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在线全文

血流感染碳青霉烯耐药肺炎克雷伯菌的耐药基因和毒力基因及分子流行病学研究

廖全凤，袁 余，张为利，邓 劲，康 梅△
四川大学华西医院 实验医学科（成都 610041）

【摘要】 **目的** 本研究旨在了解西南地区某大型三甲综合医院血流感染碳青霉烯耐药肺炎克雷伯菌（carbapenem-resistant *Klebsiella pneumoniae*, CRKP）患者的临床特征和CRKP的分子流行病学。**方法** 收集2015–2019年血流感染患者血培养分离的131株非重复CRKP，采用VITEK-2全自动微生物分析仪和质谱MALDI-TOF进行菌株鉴定，微量肉汤稀释法检测最低抑菌浓度值（minimum inhibitory concentration, MIC），PCR检测常见碳青霉烯酶耐药基因和毒力因子，多位点序列分型对菌株进行同源性分析，全基因组测序方法检测不携带碳青霉烯酶菌株的基因组特征。**结果** 131株CRKP除多粘菌素B（耐药率1.6%）和替加环素（耐药率8.0%）外，对常见抗菌药物均耐药。105株（80.2%）CRKP携带*Klebsiella pneumoniae* carbapenemase（*KPC*）耐药基因，15株（11.4%）携带New Delhi Metallo-β-lactamase（*NDM*）基因，4株（3.1%）同时携带*KPC*和*NDM*基因，以序列（sequence typing, ST）11（74.0%）为优势序列类型，*mrkD*（96.2%）、*fimH*（98.5%）、*entB*（100%）等毒力基因检出率较高，检出1株高毒力CRKP。全基因组测序显示7株不产碳青霉烯酶的CRKP携带*ESBL*或*AmpC*基因，同时存在膜孔蛋白OMPK35和OMPK36的异常。**结论** 某大型三甲综合医院CRKP主要携带*KPC*基因，对多种抗菌药物耐药率高，同时携带多种毒力基因，具有高毒力特性的CRKP应引起重视。

【关键词】 碳青霉烯耐药肺炎克雷伯菌 碳青霉烯酶基因 毒力基因 MLST分型 血流感染

Carbapenemase Genes, Virulence Genes, and Molecular Epidemiology of Carbapenem-Resistant *Klebsiella pneumoniae* Derived From Bloodstream Infections

LIAO Quanfeng, *YUAN Yu*, *ZHANG Weili*, *DENG Jin*, *KANG Mei*△. *Department of Experimental Medicine*, *West China Hospital*, *Sichuan University*, Chengdu 610041, China
△ Corresponding author, E-mail: kangmei@sina.com

【**Abstract**】 **Objective** To investigate the clinical characteristics and molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolated from patients with bloodstream infections in a large tertiary-care general hospital in Southwest China. **Methods** A total of 131 strains of non-repeating CRKP were collected from the blood cultures of patients who had bloodstream infections in 2015-2019. The strains were identified by VITEK-2, a fully automated microbial analyzer, and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. The minimum inhibitory concentration (MIC) was determined by microbroth dilution method. The common carbapenemase resistant genes and virulence factors were identified by PCR. Homology analysis was performed by multilocus sequencing typing. Whole genome sequencing was performed to analyze the genomic characteristics of CRKP without carbapenemase. **Results** The 131 strains of CRKP showed resistance to common antibiotics, except for polymyxin B (1.6% resistance rate) and tigacycline (8.0% resistance rate). A total of 105 (80.2%) CRKP strains carried the *Klebsiella pneumoniae* carbapenemase (*KPC*) resistance gene, 15 (11.4%) strains carried the New Delhi Metallo-β-lactamase (*NDM*) gene, and 4 (3.1%) isolates carried both *KPC* and *NDM* genes. Sequence typing (ST) 11 (74.0%) was the dominant sequence type. High detection rates for *mrkD* (96.2%), *fimH* (98.5%), *entB* (100%), and other virulence genes were reported. One hypervirulent CRKP strain was detected. The seven strains of CRKP that did not produce carbapenemase were shown to carry *ESBL* or *AmpC* genes and had anomalies in membrane porins OMPK35 and OMPK36, according to whole genome sequencing. **Conclusion** In a large-scale tertiary-care general hospital, CRKP mainly carries the *KPC* gene, has a high drug resistance rate to a variety of antibiotics, and possesses multiple virulence genes. Attention should be paid to CRKP strains with high virulence.

【**Key words**】 Carbapenem-resistant *Klebsiella pneumoniae* Carbapenemase genes Virulence genes Multilocus sequence typing Bloodstream infection

肺炎克雷伯菌广泛存在于宿主相关的环境中，是临床上常见的条件致病菌。它可以不断获得新的遗传物质，产生多重耐药和广泛耐药表型。抗生素耐药肺炎克雷伯菌的广泛传播与较高的死亡率相关，尤其是碳青霉烯耐药肺炎克雷伯菌（carbapenem-resistant *Klebsiella pneumoniae*, CRKP）引起的血流感染[1-2]。CRKP的碳青霉烯酶编码基因多位于细菌质粒，可通过转座子或结合质粒等可移动元件在菌株间传播[3]，造成流行或爆发性感

△ 通信作者，E-mail: kangmei@sina.com
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染,因其广泛耐药,治疗费用高[4]。而对抗菌药物耐药率较低的高毒力肺炎克雷伯菌(hypervirulent *Klebsiella pneumonia*, HVKP)虽然在治疗方案上选择较多,但其多引起侵袭性、播散性感染,治疗的挑战在于及时遏制源头感染,避免播散至中枢神经系统或血液等部位[5-6]。近年来出现CRKP同时拥有高毒力特点的报道[7],以ST11为主[8]。本研究旨在揭示我院分离自血流感染患者CRKP的临床特征,采用多位点序列分型(multilocus sequence typing, MLST)、全基因组测序(whole genome sequencing, WGS)等方法,确定其耐药机制、毒力因素和分子分型,为感染防控和治疗提供依据。

1 材料和方法

1.1 样本收集

从四川大学华西医院收集2015–2019年血流感染患者血培养中的131株CRKP。通过电子病历查阅患者年龄、性别、住院时间、诊断和预后等资料。本研究获得四川大学生物医学伦理审查委员会批准,批准号2021年审(623)号。所有菌株采用MALDI-TOF MS(Bruker Daltonics, Bremen, 德国)和Vitek 2全自动微生物分析仪器(BioMerieux, Marcy-l'Étoile, 法国)在物种水平上进行鉴定。

1.2 药物敏感性试验

采用VITEK-2全自动微生物分析仪测定药敏。最低抑制浓度(minimum inhibitory concentration, MIC)结果根据临床实验室标准协会(CLSI-M100)指南进行解释,替加环素结果根据欧洲抗微生物药敏试验委员会的建议进行解释。质控菌株为大肠杆菌ATCC 25922和肺炎克雷伯菌ATCC 700603。

1.3 碳青霉烯酶基因和毒力因子检测

PCR检测常见的碳青霉烯酶基因(bla_{KPC}、bla_{NDM}、bla_{VIM}、bla_{IMP}和bla_{OXA})。采用拉丝试验[9]鉴定肺炎克雷伯菌的高黏表型,标准接种环挑取单个菌落,拉丝≥5 mm时视为阳性。6种荚膜血清型(*K*1、*K*2、*K*5、*K*20、*K*54、*K*57)和10种毒力基因(*fimH*、*magA*、*entB*、aerobictin、*alls*、*iutA*、*kfu*、*mrkD*、*rmpA*和*ybts*)通过PCR扩增检测[10-12]。对阳性扩增产物测序,测序结果进行BLAST比对,网址为https://blast.ncbi.nlm.nih.gov/Blast.cgi。5种碳青霉烯酶基因引物(5′-3′)如下[13-14]: bla_{KPC}, F: TGTCACTGTATCGCCGTC, R: CTCAGTGCTCTACAGAAAACC; bla_{NDM}, F: ATGGAATTGCCCAATATTATGCACCCG, R: TCAGCGCAGCTTGTCGGCCATGCG; bla_{IMP}, F: CTACCGCAGCAGAGTCTTTG, R: AACCAGTTTTGCCTTACCAT; bla_{VIM}, F: GATGGTGTTTGGTCGCATA, R: CGAATGCGCAGCACCAG; bla_{OXA}, F: GCGTGGTTAAGGATGAACAC, R: CATCAAGTTCAACCCAACCG。6种荚膜血清型引物[15]: *K*1, F: GGTGCTCTTTACATCATTGC, R: GCAATGGCCATTTGCGTTAG; *K*2, F: GACCCGATATTCATACTTGACAGAG, R: CCTGAAGTAAAATCGTAAATAGATGGC; *K*5, F: TGGTAGTGATGCTCGCGA, R: CCTGAACCCACCCCAATC; *K*20, F: CGGTGCTACAGTGCATCATT, R: GTTATACGATGCTCAGTCGC; *K*54, F: CATTAGCTCAGTGGTTGGCT, R: GCTTGACAAACACCATAGCAG; *K*57, F: CTCAGGGCTAGAAGTGTCAT, R: CACTAACCCAGAAAGTCGAG。10种毒力基因引物[15]: *ybtS*, F: GACGGAAACAGCACGGTAAA, R: GAGCATAATAAGGCGAAAGA; *mrkD*, F: AAGCTATCGCTGTACTTCCGGCA, R: GGCGTTGGCGCTCAGATAGG; *entB*, F: GTCAACTGGGCCTTTGAGCCGTC, R: TATGGGCGTAAACGCCGGTGAT; *rmpA*, F: ACTGGGCTACCTCTGCTTCA, R: CTTGCATGAGCCATCTTTCA; *Kfu*, F: GGCCTTTGTCCAGAGCTACG, R: GGGTCTGGCGCAGAGTATGC; *alls*, F: CATTACGCACCTTTGTCAGC, R: GAATGTGTCGGCGATCAGCTT; *fimH*, F: GCCAACGTCTACGTTAACCTG, R: ATATTTCACGGTGCCTGAAAA; aerobictin, F: GCATAGGCGGATACGAACAT, R: CACAGGGCAATTGCTTACCT; *iutA*, F: GGGAAAGGCTTCTCTGCCAT, R: TTATTCGCCACCACGCTCTT; *magA*, F: GGTGCTCTTTACATCATTGC, R: GCAATGGCCATTTGCGTTAG。

1.4 MLST

根据肺炎克雷伯菌多位点序列分型网站(http://bigsdb.pasteur.fr/klebsiella/primers_used.html)上的方案,对CRKP菌株MLST,使用在线数据库工具https://bigsdb.pasteur.fr/klebsiella/klebsiella.html对结果进行分析。

1.5 WGS

为更好地了解不产碳青霉烯酶的CRKP(non-carbapenemase-producing CRKP, non-CP-CRKP)菌株对碳青霉烯类药物的耐药机制,本研究对7株不携带碳青霉烯酶基因的CRKP进行WGS分析。使用商品DNA提取试剂盒(Qiagen, 德国),提取CRKP的基因组DNA,通过Illumina HiSeq X10平台进行基因组测序,采用sickle(GitHub)和SPAdes 3.8修剪并组装基因组,MLST和荚膜分型通过Institut Pasteur (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html)和Kaptive (https://github.com/katholt/Kaptive)进行。采用ABRicate程序

(https://github.com/tseemann/abricate)查询ResFinder数据库(http://genomicepidemiology.org/)鉴定耐药基因。利用巴斯德研究所数据库(http://bigsdb.pasteur.fr/klebsiella/klebsiella.html)鉴定毒力基因[16]。

2　结果

2.1　临床资料

18个医院科室共分离出131株CRKP, 其中ICU(82株, 62.6%)、血液科(11株, 8.4%)和中西医结合科(10株, 7.6%)排在前3位。67.4%的患者为男性, 32.6%的患者为女性, 住院时间中位数为33 d, 大多数患者有肺部疾病(77.8%)、低蛋白血症(75.7%), 同时存在有创操作治疗, 如血管插管(64.6%)、气管插管(65.3%), 51.4%的患者发生感染性休克。所有患者均接受了抗菌药物治疗, 其中74例(51.4%)使用了碳青霉烯类药物。患者血液中检测到CRKP后的30 d死亡率为13.2%。

CRKP对常用抗菌药物的耐药率较高: 美罗培南耐药率为99.2%, 亚胺培南耐药率为98.6%, 厄他培南耐药率为100%, 多尼培南耐药率为98.1%。对多粘菌素B和替加环素的耐药率分别为1.6%和8.0%。见图1。

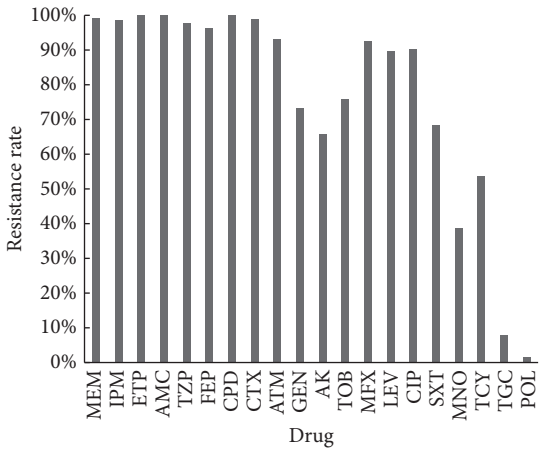

图 1 CRKP的药敏信息

Fig 1 Antimicrobial susceptibility of CRKP

MEM: meropenem; IPM: imipenem; ETP: ertapenem; AMC: amoxicillin-clavulanate; TZP: piperacillin-tazobactam; FEP: cefepime; CPD: cefpoxime; CTX: ceftriaxone; ATM: aztreonam; GEN: gentamicin; AK: amikacin; TOB: tobramycin; MFX: moxifloxacin; LEV: levofloxacin; CIP: ciprofloxacin; SXT: trimethoprim-sulfamethoxazole; MNO: minocycline; TCY: tetracycline; TGC: tigecycline; POL: polymyxin B.

2.2　CRKP菌株的MLST基因分型

见表1。共鉴定出21个序列(sequence typing, ST)型别, 其中ST11 97个(74.0%), ST15 3个(2.3%), ST16 5个(3.8%), ST23 2个(1.5%), ST45 7个(5.3%), ST1035 2个(1.5%), 其他ST (ST37、ST54、ST265、ST273、ST307、ST412、ST524、ST789、ST1128、ST2407、ST3034)各1个。

表 1　不同ST型别的耐药和毒力基因

Table 1　Resistance and virulence genes in different ST

Gene	Strain (%)						
	ST11 (*n*=97)	ST45 (*n*=7)	ST16 (*n*=5)	ST15 (*n*=3)	ST23 (*n*=2)	ST1035 (*n*=2)	Other STs (*n*=15)
KPC-2	89 (67.9)	5 (3.8)	0	3 (2.3)	2 (1.5)	0	6 (4.6)
NDM-5	2 (1.5)	0	5 (3.8)	0	0	2 (1.5)	4 (3.1)
NDM-1	0	0	0	0	0	0	2 (1.5)
KPC-2+*NDM*-1	3 (2.3)	0	0	0	0	0	0
KPC-2+*NDM*-5	1 (0.8)	0	0	0	0	0	0
None	2 (1.5)	2 (1.5)	0	0	0	0	3 (2.3)
Virulence gene							
entB	97 (74.0)	7 (5.3)	5 (3.8)	3 (2.3)	2 (1.5)	2 (1.5)	15 (11.5)
markD	94 (71.8)	7 (5.3)	5 (3.8)	3 (2.3)	2 (1.5)	0	15 (11.5)
fimH	97 (74.0)	7 (5.3)	5 (3.8)	3 (2.3)	2 (1.5)	0	15 (11.5)
aerobictin	0	0	0	0	1 (0.8)	0	0
rmpA	1 (0.8)	0	0	0	1 (0.8)	0	2 (1.5)
iutA	1 (0.8)	1 (0.8)	0	0	1 (0.8)	0	1 (0.8)
ybtS	0	0	0	0	0	0	0
Kfu	0	0	0	0	2 (1.5)	0	3 (2.3)
alls	0	0	0	0	2 (1.5)	2 (1.5)	0
magA	0	0	0	0	1 (0.8)	0	0
*K*1	0	0	0	0	1 (0.8)	0	0
*K*57	0	0	0	0	0	2 (1.5)	1 (0.8)
*K*2	0	0	0	0	0	0	0
*K*5	0	0	0	0	0	0	0
*K*20	0	0	0	0	0	0	0
*K*54	0	0	0	0	0	0	0

KPC: *Klebsiella pneumoniae* carbapenemase; *NDM*: New Delhi Metallo-β-lactamase; *rmpA*: regulator of mucoid phenotype A; ST: sequence typing.

还有4个新的ST型菌株，分别命名为ST4520、ST4521、ST4522和ST4523 。

2.3 碳青霉烯酶基因和毒力基因

124株（94.7%）CRKP携带碳青霉烯酶基因，其中105株（80.2%）携带bla_{KPC-2}，15株（11.4%）携带bla_{NDM}（13株携带bla_{NDM-5}，2株携带bla_{NDM-1}），4株CRKP同时携带2种基因（3株为bla_{KPC-2}和bla_{NDM-1}，1株为bla_{KPC-2}和bla_{NDM-5}），其余7株CRKP不携带碳青霉烯酶基因。

毒力测定结果见表2。131株CRKP中有4株CRKP拉丝试验阳性。CRKP荚膜血清型结果显示，K1型1株（0.8%），K57型3株（2.3%）。本研究将*rmpA*联合*iutA*基因阳性且具有高黏液表型的菌株定义为HVKP，发现1株HVKP。

表 2 131株CRKP的毒力结果

Table 2 Virulence results of 131 strains of CRKP

Virulence gene	Feature	Percentage/%
entB	Siderophore	100
markD	Pili	96.2
fimH	Pili	98.5
aerobictin	Siderophore	0.8
rmpA	Capsular polysaccharide regulator genes	3.1
iutA	Siderophore	3.1
ybtS	Siderophore	0
Kfu	Siderophore	7.6
alls	Allantoin metabolism	3.1
magA	Capsular polysaccharide regulator genes	0.8
*K*1	CPS	0.8
*K*2	CPS	0
*K*5	CPS	0
*K*20	CPS	0
*K*54	CPS	0
*K*57	CPS	2.3

CPS: capsule polysaccharide; *rmpA*: regulator of mucoid phenotype A.

2.4 碳青霉烯酶基因、毒力基因与ST型的关系

携带bla_{KPC-2}碳青霉烯酶基因的菌株主要ST型别为ST11，其次为ST45，携带bla_{NDM-5}基因的菌株主要ST型别为ST16；毒力基因 *entB*、*markD*和*fimH*主要分布于ST11的CRKP菌株，其次是ST45、ST16和ST15。具体见表1。

2.5 7株non-CP-CRKP的结果

7株non-CP-CRKP经全基因组测序分析后，其携带耐药基因情况见表3。与参考序列（肺炎克雷伯菌KCTC 2242和NTUH-K2044，NCBI编号No. CP002910和No. AP006725）相比，7株肺炎克雷伯菌均存在*OmpK*35基因突变，部分菌株存在*OmpK*36基因突变。

3 讨论

在本研究中，CRKP分离率最高的科室为ICU（62.6%），这可能与ICU患者进行有创手术、住院时间长、免疫力差、长期使用广谱抗生素、基础疾病严重等多重危险因素有关。研究表明，CRKP相关的血流感染主要发生在老年患者中，多为冠心病、脑梗死、肾功能不全、器官移植、低蛋白血症等，均有非手术性的有创操作和血液透析治疗史[17-19]。本研究结果与上述数据一致，老年男性占67.4%，所有患者均接受过不同的非手术有创操作，主要基础疾病包括肺部疾病和低蛋白血症。51.4%的患者发生脓毒性休克，这也是肺炎克雷伯菌血流感染患者死亡的独立危险因素[20]。

在耐药基因中，*KPC*-2是主要的碳青霉烯酶基因，且ST型别多为ST11，这与目前的流行情况一致[21]。此外，应

表 3 7株non-CP-CRKP菌株的测序结果

Table 3 Results of 7 non-CP-CRKP strains

Isolate	MIC/(mg/L)			ST	Resistance gene	Virulence gene	Outer membrane porin
	MEM	IPM	ETP				
3R	1	4	≥8	54	bla_{SHV-12}, $bla_{CTX-M-14}$, bla_{DHA-1}, *oqxA*10, *oqxB*19, *qnrB*4, *fosA*, *sul*1, *aph*(3")-*Ib*, *aph*(6)-*Id*	*entB*, *markD*, and *fimH*	*OMPK*35, *OMPK*36
4R	2	4	≥8	11	$bla_{SHV-158}$, bla_{OXA-1}, bla_{DHA-1},*oqxA*, *oqxB*, *qnrB*4, *fosA*6, *sul*1, *sul*2, *aac*(3)-*Iva*, *aph*(4)-*Ia*, *mph*(*A*), *floR*, *catB*3, *arr*-3, *aac*(6')-*Ib-cr*5, *aadA*2, *aadA*12, *dfrA*12, *aph*(3')-*Ia*	*entB*, *markD*, and *fimH*	*OMPK*35, *OMPK*36
5R	2	4	≥8	11	$bla_{SHV-158}$, bla_{OXA-1}, bla_{DHA-1}, *oqxB*, *oqxA*, *qnrB*4, *fosA*6, *sul*1, *sul*2, *aph*(4)-*Ia*, *aac*(3)-*Iva*, *mph*(*A*), *floR*, *catB*3, *aac*(6')-*Ib-cr*5, *arr*-3, *dfrA*12, *aadA*2, *aadA*12, *aph*(3')-*Ia*	*entB*, *markD*, and *fimH*	*OMPK*35, *OMPK*36
32R	4	2	≥8	412	$bla_{SHV-145}$, bla_{TEM-1}, $bla_{CTX-M-3}$, *fosA*6, *oqxA*5, *oqxB*26, *aac*(3)-*IId*	*rmpA*, *iutA*, *entB*, *markD*, and *fimH*	*OMPK*35, *OMPK*36
70R	4	8	≥8	3034	$bla_{SHV-106}$, $bla_{CTX-M-65}$, bla_{TEM-1}, bla_{DHA-1}, *oqxA*6, *oqxB*20, *qnrB*4, *fosA*6, *aac*(6')-*Ib-cr*, *arr*-3, *dfrA*12, *aadA*16, *sul*1, *sul*2, *floR*, *tet*(*A*), *mph*(*A*), *aph*(3')-*Ia*	*Kfu*, *entB*, *markD*, and *fimH*	*OMPK*35, *OMPK*36
83R	4	2	≥8	45	bla_{SHV-1}, *oqxA*11, *oqxB*20, *fosA_gen*	*entB*, *markD*, and *fimH*	*OMPK*35, *OMPK*36
103R	1	1	4	45	bla_{SHV-1}, $bla_{CTX-M-104}$, *oqxB*20, *oqxA*11, *qnrB6*, *fosA_gen*, *aac*(6')-*Ib-cr*, *arr*-3, *dfrA*27, *aadA*16, *sul1*, *aac*(3)-*IId*	entB, *markD*, and *fimH*	*OMPK*35, *OMPK*36

MIC: minimum inhibitory concentration; ETP: ertapenem; IPM: imipenem; MEM: meropenem; non-CP-CRKP: non-carbapenemase-producing CRKP. The other abbreviations are explained in the note to Table 1.

注意同时产KPC和NDM的CRKP分离株, 因为它们具有良好的适应能力和在患者之间的可转移性, 这可能是医院和社区感染中一个新的日益增长的威胁[22]。对于不含碳青霉烯酶的CRKP菌株, 另一个重要的耐药机制是高产ESBL酶或AmpC酶合并孔蛋白编码基因的突变, 导致菌株对厄他培南耐药, 引起美罗培南和亚胺培南的MIC升高[23]。在本研究中, 7株不含碳青霉烯酶的CRKP菌株均产生ESBL酶, 同时存在*OMPK*35和*OMPK*36的突变。此外, 4株CRKP携带编码AmpC酶的基因$bla_{DHA\text{-}1}$, 厄他培南的MIC高于美罗培南和亚胺培南, 推测7株不产碳青霉烯酶的CRKP对碳青霉烯类耐药的机制为产AmpC/ESBL酶合并膜孔蛋白的异常表达, 有待进一步证实。

在毒力基因方面, 131株CRKP主要携带编码Ⅲ型菌毛的*mrkD*基因(96.2%)、编码Ⅰ型菌毛的*fimH*基因(98.5%)和编码肠杆菌素的*entB*毒力基因(100%), 这与El FERTAS-AISSANI等[24]研究结果一致, 表明肺炎克雷伯菌普遍存在Ⅰ型和Ⅲ 型菌毛。铁是细菌生长所必需的金属, 摄铁能力的增强可以增加菌株毒力, 肺炎克雷伯菌可以分泌4种铁载体(气杆菌素、沙门菌素、肠杆菌素和耶尔森菌素)获取铁离子, CRKP均携带编码肠杆菌素的*entB*毒基因。Kfu蛋白通过螯合作用促使细菌摄取游离的三价铁, 7.6%的CRKP携带Kfu, 与肠杆菌素一起促进肺炎克雷伯菌的生长和定植。研究显示[25-26]HVKP的荚膜血清型别多为K1和K2, 老鼠模型证实K1和K2菌株的毒力远高于非K1/K2菌株, 因其具有较强的宿主逃逸能力, YU等[26]发现尿囊素基因*alls*和编码Kfu蛋白的基因, 这两种与侵袭性感染强相关的基因都只存在于荚膜血清型为K1的肺炎克雷伯菌中, 或许这是K1型为最强毒力型的原因。本次研究发现这两种基因不仅存在于K1型CRKP中, K57型CRKP也有检出, 与YU等的研究存在差异, 或是地域差异引起的不同。

已有研究表明, 共同表达黏液表型编码基因*rmpA*和铁载体受体iutA的菌株可被认为是HVKP[25, 27-28], 本研究仅发现1株ST412 HVKP, 提示我院从血培养分离到的CRKP毒力尚不高。LIU等[12]发现HVKP主要型别是ST23, 本次研究检出2株ST23 CRKP菌株, 皆非HVKP, 1株具有黏液表型, 携带*Kfu*、*entB*、*alls*、*markD*、*magA*和*fimH*毒力基因, 荚膜分型为K1, 且携带耐药基因*KPC*-2, 另一株携带*Kfu*、*entB*、*alls*、*rmpA*、*markD*、aerobictin、*iutA*和*fimH*基因, 同时携带耐药基因*KPC*-2, 与其他MLST型别菌株相比, 携带更多的毒力基因, 推测这两株菌具有较强的毒力水平, 需进一步毒力表型试验验证。仅检出1株non-CP-CRKP菌株为HVKP, 多位点序列分型为ST412, YANG等[29]的研究显示具有高毒力的CRKP菌株为ST11型, 本次研究结果与此不符, 提示新的ST型别高毒力CRKP也需引起关注。

综上所述, 我院血流感染患者血培养分离到的CRKP主要产KPC-2碳青霉烯酶, 其次是NDM-5, 多位点序列分型以ST11为主, 菌株主要携带*entB*、*mrkD*和*fimH*毒力基因, 高毒力CRKP菌株检出率低, 应密切关注CRKP的耐药、毒力和分子流行病学情况, 为临床诊治提供支持数据。

*　　*　　*

作者贡献声明　廖全凤负责论文构思、数据审编、正式分析、研究方法和初稿写作, 袁余和邓劲负责提供资源, 张为利负责可视化, 康梅负责论文构思、研究方法和审读与编辑写作。所有作者已经同意将文章提交给本刊, 且对将要发表的版本进行最终定稿, 并同意对工作的所有方面负责。

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参　考　文　献

[1] YUAN Y H, YAO Z H, MA B, *et al*. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections and outcomes. Infect Drug Resist, 2020, 13: 207–215. doi: 10.2147/IDR.S223243.

[2] TSIOUTIS C, EICHEL V M, MUTTERS N T. Transmission of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae*: the role of infection control. J Antimicrob Chemother, 2021, 76(Suppl 1): i4–i11. doi: 10.1093/jac/dkaa492.

[3] LIU C, DONG N, CHAN E W C, *et al*. Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in China, 2016-20. Lancet Infect Dis, 2022, 22(2): 167–168. doi: 10.1016/S1473-3099(22)00009-3.

[4] JEAN S S, HARNOD D, HSUEH P R. Global Threat of carbapenem-resistant Gram-negative bacteria. Front Cell Infect Microbiol, 2022, 12: 823684. doi: 10.3389/fcimb.2022.823684.

[5] LEE C R, LEE J H, PARK K S, *et al*. Antimicrobial resistance of hypervirulent *Klebsiella pneumoniae*: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. Front Cell Infect Microbiol, 2017(7): 483. doi: 10.3389/fcimb.2017.00483.

[6] RUSSO T A, MARR C M. Hypervirulent *Klebsiella pneumoniae*. Clin Microbiol Rev, 2019, 32(3): e00001–19. doi: 10.1128/CMR.00001-19.

[7] ZHAN L, WANG S, GUO Y, *et al*. Outbreak by Hypermucoviscous *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in a

tertiary hospital in China. Front Cell Infect Microbiol, 2017, 7: 182. doi: 10.3389/fcimb.2017.00182.

[8] GU D, DONG N, ZHENG Z, *et al*. A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. Lancet Infect Dis, 2018, 18(1): 37–46. doi: 10.1016/S1473-3099(17)30489-9.

[9] SHON A S, BAJWA R P, RUSSO T A. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence, 2013, 4(2): 107–118. doi: 10.4161/viru.22718.

[10] CHUANG C, FAN W C, LIN Y T, *et al*. The emergence of *Klebsiella pneumoniae* liver abscess in non-diabetic patients and the distribution of capsular types. Gut Pathog, 2016, 8: 46. doi: 10.1186/s13099-016-0128-y.

[11] HYUN M, LEE J Y, RYU S Y, *et al*. Antibiotic resistance and clinical presentation of health care-associated hypervirulent *Klebsiella pneumoniae* infection in Korea. Microb Drug Resist, 2019, 25(8): 1204–1209. doi: 10.1089/mdr.2018.0423.

[12] LIU C, GUO J. Hypervirulent *Klebsiella pneumoniae* (hypermucoviscous and aerobactin positive) infection over 6 years in the elderly in China: antimicrobial resistance patterns, molecular epidemiology and risk factor. Ann Clin Microbiol Antimicrob, 2019, 18(1): 4. doi: 10.1186/s12941-018-0302-9.

[13] 吕继芳. 碳青霉烯类耐药肺炎克雷伯菌耐药基础及毒力因子分析. 杭州: 浙江大学, 2017.
LYU J F. Analysis of drug resistance basis and virulence factors of carbapenem resistant *Klebsiella pneumoniae*. Hangzhou: Zhejiang University, 2017.

[14] 黄丹艳. 浙江省碳青霉烯类抗生素耐药肺炎克雷伯菌流行特征及耐药基因分析. 杭州: 浙江大学, 2018.
HUANG D Y. Epidemiological characteristics and gene analysis of carbapenem-resistant *Klebsiella pneumoniae* in Zhejiang Province. Hangzhou: Zhejiang University, 2018.

[15] 俞凤. 耐碳青霉烯类肺炎克雷伯菌临床分离株耐药机制及毒力基因的研究. 南昌: 南昌大学, 2019.
YU F. Study on drug resistance mechanism and virulence genes of carbapenem-resistant *Klebsiella pneumoniae* isolates. Nanchang: Nanchang University, 2019.

[16] LIAO Q, DENG J, FENG Y, *et al*. Emergence of ceftazidime-avibactam resistance due to a novel blaKPC-2 mutation during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. J Infect Public Health, 2022, 15(5): 545–549. doi: 10.1016/j.jiph.2022.04.002.

[17] CHEN J, MA H, HUANG X, *et al*. Risk factors and mortality of carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection in a tertiary-care hospital in China: an eight-year retrospective study. Antimicrob Resist Infect Control, 2022, 11(1): 161. doi: 10.1186/s13756-022-01204-w.

[18] CHANG D, SHARMA L, DELA CRUZ C S, *et al*. Clinical epidemiology, risk factors, and control strategies of *Klebsiella pneumoniae* infection. Front Microbiol, 2021, 12: 750662. doi: 10.3389/fmicb.2021.750662.

[19] PAN H, LOU Y, ZENG L, *et al*. Infections caused by carbapenemase-producing *Klebsiella pneumoniae*: microbiological characteristics and risk factors. Microb Drug Resist, 2019, 25(2): 287–296. doi: 10.1089/mdr.2018.0339.

[20] CHANG H, WEI J, ZHOU W, *et al*. Risk factors and mortality for patients with bloodstream infections of *Klebsiella pneumoniae* during 2014-2018: clinical impact of carbapenem resistance in a large tertiary hospital of China. J Infect Public Health, 2020, May,13(5): 784–790. doi: 10.1016/j.jiph.2019.11.014.

[21] WANG Q, WANG X, WANG J, *et al*. Phenotypic and genotypic characterization of carbapenem-resistant enterobacteriaceae: data from a longitudinal large-scale CRE study in China (2012-2016). Clin Infect Dis, 2018, 67(Suppl 2): S196–S205. doi: 10.1093/cid/ciy660.

[22] GAO H, LIU Y, WANG R, *et al*. The transferability and evolution of NDM-1 and KPC-2 co-producing *Klebsiella pneumoniae* from clinical settings. EBioMedicine, 2020, 51: 102599. doi: 10.1016/j.ebiom.2019.102599.

[23] GARCÍA-FERNÁNDEZ A, MIRIAGOU V, PAPAGIANNITSIS C C, *et al*. An ertapenem-resistant extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* clone carries a novel OmpK36 porin variant. Antimicrob Agents Chemother, 2010, 54(10): 4178–4184. doi: 10.1128/AAC.01301-09.

[24] El FERTAS-AISSANI R, MESSAI Y, ALOUACHE S, *et al*. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Pathol Biol (Paris), 2013, 61(5): 209–216. doi: 10.1016/j.patbio.2012.10.004.

[25] RUSSO T A, OLSON R, FANG C T, *et al*. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from Classical *K. pneumoniae*. J Clin Microbiol, 2018, 56(9): e00776-18. doi: 10.1128/JCM.00776-18.

[26] YU W L, KO W C, CHENG K C, *et al*. Comparison of prevalence of virulence factors for *Klebsiella pneumoniae* liver abscesses between isolates with capsular K1/K2 and non K1/K2 serotypes. Diagn Microbiol Infect Dis, 2008, 62(1): 1–6. doi: 10.1016/j.diagmicrobio.2008.04.007.

[27] ZOU Q, LI Y. Hypervirulent *Klebsiella pneumoniae*. N Engl J Med, 2021, 385(9): 833. doi: 10.1056/NEJMicm2101602.

[28] HWANG J H, LEE S Y, LEE J, *et al*. Pyogenic spondylitis caused by *Klebsiella pneumoniae*: should the possibility of hypervirulent *Klebsiella pneumoniae* be considered? BMC Infect Dis, 2022, 22(1): 801. doi: 10.1186/s12879-022-07785-6.

[29] YANG Q, JIA X, ZHOU M, *et al*. Emergence of ST11-K47 and ST11-K64 hypervirulent carbapenem-resistant *Klebsiella pneumoniae* in bacterial liver abscesses from China: a molecular, biological, and epidemiological study. Emerg Microbes Infect, 2020, 9(1): 320–331. doi: 10.1080/22221751.2020.1721334.

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