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ORIGINAL RESEARCH

Rapid Identification of Carbapenem-Resistant *Klebsiella pneumoniae* and Carbapenemase Genes via PTR-MS

Xuan Teng[1,2], Kexue Yu[1,2], Qi Zhang[3], Chengcheng Ma[1,2], Wenwen Chu[2], Chengyin Shen[3], Zhou Liu[1]

[1]Department of Clinical Laboratory Center, Anhui Chest Hospital, Hefei, 230031, People's Republic of China; [2]Department of Clinical Laboratory, the Second Affiliated Hospital of Anhui Medical University, Hefei, 230601, People's Republic of China; [3]Anhui Province Key Laboratory of Medical Physics and Technology, Institute of Health and Medical Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, 230031, People's Republic of China

Correspondence: Zhou Liu; Chengyin Shen, Email liuzhou_ac_ahmu@126.com; chyshen@aiofm.ac.cn

Purpose: To systematically analyse and identify volatile organic compounds (VOCs) released by clinical *Klebsiella pneumoniae* (*K. pneumoniae*) during growth via proton transfer reaction–mass spectrometry (PTR-MS), aiming to establish a rapid and accurate method for differentiating and identifying carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and KPC and NDM producers.

Methods: Nonrepetitive clinical strains isolated from patient specimens were collected from September 2021 to May 2025. The strains were subjected to drug susceptibility testing and carbapenemase genotype identification via the VITEK2 system and polymerase chain reaction (PCR). The clinical strains were incubated in a closed system under the combined pressure of meropenem (MEM) and carbapenemase inhibitors for 3 h. PTR-MS was used to monitor the inhibition rate of the characteristic VOC ion signal intensity to obtain drug susceptibility information of KP and carbapenemase type. Characteristic ions were characterized via fast gas chromatography (FGC)-PTR-MS.

Results: A total of 105 clinical isolates, including 53 carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) isolates and 52 CRKP (43 KPC-positive and 9 NDM-positive) isolates, were collected. With MEM, the sensitivity and specificity of PTR-MS for monitoring CRKP were 98.08% and 100.00%, respectively. In the case of MEM combined with different carbapenemase inhibitors, the assay was evaluated using a subset of isolates (n=31), comprising 22 KPC-positive and 9 NDM-positive strains. The sensitivity and specificity of PTR-MS for monitoring single KPC producers were 90.91% and 100.00%, respectively, and those for single NDM producers were 88.89% and 100.00%, respectively (kappa= 0.853 and 0.919 for KPC- and NDM-positive strains, respectively). FGC–PTR-MS analysis indicated that the VOCs corresponding to these characteristic ions were acetaldehyde, ethanol and acetic acid.

Conclusion: Real-time monitoring by PTR-MS of the dynamic release characteristics of specific VOC ions in the headspace of CRKP within 3 h under the combined stress of antibiotics and carbapenemase inhibitors can provide important information for rapidly identifying CRKP and the main clinical carbapenemase types.

Keywords: proton transfer reaction-mass spectrometry, volatile organic compounds, *K. pneumoniae*, meropenem, carbapenemase inhibitors

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is among the main pathogens that cause hospital-acquired infections. The proliferation of carbapenem-resistant strains worldwide has elevated them to the status of a critical public health challenge.[1] The emergence and spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have significantly limited clinical treatment options, leading to a significant increase in patient mortality.[2] Moreover, CRKP exhibits high transmission ability, rendering it highly prone to outbreaks and epidemics in hospital environments, especially in intensive care units (ICUs) and among immunocompromised patient populations.[3] Notably, CRKP is exhibiting a rapid evolutionary trend, and the boundary between CRKP and highly virulent *Klebsiella pneumoniae* (HvKP) is becoming increasingly blurred. The emergence of

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carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) in Asian clinical settings has significantly complicated efforts to prevent and control CRKP.[4] The main resistance mechanism of CRKP is the production of carbapenemases,[5] but its population is highly diverse and regionally specific, with significant differences in molecular characteristics and resistance mechanisms among strains derived from different regions. A genomic epidemiological study of cpKP isolates from multiple centres in China from 2009 to 2017 revealed that the main genotype of the isolates was *Klebsiella pneumoniae* carbapenemases (KPC,375/420,89.29%), followed by New Delhi metallo-β-lactamases (NDM,29/420,6.90%) and imipenem-hydrolysing MBL (IMP,19/420,4.52%).[6] Although Teo et al also attributed the primary resistance mechanism of CRKP to the production of carbapenemases, genomic differences exist, with KPC producers as the most common type (235/497, 40.9%), followed by OXA-48-like (128/497, 22.3%) and NDM producers (93/497, 16.2%).[7]

Currently, laboratory identification of CRKP relies mainly on traditional microbiological methods,[8] such as drug sensitivity and modified carbapenem inactivation (mCIM) tests, as well as molecular biological techniques, such as polymerase chain reaction (PCR) detection targeting carbapenemase genes such as bla_{KPC} and bla_{NDM}.[9] Although these methods provide high sensitivity and specificity, they are limited by their complex operation, long detection times, and high cost, which hinders their ability to meet the clinical need for the rapid diagnosis of CRKP. Especially at the early stage of infection, delayed diagnosis can easily lead to empirical antibiotic use or irrational use, thereby exacerbating the spread of drug-resistant bacteria. Given the severe situation of CRKP and the shortcomings of classic detection methods, the development of new detection methods that are rapid, accurate, convenient, and cost effective is urgent.

In recent years, many studies have revealed that volatile organic compounds (VOCs) exhibit significant application potential in multiple fields, such as environmental monitoring,[10,11] bacterial species identification,[12,13] and clinical disease diagnosis.[14,15] In the field of microbial research, analytical methods that are based on VOCs have provided new research ideas for antibiotic sensitivity testing, and the application value of volatile metabolites in drug sensitivity tests is becoming increasingly prominent. Researchers have used gas chromatography–ion mobility spectrometry (GC–IMS) to analyse the characteristic metabolic spectrum of 3-hydroxy-2-butanone under imipenem stress, and the results indicate that this method can be used to identify CRKP early.[16]

Proton transfer reaction-mass spectrometry (PTR-MS) is a technique with high sensitivity and real-time online detection capabilities that has been widely applied in fields such as food safety and public security.[17,18] Via the use of PTR-MS, Wang et al systematically profiled VOC release during *Pyrus communis* ripening. Their work enabled the rapid, nondestructive discrimination of maturity stages, providing a valuable tool for optimizing harvest timing and minimizing economic losses. In recent years, PTR-MS has also shown significant application potential in medical research.[13,19,20] For example, Xu et al successfully applied this technology to monitor the characteristic VOCs released by common pathogens of ventilator-associated pneumonia, and through the analysis of the differences in characteristic VOC spectra, they accurately identified 6 common pathogens.[13] The above studies indicate that PTR-MS technology offers significant advantages in the real-time monitoring of bacterial VOCs and construction of chemical fingerprint diagrams and has broad application prospects.

In this study, PTR-MS was used to monitor the dynamic changes in the intensity of characteristic VOC ion signals released by CSKP and CRKP under meropenem (MEM) pressure, the differences in the characteristic ion signal intensities of the two strains under antibiotic stress conditions were compared, and their metabolic response characteristics were preliminarily revealed. Furthermore, in conjunction with the results of a carbapenemase inhibition test, the carbapenemase type in CRKP was identified. The aim was to establish a rapid CRKP identification method that is based on real-time analysis of VOCs and provide a new experimental basis and technical support for the early clinical differentiation of CRKP, KPC and NDM producers.

Materials and Methods

Source of Bacteria

A collection of 105 *K. pneumoniae* isolates was obtained from the Second Affiliated Hospital of Anhui Medical University and Anhui Provincial Chest Hospital between September 2021 and May 2025. On the basis of the results

of drug sensitivity and PCR tests, the isolates comprised 53 CSKP isolates and 52 CRKP isolates (43 KPC-positive strains and 9 NDM-positive strains).

The inclusion criteria for the study strains were blood cultures or bacterial cultures from other sites of hospitalized patients with *K. pneumoniae*, and the drug sensitivity test verified the diagnostic criteria for CSKP/CRKP; the exclusion criterion was the occurrence of mixed infections with two or more types of bacteria found in the same specimen. The CRKP group was defined as the strains that were resistant to at least one carbapenem drug (ertapenem MIC ≥ 2 μg/mL, imipenem MIC ≥ 4 μg/mL, or meropenem MIC ≥ 4 μg/mL).

Bacterial Drug Sensitivity Test and Genotype Characterization of Strains

All the experimental strains were subjected to in vitro drug sensitivity tests using a VITEK2 fully automatic microbial identification system (Bruker, Germany) and a drug sensitivity analysis system (Bio-Merieux, Inc., France). The drug sensitivity results were strictly interpreted according to the latest standards of the Clinical and Laboratory Standards Institute (CLSI).[21] To detect the five major carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{OXA-48}), specific primers (Sangon, China; Table 1) were employed for PCR amplification (Supplementary Tables 1 and 2),[22] with the resulting products separated via 1% agarose gel electrophoresis (120 V, 30 min) and visualized under a gel imaging system (Supplementary Figure 1).

Bacterial Culture

One strain was selected from each of the CSKP and CRKP groups and used as the model strain to explore the experimental conditions. First, prior to experimentation, the bacterial strains were subcultured on Columbia blood agar plates and incubated overnight at 37 °C to ensure robust growth. The fresh colonies from the above cultures were used to prepare bacterial suspensions and added to tryptic soy broth (TSB; Hopebio, China) culture bottles (total volume 10 mL; bacterial concentration 10^8 CFU/mL), which were sealed tightly and incubated at 37 °C and 200 rpm under oscillation. The blank medium was used as the background. PTR-MS was used to continuously monitor the dynamic changes in headspace VOCs of KP under different culture conditions. The time point of KP culture initiation was set to T0, and continuous detection was conducted until 9 h (T9), with each group of experiments repeated 3 times. After reproducible initial results were obtained, the effectiveness of the method was systematically verified on the basis of clinical strains.

To assess the impact of MEM on CSKP and CRKP, bacterial suspensions were exposed to MEM (Macklin, China) at a final concentration of 4 μg/mL starting at time point T0. In addition, to evaluate the effects of carbapenemase inhibitors on the release of headspace volatile gases from CRKP, MEM and the carbapenemase inhibitors vaborbactam (VAB, Aladdin, China) at a final concentration of 4 μg/mL and a bacterial concentration of 10^7 CFU/mL[23] and ethylenediaminetetraacetic acid (EDTA, Sangon, China) at a final concentration of 0.05 mM and a bacterial concentration of 10^8 CFU/mL[24] were added at the T0 time point, and a blank medium was adopted as the background. All experimental operations were carried out strictly

Table 1 Antimicrobial Resistance Gene Detection Primers

Primer name	Primers Sequence (5′-3′)	Product Size(bp)
bla_{KPC}	Forward, TGTCACTGTATCGCCGTC Reverse, CAGTGCTCTACAGAAAACC	1009
bla_{NDM}	Forward, GCAGCTTGTCGGCCATGCGGGC Reverse, GGTCGCGAAGCTGAGCACCGCAT	782
bla_{IMP}	Forward, GAAGGCGTTTATGTTCATAC Reverse, GTACGTTTCAAGAGTGATGC	587
bla_{OXA-48}	Forward, GCGTGGTTAAGGATGAACAC Reverse, CATCAAGTTCAACCCAACCG	438
bla_{VIM}	Forward, GTTTGGTCGCATATCGCAAC Reverse, AATGCGCAGCACCAGGATAG	389

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according to the established experimental protocol for the strains to ensure the consistency of the experimental conditions and comparability of the results.

Quantitative and Qualitative Analysis of Characteristic Ions

The headspace VOCs of the strains were identified using a PTR-MS device. The PTR-MS parameters were as follows: ion source voltage, 420.1 V; reaction chamber pressure, 0.912 mbar; drift tube temperature, 70 °C; detection mass range, *m/z* 20–29, *m/z* 33–36 and *m/z* 38–150; residence time, 1 s; settling time, 0.1 s; and injection flow rate, 10 mL/min.

The characteristic ions were quantitatively analysed using FGC–PTR-MS.[13] The instrument was configured with a TG-624SILMS column (30 m × 0.53 mm × 3 μm). The inlet, transfer line, and column temperatures were set at 120 °C, 70 °C, and 70 °C, respectively. Separation was achieved under a constant nitrogen carrier gas flow of 10 mL/min, with eluting compounds subsequently analysed by mass spectrometry.

Data Analysis

Statistical analysis was conducted using SPSS 27.0, and $P<0.05$ indicated a statistically significant difference. GraphPad Prism 10.1.0 and Origin Lab 2024 were used for plotting. The experimental results were of independent samples with a nonnormal distribution. Comparisons of differences in VOCs between groups were performed using the Mann–Whitney *U*-test. The sensitivity and specificity were calculated using a 2×2 contingency table (PTR-MS and PCR results), and the consistency was assessed on the basis of Cohen's kappa. The VOCs were quantitatively analysed by referring to the NIST mass spectrometry database (https://webbook.nist.gov/chemistry/name-ser/).

The creation of a principal component analysis (PCA) score chart facilitated the observation of sample aggregation and dispersion. The PCA plug-in in Origin Lab was used for PCA statistical analysis. The selection of principal components was based on the scree plot test and the contribution rate of variance (Supplementary Table 3 and Supplementary Figure 2).

Results

Continuous Monitoring of VOCs in the Headspace of *K. pneumoniae* via PTR-MS

The PTR-MS detection results are presented as *m/z* values. First, the characteristic ion signals of VOCs released by *K. pneumoniae* under different culture conditions were investigated. The inclusion criteria for characteristic ions were as follows: (1) ions with an average signal intensity of less than 1000 cps after subtraction of the medium background were filtered out and (2) the ion signal intensity of the bacterial headspace VOCs increased over time. PTR-MS revealed information on a total of 126 *m/z*. After individual analysis, 6 characteristic ions (*m/z* 27, 44, 45, 46, 47, and 61) were ultimately determined.

The VOC signal intensity of the blank culture medium remained stable over time (Figure 1A–F). The signal intensities of *m/z* 27, 44, 45, 46, 47, and 61 in the CSKP and CRKP groups significantly increased over time and were greater than 1000 cps. Among them, the signal intensities of m/z 44, 45, 46, and 47 peaked after 3 h of culture and remained relatively stable (Figure 1B–E). When MEM was added at T0, the headspace VOC release trends of the CRKP group and non-MEM-supplemented group were similar, but the signal intensity of characteristic VOC ions in the CSKP group significantly decreased compared with that in the non-MEM-supplemented group and did not increase over time (Figure 1A–F). The PCA results indicated that at 3 h of culture, the CSKP group and the MEM-supplemented CSKP group separated in terms of the first principal component, and this characteristic remained stable during the subsequent culture periods (Figure 2C–F). The MEM-supplemented CRKP group and the CRKP group remained overlapping, and no separation occurred (Figure 2A–F). On the basis of the above results, the subsequent analysis focused mainly on the changes in the signal intensities of the 6 characteristic ions (*m/z* 27, 44, 45, 46, 47, and 61) at the T3 time point.

MEM Affects the Signal Intensity of the Characteristic VOC Ions of *K. pneumoniae*

When MEM was added, the signal intensity of characteristic VOC ions in the CSKP group was significantly inhibited, with an inhibition rate of >80%, while the signal intensity of characteristic ions in the CRKP group was not significantly

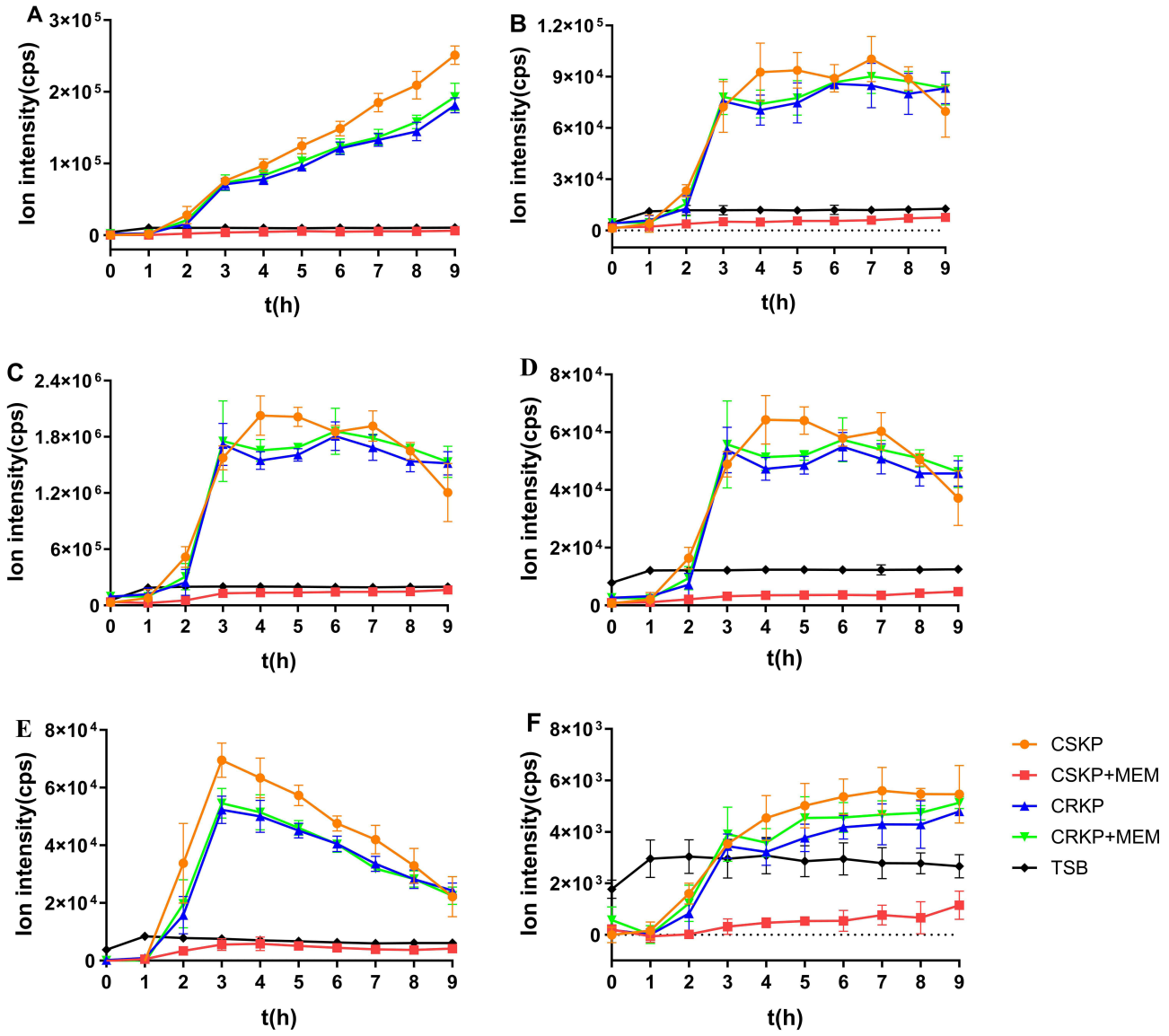

Figure 1 Curve graph showing the time variation of VOCs ion signal intensity of clinical strains with or without MEM. CSKP and CRKP were cultured in TSB medium with and without MEM respectively, with the blank medium serving as the background. The signal intensities of characteristic ions (*m/z* 27 (**A**), 44 (**B**), 45 (**C**), 46 (**D**), 47 (**E**) and 61 (**F**)) were continuously monitored by PTR-MS from 0 to 9 hours.

inhibited, some characteristic ions increased in signal intensity. The significant difference in the inhibition rate of signal intensity occurred between the two groups ($P<0.001$) (Figure 3).

Carbon Penicillinase Inhibitors Affect the Signal Intensity of Characteristic VOC Ions of CRKP

On the basis of the results of the previous experiments, this section focuses on monitoring and analysing the key characteristic ion signals at 3 h after the addition of MEM and the corresponding enzyme inhibitors. After 3 h of closed culture in MEM, the signal intensities of the characteristic ions of the KPC- and NDM-positive strains were not significantly inhibited (Figure 4). The combination of VAB reduced the signal intensity of the characteristic ions of KPC-positive strains by more than 75% (Figure 4A). PCA revealed that after VAB was added to the KPC-positive strains, they could be effectively distinguished from those using MEM alone, while different drug treatments did not significantly affect the signal intensity of the characteristic ions of NDM-positive strains (Figure 5A). Similarly, the addition of EDTA significantly inhibited the signal intensity of the characteristic ions of NDM-positive strains (Figure 4B), and PCA revealed that after EDTA was added to NDM-positive

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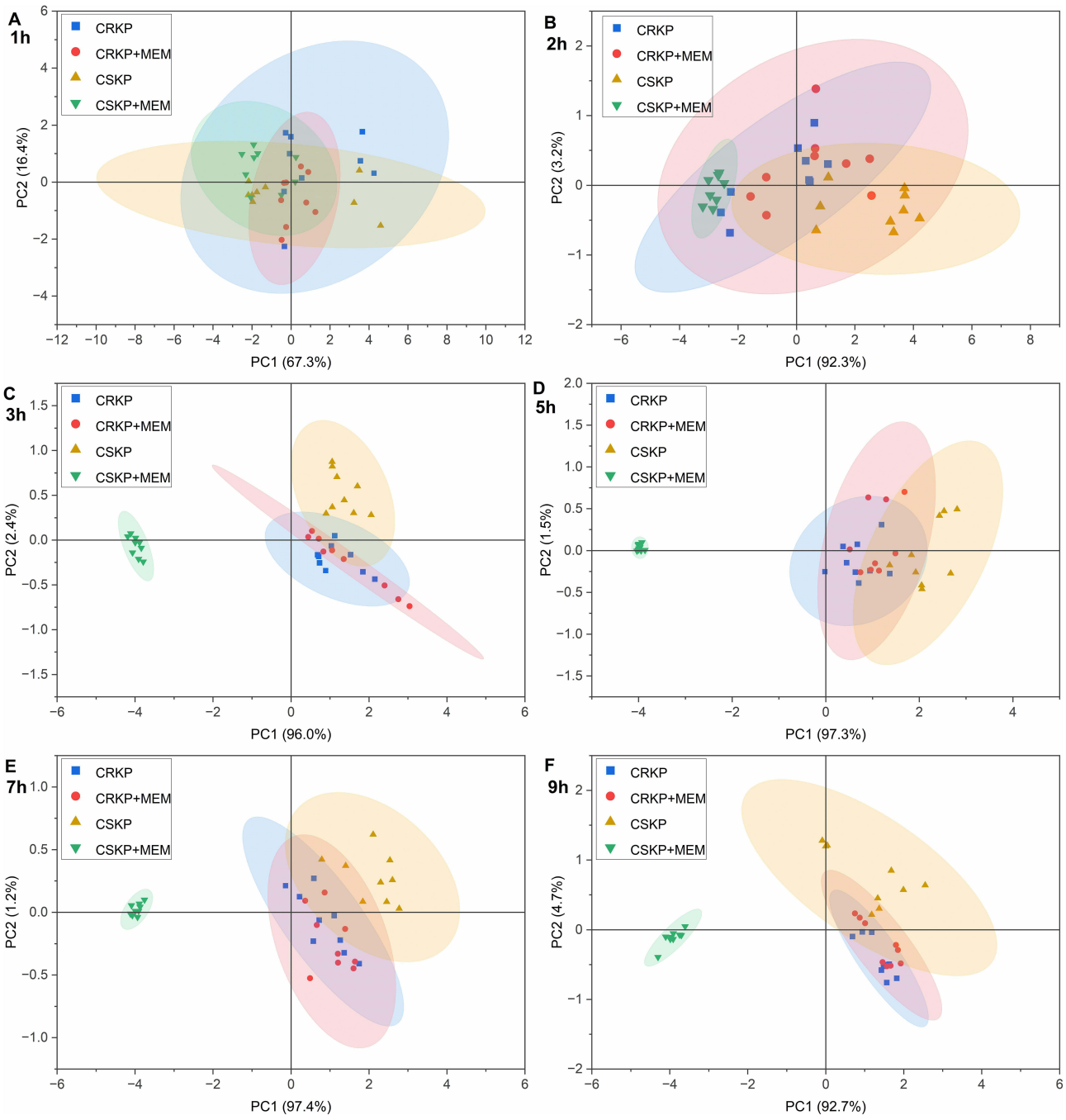

Figure 2 PCA based on volatile metabolites of CSKP and CRKP strains with or without MEM exposure at different culture times. CSKP and CRKP were cultured in TSB with or without MEM, metabolic profiles were monitored by tracking the signal intensities of specific volatile organic compound ions (m/z 27, 44, 45, 46, 47, 61), with blank medium as the background control. PCA was performed using the first two principal components to show the dynamic metabolic profiles of CSKP and CRKP strains at selected time points (1h (**A**), 2h (**B**), 3h (**C**), 5h (**D**), 7h (**E**), and 9h (**F**)), which were chosen for a representative overview of the process.

strains, the strains could be effectively distinguished from using MEM alone, while KPC-positive strains did not indicate the above changes (Figure 5B).

Verification of the Diagnostic Value of VOC Ions as Features

After MEM was added, the sensitivity for the CRKP strains was 98.08%, indicating favourable specificity (100%) for the CSKP strains. Under the conditions of MEM combined with VAB treatment, both the sensitivity (90.91%) and specificity

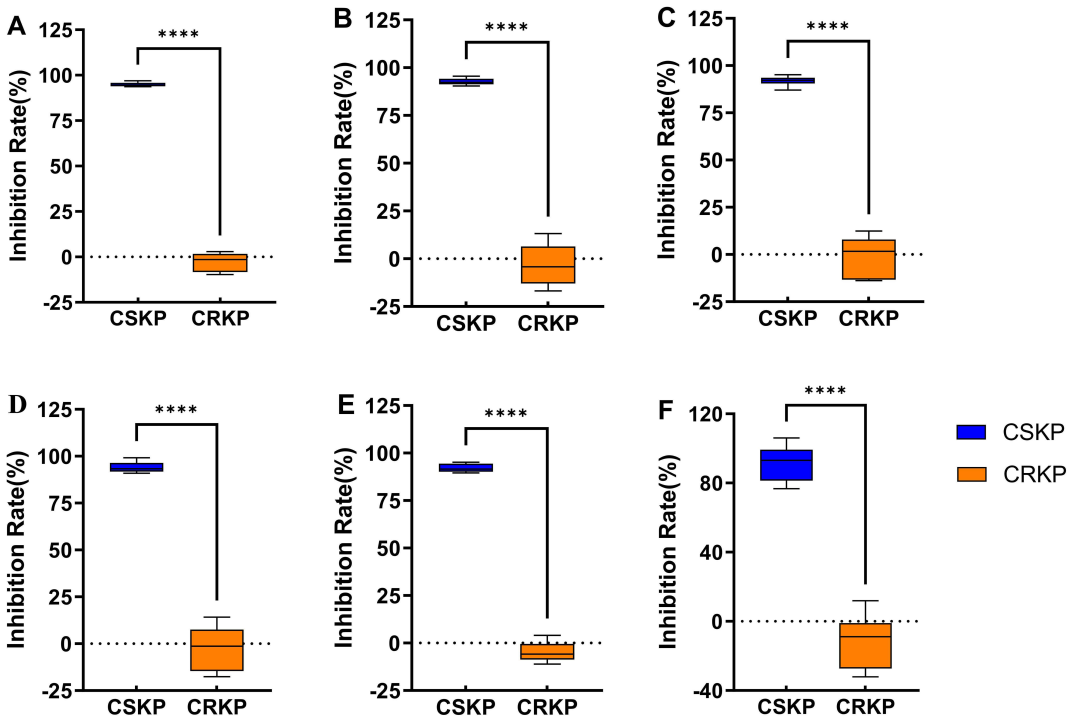

Figure 3 Inhibition rates of characteristic VOC ion signals in CSKP and CRKP strains after 3 hours of MEM exposure. CSKP and CRKP were cultured for 3 hours in TSB broth with or without 4 μg/mL MEM (using blank medium as a background), after which the inhibition rates of characteristic ions (m/z 27(**A**), 44(**B**), 45(**C**), 46(**D**), 47(**E**), and 61(**F**)) were monitored and statistically analyzed (Inhibition rate (%) =[(signal intensity without MEM - signal intensity with MEM) / signal intensity without MEM]× 100%; ****$P < 0.001$).

(100%) for the NDM-positive strains were suitable In the presence of MEM combined with EDTA treatment, both the sensitivity (88.89%) for NDM-positive strains and specificity (100%) for KPC-positive strains were good (Table 2).

Qualitative Analysis of Characteristic Ions via FGC–PTR-MS

The characteristic ions were quantitatively analysed via FGC–PTR-MS. When the ion at *m/z* 47 was used as an illustrative example, its retention time in the headspace gas of *K. pneumoniae* matched that of an authentic ethanol standard (Aladdin, China), as shown in Figure 6. This correspondence allowed the ion to be unambiguously identified as ethanol. The remaining 5 characteristic ions released by *K. pneumoniae* were subsequently identified (Table 3). The *m/z* 27 signal was identified as the fragment ion of acetaldehyde (*m/z* 45), with a retention time of 18.6 s. The signal changes at *m/z* 44 and 46 were also confirmed to be related to acetaldehyde, and the retention time of acetic acid (*m/z* 61) was 31.0 s.

Discussion

In recent years, the global prevalence of CRKP has posed a severe challenge for clinical anti-infective treatment. Its high mortality rate and rapid transmission ability urgently necessitate efficient detection methods.[3,25] In this study, PTR-MS technology was used to quantitatively analyse the VOCs produced by *K. pneumoniae* under MEM pressure in a 3-h sealed culture process. The results revealed that the signal intensities of 6 characteristic VOC ions were significantly correlated with the sensitivity of the strain to carbapenem drugs. On the basis of these characteristics, we successfully achieved rapid differentiation between CSKP and CRKP and further combined carbapenemase inhibitors to identify KPC- and NDM-producing bacteria. The method demonstrated satisfactory sensitivity and specificity.

Furthermore, FGC-PTR-MS was used to precisely identify the characteristic ions, and the key VOCs related to carbapenem drug sensitivity were determined to be acetaldehyde, ethanol, and acetic acid. Although these VOCs are not specific to *K. pneumoniae*,[26,27] the changes in their dynamic abundance patterns under MEM pressure have significant discriminatory significance. Under nonantibiotic conditions, the VOC spectra of CRKP and CSKP were similar; however, under MEM pressure, significant differences were observed in the spectra of the two strains, suggesting that

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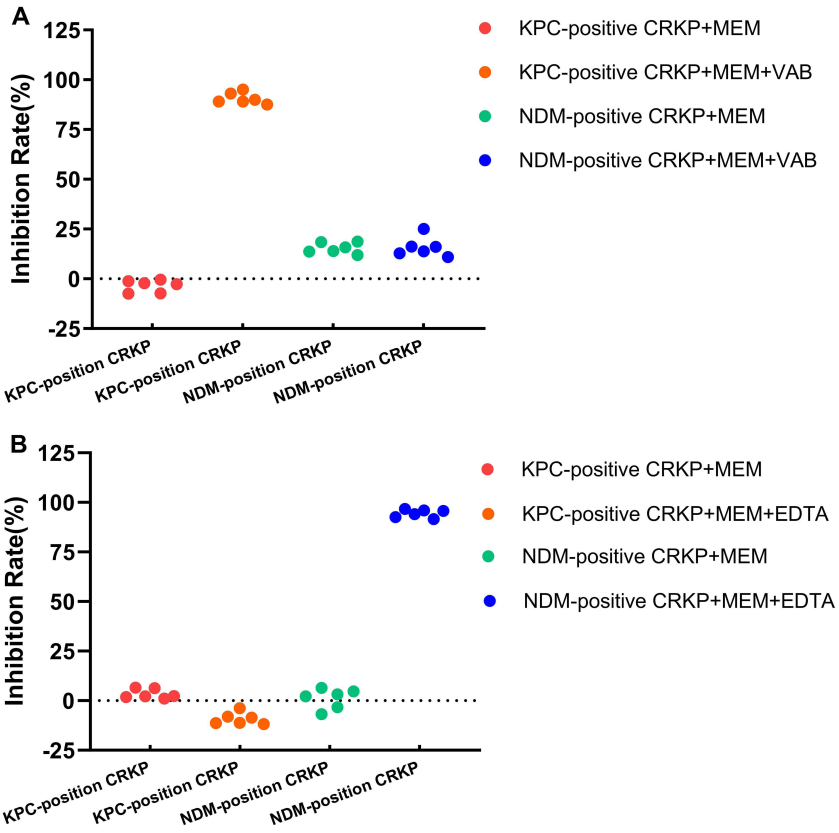

Figure 4 Scatter plot of the inhibition of ion signals in CRKP strains after 3-hour exposure to different treatments. KPC-positive CRKP and NDM-positive CRKP were cultured in TSB (control), TSB with 4 μg/mL meropenem (MEM), or TSB with MEM plus a carbapenemase inhibitor (VAB(**A**) or EDTA (**B**)). The inhibition rate for each of six characteristic ions (m/z 27, 44, 45, 46, 47, 61) was calculated as (Inhibition rate (%) = [(Signal intensity in Control − Signal intensity in Treatment) / Signal intensity in Control] × 100%, Control: TSB broth without MEM; Treatment: TSB with MEM alone or MEM combined with inhibitors, using blank medium as background). Each scatter point in panels (**A**) and (**B**) represents the inhibition rate of a single characteristic ion under the specified condition.

the observed changes in VOCs resulted mainly from the metabolic responses of bacteria under carbapenem antibiotic stress rather than differences in the inherent metabolic background between the strains, thereby reflecting their drug resistance phenotypes. Studies have shown that the acetaldehyde dehydrogenase-encoding gene is widely present in various bacteria and that different bacterial species may exhibit specific patterns of acetaldehyde metabolism and release.[13] Notably, high-yielding ethanol-producing *Klebsiella pneumoniae* (HiAlc Kpn) is associated with the occurrence of various diseases. For example, 91.2% of KP strains isolated from patients with respiratory system diseases can produce ethanol. The endogenous ethanol produced by HiAlc Kpn can induce oxidative stress damage in lung tissue and interfere with the function of neutrophils, thereby promoting bacterial immune evasion and increasing host susceptibility and lung tissue damage.[28] On the other hand, through animal experiments, Li et al reported that HiAlc Kpn can utilize the 2,3-butanediol fermentation pathway to generate high levels of endogenous ethanol.[29] This metabolic characteristic has been further confirmed to be related to the pathogenesis of nonalcoholic fatty liver disease, indicating that HiAlc Kpn may be a potential pathogenic factor for this disease.

The detection system established in this study provides significant advantages over traditional microbiological methods. First, the use of this method significantly reduced the detection time, facilitating the identification of CRKP and its drug resistance genes within a few hours. Antibiotic susceptibility testing (AST), which is commonly used in clinical settings abroad and domestically, takes 18–24 h and cannot be used to detect drug resistance genes.[30] Although the newly launched Carba NP test exhibits high sensitivity and specificity and is a rapid, simple and cost-effective method for detecting carbapenemase phenotypes,[31] its interpretation has the limitations of being highly subjective and prone to interference from colour transition intervals. In contrast, PTR-MS technology can achieve continuous sample injection and automated data analysis and may demonstrate better detection efficiency and result in consistency in large-

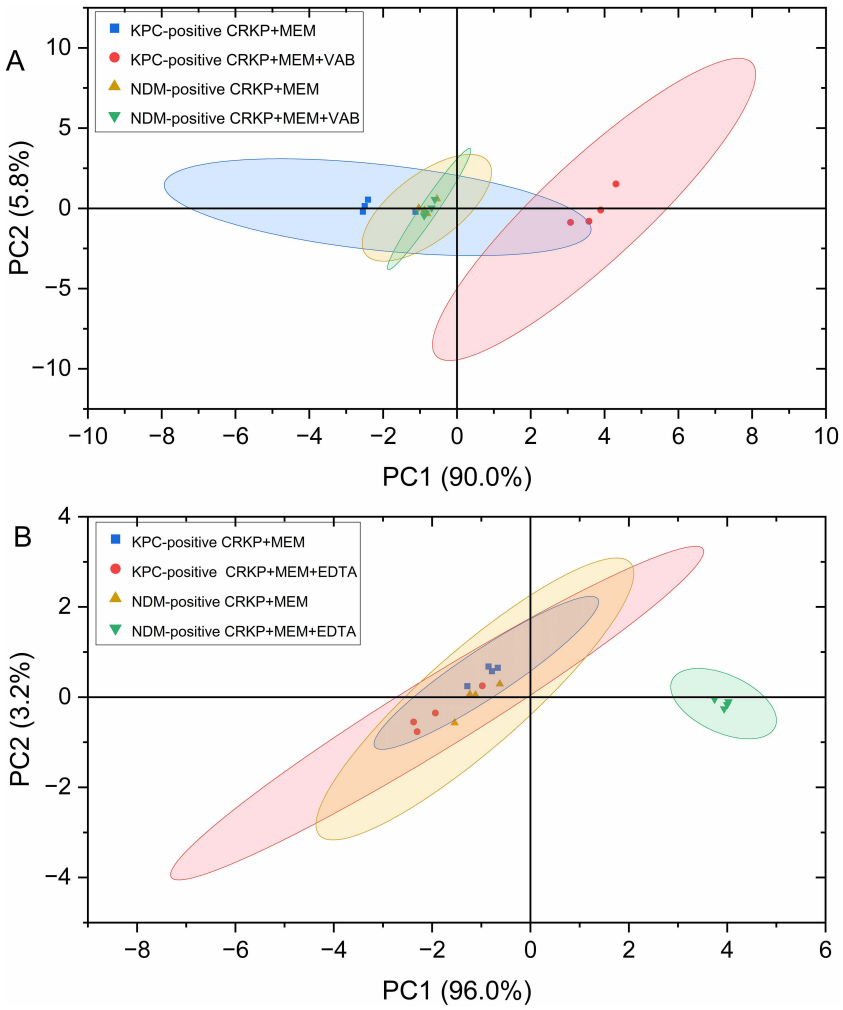

Figure 5 PCA of the bacterial metabolic response to MEM and inhibitor combinations after 3-hour exposure. The score plot resolves the distinct metabolic profiles of KPC- and NDM-positive CRKP strains following 3-hour culture in TSB with no drug, 4 μg/mL meropenem (MEM), or MEM combined with a carbapenemase inhibitor (VAB(**A**) or EDTA (**B**)). Metabolic profiles were derived from six characteristic volatile organic compound ions (m/z 27, 44, 45, 46, 47, 61), using blank medium as a background control, and analyzed via PCA based on the first two principal components.

scale sample screening scenarios. Rapid diagnosis helps with early clinical intervention, optimizing strategies for antibiotic use, improving patient prognosis and reducing the spread of drug-resistant bacteria. Previously, studies have applied VOC analysis to the field of antibiotic susceptibility testing.[32–34] Li et al used GC–IMS and reported that after 5 h of culture under imipenem pressure, the VOC spectra of CRKP and CSKP significantly differed.[33] Dixon et al used GC–

Table 2 Results of PTR-MS Analysis for *K. pneumoniae*, KPC/NDM-Positive CRKP

Scheme	Total, n	Experimental Group	Control Group	Sensitivity, n (%)	Specificity, n (%)	Kappa
Meropenem Added[a]	105	CRKP	CSKP	98.08 (51/52)	100.00 (53/53)	0.981
Meropenem and VAB Added[b]	31	KPC-positive CRKP	NDM-positiveCRKP	90.91 (20/22)	100.00 (9/9)	0.853
Meropenem and EDTA Added[b]	31	NDM-positive CRKP	KPC-positive CRKP	88.89 (8/9)	100.00 (22/22)	0.919

Notes: [a]Positive determination: After adding MEM, the inhibition rate of any 5 characteristic VOC ion signal intensities is less than 40%; [b]Positive determination: After combining MEM with carbapenemase inhibitors, the inhibition rate of any 5 characteristic VOC ion signal intensities is greater than or equal to 40%. This table demonstrates the performance of the PTR-MS method in classifying strains pre-confirmed by PCR as KPC-positive (n=22) and NDM-positive (n=9).

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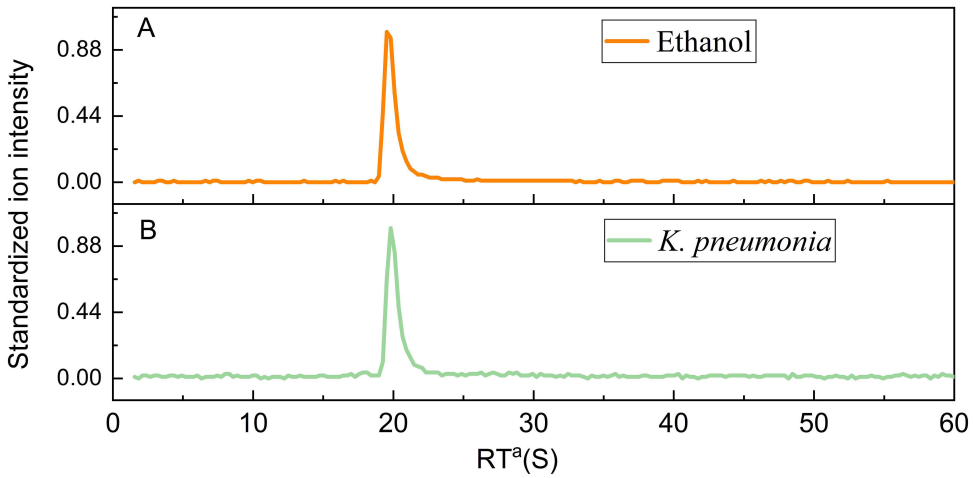

Figure 6 Example diagram for qualitative analysis of *m/z* 47. Using FGC-PTR-MS to monitor the retention time at m/z 47 of the ethanol standard ((**A**) Aladdin, China) and *K. pneumoniae* (**B**), the retention times of both were consistent. Therefore, it can be concluded that the VOC represented by m/z 47 is ethanol.
Note: [a]Retention time.

MS technology to analyse the topological VOC spectra of ampicillin-resistant and ampicillin-sensitive *Escherichia coli* treated with different concentrations of ampicillin for 6 h, enabling the differentiation of the above resistant and susceptible bacteria.[34] Compared with previous studies, the overall detection period was reduced in this study, which may be attributed to the intervention strategy of adding MEM at the beginning of the experiment, and the real-time monitoring advantage of PTR-MS technology also provided notable support for rapid data acquisition. In addition, exhalation VOC detection has significant advantages in terms of detection speed, noninvasiveness, and simultaneous multi-index analysis. This detection method has also been studied for use in early disease screening and shows promising potential in clinical applications.[35,36] However, its promotion still faces many challenges, such as the detection results being easily affected by diet, drug intake, and the surrounding environment. The existing detection technologies have high professional requirements for operators, including regular instrument calibration and maintenance. Nevertheless, with the increasing application of PTR-MS in the medical field and the continuous improvement in clinical VOC detection guidelines, the operation process is projected to be simplified, and human errors can be reduced through the development of fully automatic sample input–result output equipment, thereby promoting the large-scale clinical application of this technology.

Table 3 Putative Identities of Characteristic Ions

m/z	Possible Substance	RI[a](s)	CAS	Formula	Molecular Weight	PA[b] (kJ mol^{-1})
27	Acetaldehyde (fragment)	18.6(18.6)	75-07-0	C2H4O	44.053	768.5
44	Acetaldehyde	18.6(18.6) 16.4	75-07-0	C2H4O	44.053	768.5
45	Acetaldehyde	18.6(18.6)	75-07-0	C2H4O	44.053	768.5
46	Acetaldehyde	18.6(18.6) 16.4	75-07-0	C2H4O	44.053	768.5
47	Ethanol	19.8(19.8)	64-17-5	C2H6O	46.07	776.4
61	Acetic Acid	31.0(31.0)	64-19-7	C2H4O2	60.052	783.7

Notes: [a]FGC-PTR-MS chromatographic retention time, bacterial headspace (standard reagent); [b]According to the NIST mass spectrometry database, query the proton affinity potential (PA value) of the substance corresponding to the substance, and the PA value of the substance should be higher than that of water (691 kJ mol^{-1}).

Although the initial data showed a positive trend, the current experimental study included only clinical isolates from two hospitals and did not cover all resistance mechanisms. The number of research centres is small, making evaluating the stability and repeatability of this method under different laboratory conditions difficult. Owing to the possible differences in detection processes, sample handling methods, and resistance spectra among various medical institutions, further verification of the universality of this method is needed through multicentre cooperation. CRKP exhibits genetic diversity and regional distribution characteristics. Different regions may encompass different CRKP epidemic strains with genetic differences. For example, some studies have reported that the typing of clinical isolates mainly includes $bla_{\text{OXA-48}}$ and bla_{IMP},[37] and some CRKP strains may also mediate resistance through nonenzymatic mechanisms such as membrane pore protein deficiency.[38] This study focuses mainly on KPC- and NDM-positive CRKP. Therefore, the small sample size and the focus on only the main genotypes may lead to overestimation of the sensitivity and specificity of the detection method. Moreover, more clinical samples (such as respiratory specimens and blood samples) should be included, more resistance gene targets (such as $bla_{\text{OXA-48}}$ and bla_{IMP}) should be integrated, and their sensitivity in mixed infections or low-bacterial-load samples should be evaluated.

Conclusion

The new rapid detection method developed in this study exhibits a satisfactory application potential for identifying CRKP, including KPC- and NDM-positive CRKP. Preliminary verification revealed that this method has good specificity, sensitivity, and timeliness and has important clinical application potential. However, the current sample size is small, and because this study only focused on the main genotypes, further verification of its reliability through larger-scale, multicentre clinical studies is needed. In the future, through systematic and comprehensive validation studies, this method is expected to become an important tool for the rapid diagnosis and resistance monitoring of CRKP, providing strong support for curbing the spread of drug-resistant bacteria.

Abbreviations

PTR/GC–MS, proton transfer reaction/gas chromatography–mass spectrometry; VOCs, volatile organic compounds; CSKP, carbapenem-susceptible *K. pneumoniae*; CRKP, carbapenem-resistant *K. pneumoniae*; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; IMP, imipenem hydrolysing MBL; RT, retention time; hvKP, hypervirulent *K. pneumoniae*; ICU, intensive care unit.

Ethics Approval and Informed Consent

Given that the clinical strains of *K. pneumoniae* were isolated from clinical specimens, the isolation has no identifiable patient data, and the Ethics Committee of Anhui Medical University and Anhui Chest Hospital exempted this research for review, with approval numbers KJ2024-108 and 2023100.

Author Contributions

All the authors significantly contributed to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; participated in drafting, revising or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Makharita RR, El-Kholy I, Hetta HF, et al. Antibiogram and genetic characterization of carbapenem-resistant gram-positive pathogens incriminated in healthcare-associated infections. *Infect Drug Resist*. 2020;13:3991–4002. doi:10.2147/IDR.S276975
2. Liu KS, Tong YS, Lee MT, et al. Risk factors of 30-day all-cause mortality in patients with carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection. *J Pers Med*. 2021;11(7):616. doi:10.3390/jpm11070616
3. Li J, Li Y, Song N, Chen Y. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection: a meta-analysis. *J Glob Antimicrob Resist*. 2020;21:306–313. doi:10.1016/j.jgar.2019.09.006
4. Pu D, Zhao J, Chang K, et al. "Superbugs" with hypervirulence and carbapenem resistance in *Klebsiella pneumoniae*: the rise of such emerging nosocomial pathogens in China. *Sci Bull*. 2023;68(21):2658–2670. doi:10.1016/j.scib.2023.09.040
5. Karampatakis T, Tsergouli K, Behzadi P. Carbapenem-resistant *Klebsiella pneumoniae*: virulence factors, molecular epidemiology and latest updates in treatment options. *Antibiotics*. 2023;12(2):234. doi:10.3390/antibiotics12020234
6. Li C, Jiang X, Yang T, et al. Genomic epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in China. *Genomics Proteomics Bioinf*. 2022;20(6):1154–1167. doi:10.1016/j.gpb.2022.02.005
7. Teo JQ, Tang CY, Tan SH, et al. Genomic surveillance of carbapenem-resistant *Klebsiella pneumoniae* from a major public health hospital in Singapore. *Microbiol Spectr*. 2022;10(5):e0095722. doi:10.1128/spectrum.00957-22
8. Khalifa HO, Okanda T, Abd El-Hafeez AA, et al. Comparative evaluation of five as says for detection of carbapenemases with a proposed scheme for their precise application. *J Mol Diagn*. 2020;22:1129e38. doi:10.1016/j.jmoldx.2020.05.012
9. Zhang P, Shi Q, Hu H, et al. Emergence of ceftazidime/avibactam resistance in carbapenem-resistant *Klebsiella pneumoniae* in China. *Clin Microbiol Infect*. 2020;26(1):124.e1–124.e4. doi:10.1016/j.cmi.2019.08.020
10. Liu M, Wu T, Zhao X, et al. Cyanobacteria blooms potentially enhance volatile organic compound (VOC) emissions from a eutrophic lake: field and experimental evidence. *Environ Res*. 2021;202:111664. doi:10.1016/j.envres.2021.111664
11. Pan Q, Liu QY, Zheng J, et al. Volatile and semi-volatile organic compounds in landfill gas: composition characteristics and health risks. *Environ Int*. 2023;174:107886. doi:10.1016/j.envint.2023.107886
12. Bous M, Tielsch M, Papan C, et al. Detection of volatile organic compounds in headspace of *Klebsiella pneumoniae* and *Klebsiella oxytoca* colonies. *Front Pediatr*. 2023;11:1151000. doi:10.3389/fped.2023.1151000
13. Xu W, Zou X, Ding Y, et al. Qualitative and quantitative rapid detection of VOCs differentially released by VAP-associated bacteria using PTR-MS and FGC-PTR-MS. *Analyst*. 2024;149(5):1447–1454. doi:10.1039/D3AN02011H
14. Zhou M, Wang Q, Lu X, et al. Exhaled breath and urinary volatile organic compounds (VOCs) for cancer diagnoses, and microbial-related VOC metabolic pathway analysis: a systematic review and meta-analysis. *Int J Surg*. 2024;110(3):1755–1769. doi:10.1097/JS9.0000000000000999
15. Liu Q, Li S, Mao M, et al. Serum-volatile organic compounds in the diagnostics of esophageal cancer. *Sci Rep*. 2024;14(1):17722. doi:10.1038/s41598-024-67818-9
16. Li F, Zheng Y, Liu Y, et al. Gas chromatography-ion mobility spectrometry reveals acetoin as a biomarker for carbapenemase-producing *Klebsiella pneumoniae*. *Med Sci Monit*. 2024;30:e944507. doi:10.12659/MSM.944507
17. Liu Y, Zuo M, Wang K, et al. Rapid identification of artificial fragrant rice based on volatile organic compounds: from PTR-MS to FTIR. *Food Chem*. 2023;418:135952. doi:10.1016/j.foodchem.2023.135952
18. Wang Y, Zhu Q, Liu S, et al. Rapid determination of different ripening stages of occidental pears (*Pyrus communis* L.) by volatile organic compounds using proton-transfer-reaction mass spectrometry (PTR-MS). *Foods*. 2024;13(4):620. doi:10.3390/foods13040620
19. Roslund K, Lehto M, Pussinen P, et al. Identifying volatile in vitro biomarkers for oral bacteria with proton-transfer-reaction mass spectrometry and gas chromatography-mass spectrometry. *Sci Rep*. 2021;11(1):16897. doi:10.1038/s41598-021-96287-7
20. Jia Z, Ong WQ, Zhang F, et al. A study of 9 common breath VOCs in 504 healthy subjects using PTR-TOF-MS. *Metabolomics*. 2024;20(4):79. doi:10.1007/s11306-024-02139-6
21. Standards Committee. *Performance Standards for Antimicrobial Susceptibility Testing*. 34th ed ed. Wayne(PA): Clinical and Laboratory Standards Institute; 2024. CLSI supplement M100.
22. Xb H. Clinical infection characteristics, molecular typing and the differences of fitness after acquisition of carbapenem-resistance gene of hypervirulent *Klebsiella pneumoniae* by bloodstream infection. *Anhui Medical University*. 2023.
23. Yan R, Shen H, Cao X. Progress on the novel β-lactamase inhibitors. *Chin J Antibiot*. 2024;49(12):1360–1367.
24. Li G, Ye Z, Zhang W, et al. Rapid LC-MS/MS detection of different carbapenemase types in carbapenemase-producing *Enterobacterales*. *Eur J Clin Microbiol Infect Dis*. 2022;41(5):815–825. doi:10.1007/s10096-022-04440-5
25. Li D, Huang X, Rao H, et al. *Klebsiella pneumoniae* bacteremia mortality: a systematic review and meta-analysis. *Front Cell Infect Microbiol*. 2023;13:1157010. doi:10.3389/fcimb.2023.1157010
26. Filipiak W, Sponring A, Baur MM, et al. Molecular analysis of volatile metabolites released specifically by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *BMC Microbiol*. 2012;12:113. doi:10.1186/1471-2180-12-113
27. Cao R, Xue C, Li X, et al. Profiling of volatile organic compounds in clinical isolate cultures and stools from children with *Clostridioides difficile* infection by HS-SPME-GC-MS. *J Breath Res*. 2025;19(4):046008. doi:10.1088/1752-7163/ae0511
28. Cui J, Xu Z, Yu Z, et al. High-alcohol-producing *Klebsiella pneumoniae* aggravates lung injury by affecting neutrophils and the airway epithelium. *Cell Rep Med*. 2025;6(1):101886. doi:10.1016/j.xcrm.2024.101886
29. Li NN, Li W, Feng JX, et al. High alcohol-producing *Klebsiella pneumoniae* causes fatty liver disease through 2,3-butanediol fermentation pathway in vivo. *Gut Microbes*. 2021;13(1):1979883. doi:10.1080/19490976.2021.1979883
30. Jiang G, Yu S. Yu W, et al.Research progress in the detection techniques and methods of bacterial drug resistance and drug-resistant genes. *Chin J New Clin Med*. 2022;15(10):907–913.
31. Literacka E, Herda M, Baraniak A, et al. Evaluation of the Carba NP test for carbapenemase detection in *Enterobacteriaceae, Pseudomonas spp*. and *Acinetobacter spp*. and its practical use in the routine work of a national reference laboratory for susceptibility testing. *Eur J Clin Microbiol Infect Dis*. 2017;36(11):2281–2287. doi:10.1007/s10096-017-3062-0
32. Luo H, Hang Y, Zhu H, et al. Rapid identification of carbapenemase-producing *Klebsiella pneumoniae* using headspace solid-phase microextraction combined with gas chromatography-mass spectrometry. *Infect Drug Resist*. 2023;16:2601–2609. doi:10.2147/IDR.S404742

33. Li F, Gu S, Zhao C, et al. The detection and utilization of volatile metabolomics in *Klebsiella pneumoniae* by gas chromatography-ion mobility spectrometry. *Sci Rep*. 2024;14(1):26122. doi:10.1038/s41598-024-77746-3
34. Dixon B, Ahmed WM, Mohamed AA, et al. Metabolic phenotyping of acquired ampicillin resistance using microbial volatiles from *Escherichia coli* cultures. *J Appl Microbiol*. 2022;133(4):2445–2456. doi:10.1111/jam.15716
35. Vinhas M, Leitão PM, Raimundo BS, et al. AI applied to Volatile Organic Compound (VOC) profiles from exhaled breath air for early detection of lung cancer. *Cancers*. 2024;16(12):2200. doi:10.3390/cancers16122200
36. Heers H, Chwilka O, Huber J, et al. VOC-based detection of prostate cancer using an electronic nose and ion mobility spectrometry: a novel urine-based approach. *Prostate*. 2024;84(8):756–762. doi:10.1002/pros.24692
37. Coşkun USŞ, Dagcioğlu Y. Investigation of genotyping and phenotyping characteristics of carbapenem-resistant *Klebsiella pneumoniae* isolates. *An Acad Bras Cienc*. 2024;96(2):e20231322. doi:10.1590/0001-3765202420231322
38. Kumar S, Anwer R, Azzi A. Molecular typing methods & resistance mechanisms of MDR *Klebsiella pneumoniae*. *AIMS Microbiol*. 2023;9(1):112–130. doi:10.3934/microbiol.2023008