with acute lower respiratory tract infections in 2024.

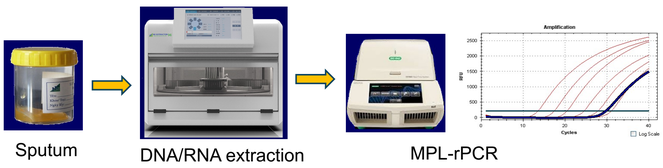

Figure 1. Workflow used to detect pathogens and antibiotic-resistant genes using MPL-rPCR

Table 1. Pathogens and antibiotic-resistantgenes detected by the MPL-rPCR developed by the VCM

S. aureus	*L. pneumophila*	*C. neoformans*	*Influenza virus A*
S. epidermidis	*C. psittaci*	*P. jirovecci*	*Influenza virus B*
E. faecium	*C. trachomatis*	*C. glabrata*	*Influenza virus C*
S. maltophilia	*B. pertussis*	*P. marneffei*	*Parainfluenza 1 virus*
E. faecalis	*Mycoplasma genitalium*	*C. albicans*	*Parainfluenza 2 virus*
K. pneumoniae	*M. pneumoniae*	*C. tropicalis*	*Parainfluenza 3 virus*
E. coli	*B. parapertussis*	*C. krusei*	*Rhinovirus*
E. cloacae		*C. kefyr*	*RSV*
E. aerogenes	*C. pneumoniae*	*A. fumigatus*	*hMPV*
Citrobacter freundii	*S. agalactiae*	*A. niger*	*Measle virus*
Proteus mirabilis	*S. pyogenes (GAS)*	*A. flavus*	*Corona virus*
Morganella morgani	*S. suis*	*Candida auris*	*CMV*
Providencia	*H. influenzae B*	*F.oxysporum+F.vertisillioides*	*Bocavirus*
B. cepacia	*M. catarrhalis*	*A. terrus*	*EBV (HHV4)*
E. meningoseptica	*S. pneumoniae*	*H. capsulatum*	*Adenovirus*
P. aeruginosa	*H. influenzae*	*F. solani*	*Corona SARS*
A. baumannii	*M. tuberculosis*	*Coccidioides immitis/posadasii*	*Varicella/Zoster virus*
F. nucleatum	*Nocardia asteroides*	*Sporothrix globosa (HEX)*	*SARS-COV2 (N2)*
B. pseudomallei	*Non-Tuberculosis Mucobacteria*	*Sporothrix schenckii/brasiliensis*	*Rubella virus*
		Mucormycosis (Rhizopus oryzae)	

Methicillin-resistant	MecA
Vancomycin-resistant	VanA, VanB
ESBL	TEM, SHV, CMY, CTX-M
AmpC	CIT, ACC, FOX, MÕ, DHA, EBC
Carpabenemase	OXA-23, OXA-24, OXA-51, OXA-58
Colistin-resistant	MCR1-10

Results

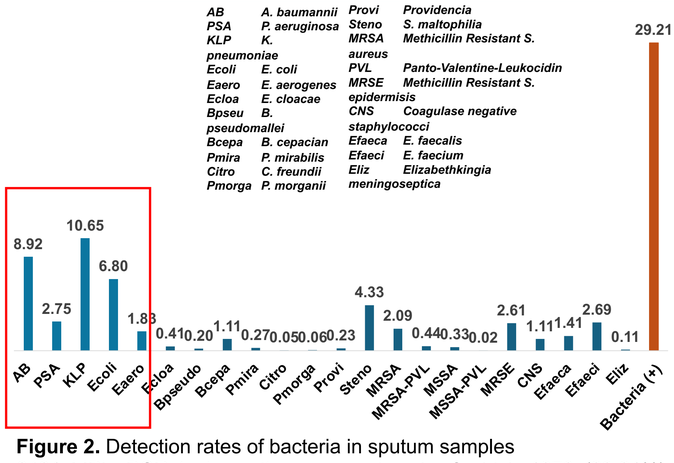

Figure 2. Detection rates of bacteria in sputum samples

6401 MPL-rPCR test results were analysed, of which 1870 (29.21%) detected bacterial agents. The detection rates for Gram-negative bacilli potentially carrying antibiotic-resistant genes were A. *baumannii* (8.92%), *P. aeruginosa* (2.75%), *K. pneumoniae* (10.65%), *E. coli* (8.80%), and *K. aerogenes* (1.83%).

Table 2. Detection rates of antibiotic-resistant gene groups in Gram-negative bacilli

CBR genes were highly prevalent in *A. baumannii* (81.09%) and *K. aerogenes* (80.34%), followed by *K. pneumoniae* (54.69%), *P. aeruginosa* (46.02%), and *E. coli* (29.89%). ESBL/AmpC rates were 84.62%/9.41% for *K. aerogenes*, 56.60%/14.22% for *K. pneumoniae*, and 44.60%/23.45% for *E. coli*

	Bacteria		CBR		ESBL		AmpC	
	N	%	N	%	N	%	N	%
AB	571	8.92	463	81.09				
PSA	176	2.75	81	46.02				
KLP	682	10.65	373	54.69	386	56.60	97	14.22
Ecoli	435	6.80	130	29.89	194	44.60	102	23.45
Eaero	117	1.83	94	80.34	99	84.62	11	9.40

CBR : Carbapenem Resistant Gene
ESBL: Extended-spectrum β-lactamase

Table 3. Ambler classification in specimens carrying CBR-genes

For specimens where CBR genes were detected but *A. baumannii* and *P. aeruginosa* were absent, the detection rates of Ambler class B, D, and A of CRB genes were 38.6%, 45.96%, and 15.44%

CBR-gene (+)							
N		(N) CBRgene			(%) CBRgene		
		Class B	Class D	Class A	Class B	Class D	Class A
AB/PSA	518	148	361	9	28.57	69.69	1.74
Not AB/PSA	272	105	125	42	38.60	45.96	15.44

Table 4. ESBL and AmpC detection guiding antibiotic choice

In specimens where CBR gene were not detected but ESBL and AmpC were present, the detection rates for ESBL+AmpC were 22.36%, ESBL 50.31%, and AmpC 27.33%.

	N	%	Carbapenem	Cefepim
ESBL+AmpC	36	22.36	[+]	
ESBL	81	50.31	[+]	
AmpC	44	27.33		[+]
Total	161	100.00		

Conclusions

By comprehensively analyzing information on the direct detection of bacterial agents, particularly Gram-negative bacilli, from lower respiratory tract specimens, this study highlights the assay's value in helping clinicians optimize antibiotic selection

References

1. Principe, L., Lupia, T., Andriani, L., Campanile, F., Carcione, D., Corcione, S., ... & Di Bella, S. (2022). Microbiological, clinical, and PK/PD features of the new anti-Gram-negative antibiotics: β-lactam/β-lactamase inhibitors in combination and cefiderocol—an all-inclusive guide for clinicians. *Pharmaceuticals*, *15*(4), 463.2. Hall, B. G., & Barlow, M. (2005). Revised Ambler classification of β-lactamases. Journal of Antimicrobial Chemotherapy, 55(6), 1050-1051.