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◇临床医学研究◇

肺泡灌洗液宏基因组二代测序在检测肺部感染病原菌中的应用

张 鹤,罗欣悦,衡 鑫,张 沄,王宋平,邓 俊

(西南医科大学附属医院呼吸与危重症医学科,泸州 646000)

摘要 目的 探讨宏基因组二代测序(mNGS)对肺部感染患者病原学检测的应用价值。**方法** 回顾性分析近4年收治的434例肺部感染患者资料,根据有无基础疾病,分为基础疾病组($n=262$)和无基础疾病组($n=172$)。分别采用mNGS和常规检测获取各组患者的病原菌,分析患者的临床及实验室资料、影像学资料及病原菌检测结果,比较两种检测方法对肺部感染病原菌的诊断效能。**结果** 434例患者mNGS检出阳性率高于常规检测,差异有统计学意义($P<0.05$)。mNGS检出细菌、病毒效能明显高于常规检测,差异有统计学意义($P<0.05$);而mNGS真菌检出率虽高于常规检测,但差异无统计学意义。其中结核分枝杆菌、肺炎支原体、流感嗜血杆菌、肺炎链球菌、星座链球菌及金黄色葡萄球菌、烟曲霉检出率明显高于常规检测,差异有统计学意义($P<0.05$)。亚组分析显示,基础疾病组的男性比例、住院时间、吸烟率及平均年龄均高于无基础疾病组,差异有统计学意义($P<0.05$),而两组抗生素使用及气管插管率差异无统计学意义。mNGS检出基础疾病组最常见病原菌为结核分枝杆菌、流感嗜血杆菌、肺炎链球菌、铜绿假单胞菌、人类疱疹病毒4型及烟曲霉,无基础疾病组最常见病原菌为结核分枝杆菌、流感嗜血杆菌、肺炎链球菌、肺炎支原体及肺炎克雷伯菌。两组mNGS阳性率均显著高于常规检测,差异有统计学意义($P<0.05$),而两组mNGS阳性率对比差异无统计学意义。**结论** mNGS在肺部感染病原菌检测中较常规检测具有显著优势,受基础疾病的影响小,可为肺部感染提供病原学依据。

关键词 肺部感染;宏基因组二代测序;mNGS;常规检测;肺泡灌洗液;基础疾病;病原体

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肺部感染是最常见的感染性疾病,严重威胁人类健康,尽早明确肺部感染的病原体及确定抗感染方案对改善患者预后、减轻医疗负担有极大的帮助[1]。临床上对病原体的常规检测包括一般细菌/真菌培养、抗原抗体检测或基于PCR技术等。病原体培养通常时间长,阳性率低,尤其是使用抗生素后,较多病原体无法培养,阳性率更低,PCR检测虽然灵敏度和特异度较高,但仅针对有限的病原体,存在较多病原体无法确定,多种病原体混合感染难以鉴定等缺点[2],因此需要一种更快速、准确的检测方法。宏基因组二代测序(metagene next-generation sequencing, mNGS)能够覆盖更广泛的病原体,结合病原微生物数据库及特定算法,快速检测样本中的

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作者简介:张 鹤,女,硕士研究生,住院医师;

邓 俊,女,副主任医师,硕士生导师,通信作者,E-mail:dj2882@sina.com

EMT in ESCC cells *via* the EGFR/GSK3β signaling pathway. Notably, Ctx exhibited markedly weaker inhibitory effects on mesenchymal-like cells compared to epithelial ESCC cells. ***Conclusion*** Pg promotes ESCC cells proliferation, invasion and migration by regulating EMT through the EGFR/GSK3β signaling pathway, and enhances chemoresistance to Ctx.

Key words *Porphyromonas gingivalis*; ESCC; EGFR; EMT; Ctx; cancer drug resistance

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Corresponding author Gao Shegan, E-mail: gsg112258@163.com

可能病原体,包括病毒、细菌、真菌、寄生虫等[3]。本研究回顾性选择434例肺部感染患者作为研究对象,旨在比较mNGS和常规检测在病原体方面的诊断效能,探讨mNGS在肺部感染病原学检测中的应用价值。

1 材料与方法

1.1 病例资料 选择2020年9月—2023年12月在西南医科大学附属医院呼吸与危重症医学科收治的434例肺部感染患者作为研究对象,并对患者的病历进行审查,以收集基线信息,包括年龄、性别、是否合并基础疾病、常规检测和mNGS检测结果。纳入标准:① 年龄≥14岁;② 临床及影像学资料完整;③ 肺部感染诊断符合成人社区获得性肺炎诊断标准[4];④ 所有患者均能完成支气管镜检查及肺泡灌洗术,收集肺泡灌洗液做mNGS和常规检测。排除标准:① 未收集肺泡灌洗液做mNGS或常规检测的患者;② 肺泡灌洗液样本收集或检测过程不符合mNGS或常规检测的质量控制;③ 临床和实验室检查资料不完整。本研究纳入的患者均签署知情同意书,并经本院伦理委员会审核通过(受理号:KY2024373)。

1.2 方法

1.2.1 常规检测 常规检测类型包含常规培养(痰培养/真菌培养)、呼吸道病原体核酸检测(细菌/病毒)、抗酸染色、新型冠状病毒核酸检测及弓形虫、风疹病毒、巨细胞病毒和单纯疱疹病毒检测(TORCH4项),送检标本类型包括肺泡灌洗液、痰液、咽拭子及血液。

常规培养:采集合格痰或肺泡灌洗液分别接种于血平板、麦康凯平板、巧克力平板中,于36 ℃下,培养18~48 h,其中巧克力培养基置于7% CO_2浓度培养箱内培养。培养后经分离纯化,MicrofiexLT/SHMALDI-TOF-MS质谱仪(德国布鲁克公司)进行鉴定,VITEK 2 Compact全自动分析系统进行药敏试验(法国梅里埃公司),试验结果依据美国临床与实验室标准化协会2019年标准(2019 Clinical and Laboratory Standards Institute, CLSI 2019)进行判读。真菌培养:合格痰或肺泡灌洗液接种于沙保弱琼脂平板,置37 ℃培养5 d。常见的酵母菌在念珠菌培养基显色即可报告。如为真菌,在沙保弱菌培养基中培养,根据菌落形态、色素及镜下结构鉴别。

呼吸道病原体核酸检测:采集合格痰或肺泡灌洗液,采用湖南圣湘生物科技有限公司配套核酸提取试剂盒提取核酸,采用呼吸道病原体核酸检测试剂盒(PCR-荧光探针法)在ABI7500荧光定量PCR进行扩增,对常见病原菌进行检测包括呼吸道合胞病毒、甲/乙型流感病毒、人鼻病毒、腺病毒、肺炎支原体、肺炎链球菌、金黄色葡萄球菌、耐甲氧西林金黄色葡萄球菌、肺炎克雷伯杆菌、鲍曼不动杆菌、嗜麦芽寡食单胞菌、流感嗜血杆菌。

新型冠状病毒核酸检测:采集患者咽拭子,采用核酸提取试剂盒(湖南圣湘生物科技有限公司)提取RNA,采用新型冠状病毒2019-nCoV核酸检测试剂盒(荧光PCR法)在ABI7500荧光定量PCR进行扩增。

抗酸:采集合格痰或肺泡灌洗液经涂片自然干燥后,在生物安全柜内紫外照射30 min,加石炭酸复红染液染色10 min后细流水冲洗甩干,加酸性酒精脱色1~2 min或至无可见红色为止,水洗,滴加亚甲基蓝复染30~60 s,自然干燥后镜检。

TORCH4项定量测定:采集静脉血3 mL,在LIAISON XL全自动化学发光免疫分析系统(意大利索灵公司)进行检测包括弓形虫、风疹病毒、巨细胞病毒、单纯疱疹病毒。

1.2.2 mNGS检测及数据解读 取无菌密封的肺泡灌洗液为送检样本,低温冰袋运送24 h内送达标本,由艾迪康医学检验实验室进行样本检测。将肺泡灌洗液破壁离心后取600 μL上清液,使用核酸提取试剂盒提取DNA,使用测序反应制备通用试剂盒进行片段化、末端修复、接头连接和PCR扩增、磁珠纯化等操作。Agilent2100生物分析仪(美国Agilent公司)对文库及插入片段大小进行质控,使用荧光定量PCR测定文库浓度。使用MGI200/2000测序仪进行上机测序。测序数据用Snap Gene软件与微生物基因组数据库[从美国国家生物技术信息中心数据库(national center for biotechnology information database,NCBI)中筛选组成]进行比对,从而对微生物进行鉴定。

数据解读:由于mNGS缺乏统一标准,经大量文献检索满足以下条件可判定为阳性:对于不同类型的微生物,在种/属水平上严格序列数设置如下:细菌、病毒、真菌、支原体、衣原体序列数≥50,寄生虫序列数≥100,结核分枝杆菌序列数≥1,对于序列数<50的,与患者临床相符,经3名医师独立审核后仍可考虑为致病原。

1.3 统计学处理 运用SPSS 29.0统计软件进行统计分析,对于非正态分布或方差不齐的计量资料采用非参数检验,以$M(P_{25},P_{75})$表示,符合正态分布计量资料用均数±标准差($\bar{x}\pm s$)表示;计数资料

以例数(百分数)表示,组间比较采用 χ^2 检验,或 Fisher 精确检验,$P<0.05$ 为差异有统计学意义。

2 结果

2.1 一般基线资料 如表 1 所示,基础疾病组的男性比例、住院时间、吸烟率及平均年龄均高于无基础疾病组,差异有统计学意义($P<0.05$),而两组气管插管率及抗生素使用差异无统计学意义($P>0.05$)。

2.2 mNGS 与常规检测的病原体分布及阳性率比较

2.2.1 mNGS 与常规检测的病原体分布特点 如表 2 所示,mNGS 检出细菌、病毒效能明显高于常规检测,差异有统计学意义($P<0.05$);而 mNGS 真菌检出率虽高于常规检测,但差异无统计学意义($P>0.05$)。其中结核分枝杆菌、肺炎支原体、流感嗜血杆菌、肺炎链球菌、星座链球菌及金黄色葡萄球菌、烟曲霉检出率明显高于常规检测,差异有统计学意义($P<0.05$)。mNGS 与常规检测检出常见病原体如图 1-3 所示,其中 mNGS 还检出少见病原体包括耶氏肺孢子菌、鹦鹉热衣原体、鸟-胞内分枝杆菌、解鸟氨酸拉乌尔菌、马尔尼菲篮状菌等。

2.2.2 mNGS 与常规检测阳性率比较 如表 3 所示,mNGS 检出阳性率(70.97%)高于常规检测(32.49%),差异有统计学意义($\chi^2=11.374$, $P<0.001$)。mNGS 与常规检测的一致性见图 4。

2.3 两种方法对有无基础疾病患者常见病原体及检测阳性率的比较 如表 4、5 所示,有无基础疾病患者中 mNGS 检出阳性率均明显高于常规检测,差异均有统计学意义($P<0.05$)。对两组常规检测的阳性率对比分析显示,基础疾病组检出阳性率高于无基础疾病组,差异有统计学意义($P<0.05$),而两组 mNGS 检出阳性率差异无统计学意义($P>0.05$)。两组常见病原体见图 5。

2.4 两种检测方法检出混合感染特征 如表 6 所示,mNGS检出混合感染阳性率高于常规检测,差异

表 1 肺部感染患者的基线资料[n(%),$M(P_{25},P_{75})$]

Tab. 1 Baseline data of patients with pulmonary infection[n(%), $M(P_{25},P_{75})$]

Item	Underlying disease group($n=262$)	Non-underlying disease group($n=172$)	χ^2/Z value	P value
Gender			9.957	0.002
Man	178(67.94)	91(52.91)		
Woman	84(32.06)	81(47.09)		
Age (years)	65(53,72)	53(39,64)	-7.266	<0.001
Number of days in hospital	12.0(9,16)	10.5(7,14)	-3.573	<0.001
Smoking			8.888	0.003
Yes	110(41.98)	48(27.91)		
No	152(58.02)	124(72.09)		
Endotracheal incubation			0.454	0.501
Yes	21(8.02)	17(9.88)		
No	241(91.98)	155(90.12)		
Antibiotic use			1.575	0.210
Yes	40(15.27)	19(11.05)		
No	222(84.73)	153(88.95)		

表 2 mNGS 与常规检测的病原菌分布情况[$n=434$,n(%)]

Tab. 2 Distribution of pathogens detected by mNGS and conventional tests[$n=434$, n(%)]

Detection method	mNGS	Conventional tests	χ^2 value	P value
Bacteria	270(62.21)	104(23.96)	129.461	<0.001
Mycobacterium tuberculosis complex	85(19.59)	22(5.07)	42.309	<0.001
Mycoplasma pneumoniae	15(3.46)	6(1.38)	3.953	0.047
Haemophilus influenzae	60(13.82)	10(2.30)	38.847	<0.001
Streptococcus pneumoniae	41(9.45)	3(0.69)		<0.001
Streptococcus constellation	19(4.38)	1(0.23)		<0.001
Staphylococcus aureus	16(3.69)	4(0.92)		0.011
Fungi	71(16.36)	56(12.90)	2.075	0.150
Aspergillus fumigatus	27(6.22)	9(2.07)	9.389	0.002
Virus	74(17.05)	10(2.30)	53.986	<0.001

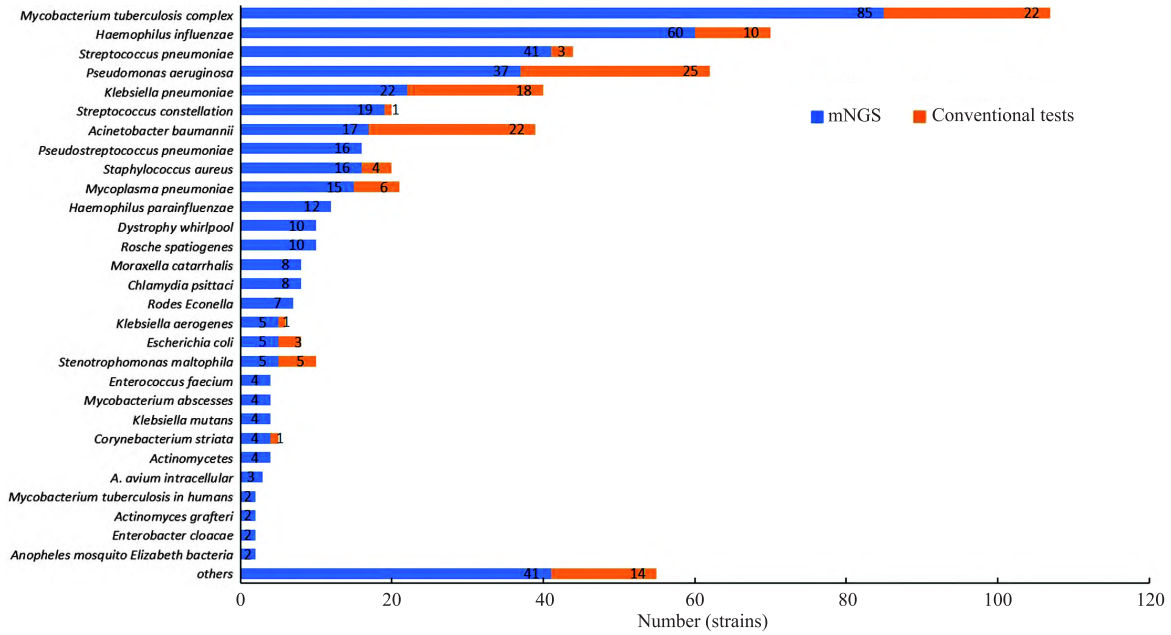

图 1　两种检测方法检出常见细菌种类分布图

Fig. 1　Distribution of common bacterial species detected by two detection methods

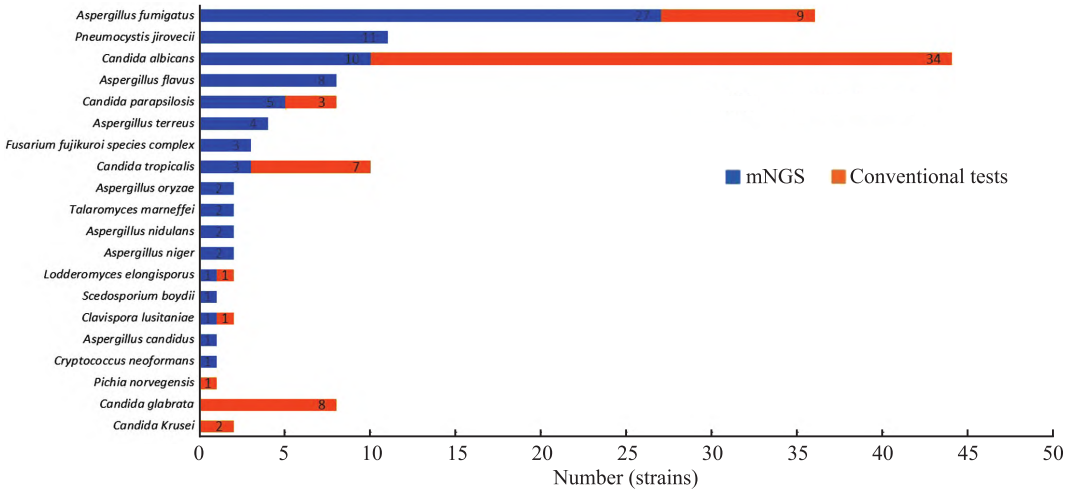

图 2　两种检测方法检出真菌种类分布图

Fig. 2　Distribution of fungi species detected by two detection methods

表 3　mNGS 与常规病原学检测阳性率比较

Tab. 3　Comparison of positive rate of mNGS detection and conventional etiological detection

mNGS	Conventional tests		Total
	Positive	Negative	
Positive	115	193	308
Negative	26	100	126
Total	141	293	434

有统计学意义($\chi^2=40.021$, $P<0.05$)。

3　讨论

肺部感染抗菌治疗的选择需要了解引起感染的病原菌,从微生物学的角度来看,呼吸道感染的经验性和靶向抗菌治疗都是基于分离微生物的敏感性特征和可能呈现的耐药机制[5]。mNGS 在病原学检测的应用越来越广泛,可以覆盖绝大部分病原微生物,尤其是罕见和新出现的病原微生物的检测,能有效地进行下呼吸道感染的临床管理和治疗[6]。采用 mNGS 分析肺部感染患者的下呼吸道微生物特征,对该类感染患者的病原诊断和精准治疗具有重要的临床意义,即使患者有痰培养和多重 PCR 结果可用,也可能在识别肺炎病因方面发挥作用[7]。

本研究结果显示,经 mNGS 检出细菌、病毒明显

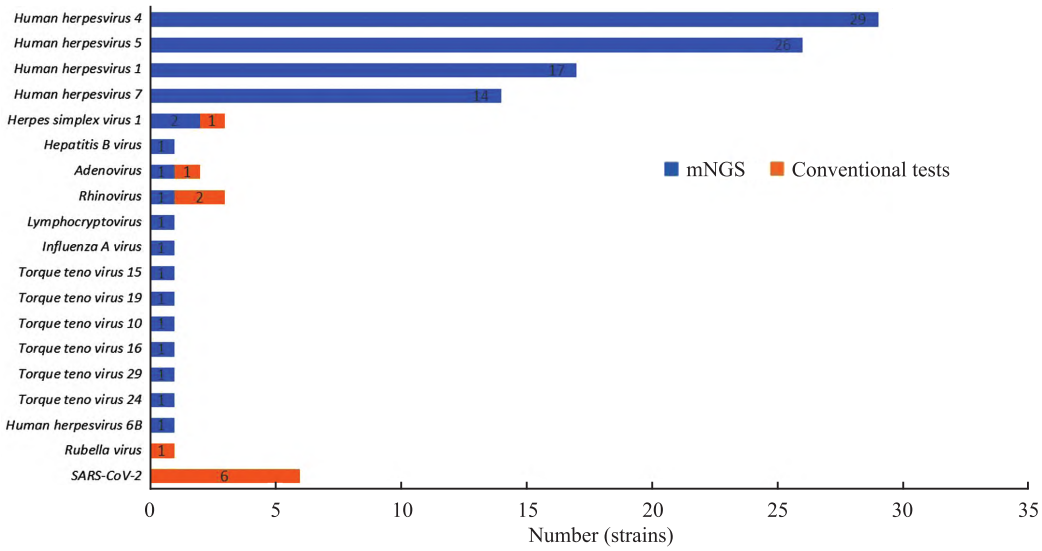

图 3　两种检测方法检出真菌种类分布图

Fig. 3　Distribution of virus species detected by two detection methods

表 4　两种检测方法对有无基础疾病患者检出阳性率的比较

Tab. 4　Comparison of the positive rate of the two tests in patients with and without underlying disease

Underlying disease group		Conventional tests		Total	Non-underlying disease group		Conventional tests		Total
		Positive	Negative				Positive	Negative	
mNGS	Positive	75	110	185	mNGS	Positive	40	84	124
	Negative	21	56	77		Negative	5	43	48
	Total	96	166	262		Total	45	127	172

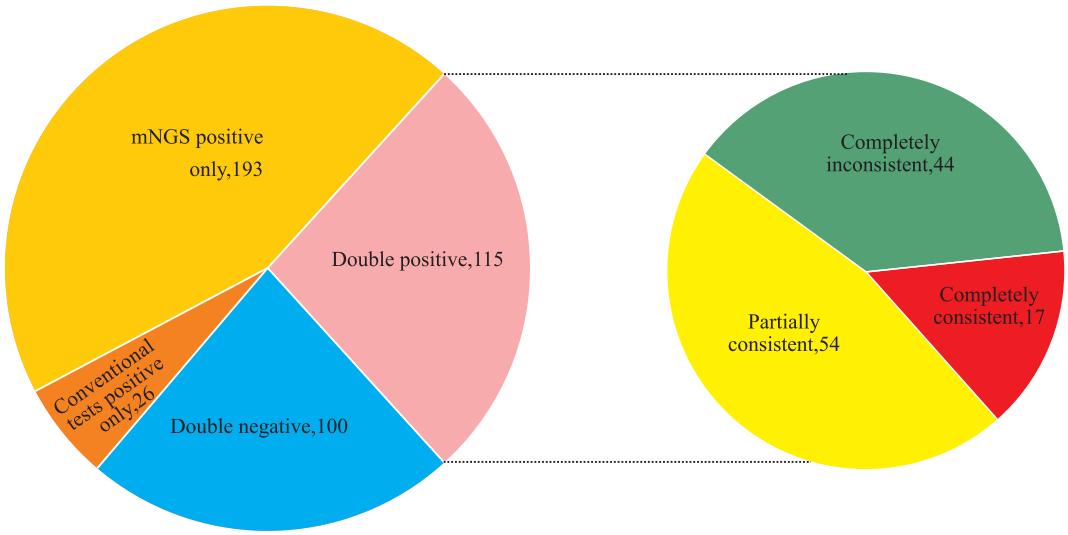

图 4　mNGS 与常规检测的一致性比较

Fig. 4　Comparison of consistency between mNGS and conventional tests

高于常规检测,而在检测真菌方面与常规检测差异不大,这与吴媛媛 等[8]研究相似。mNGS 检出混合感染阳性率高于常规检测,提示 mNGS 在混合感染患者中的诊断价值更高[9]。本研究中 mNGS 检出最常见的革兰阳性菌前三为肺炎链球菌、星座链球菌、金黄色葡萄球菌,最常见的革兰阴性菌前三为流感嗜血杆菌、铜绿假单胞菌、肺炎克雷伯菌,细菌谱特征与刘静 等[10]研究基本相似。最常见的真菌是烟曲霉、耶氏肺孢子菌、白念珠菌,真菌谱特征与 Tiew et al[11]基本相似。最常见的病毒是人类疱疹

表 5 mNGS 和常规检测分别在不同组的阳性率比较

Tab. 5 The positive rates of mNGS and conventional tests were compared in different groups

Detection method	Group	Positive rate(%)	χ^2 value	*P* value
Conventional tests	Underlying disease group	36.64	5.198	0.023
	Non-underlying disease group	26.16		
mNGS	Underlying disease group	70.61	0.111	0.739
	Non-underlying disease group	72.09		

表 6 两种检测方法对混合感染检出率的比较

Tab. 6 Comparison of mixed infection detection rates between the two tests

Type of infection	mNGS	Conventional tests	Total
Bacteria + Viruses	38	3	41
Bacteria + Fungi	37	24	61
Bacteria + Fungi + Viruses	13	1	14
Fungi + Viruses	6	1	7
Total[*n*(%)]	94(21.66)	29(6.68)	123(14.17)

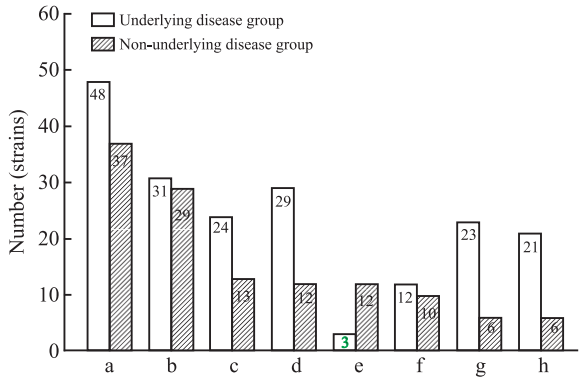

图 5 两组常见病原体分布图

Fig. 5 Pathogen distribution of common pathogens in the two groups

a: *Mycobacterium tubercu*; b: *Haemophilus influenzae*; c: *Pseudomonas aeruginosa*; d: *Streptococcus pneumoniae*; e: *Mycoplasma pneumoniae*; f: *Klebsiella pneumoniae*; g: *Human herpesvirus* 4; h: *Aspergillus fumigatus*.

病毒 4 型、人类疱疹病毒 5 型、人类疱疹病毒 1 型。呼吸道病毒是导致肺部感染的最常见原因之一，NGS 技术可不依赖培养的机制检测病毒，并能检测以前未表征的病原体[12]。具体分析显示 mNGS 在检测肺炎支原体、流感嗜血杆菌、肺炎链球菌、星座链球菌及金黄色葡萄球菌方面优于常规检测，其中 mNGS 检出结核分枝杆菌相较常规检测具有明显优势，这与 Acharya et al[13] 研究相似。在真菌检测中烟曲霉的检测效率也明显优于常规检测。McTaggart et al[14] 研究表明 mNGS 在真菌检测方面改进了对各种不可培养真菌的评估，能够从临床标本中检测多种真菌，是呼吸道真菌病的潜在有用辅助手段。mNGS 也检测出鹦鹉热衣原体、鸟 - 胞内分枝杆菌、解鸟氨酸拉乌尔菌、马尔尼菲篮状菌等特殊病原菌，在鉴定特殊类型病原体感染中优势较为明显，提示 mNGS 相较常规检测具有更广泛的病原检测谱，这与 Gaston et al[15] 研究相似。mNGS 可以在短时间内快速检查病原体，揭示肺部病原体微生物的特征，还可以揭示不同病原体的存在，对常规微生物学方法具有互补作用[16]。

本研究纳入的所有患者 mNGS 阳性率明显高于常规检测，提示 mNGS 具有高检出率，可以在短时间内对标本中全部核酸信息进行测序，在明确感染病原体方面有着明显的优势。进一步将所有患者根据有无基础疾病进行分组后，结果显示，基础疾病组的男性患者多于女性、住院时间、吸烟率以及平均年龄均大于无基础疾病组，提示基础疾病可能影响肺部感染患者住院时长，与女性患者相比，基础疾病多见于男性，多与吸烟行为相关，尤其在老年社区获得性肺炎住院患者，均以男性居多[17]。mNGS 检出基础疾病组最常见病原菌为结核分枝杆菌、流感嗜血杆菌、肺炎链球菌、铜绿假单胞菌、人类疱疹病毒 4 型及烟曲霉，而无基础疾病组最常见病原菌为结核分枝杆菌、流感嗜血杆菌、肺炎链球菌、肺炎支原体及肺炎克雷伯菌，可见社区获得性肺炎多由流感嗜血杆菌、肺炎链球菌等病原菌引发，而四川因地处盆地，气候潮湿，属于结核感染的高发区之一。本研究结果显示，基础疾病组患者常规检测阳性率明显高于无基础疾病组常规检测阳性率，而两组 mNGS 检测阳性率对比差异不大，基础疾病组和无基础疾病组 mNGS 阳性率均明显高于常规检测阳性率，提示 mNGS 在病原菌检测方面受患者本身存在基础疾病的影响小，是客观全面的检测手段。

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Application of metagene next-generation sequencing of alveolar lavage fluid in the detection of pathogenic bacteria of pulmonary infection

Zhang He, Luo Xinyue, Heng Xin, Zhang Yun, Wang Songping, Deng Jun
(*Dept of Respiratory and Critical Care Medicine, Affiliated Hospital of Southwest Medical University, Luzhou* 646000)

Abstract ***Objective*** To investigate the value of metagene next-generation sequencing (mNGS) in the detection of pathogens in patients with pulmonary infection. ***Methods*** A retrospective analysis was performed on clinical data from 434 patients with pulmonary infections admitted over the past four years. Based on the presence of underlying comorbidities, patients were divided into underlying disease group ($n=262$) and non-underlying disease group ($n=172$). Pathogen detection was conducted using both mNGS and conventional tests. Clinical and laboratory parameters, radiographic findings, and pathogen detection results were systematically analyzed. The diagnostic performance of the two methods in identifying causative pathogens of pulmonary infections was compared. ***Results*** The positive rate of mNGS in 434 patients was higher than that of conventional tests, and the difference was statistically significant ($P<0.05$). The efficacy of mNGS in detecting bacteria and viruses was significantly higher than

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sis was performed to plot survival curves, and Cox proportional hazards regression analysis was conducted to identify factors affecting the prognosis of sepsis patients. ***Results*** Data set analysis revealed that RUNX3 was a differentially methylated gene associated with the prognosis of sepsis. The mRNA expression level of RUNX3 was lower in the non-survivor group compared to the survivor group ($P<0.05$), and the methylation ratio of RUNX3 was higher in the non-survivor group than in the survivor group ($P<0.05$). In sepsis patients, RUNX3 mRNA expression levels were negatively correlated with interleukin-6 (IL-6), procalcitonin (PCT), C-reactive protein (CRP), acute physiology and chronic health evaluation (APACHE Ⅱ) score, and sequential organ failure assessment (SOFA) score. Kaplan-Meier analysis showed that the 28-day survival rate in the methylated group was lower than that in the unmethylated group ($P<0.05$). Cox regression analysis results indicated that RUNX3 promoter methylation was an independent risk factor for predicting the 28-day prognosis of sepsis patients. ***Conclusion*** In sepsis patients, the mRNA levels of RUNX3 were reduced, and the degree of promoter methylation was higher. RUNX3 promoter methylation was an independent risk factor for the 28-day prognosis of sepsis patients and could serve as a prognostic biomarker for sepsis.

Key words sepsis; RUNX family transcription factor 3; methylation; inflammatory response; biomarker

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Corresponding author Zhong Yi, E-mail: 490173559@qq.com

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that of conventional tests, and the difference was statistically significant ($P<0.05$). Although the fungal detection rate of mNGS was higher than that of conventional tests, the difference was not statistically significant. Among them, the detection rates of *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus constellation*, *Staphylococcus aureus* and *Aspergillus fumigatus* were significantly higher than those of conventional tests, and the difference was statistically significant ($P<0.05$). Subgroup analysis showed that the proportion of males, hospital stay, smoking prevalence and average age in the underlying disease group were higher than those in the non-underlying disease group, and the difference was statistically significant ($P<0.05$), while there were no significant differences in antibiotic use and endotracheal intubation rate between the two groups. The most common pathogens detected by mNGS in the underlying disease group were *Mycobacterium tuberculosis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *human herpesvirus type 4* and *Aspergillus fumigatus*, while the most common pathogens in the non-underlying disease group were *Mycobacterium tuberculosis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae* and *Klebsiella pneumoniae*. The positive rate of mNGS in the two groups was significantly higher than that of conventional tests, and the difference was statistically significant ($P<0.05$), while the difference in the positive rate of mNGS between the two groups was not statistically significant. ***Conclusion*** mNGS has significant advantages over conventional tests of pathogen in lung infection, and is less affected by underlying diseases, which can provide an etiological basis for lung infection.

Key words pulmonary infection; metagenomic second generation sequencing; mNGS; conventional detection; alveolar lavage fluid; underlying medical conditions; pathogen

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Corresponding author Deng Jun, E-mail: dj2882@sina.com