

ORIGINAL ARTICLE

Antibiotic Resistance Determinants and Virulence Factors of Hypervirulent and Carbapenem Non-Susceptible *Pseudomonas aeruginosa*

Jian-Ming Zhu¹, Ru-Jin Jiang², Jin-Lan Wu³, Xing-Bei Weng⁴, Li-Ping Ling¹

¹ Department of Medical Laboratory, Hangzhou Linping Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China

² Department of Medical Laboratory, Hangzhou Linping First Hospital, Hangzhou, Zhejiang, China

³ Department of Traditional Chinese Medicine, Hangzhou Linping Hospital of Integrated Traditional Chinese and Western Medicine, Hangzhou, Zhejiang, China

⁴ Department of Medical Laboratory, Ningbo First Hospital, Ningbo, Zhejiang, China

SUMMARY

Background: The aim was to investigate the distribution of antibiotic resistance determinants and virulence factors in a group of carbapenem non-susceptible *Pseudomonas aeruginosa* (*P. aeruginosa*).

Methods: From March 2018 to May 2019, a total of 98 *P. aeruginosa* samples were collected from 6 hospitals in Ningbo and Hangzhou, Zhejiang Province, China. Drug susceptibility tests to 13 antimicrobial agents were conducted. The presence of antibiotic resistance determinants and virulence factors were investigated by PCR, including 39 β -lactamase genes, 14 aminoglycoside modifying enzyme genes, 10 16SrRNA methylase genes, and 11 virulence genes. Phylogenetics of 98 *P. aeruginosa* was analyzed by sample cluster analysis (UPGMA).

Results: PCR revealed the presence of 7 β -lactamase genes, 5 aminoglycoside modifying enzymes, 1 16S rRNA methylase gene, and 8 virulence genes in total, at least 2 β -lactamase genes and 4 virulence genes were positive in every isolate. In addition, regional differences in distributions of resistance and virulence genes remained between 2 cities. Sample cluster analysis showed that the strains had obvious aggregation and were divided into several clusters, strains in the same cluster were isolated from different hospitals, even from different cities.

Conclusions: Carrying resistance genes *bla*PDC and *bla*OXA-50 group and virulence genes *plcH*, *aprA*, and *algD* were the important epidemiological characteristics of this group of *P. aeruginosa*. The present findings provide insights into the mechanisms of hypervirulence as well as resistance to β -lactams and aminoglycosides. To the best of our knowledge, this is the first report of *bla*PDC, *bla*OXA-50, and *aph*(3')-XV in *P. aeruginosa* in China. (Clin. Lab. 2022;68:1-4. DOI: 10.7754/Clin.Lab.2021.210801)

Correspondence:

Jian-Ming Zhu
Department of Medical Laboratory
Hangzhou Linping Hospital of Traditional Chinese Medicine
No. 101 Yuncheng Street
Tangqi Town
Liping District
Hangzhou, 311106
Zhejiang
China
Phone: + 86 571 89020552
Fax: + 86 571 86381115
Email: zhujianming001@126.com

Xing-Bei Weng
Department of Medical Laboratory
Ningbo First Hospital
No. 31 Guangji Street
Haishu District
Ningbo, 315010
Zhejiang
China
Phone: + 86 57487085148
Email: wxb6006@hotmail.com

and

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Supplementary Tables and Figures

Table S1. PCR primer sequences of resistance determinants and virulence factors in *Pseudomonas aeruginosa*.

Class	Gene	Primer sequence (5' → 3') [product length]
Class A β - lactamases	<i>bla</i> TEM	P1: AGGAAGAGTATGATTCAACA P2: CTCGTCGTTTGGTATGGC [512bp]
	<i>bla</i> SHV	P1: TGC GCAAGCTGCTGACCAGC P2: TTAGCGYTGCCAGTGCTCGA [305bp]
	<i>bla</i> CTX-M-1 group	P1: ATGGTTAAAAAATCACTGCGYCAGTTC P2: TCACAAACCGTYGGTGACGATTTAGCCGC [876bp]
	<i>bla</i> CTX-M-2 group	P1: ATGATGACGCAGAGCATTCGCCGCTCA P2: TCAGAAACCGTGGGTTACGATTTTCGC [876bp]
	<i>bla</i> CTX-M-8 group	P1: ATGATGAGACATCGCGTTAAGCGG P2: TTAATAACCGTCGGTGACGATTTTCGC [876bp]
	<i>bla</i> CTX-M-9 group	P1: ATGGTGACAAAGAGAGTGCAACGG P2: TTACAGCCCTTCGGCGATGATTCTCGC [876bp]
	<i>bla</i> CTX-M-25 group	P1: ATGATGAGAAAAAGCGTAAGGCGGGCG P2: TTAATAACCGTCGGTGACAATTCTGGC [876bp]
	<i>bla</i> PER	P1: AGTCAGCGGCTTAGATA P2: CGTATGAAAAGGACAATC [978bp]
	<i>bla</i> GES	P1: ATGCGCTTCATTACGCAC P2: CTATTTGTCCGTGCTCAGG [846bp]
	<i>bla</i> VEB	P1: GCGGTAATTTAACCAGA P2: GCCTATGAGCCAGTGTT [961bp]
	<i>bla</i> CARB	P1: AAAGCAGATCTTGTGACCTATTC P2: TCAGCGGACTGTGATGTATAAAC [588bp]
	<i>bla</i> KPC	P1: ATGTCACTGTATCGCCGTCTA P2: TTACTGCCCGTTGACGCCAA [882bp]
	<i>bla</i> PME-1	P1: GCGCGAACACTTGGCCCTCG P2: CGCCGGCCTGCCAGTGCGGT [380bp]
	<i>bla</i> BEL-1	P1: CGACAATGCCGCAGCTAACC P2: CAGAAGCAATTAATAACGCC [449bp]
Class B β - lactamases	<i>bla</i> IMP	P1: CGGCCKCAGGAGMGKCTTT P2: AACCAGTTTTGCTTACYAT [588]
	<i>bla</i> VIM	P1: CTTTACCAGATTGCTGATGG P2: GGYAGRCCGTGCCSSGGAAT [593]
	<i>bla</i> SIM	P1: ACAAGGGATTCGGCATCGTT P2: TTATCTTGAGTGTGCTCTGG [355]
	<i>bla</i> SPM	P1: CTGCTTGGATTCATGGGCGCG P2: CCTTTCCGCGACCTTGATCG [786]
	<i>bla</i> GIM	P1: CCTGTAGCGTTGCCAGCTTTA P2: CAGCCCAAGAGCTAATTGAGG [562]
	<i>bla</i> AIM	P1: CGTCGCTTCACCCTGCTGGGCAGC P2: AGGCGAGGCGACCGCCGTCAGGCC [535]
	<i>bla</i> NDM	P1: TCAGCGCAGCTTGTGCGCCATGCG P2: GCAACCGCGCCCACTTTGGCCCG [300]
	<i>bla</i> KHM	P1: ATGAAAATAGCTCTTGTATATCG P2: TCACTTTTAGCTGCAAGCGCTTC [726bp]
	<i>bla</i> DIM	P1: ATGAGAACACATTTACAGCGTTA P2: TCAATCAGCCGACGCGTTAGCGTT [756bp]
	<i>bla</i> TMB	P1: TATGCCTCAGCGCTGACTAAT P2: TCAGCGGTCCCGTGATTGGC [400bp]
	<i>bla</i> HMB	P1: TGACAGCACCATCGGCATTG P2: TGCATCGCCATTGTCCAT [320bp]
	<i>bla</i> FIM	P1: GCCGCCTTAACACCCGTCGT P2: CATTGATTAGAGTCGGCCG [360bp]

Class	Gene	Primer sequence (5' → 3') [product length]
Class C β - lactamases	<i>blaDHA</i> group	P1: AACTTTCACAGGTGTGCTGGGT P2: CCGTACGCATACTGGCTTTGC [405bp]
	<i>blaACT</i> group	P1: TCGGTAAAGCCGATGTTGCG P2: CTTCCACTGCGGCTGCCAGT [302bp]
	<i>blaCMY/LAT</i> group	P1: TGGCCAGAACTGACAGGCAA P2: TTTCTCTGAACGTGGCTGG [462bp]
	<i>blaCMY/MOX</i> group	P1: GCTGCTCAAGGAGCACAGGAT P2: CACATTGACATAGGTGTGGTG [520bp]
	<i>blaACC</i> group	P1: ACAGCCTCAGCAGCCGGTTA P2: TTCGCCGCAATCATCCCTAG [345bp]
	<i>blaFOX</i> group	P1: AACATGGGGTATCAGGGAGAT P2: CAAAGCGCGTAACCGGATTGG [190bp]
	<i>blaPDC</i>	P1: CTGCCTGTGCGGCATCGCCG P2: TCCTGGGCCAGGCATAG [300bp]
Class D β - lactamases	<i>blaOXA-10</i> group	P1: GTCTTTCRAGTACGGCATT P2: GATTTTCTTAGCGCAACTTA [822bp]
	<i>blaOXA-13</i> group	P1: CGGCATTAGCTAGTTCAATTACAG P2: AATGCACAAGATATTCAGGCGCAG [564bp]
	<i>blaOXA-18</i> group	P1: ATGCAACGGAGCCTGTCCAT P2: CTGCGAATACCAGACGATGG [360bp]
	<i>blaOXA-20</i> group	P1: GAATCCCGGGCTCAGCAGCG P2: CCACCAACCCATTTGACCAT [480bp]
	<i>blaOXA-46</i> group	P1: CCGTTCGTGATGAGTTCAGG P2: TCCGTTCTGTTGGCGTATCA [470bp]
	<i>blaOXA-50</i> group	P1: ATGCGCCCTCTCCTCTTCAG P2: GTAACCCAGGCGGAGACAT [420bp]
Aminoglycoside modifying enzymes	<i>aac(3)-I</i>	P1: ACCTACTCCCAACATCAGCC P2: ATATAGATCTCACTACGCGC [169bp]
	<i>aac(3)-II</i>	P1: ACTGTGATGGGATACGCGTC P2: CTCCGTCAGCGTTTCAGCTA [237bp]
	<i>aac(3)-III</i>	P1: CACAAGAACGTGGTCCGCTA P2: AACAGGTAAGCATCCGCATC [185bp]
	<i>aac(3)-IV</i>	P1: CTTCAGGATGGCAAGTTGGT P2: TCATCTCGTTCCTCCGCTCAT [286bp]
	<i>aac(6')-Ib</i>	P1: ATGACTGAGCATGACCTTGC P2: TTAGGCATCACTGCGTGTTT [519bp]
	<i>aac(6')-II</i>	P1: TTCATGTCCGCGAGACCCC P2: GACTCTCCGCCATCGCTCT [178bp]
	<i>ant(3'')-I</i>	P1: TGATTTGCTGGTTACGGTGAC P2: CGCTATGTCTCTTGCTTTTG [284bp]
	<i>ant(2'')-I</i>	P1: GAGCGAAATCTGCCGCTCTGG P2: CTGTTACAACGGACTGGCCGC [320bp]
	<i>ant(4')-I</i>	P1: CGTGGAGCGATATCGATTTTCG P2: TCTGGTTCGGCGGCCGGATGC [266bp]
	<i>aph(3')-Ib</i>	P1: TGCCGACGACGTGACTGATG P2: CTCCCAAACCTGTTCCGCCG [358bp]
	<i>aph(3')-IIb</i>	P1: ATGCATGATGCAGCCACCTCC P2: CTAGAAGAACTCGTCCAATAGCCT [804bp]
	<i>aph(3')-VIa</i>	P1: ATACAGAGACCACCATACAGT P2: GGACAATCAATAATAGCAAT [234bp]
	<i>aph(3')-XV</i>	P1: ATGACAGTCGCCCTCGACGAAGTA P2: TCGCAGGTGGAGCCTTTCGTCGAA [420bp]
	<i>aadA5</i>	P1: ATGGGTGAATTYTTCCTGCACAA P2: TCAACGCAAGATTCTCATTCGT [789bp]

Class	Gene	Primer sequence (5' → 3') [product length]
16S rRNA methylase	<i>armA</i>	P1: ATGGATAAGAATGATGTTGTTAAG P2: TTATTTCTGAAATCCACTAGTAATTA [315bp]
	<i>npmA</i>	P1: TTGGGTACTGGAGACGGTAG P2: CAGCTTTGTATTGTTCCGCTC [421bp]
	<i>rmtA</i>	P1: CCTAGCGTCCATCCTTTCCTC P2: AGCGATATCCAACACACGATGG [315bp]
	<i>rmtB</i>	P1: TGCGGCGCATCCTGACTGAGGA P2: GGGAAAGCTGACAGCCATGCGC [542bp]
	<i>rmtC</i>	P1: ATGAAAACCAACGATAATTATC P2: TTACAATCTCGATACGATAAAATAC [846bp]
	<i>rmtD</i>	P1: ATGAGCGAACTGAAGGAAAACTGCT P2: TCATTTTCGTTTCAGCACGTAAAACAG [744bp]
	<i>rmtE</i>	P1: TTGATCGATATTACTATGCAAATC P2: GTTTTGTATCACATAGAACATCTC [541bp]
	<i>rmtF</i>	P1: ATGGATGAACGAGCGCAGGC P2: GGCCAGATCGAGCACGCGCG [360bp]
	<i>rmtG</i>	P1: GCGCACGCATTGGCCGCTGA P2: GCGCACGCATTGGCCGCTGA [370bp]
	<i>rmtH</i>	P1: ATGACCATTGAACAGGCAGC P2: GCACAGCACATCGTGCAGGG [480bp]
Virulence factors	<i>plcH</i>	P1: ATGACCGAAAACTGGAAATTCGCG P2: TGGTCTGCGAACTCACCCAC [359bp]
	<i>aprA</i>	P1: ATGTCCAGCAATTCTCTTGCAATTG P2: GGCGCTGAACTGCCAGCGA [288bp]
	<i>algD</i>	P1: ATGCGAATCAGCATCTTTGGTTTG P2: GCCAGGTCCAGGTCGCCGT [291bp]
	<i>exoS</i>	P1: TCAGCAGAGTCCGTCTTTTCGCC P2: GCCAGGCGGGAGTGCTCCCGG [407bp]
	<i>exoT</i>	P1: TCAGCAGAACCCGTCTTTTCGT P2: GCCAGGCGGTGTGATCCTTC [407bp]
	<i>exoU</i>	P1: CCGTCGCAGGCAGCGCATAAGTCC P2: GAACGCCGCCGGCTCATACCTGA [420bp]
	<i>exoY</i>	P1: ACCATGCGTATCGACGGTCATC P2: TTGCTGAGATGCTGGTCGACAC [281bp]
	<i>toxA</i>	P1: GGCTGCTCCAGGCGCACCGCC P2: GCCGATCAGCCGTTTCGACCTC [438bp]
	<i>pyo</i>	P1: TGCCGGTACGACTCACGAGTG P2: GTTCTGGCTTCTGGAGGGGT [231bp]
	<i>oacIR</i>	P1: ATGAGTGGGTTGTGGTCCCTG P2: AGTCTCTTCGGACTGGAGATT [240bp]
	<i>norC</i>	P1: GGCATGGCCAGGAACATCTAT P2: TCAACCCTCCTTGTTCGGCGG [420bp]