

# 重症监护室患者耐碳青霉烯类肺炎克雷伯菌流行病学研究及同源性分析\*

周朝琼<sup>1</sup>, 邓劲<sup>2</sup>, 刘翔<sup>1</sup>, 孔丽蕊<sup>1</sup>

[1. 成都市郫都区中医医院(成都中医药大学附属第三医院)检验科, 成都 611730;  
2. 四川大学华西医院实验医学科, 成都 610041]

**摘要:**目的 对重症监护室患者分离出的耐碳青霉烯类肺炎克雷伯菌进行流行病学研究,为临床治疗及监测提供参考依据。**方法** 收集2018年1月~2019年1月成都市郫都区中医医院重症监护室患者标本共24例,对分离到的耐碳青霉烯类肺炎克雷伯菌进行全自动细菌生化鉴定药敏分析,并通过脉冲场凝胶电泳DNA指纹图谱对菌株进行比对,确定菌株同源性,同时结合临床资料进行分析。**结果** 该研究耐碳青霉烯类肺炎克雷伯菌患者下呼吸道感染占58.3%,仅极少数感染者(小于30%)对多黏菌素B敏感,对其余药物均耐药。该菌院内获得感染者23例占95.83%。24例耐碳青霉烯类肺炎克雷伯菌感染者死亡13例占54.2%。通过脉冲场凝胶电泳DNA指纹图谱对菌株进行比对发现,24例耐碳青霉烯类肺炎克雷伯菌菌株亲缘关系较强,仅极少数菌株间无亲缘关系或亲缘关系较差。**结论** 该院耐碳青霉烯类肺炎克雷伯菌患者主要感染部位为下呼吸道感染,药物敏感性极差,菌株同源性较强,院内感染极为严重,治疗困难,死亡率较高。

**关键词:**重症监护室患者;耐碳青霉烯类肺炎克雷伯菌;流行病学研究;同源性分析

中图分类号:R378.996;R181.32 文献标志码:A 文章编号:1671-7414(2019)04-071-04

doi:10.3969/j.issn.1671-7414.2019.04.017

## Epidemiological Study and Homology Analysis of Carbapenem-Resistant *Klebsiella Pneumoniae* in Intensive Care Unit Patients

ZHOU Chao-qiong<sup>1</sup>, DENG Jin<sup>2</sup>, LIU Xiang<sup>1</sup>, KONG Li-rui<sup>1</sup> [1. Department of Clinical Laboratory, Chinese Medicine Hospital of Pidu (the Third Affiliated Hospital of Chengdu University of Traditional Chinese Medicine), Chengdu 611730, China; 2. Department of Clinical Laboratory, West China Hospital of Sichuan University, Chengdu 610041, China]

**Abstract: Objective** To investigate the epidemiological investigation of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolated from intensive care unit patients, and provide reference for clinical treatment and monitoring. **Methods** A total of 24 specimens from the intensive care unit of the Chinese Medicine Hospital of Pidu in Chengdu, from January 2018 to January 2019, the isolated CRKP was subjected to a automatic bacterial biochemical identification drug sensitivity analysis, and the strains were compared by pulsed gel electrophoresis DNA fingerprinting to determine the homology of the strain. At the same time, combined with clinical data for analysis. **Results** In this study, patients with CRKP accounted for 58.3% of lower respiratory tract infections, and patients with CRKP only had a resistance rate of less than 30% for polymyxin B, and 100% for the remaining drugs. One case of CRKP was infected with infected patients, accounting for 4.17%. 23 cases of infected patients in the hospital accounted for 95.83%, and 24 patients with CRKP infection died (54.2%). The strains were aligned by pulse gel electrophoresis DNA fingerprinting. 24 cases of CRKP strains had strong genetic relationship. Only a few strains were unrelated or had poor genetic relationship. **Conclusion** The main infection site of patients with CRKP infection was lower respiratory tract infection in this hospital, drug sensitivity was very poor, nosocomial infection was serious, strain homology was strong, treatment was difficult, and mortality was extremely high.

**Keywords:** intensive care unit patient; carbapenem-resistant *klebsiella pneumoniae*; epidemiological investigation; homology analysis

肺炎克雷伯菌作为临床常见和易于分离的条件致病菌,现阶段该菌因其高耐药率、高死亡率、高难度治愈率及高院内感染率等特点被广泛关注<sup>[1]</sup>。耐碳青霉烯类肺炎克雷伯菌(carbapenem-resistant *klebsiella pneumoniae*, CRKP)出现且呈递增趋势的原因是碳青霉烯类抗生素使用不当所致<sup>[2]</sup>。有

研究表明<sup>[3-5]</sup>医院重症监护室(intensive care unit, ICU)患者因其基础疾病较多故CRKP感染率较高。本研究利用VITEK 2 Compact鉴定系统及细菌分型金标准[脉冲场凝胶电泳(pulsed field gel electrophoresis, PFGE)]<sup>[6]</sup>对ICU感染CRKP患者进行流行病学调查,为医院获得性感染、临床用

\* 作者简介:周朝琼(1995-),女,本科,技师,主要研究方向:临床微生物学检验及临床生物化学检验,E-mail:1340709790@qq.com。

药及减少患者病死率等提供参考依据,现报道如下:

## 1 材料与与方法

1.1 研究对象 选取成都市郫都区中医医院2018年1月~2019年1月ICU患者24例,男性16例,女性8例,年龄16~85岁,24例菌株标本类型有:痰(来自下呼吸道)、脑脊液、血液、尿液、尖端导管、腹腔积液及体表浅切口分泌物。

1.2 仪器与试剂 细菌鉴定采用布鲁克公司的基质辅助激光解析飞行时间质谱仪(MALDI-TOF MS),细菌药敏采用梅里埃的全自动细菌生化鉴定药敏分析仪(VITEK 2 Compact),补充药敏试验纸片购自赛默飞公司,培养和药敏所需的平板购于安图生物有限公司,二氧化碳孵箱采用上海博迅HH-CP-7W,血培养仪为梅里埃公司的全自动血液培养仪Bact Alt 3D,PFGE仪器及配套设备、紫外线透照仪及一次成像照相系统均购自美国Bio-Rad公司。

## 1.3 方法

1.3.1 标本采集与接种方法:深部痰标本通过吸痰器采集,尿液采集清洁中段尿,血液采集来自不同穿刺点的外周血或导管血,脑脊液经脑室引流或腰椎穿刺,腹腔积液来自腹腔穿刺液,导管为撤除置管的尖端5 cm,分泌物标本为冲洗后深部采集物。痰液、脑脊液、腹腔积液和分泌物为四区划线,尿液标本为“丰”字型划线,导管尖端为半定量滚动培养,血液为全自动仪器培养。

1.3.2 细菌药敏试验方法:细菌药敏试验采用VITEK 2 Compact进行。针对肺炎克雷伯菌敏感性试验选用的药物为替加环素、多黏菌素B、阿米卡星、厄他培南、亚胺培南、美罗培南、哌拉西林/他唑巴坦、头孢哌酮/舒巴坦、头孢他啶、头孢噻肟、头孢吡肟、头孢呋辛、头孢西丁、庆大霉素、复方磺胺甲恶唑、环丙沙星、哌拉西林和氨苄西林/舒巴坦。药敏试验判断参照2018年美国临床实验室标准化委员会(CLSI)制定的标准(<https://clsi.org>)。

1.3.3 PFGE方法:PFGE操作步骤依次为细菌准备,胶块制备,细胞裂解,清洗胶块,胶块内DNA的酶切,加样,电泳,图像获取,数据分析,详细步骤参照李秀清等<sup>[1]</sup>文中PFGE操作方法进行。

1.3.4 菌株同源性判断标准的方法:PFGE结果按TENOVER等<sup>[7]</sup>的标准判读,如下:①同一克隆型:具有相同条带数目和位置,则为同一菌株。②高度相关:仅有2~3个条带变化,被认为有亲缘关系。③可能相关:通常为4~6个条带变化。④无关:7个及以上条带变化。

1.3.5 院内感染判断标准的方法:病人入院前无

明确潜伏期的感染,入院后48 h在院内获得的感染,包括住院期间获得和在院内获得出院后发生的感染。

## 2 结果

2.1 流行病学结果 24例CRKP菌株中58.33%来自痰(14株),12.5%来自脑脊液(3株),血液及尿液各2株,各占8.3%,分泌物、腹腔积液和尖端导管各1例,各占4.2%。24株感染CRKP的患者死亡率为54.2%,出院率为29.2%,转归率为16.7%(死亡:13/24,出院:7/24,转归:4/24),该院院内感染率极高,占95.8%(23/24),外院带入感染者1例,占4.2%(1/24)。

2.2 细菌药敏结果 采用全自动细菌生化鉴定药敏分析仪对24株细菌进行药敏试验,结果显示,仅小于30%的感染者对多黏菌素B敏感,对其余抗生素均100%耐药。

2.3 PFGE结果 按菌株同源性判断标准,24株CRKP的PFGE图谱结果为:1,3,5,6,7,8,11,12,13和14号标本为同一克隆型,2号与10号为同一克隆型,16,17,18和20号为同一克隆型,19,21和23号为同一克隆型。4号、9号与1~3号、5~8号及11~14号菌株间有亲缘关系,15,22和24号三者间也存在亲缘关系,其他菌株基因组间同源性很差或无亲缘关系,应来自于不同克隆菌株,24株CRKP的PFGE图谱见图1。

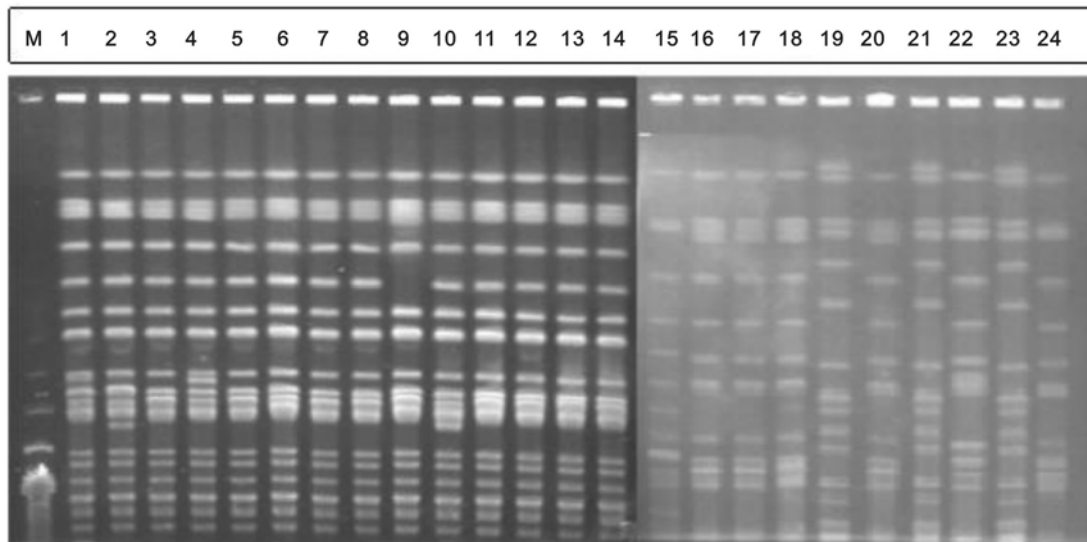
## 3 讨论

3.1 治疗多重耐药肺炎克雷伯菌的首选药物为碳青霉烯类抗生素,CRKP感染率因临床对该药应用增加不断上升<sup>[2,8]</sup>,该菌被美国疾病预防控制中心列为目前“最棘手细菌”<sup>[9]</sup>,有研究报道泛耐药肺炎克雷伯菌(pan-drug resistant *Klebsiella pneumoniae*,PDRKPN)死亡率高达40%<sup>[10]</sup>,KARAIKSKO-SL等<sup>[11]</sup>研究发现CRKP感染引起的死亡率可达23%~75%,感染CRKP的ICU患者院内死亡率增高<sup>[12]</sup>,本研究24例ICU患者经积极治疗后仍有13例死亡,死亡率高达54.2%,可见该类患者治疗困难,预后较差,死亡率极高<sup>[13-14]</sup>,有效抗生素的缺乏、较多的基础疾病及免疫力降低均可增加患者死亡率。

3.2 CRKP作为院内感染常见致病菌,2015年中国细菌耐药监测网(China highspeed information network,CHINET)结果显示,其感染率已高达14%<sup>[15-16]</sup>,有国外报道称其在医院获得性感染中位居第四<sup>[17]</sup>。医院感染传播途径以空气、接触及媒介为主<sup>[18]</sup>,LI等<sup>[19]</sup>对NICU空气环境及其患者病房环境进行研究发现,病房环境可成为CRKP的重要传播途径,同时也可成为潜在的感染源。PF-

GE 因其高分辨率及高重复性,目前被公认为是从基因水平上确定菌株间亲缘关系的细菌分型金标准<sup>[6]</sup>。本研究 PFGE 基因分型结果显示,24 株 CRKP 菌株同源性较高,大多数菌株均属同一克隆型,且不为同一克隆型部分菌株间存在亲缘关系,仅少量菌株同源性较差或无亲缘关系,从基因学角度提示院内感染较严重,与实验中对患者是否为院

内感染的调查数据相吻合,该结果提示我院需采取有效的院感防控措施,加强医护人员、患者对院内感染的防护及认知,对病房环境及物品严格进行相应消毒及灭菌处理,常开窗保持空气流通,对 CRKP 感染患者采取一定治疗防护措施,避免引起其他患者或医护人员的感染。



注:M为质控菌株,1~24为1~24号菌株。

图1 24株肺炎克雷伯菌脉冲场凝胶电泳指纹图

3.3 本研究药敏结果显示,临床分离的24株 CRKP 除30%对多黏菌素B敏感外,对其余抗生素均耐药,符合泛耐药菌株定义<sup>[20]</sup>,较张丽等<sup>[21]</sup>研究中耐药率更高,该结果提示我院应进一步规范控制抗生素的使用。CRKP 泛耐药导致临床很难找到合适的抗感染治疗方案,患者最终治疗效果与临床所选治疗方案密切相关。临床治疗该类细菌感染可根据药敏结果选用抗生素,以指导临床合理应用抗生素,对其进行耐药监测;同时遵循抗感染治疗的基本原则(经济、安全、有效),合理选药、合理给药,预防和避免抗生素间的不良反应和相互作用,预防和延缓细菌耐药性的产生,合理用药及治疗可极大提高患者生存率。

3.4 肺炎克雷伯菌主要存在于人体呼吸道及肠道内,当免疫力降低时,可引发肺部相关疾病,如大叶或小叶融合性实变则可因该菌经呼吸道进入肺部而诱发<sup>[22]</sup>,本研究中 CRKP 主要分离自痰<sup>[23]</sup>,表明其主要定植在呼吸道,故应加强感染 CRKP 可疑患者呼吸道的细菌检测,及时发现 CRKP 对患者临床治疗及用药指导尤为重要。

3.5 综上所述,我院 CRKP 菌株同源性较强,耐药率极高,院内感染极为严重,且感染 CRKP 患者死亡率极高,故应加强并采取有效的院感防护措施,进一步规范抗生素的使用,制定我院 CRKP 感

染的治疗方案和抗生素临床应用策略,减少患者感染率,提高感染患者生存率。

#### 参考文献:

- [1] 李秀清,高磊,崔兰卿,等. 急诊重症监护室患者感染碳青霉烯类耐药肺炎克雷伯菌分子流行病学的研究[J]. 中华医院感染学杂志,2017,27(15):3404-3407. LI Xiuqing, GAO Lei, CUI Lanqing, et al. Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in patients in emergency intensive care unit[J]. Chinese Journal of Nosocomiology, 2017, 27(15):3404-3407.
- [2] 刘云,万玉香,马炜,等. VITEK 2 Compact 检测耐碳青霉烯类肺炎克雷伯菌对阿米卡星药敏结果的准确性评价[J]. 现代检验医学杂志,2018,33(1):133-136. LIU Yun, WAN Yuxiang, MA Wei, et al. Evaluation on the accuracy of VITEK 2 compact for the susceptibility of carbapenem resistant *Klebsiella pneumoniae* to amikacin results[J]. Journal of Modern Laboratory Medicine, 2018, 33(1):133-136.
- [3] 李继红,时东彦,李媛媛,等. 2015~2017年耐碳青霉烯类肺炎克雷伯菌耐药变迁及流行调查[J]. 中国抗生素杂志,2018,43(5):572-576. LI Jihong, SHI Dongyan, LI Yuanyuan, et al. Epidemiological characteristics of nosocomial infections and resistance surveillance of carbapenem-resistant *Klebsiella pneumoniae* from 2015 to 2017[J]. Chinese Journal of Antibiotics, 2018, 43(5):572-576.

- [4] 何珊娜,陈敏,金海英,等.肺炎克雷伯菌临床分布特点与耐药性分析[J].中华医院感染学杂志,2015,25(19):4345-4346,4555.  
HE Shanna, CHEN Min, JIN Haiying, et al. Analysis of clinical distribution and drug resistance of *Klebsiella pneumoniae*[J]. Chinese Journal of Nosocomiology, 2015, 25(19): 4345-4346, 4555.
- [5] 裘莉佩,常燕子,竺军洋,等.肺炎克雷伯菌耐药基因及医院感染控制研究[J].中华医院感染学杂志,2013,23(19):4605-4608.  
QIU Lippei, CHANG Yanzi, ZHU Junyang, et al. Drug resistance genes in *Klebsiella pneumoniae* and control of nosocomial infections[J]. Chinese Journal of Nosocomiology, 2013, 23(19): 4605-4608.
- [6] HU Honghua, MANOS J. Pulsed-field gel electrophoresis of *Pseudomonas aeruginosa* [J]. Methods Mol Biol, 2015, 1301: 157-170.
- [7] TENOVER F C, ARBEIT R D, GOERING R V, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing[J]. J Clin Microbiol, 1995, 33(9): 2233-2239.
- [8] MA Ling, LU Poliang, SIU L K, et al. Molecular typing and resistance mechanisms of imipenem-non-susceptible *Klebsiella pneumoniae* in Taiwan; results from the Taiwan surveillance of antibiotic resistance (TSAR) study, 2002 ~ 2009 [J]. J Med Microbiol, 2013, 62(Pt1): 101-107.
- [9] BOWERS J R, KITCHEL B, DRIEBE E M, et al. Genomic analysis of the emergence and rapid global dissemination of the clonal group 258 *Klebsiella pneumoniae* pandemic [J]. PLoS One, 2015, 10 (7): e0133727.
- [10] ZHOU Tieli, ZHANG Yapei, LI Meimei, et al. An outbreak of infections caused by extensively drug-resistant *Klebsiella pneumoniae* strains during a short period of time in a Chinese teaching hospital: epidemiology study and molecular characteristics [J]. Diagn Microbiol Infect Dis, 2015, 82(3): 240-244.
- [11] KARAIKOS I, GIAMARELLOU H. Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: Current and emerging therapeutic approaches[J]. Expert Opin Pharmacother, 2014, 15(10): 1351-1370.
- [12] SAIDEL-ODES L, BORER A. Limiting and controlling carbapenem-resistant *Klebsiella pneumoniae* [J]. Infect Drug Resist, 2013, 7: 9-14.
- [13] BORER A, SAIDEL-ODES L, RIESENBERG K, et al. Attributable mortality rate for carbapenem-resistant *Klebsiella pneumoniae* bacteremia[J]. Infect Control Hosp Epidemiol, 2009, 30(10): 972-976.
- [14] MUNOZ-PRICE L S, POIREL L, BONOMO R A, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases [J]. Lancet Infect Dis, 2013, 13(9): 785-796.
- [15] 胡付品,朱德妹,汪复,等.2015年CHINET细菌耐药性监测[J].中国感染与化疗杂志,2016,16(6):685-694.  
HU Fupin, ZHU Demei, WANG Fu, et al. Report of CHINET antimicrobial resistance surveillance program in 2015 [J]. Chinese Journal of Infection and Chemotherapy, 2016, 16(6): 685-694.
- [16] NORDMANN P, CUZON G, NAAS T, et al. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria [J]. Lancet Infect Dis, 2009, 9(4): 228-236.
- [17] NADKARNI A S, SCHLIEP T, KHAN L, et al. Cluster of bloodstream infections cause by KPC-2 carbapenemase-producing *Klebsiella pneumoniae* in Manhattan [J]. Am J Infect Control, 2009, 37(2): 121-126.
- [18] MALACARNE P, BOCCALATTE D, ACQUAROLO A, et al. Epidemiology of nosocomial infection in 125 Italian intensive care units [J]. Minerva Anestesiologica, 2010, 76(1): 13-23.
- [19] LI Junjie, SHENG Zike, DENG Mei, et al. Epidemic of *Klebsiella pneumoniae* ST11 clone coproducing KPC-2 and 16S rRNA methylase RmtB in a Chinese University Hospital [J]. BMC Infect Dis, 2012, 12(1): 373-378.
- [20] MAGIORAKOS A P, SRINIVASAN A, CAREY R B, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance [J]. Clin Microbiol Infect, 2012, 18(3): 268-281.
- [21] 张丽,齐军,吴宗勇,等.耐碳青霉烯类肺炎克雷伯菌临床分离株中喹诺酮类及16SrRNA甲基化酶基因的检测[J].现代检验医学杂志,2018,33(5):27-30.  
ZHANG Li, QI Jun, WU Zongyong, et al. Detection of quinolones and 16SrRNA methyltransferase genes of clinical laboratory isolated of carbapenem-resistant *Klebsiella pneumoniae* [J]. Journal of Modern Laboratory Medicine, 2018, 33(5): 27-30.
- [22] 张丽,朱元祺,张小兵,等.耐碳青霉烯类肺炎克雷伯菌的耐药基因检测[J].中华医院感染学杂志,2014,24(23):5734-5736,5745.  
ZHANG Li, ZHU Yuanqi, ZHANG Xiaobing, et al. Detection of genotypes of carbapenem-resistant *Klebsiella pneumoniae* isolates [J]. Chinese Journal of Nosocomiology, 2014, 24(23): 5734-5736, 5745.
- [23] 张志军,鹿麟,牛法霞,等.耐碳青霉烯类肺炎克雷伯菌的耐药机制与分子流行病学特征[J].中国感染控制杂志,2018,17(9):759-763.  
ZHANG Zhijun, LU Lin, NIU Faxia, et al. Antimicrobial resistance mechanism and molecular epidemiological characteristics of carbapenem-resistant *Klebsiella pneumoniae* [J]. Chinese Journal of Infection Control, 2018, 17(9): 759-763.

收稿日期:2019-03-22

修回日期:2019-04-06