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· 临床研究 ·

医院肺炎克雷伯菌耐药及分子流行病学特征研究 *

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摘要 目的:了解医院肺炎克雷伯菌的耐药及分子流行特点,分析其基因同源性,为医院感染控制提供实验室依据。**方法:**收集2017年10月-2018年12月医院分离的耐碳青霉烯类肺炎克雷伯菌和碳青霉烯类敏感肺炎克雷伯菌,用梅里埃 VITEK 2 Compact 全自动微生物分析系统进行细菌鉴定和药敏分析,K-B 法药敏予以补充;采用赛沛 Xpert® Carba-R 试剂盒检测耐碳青霉烯类肺炎克雷伯菌碳青霉烯耐药基因;采用多位点序列分析技术检测菌株 7 个管家基因型别及其 ST 型别,使用 eBURST 软件对 ST 数据进行亲缘性关系分析。**结果:**共分离到 63 株耐碳青霉烯类肺炎克雷伯菌和 211 株碳青霉烯类敏感肺炎克雷伯菌(随机选取 30 株作研究);耐药组对青霉素类、大环内酯类、头孢菌素类、喹诺酮类、含酶抑制剂类、四环素类耐药率显著高于敏感组;63 株耐药株中,96.83%(61/63)产 KPC 酶,1.59%(1/63)产 KPC 酶和 NDM 酶,1.59%(1/63)未检测到 5 种碳青霉烯酶基因;耐碳青霉烯类组获得 8 个 ST 型别,包括 ST11 (54/63,85.71%)、ST15 (2/63,3.17%)、ST392 (2/63,3.17%)、ST45 (1/63,1.59%)、ST147 (1/63,1.59%)、ST659 (1/63,1.59%)、ST3228 (1/63,1.59%)、STr1 (1/63,1.59%),STr1 为新发现型别,属于克隆复合体 11;30 株碳青霉烯敏感组获得 25 个 ST 型别,包括 ST35 (3/30,10.00%)、ST659 (3/30,10.00%)、ST147 (2/30,6.67%)、ST485、ST34、ST395、ST370、ST2388、ST893、ST11、ST2176、ST221、ST86、ST380、ST65、ST920、ST268、ST25、ST2154、ST2229 以及 5 个新 ST 型别(STs1,STs2,STs3,STs4,STs5)各 1 株,分别占 3.33%,两组比较,耐药组 ST 型别集中而敏感组离散,差异明显。**结论:**医院耐碳青霉烯肺炎克雷伯菌具有基因同源相关性,并呈现水平传播的趋势。

关键词:肺炎克雷伯菌;耐碳青霉烯类肠杆菌;多位点序列分型;医院感染

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Study on Klebsiella Pneumoniae Resistance and Molecular Epidemiological Characteristics in Hospital*

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ABSTRACT Objective: To understand the molecular epidemic characteristics of Klebsiella pneumoniae in hospitals and analyze their genetic homology correlation to provide laboratory basis for nosocomial infection control. **Methods:** Carbapenem-resistant Klebsiella pneumoniae and Carbapenem-sensitive Klebsiella pneumoniae isolated from October 2017 to December 2018 were collected. Merièr VITEK 2 Compact full-automatic microbial analysis system was used for bacterial identification and drug sensitivity analysis, supplemented by K-B method; and Cyper Xpert® Carba-R kit was used to detect carbapenem-resistant genes; Seven-housekeeper genotypes and ST strains of the strains were analyzed using multi-site sequence analysis technology, and the etiologic analysis of ST data was performed using eBURST software. **Results:** A total of 63 strains of carbapenem-resistant Klebsiella pneumoniae and 211 strains of carbapenem-sensitive Klebsiella pneumoniae were collected. Compared with the sensitive group, the drug-resistant rates to penicillins, macrolides, cephalosporins, quinolones, enzyme inhibitors, and tetracyclines of resistant group were significantly higher. Of the 63 resistant strains, 96.83% (61/63) produced KPC enzyme, and 1.59% (1/63) produced KPC enzyme and NDM enzyme, 1.59% (1/63) did not detect 5 carbapenemase genes; the carbapenem-resistant group obtained 8 ST types, including ST11 (54/63, 85.71%), ST15 (2/63, 3.17%), ST392 (2/63, 3.17%), ST45 (1/63, 1.59%), ST147 (1/63, 1.59%), ST659 (1/63, 1.59%), ST3228 (1/63, 1.59%), STr1 (1/63, 1.59%), STr1 was a newly discovered type and belongs to the cloning complex 11; 30 carbapenem-sensitive groups obtained 25 ST types, including ST35 (3/30, 10.00%), ST659 (3/30, 10.00%), ST147 (2/30, 6.67%), ST485, ST34, ST395, ST370, ST2388, ST893,

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ST11, ST2176, ST221, ST86, ST380, ST65, ST920, ST268, ST25, ST2154, ST2229 and 5 new ST types (STs1, STs 2, STs3, STs4, STs5), accounting for 3.33% respectively. The ST type in the drug-resistant group was concentrated while that of the sensitive group was discrete. **Conclusion:** Hospital carbapenem-resistant *Klebsiella pneumoniae* has a gene homology correlation and shows a trend of horizontal transmission.

Key words: *Klebsiella pneumoniae*; Carbapenem-resistant Enterobacteriaceae; Multilocus sequence typing; Nosocomial infection

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前言

作为一种人类共生菌和条件致病菌,肺炎克雷伯菌逐渐成为引起院内感染和社区获得性感染的重要病原菌之一^[1]。据耐药监测网 CHINET 报道,在所有分离的阴性杆菌中,肺炎克雷伯菌分离数目仅次于大肠埃希菌,位于第 2 位,且自 2017 年起,肺炎克雷伯菌在呼吸道标本的分离率超过鲍曼不动杆菌,上升至第 1 位^[2]。更加严峻的是高毒力肺炎克雷伯菌和多耐药肺炎克雷伯菌的蔓延使得治疗更加棘手^[3,4]。2018 年 CHINET 统计肺炎克雷伯菌对美罗培南和亚胺培南的耐药率依次为 26.3%和 25%,意味着肺炎克雷伯菌感染的患者使用碳青霉烯类治疗可能有 25%的失败率,而这种高耐药率主要来源于碳青霉烯类抗生素滥用和耐药菌在医院内的传播^[5]。对院内获得性肺炎克雷伯菌感染,了解其分子流行病学以及临床特征对有效治疗和院感防控有重要意义。在多种分子分型方法中,基于核酸序列测定的多位点序列分型(MLST),以高分辨率、可重复性和可比性等优势被广泛应用于同一医院、不同医院、不同地区、不同国家的耐碳青霉烯类的肺炎克雷伯菌的序列分型,了解局部和全球流行的态势^[6]。因此我们采用 MLST 对收集到的 63 株耐碳青霉烯类肺炎克雷伯菌(carbapenem-resistant *Klebsiella pneumoniae*, CRKP)和 30 株碳青霉烯类敏感肺炎克雷伯菌(carbapenem-susceptible *Klebsiella pneumoniae*, CSKP)进行分型,了解其亲缘关系,为院感防控提供理论依据。

1 材料与方法

1.1 菌株来源

收集 2017 年 10 月-2018 年 12 月期间某医院临床分离的非重复 CRKP 菌株 63 株和随机选择的 CSKP 菌株 30 株。试验菌株均经法国生物梅里埃 Vitek MS 鉴定为肺炎克雷伯菌。质控菌株为大肠埃希菌 ATCC8739 和 ATCC25922。

1.2 仪器与试剂

Vitek2 Compact 全自动细菌鉴定及药敏分析系统(法国生物梅里埃),GeneXpert 分子诊断系统(美国赛沛),聚合酶链式反应(PCR)扩增仪(美国 Bio-Rad),凝胶电泳成像系统(美国 Bio-Rad),抗菌药敏纸片(英国 Oxoid),M-H 培养基(温州康泰生物科技有限公司),普通血琼脂平板(安图生物)。

1.3 方法

1.3.1 药物敏感性试验 取对数生长期的菌株按照《全国临床检验操作规程》,采用梅里埃 Vitek2 Compact 全自动细菌鉴定及药敏分析系统完成药敏试验,K-B 法作补充,结果判读参照 CLSI2020 年标准。

1.3.2 碳青霉烯酶基因检测 采用赛沛 Xpert® Carba-R 试剂

盒检测 5 个碳青霉烯酶基因 blaKPC, blaNDM, blaVIM, blaOXA-48 和 blaIMP,按照试剂盒说明书操作。

1.3.3 多位点序列分型 刮取适量细菌加入 100 微升蒸馏水中,振荡混匀,100℃煮沸 15 min,10000 rpm 离心 5 min,细菌 DNA 则溶解于上清中。PCR 扩增 rpoB, gapA, mdh, pgi, phoE, infB 和 tonB 7 个管家基因,引物参考 Pasteur 数据库(https://bigsdb.pasteur.fr/klebsiella/pr-imers_used.html)。PCR 条件:94℃ 2 min, 94℃ 30s, 50℃ 1 min, 72℃ 30 s 共 35 个循环, 72℃ 5 min。PCR 产物经 1.5%琼脂糖凝胶电泳鉴定为单一条带后,送上海生工生物公司测序。将管家基因的序列在 Pasteur 数据库(https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef_public&page=sequenceQuery)进行比对,获得管家基因型别,将 7 个管家基因型别写入此网页提交(https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef_public&page=profiles)获得菌株 ST 型别。

1.4 肺炎克雷伯菌基因同源性分析

使用 eBURST V3 软件对 63 株 CRKP 的 ST 数据进行亲缘关系分析,并绘制网络结构的辐射状图。

2 结果

2.1 临床资料及菌株来源

CRKP 组和 CSKP 组平均年龄分别为(49.18±17.41)岁和(58.73±19.29)岁,男女比例分别为 15:48 和 14:16。CRKP 组和 CSKP 组分别有 35(55.56%)株和 9(30.00%)株来源于重症监护病房。菌株标本来源信息见图 1。

2.2 药物敏感性试验结果

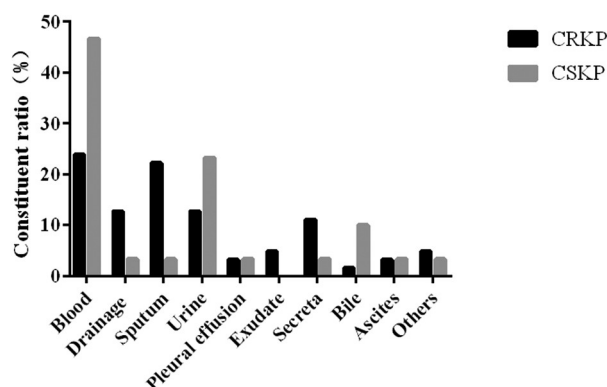


图 1 CRKP 和 CSKP 来源样本类型构成图

Fig.1 Specimen orientation composition of CRKP and CSKP

CRKP 组细菌对一、二、三、四代头孢菌素,头霉素类、氨基糖苷类、含酶抑制剂类及碳青霉烯类抗生素的耐药率均在 90%以上,氨基糖苷类抗生素的耐药率也很高,在 80%以上,

仅有四环素类抗生素的耐药率较低,四环素为 42.37%,替加环素为 7.94%,提示可用于 CRKP 感染的联合治疗。

及四环素的耐药率在 43%以上外,其他类抗生素均呈现较高的敏感性。

CSKP 组细菌除对一、二代头孢菌素,氨苄西林 / 舒巴坦以及 2.3 碳青霉烯酶基因检测结果

表 1 肺炎克雷伯菌药物敏感性结果
Table 1 Drug sensitivity results of Klebsiella pneumonia

Antibiotic	Resistance percentage(%)		Intermediate percentage(%)		Sensitivity Percentage(%)	
	CRKP	CSKP	CRKP	CSKP	CRKP	CSKP
	(n=63)	(n=30)	(n=63)	(n=30)	(n=63)	(n=30)
Cefazolin	98.36	66.67	0.00	0.00	1.64	33.33
Cefuroxime	98.36	48.39	0.00	3.23	1.64	48.38
Ceftazidime	93.65	16.13	1.59	3.23	4.76	80.64
Ceftriaxone	98.36	35.48	0.00	0.00	1.64	64.52
Cefepime	93.65	9.68	0.00	0.00	6.35	90.32
Ampicillin/sulbactam	98.36	38.71	0.00	6.45	1.64	54.84
Piperacillin/tazobactam	90.48	0.00	3.17	6.45	6.35	93.55
Cefoperazone/sulbactam	90.48	0.00	3.17	0.00	6.35	100.00
Meropenem	92.06	0.00	1.59	0.00	6.35	100.00
Imipenem	90.47	0.00	1.59	0.00	7.94	100.00
Biapenem	95.16	0.00	0.00	0.00	4.84	100.00
Tetracycline	42.37	43.33	3.39	0.00	54.24	56.67
Tigecycline	7.94	0.00	3.33	0.00	88.73	100.00
Amikacin	80.95	3.23	0.00	0.00	19.05	96.77
Gentamicin	84.13	29.03	0.00	0.00	15.87	70.97
Tobramycin	82.55	12.90	6.34	19.35	11.11	67.75
Levofloxacin	92.06	25.81	0.00	0.00	7.94	74.19
Ciprofloxacin	92.06	25.81	1.59	0.00	6.35	74.19
Cefotetan	91.80	6.45	0.00	0.00	8.20	93.55
Aztreonam	95.16	32.26	0.00	0.00	4.84	67.74
Sulfamethoxazole and trimethoprim	78.69	38.71	0.00	0.00	21.31	61.29

61 株 CRKP 检出 blaKPC 基因,1 株检出 blaKPC 和 blaNDM 基因,1 例五种耐药基因均未检测到,表明我院流行的 CRKP 以产 KPC 酶株为主,同时产 KPC 酶和 NDM 酶的 CRKP 目前仅见于烧伤外科,除了产上述 5 种碳青霉烯酶外,可能还存在其他引起碳青霉烯类耐药的机制。

2.4 PCR 扩增产物鉴定结果

PCR 扩增产物经 1.5%琼脂糖凝胶电泳鉴定,7 个管家基因的产物大小在 506bp-1119bp 之间,电泳结果见图 2。

2.5 MLST 结果

PCR 测序结果经 DNAMAN 比对,得到的序列上传到 KP-NMLST 数据库进行比对分析。63 株 CRKP 得到 8 个 ST 型,其中 ST11 是最主要的 ST 型,占 85.71%,新发现一个 ST 型(STr1)。ST 型别及其分布科室详见表 2。30 株 CSKP 得到 25 个 ST 型,包括 ST35(3),ST659(3),ST147(2),ST485(1),ST34(1),ST395(1),ST370(1),ST2388(1),ST893(1),ST11(1),

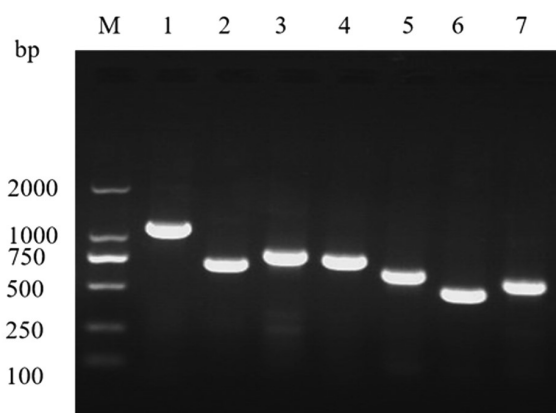


图 2 KPN 管家基因 PCR 扩增结果验证

Fig.2 PCR amplification results of KPN housekeeper genes
注:条带 1-7 依次为 rpoB、gapA、mdh、pgc、phoE、infB、tonB 基因
Note: Bands 1-7 were rpoB, gapA, mdh, pgc, phoE, infB, tonB genes in sequence

ST2176 (1),ST221 (1),ST86 (1),ST380 (1),ST65 (1),ST920 (1),ST268 (1),ST25 (1),ST2154 (1),ST2229 (1)以及 5 个新 ST 型别(STs1,STs2,STs3,STs4,STs5),其中 STs3,STs4 和 STs5 分别新发现 1 个(n1)、1 个(n2)和 5 个新等位基因(n3,n4,n5,n6,n7),具体等位基因谱见表 3。

2.6 KPN 基因同源性分析

使用 eBURST 将 CRKP 组 ST 型别及其等位基因数据与 KPNMLST 数据库比较,并绘制亲缘关系图,如图 3 所示。CRKP 组新 ST 型为 11 克隆复合体的 SLV。

表 2 63 株 CRKP 科室分布及 ST 型别构成比

Table 2 63 strains CRKP department distribution and ST type composition ratio

ST type	Allelic profile	Departments	No.	Composition ratio(%)
Gastroenterology ICU(23),Neurology ICU(7),Burns(8),				
ST11	3-3-1-1-1-1-4	Gastroenterology(4),Neurology(2),Respiratory ICU(2),Outpatient (2), Cardiac surgery ICU(1),Urology(1),Pediatrics(1),Hematology (1),Transplant Center(1),Physiotherapy(1)	54	85.71
ST15	1-1-1-1-1-1-1	Gastroenterology ICU(1),Hematology (1)	2	3.17
ST392	3-4-6-1-7-4-40	Neurology ICU(1),Physiotherapy (1)	2	3.17
ST45	2-1-1-6-7-1-12	Gastroenterology (1)	1	1.59
ST147	3-4-6-1-7-4-40	Neurology(1)	1	1.59
ST659	66-1-65-1-9-11-18	Transplant Center (1)	1	1.59
ST3228	31-3-1-37-3-27-4	Neurosurgery ICU (1)	1	1.59
STr1	3-3-1-1-1-1-34	Gastroenterology ICU(1)	1	1.59

表 3 30 株 CSKP 新发现 ST 型别特点

Table 3 Features of ST type newly discovered in CSKP strains

ST type	Allelic profile	Departments	No.
STs1	2-1-2-1-10-4-7	Hematology	1
STs2	4-1-1-1-7-1-35	Rheumatology and immunology	1
STs3	18-22-n1-22-94-20-50	Geriatrics	1
STs4	16-24-21-107-53-66-n2	Neurology	1
STs5	9-n3-n4-n5-1-n6-n7	Traditional Chinese Medicine	1

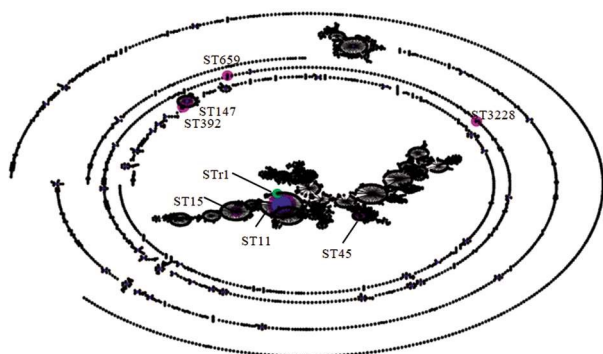


图 3 63 株 CRKP 亲缘关系分布图

Fig.3 Distribution of phylogenetic relationships of 63 strains of CRKP
注:红色点为本研究 CRKP 与 KPNMLST 数据库共有的 ST 型别,绿色点为新发现型别,黑色点为 KPNMLST 数据库的其他 ST 型别。

Note: The red dots were the ST types shared by CRKP and KPNMLST databases in the study, the green dot was the newly discovered type, and the black dots were the other ST types in the KPNMLST database.

3 讨论

肺炎克雷伯菌是人体正常定植菌,也是条件致病菌,当免疫力降低、经历有创操作手术、粘膜屏障破坏、长期服用激素和(或)免疫抑制药物等时会引起感染性疾病^[7]。自上世纪 90 年代第一株 CRKP 被分离以来,随着碳青霉烯类抗生素在住院患者中广泛应用,CRKP 的分离率不断增加^[8]。CRKP 感染患者的死亡率居高不下,有研究报道 CRKP 和 CSKP 感染的死亡率分别为 42.14%和 21.16%,血流感染时死亡率更是高达 54.30%^[9]。了解医院内 CRKP 的分子流行病学特征和耐药基因型对院内感染控制具有重要意义。本研究中的 63 株 CRKP 中有 54 株为 ST11 型,与王辉教授等报道的中国大陆 CRKP 的主要 ST 型别一致,有聚集性传播趋势^[10]。ST15、ST392、ST45、ST147、ST659、ST3228 及新型别 STr1 均为散发。从科室分布角度分析,CRKP 广泛分布于包括消化、神内、呼吸和心外在内的四个重症监护病房以及 10 多个普通病房。重症监护室由于呼吸机相关性感染和广谱抗生素的使用,多重耐药菌的分离率明显高于普通病房。理疗科分离出 CRKP 的多来源于脑出血、脑梗治疗后接受康复训练的患者,CRKP 多为定植菌,需要定期监测,管理其痰液和粪便,避免引起病区内其他患者感染。血液科、儿科和移植中心患者感染 CRKP 后往往预后不良,需要及时根据药敏结果

调整用药。值得注意的是,30株CSKP共得到25种ST型别,其中5个型别为本研究首次发现,与CRKP组相比较,主要分布于普通病房,具有明显离散趋势。

肺炎克雷伯菌耐碳青霉烯类药物机制主要是产生各种水解酶,如A类碳青霉烯酶KPC、B类金属 β 内酰胺酶NDM,KPC是中国地区分离的肺炎克雷伯菌中最常见的碳青霉烯酶^[11,12]。其次是外膜蛋白缺失致渗透性降低,药物进入细菌内困难,其它耐药机制还有外排泵高表达、靶位点改变等;通常多种耐药机制同时存在,协同导致高水平耐药^[13]。医院分离的63株CRKP有61株产KPC酶,1株同时产KPC酶和NDM酶,还有1株未检测到五种碳青霉烯酶,可能由其他未测碳青霉烯酶或外膜蛋白缺失等机制介导耐药。对于产KPC酶的CRKP可根据碳青霉烯类最低抑菌浓度增大剂量或者联合其他抗生素治疗。同时产KPC酶和NDM酶的菌株分离自烧伤科,提示该科室如果出现增加碳青霉烯类剂量或使用头孢他啶/阿维巴坦疗效不佳时,应考虑是否为产金属酶株。

对于多重耐药菌的管理,消灭来源,切断传播途径,保护易感人群同样适用。文献报道,产碳青霉烯酶细菌和其基因不仅仅存在于医院环境中,城市污水、饮用水、娱乐用水、宠物、野生动物以及农业环境中也广泛存在^[14]。在法国、埃及和中国都曾报道无近期住院史,也未在长期护理机构接受治疗而在携带耐碳青霉烯肠杆菌,表明主动对环境和患者肠道CRKP及其基因进行连续监测具有重要意义,尤其在重症监护室、外科手术室和收治免疫力低下患者的科室,Karampatakis等通过主动筛查的方式成功将儿科ICU CRKP的感染率由1.2%降低至0.1%^[15,16]。

CRKP组对青霉素类、大环内酯类、头孢菌素类、喹诺酮类、含酶抑制剂类、四环素类耐药率显著高于CSKP组。对长期住院患者,抗生素治疗效果不佳时,可能对抗生素产生耐药,需要及时调整剂量或使用高级别抗生素。本研究分离到5株(7.94%)对替加环素敏感性降低的CRKP,即耐替加环素和碳青霉烯肺炎克雷伯菌(tigecycline- and carbapenem-resistant *Klebsiella pneumoniae*, TCRKP),俞云松等研究表明CRKP中约有8.3%为TCRKP,其致死率高达44%,呼吸道感染比泌尿道感染的致死率更高^[17]。而TCRKP耐药的产生主要与acrB、oqxB和tet(A)外排泵基因和(或)ramA和rarA等调节基因表达增加有关^[18,19]。我院分离到的TCRKP均为ST11型,与国内报道一致^[20],其在院内感染监测中需要引起重视。

本研究的不足之处为TSKP组样本例数较少,需要增加样本量才能更好的反映TSKP的分子流行病学特征。

综上所述,CRKP在医院感染中呈现聚集性水平传播趋势,遏制耐药不仅要从合理使用抗生素着手,还需要做好院内感染控制,隔离感染源,切断传播途径。

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