

# Subspecies typing on the example of *Klebsiella pneumoniae* isolated from patients with pulmonary tuberculosis

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## ABSTRACT

**Aim:** The purpose of this study was to evaluate sub-species typing by matrix-activated laser desorption/ionisation mass spectrometry on the example of *Klebsiella pneumoniae* isolated from patients with pulmonary tuberculosis in Karaganda region.

**Materials and Methods:** This study evaluated the potential of MALDI-TOF MS for epidemiological identification of *Klebsiella pneumoniae* isolated from pulmonary tuberculosis patients in Karaganda region. A total of 47 strains of *Klebsiella pneumoniae* obtained between 2015 and 2019 were typed. Mass spectra were taken using Microflex LT MALDI-TOF (Bruker) according to standard procedure.

**Results:** As a result, MALDI-TOF evaluation of subspecies typing on the example of *Klebsiella pneumoniae* isolated from patients with pulmonary tuberculosis in Karaganda region, predominantly showed the diversity of isolates, indicating their out-of-hospital nature. Based on all available information, including facts, sources, and experimental results, it can be reasonably concluded that the current study of *Klebsiella pneumoniae* does not provide a complete picture of the epidemiology of this microorganism.

**Conclusions:** The current study provided a limited opportunity to investigate the genetic structure and diversity of *Klebsiella pneumoniae* subspecies more accurately. The findings suggest that the time-of-flight laser-ionisation ablation mass spectrometry method with magnetic focusing is more likely to detect differences in external characteristics between individual specimens of the same species than internal genetic variation in the microorganisms under study. The practical relevance of this study is to inform the development of more effective infection control strategies in clinical practice and infection control.

**KEY WORDS:** pathogens; microorganisms; infectious diseases; mass spectrometry; MALDI-TOF MS

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## INTRODUCTION

*Klebsiella pneumoniae* is a conditionally pathogenic microorganism belonging to the *Enterobacterialis* genus. Infections caused by *K. pneumoniae* are currently associated with hospitalised patients with weakened immune systems. *K. pneumoniae* causes a wide range of human diseases, including urinary tract infections, pneumonia, and septicaemia [1, 2]. Infection with *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* simultaneously poses a serious threat to the life and health of the patient. This combination is often under-diagnosed and under-treated in time, resulting in increased mortality [3].

Previously, various aspects of tuberculosis have been actively discussed in the context of Kazakhstan's healthcare system. For example, a recent study examined the impact of economic, organisational, veterinary, and sanitary measures on the epizootic situation of tuberculosis in animals and the epidemiological situation of tuberculosis in humans in the Republic of Kazakhstan. The authors analysed the epizootic situation of tuberculosis in livestock in Kazakhstan from 1991 to 2019. The findings showed a decrease in the incidence in animals since 2001, which had a positive impact on the epidemiological situation in humans. The study emphasises the importance of veterinary and sanitary

measures in improving the epizootic situation and reducing the risk of human tuberculosis in Kazakhstan [4]. In addition, the results of another recent study showed a decrease in tuberculosis (TB) incidence in Kazakhstan from 227 to 15.2 per 100,000 people between 2014 and 2019, while overall mortality increased from 8.4 to 15.2 per 100,000 people. The risks of death were higher for older age groups, men, urban dwellers, retirees, and those with HIV and diabetes. The study emphasises the need for targeted interventions to reduce the burden of TB, especially among older people, men, urban dwellers, and those with HIV and diabetes. The use of administrative health data is important for policy development and systemic TB control measures in Kazakhstan [5]. Comparable findings are presented in a study conducted in four regions of Kazakhstan that assessed the association between imprisonment, tobacco, alcohol, drug use, HIV, and diabetes mellitus (DM) with tuberculosis incidence. The study included 1,600 participants. The findings suggested that participants who had ever used tobacco, alcohol, had HIV or DM had a higher risk of TB. This emphasises the need for a comprehensive response to TB, including intervention and prevention for tobacco, alcohol, HIV, and DM [6]. Another study conducted in Kazakhstan focused on genomic study of *Mycobacterium tuberculosis* strains in patients with a

prior high level of drug resistance to tuberculosis. The researchers sequenced the complete genome of 10 such strains from different regions of Kazakhstan. The results are comparable to drug resistance data obtained from phenotypic tests [7, 8].

In recent years, the overuse of antimicrobials has had dire consequences worldwide, specifically the emergence of antimicrobial resistance in pathogens. This fact influences high rates of ineffective treatment and high mortality rates [9-11]. To effectively treat *K. pneumoniae* infections, it is important to make careful antibiotic choices, considering the level of resistance in a particular region or clinical setting. Continuous monitoring and surveillance of antibiotic resistance helps to combat this severe problem in medicine, as the proportion of antibiotic-resistant *K. pneumoniae* is increasing worldwide [12, 13], and the rapid spread of such isolates is becoming a major challenge. Thus, despite the accumulation of data on various aspects of tuberculosis infection in the context of public health in Kazakhstan, there has been little previous research on *K. pneumoniae* typing in tuberculosis patients using modern technologies.

## AIM

The purpose of this study was to evaluate sub-species typing using MALDI-TOF technology on the example of *K. pneumoniae* isolated from patients with pulmonary tuberculosis in Karaganda region.

## MATERIALS AND METHODS

Microbiological study, subspecies typing was carried out based on the laboratory of collective use of Karaganda Medical University. The study used a collection of *Klebsiella pneumoniae* isolates (n=47) isolated from multidrug resistant tuberculosis patients.

Microbial strains were obtained between 2015 and 2019 from multidrug-resistant tuberculosis patients in two profile hospitals in Karaganda region. Sputum samples were collected in sterile dishes and brought to the laboratory no later than 2 hours after collection. Sowing for non-specific microflora was carried out on blood agar with 5% lamb blood with isolation of pure cultures by conventional methods. The strains of opportunistic microorganisms obtained in the study were considered clinically significant at concentrations  $>10^5$  CFU/ml. Isolates were identified to species composition by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry with matrix-assisted laser desorption/ionisation (MALDI-TOF MS) using a Microflex LT system and MALDI Biotyper Compass 4.1.80 software (Bruker Daltonics, Germany). MALDI-TOF MS is based on two main processes, matrix-assisted laser desorption and ion time-of-flight analysis in the mass spectrometer. First, the sample, usually mixed with a matrix (e.g., sinapic acid), is applied to the target. The laser light then knocks ions out of the sample, which are then flown through a time-of-flight analyser. The time taken for ions to travel through the analyser is proportional to their mass-charge. A score of  $\geq 2.2$  was used as a criterion for reliable species identification [14].

The isolated and identified microorganisms were frozen at  $-70^{\circ}\text{C}$  in tryptic soy broth supplemented with 30% glycerol before subspecies typing. Using a daily culture of *K. pneumoniae* grown on blood agar with 5% lamb blood ( $37^{\circ}\text{C}$ , 18 h), samples were prepared with ethanol/formic acid extraction according to the manufacturer's recommendations (Bruker Daltonics, Bremen, Germany) [15]. Samples were processed on MBT Biotarget 96 using a solution with a high concentration of cyano-4-hydroxycinnamic acid. Mass spectra were obtained on a Microflex LT mass spectrometer with default parameters over a defined mass range. Each strain had six spectra each, and calibration was done using the Bruker bacterial test. The resulting data were analysed using FlexAnalysis software to extract peak information and saved in Excel files [16]. Discriminating peaks after processing to remove outliers were processed in Biotyper SW software and converted to create a normalised gel and correlation matrix. Antimicrobial susceptibility was determined by disc-diffusion method on Mueller-Hinton agar according to CLSI (Clinical & Laboratory Standards Institute) recommendations [17]. ESBL (extended spectrum beta-lactamase) production was detected using the antibiotic combination disc method as previously described. *Escherichia coli* ATCC®25922, *Escherichia coli* ATCC®35218, *Pseudomonas aeruginosa* ATCC®27853 were used for quality control of sensitivity determination.

## RESULTS

The following results were obtained from analysing the sample. The predominant clinical forms of tuberculosis were infiltrative pulmonary tuberculosis 18 (38.3%), pulmonary tuberculoma 11 (23.4%) and fibrotic cavernous pulmonary tuberculosis 6 (12.8%). The sample results provided important characteristics and statistics on the TB infected group, including age, sex, nature of TB (primary case), patients' incarceration, and information on drug resistance. It was expected that these data would be useful for analysing the findings of the study and further developing strategies for tuberculosis control and disease management. The mean age of the infected patients was 40 years. Gender statistics: the number of males was 39 (83%); the number of females was 8 (17%). Primary and secondary forms of tuberculosis: 39 people (83%) were TB-diagnosed for the first time. Number of patients from places of imprisonment: 4 people (8.5%). Statistics on drug resistance:

Patients with multidrug resistance – 15 patients (31.9%).

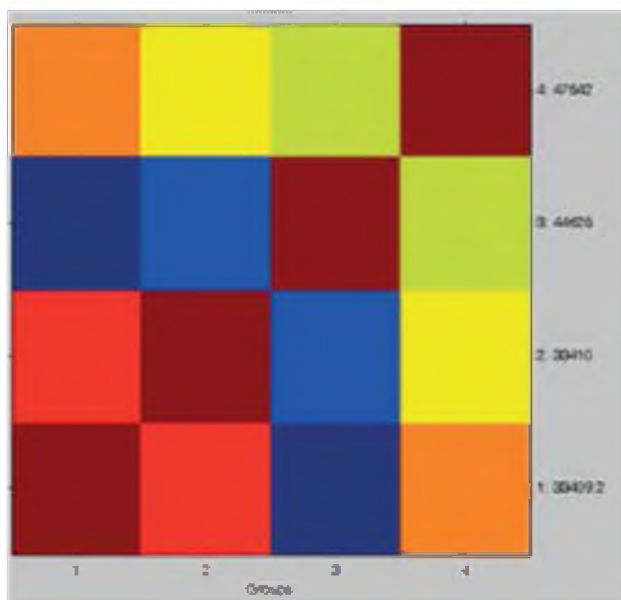
Patients without multidrug resistance – 32 patients (68.1%).

As noted earlier, the MALDI-TOF MS method claims the ability to identify and quantitatively analyse molecules in a sample. This method was expected to be useful for determining the mass of proteins, searching for almost any change in amino acid sequence, detecting post-translational modifications. In the current study, 47 isolates included in the study were identified as *K. pneumoniae* by MALDI-TOF MS with a score  $>2$  (categorised as high probability species determination) using Biotyper software analysis. Next, for each strain, a total spectrum of about 50 peaks was compiled from the MALDI recording of bacterial cell

mass spectra. All peaks of the total spectrum between 2000 and 20000 m/z were included in the analysis. By comparing the mass spectra of bacteria in the categories of interest, several clusters could be identified. The first cluster equalled 4 isolates (groups) of patients who had served prison sentences (Fig. 1). As a result, the two isolates were characterised by overlapping peaks, so that they were isolated in the same year in patients with the same sensitivity profile to broad-spectrum antibiotics diagnosed with fibrotic cavernous pulmonary tuberculosis, isolating multidrug-resistant strains of *Mycobacterium tuberculosis* and receiving only symptomatic treatment. The results of the study included mass spectra, which are graphical representations that show the intensities of ions as a function of their mass-charge. Comparison of mass spectra with standards in the database allowed for the identification of microbial subspecies. MALDI-TOF MS usually gave the probability that the sample matched a particular *Klebsiella pneumoniae* subspecies. This helped to establish the researchers' confidence in the outcome of the study conducted.

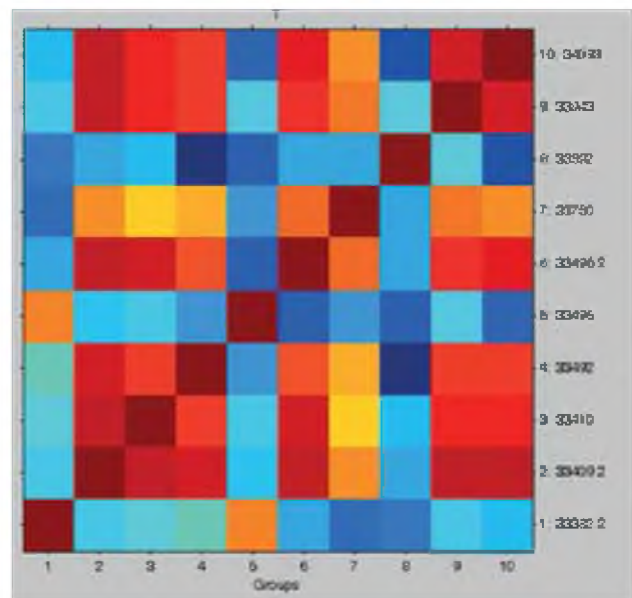
The second cluster is presented in Fig. 2. These are isolates collected in 2015. By comparing the peaks and looking at the correlation matrix, it was concluded that the samples collected in 2015 were similar in patients isolating *Mycobacterium Tuberculosis*.

In the third cluster, isolates obtained from patients in 2018 were identified (Fig. 3). Importantly, isolates 2 and 4 appeared phylogenetically similar to each other and were obtained from patients with first-diagnosed pulmonary tuberculosis, despite the absence of *Mycobacterium Tuberculosis* isolates from pulmonology and surgical wards. This may indicate a possible outbreak of the disease in the wards in question.



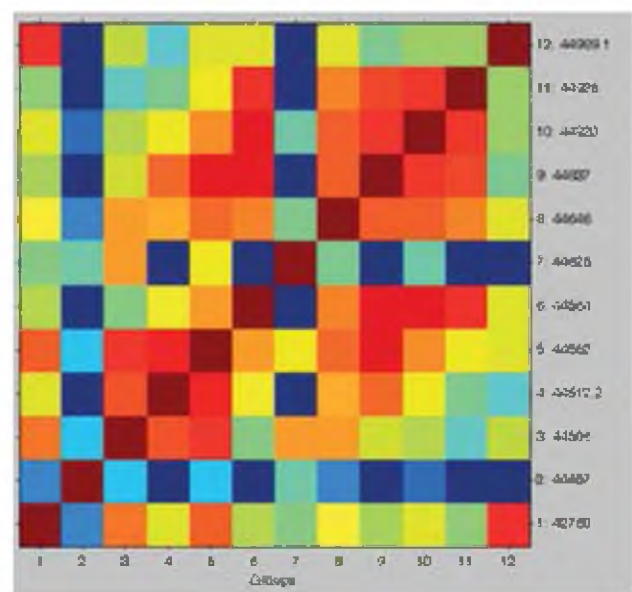
**Fig. 1.** *Klebsiella pneumoniae* mass spectra from a category of incarcerated patients.

Note: the more intense the red colour, the more likely the peaks of the matched samples, i.e., the samples themselves, will match.



**Fig. 2.** Mass spectra of *K. pneumoniae* isolated in 2015.

Note: the more intense the red colour, the more likely the peaks of the compared samples, i.e., the samples themselves, will match.

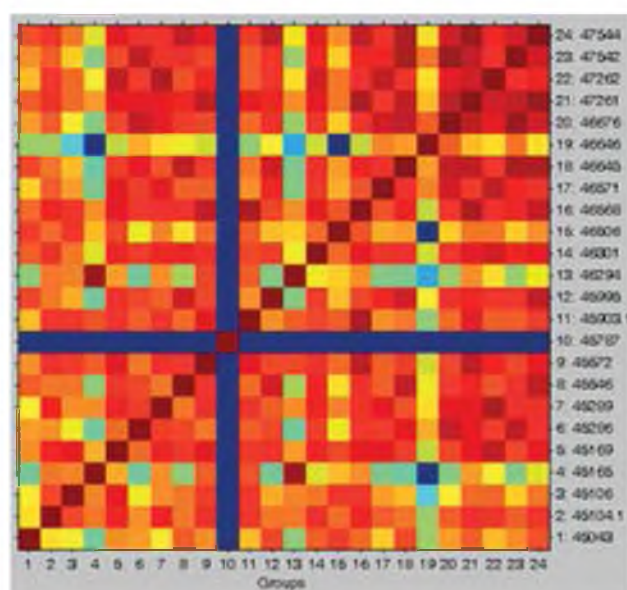


**Fig. 3.** Mass spectra of *K. pneumoniae* isolated in 2018

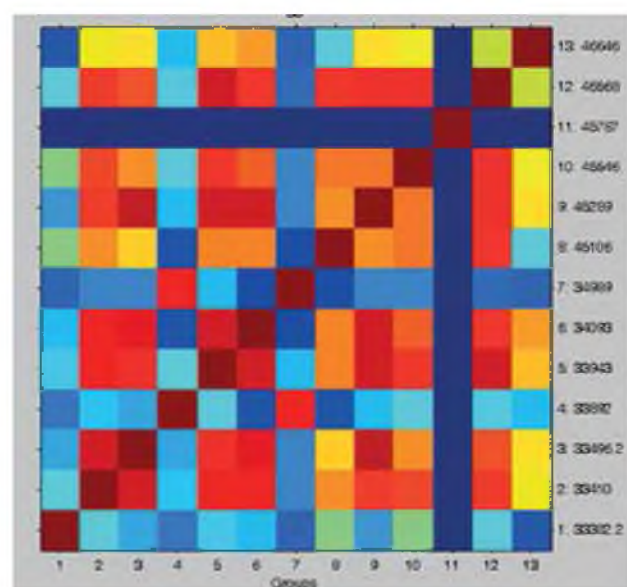
Note: the more intense the red colour, the more likely the peaks of the matched samples, i.e., the samples themselves, will match.

It is also worth noting that isolates 3 were also close and came from patients with primary pulmonary TB. Therewith, these strains remained sensitive to antituberculosis drugs. Furthermore, the study showed that antimicrobials tested on these isolates showed high efficacy against *Klebsiella pneumoniae*.

Analysing the results of the heat/correlation matrix obtained in 2019, it is clear that it was difficult to draw unambiguous conclusions (Fig. 4). Nevertheless, similarities between groups of isolates were found: seven and five strains were obtained from patients with primary tuberculosis, and



**Fig. 4.** Mass spectra of *K. pneumoniae* isolated in 2019  
 Note: the more intense the red colour, the more likely the peaks of the compared samples, i.e., the samples themselves, will match.



**Fig. 5.** Mass spectra of *K. pneumoniae* obtained from patients with open tuberculosis, city of Karaganda  
 Note: the more intense the red colour, the more likely the peaks of the compared samples, i.e., the samples themselves, will match.

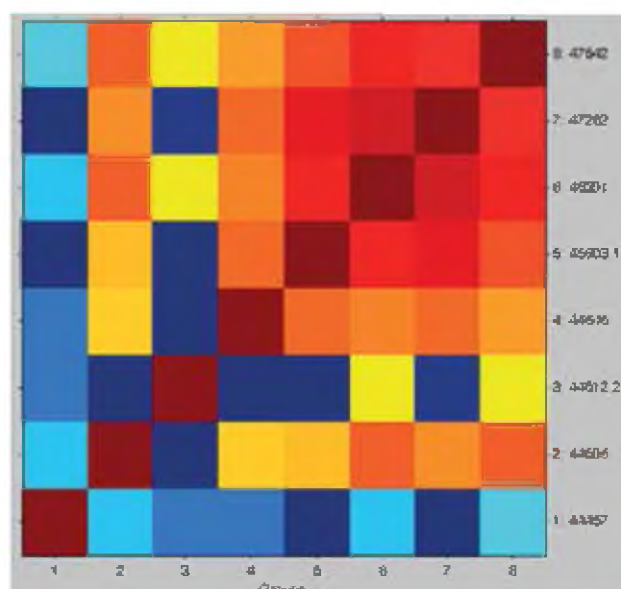
three isolates from patients with preserved sensitivity to antituberculosis drugs, but *Mycobacterium Tuberculosis* was not isolated. Importantly, there was homogeneity in antimicrobial sensitivity in this cluster, where isolates included both those isolated among patients with identified *Mycobacterium Tuberculosis* and those that stayed sensitive to antituberculosis drugs. All these data suggest a complex epidemiological picture in 2019 that requires further investigation.

When trying to investigate the similarity of *K. pneumoniae* isolates isolated from pulmonary tuberculosis patients from

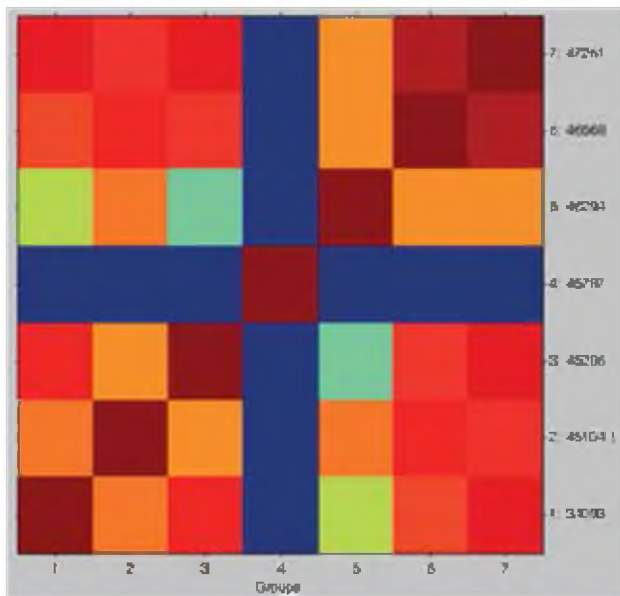
Karaganda hospital, these strains had comparable patterns of sensitivity to antibacterial drugs, and therefore the strains were sensitive to broad-spectrum antibiotics and multidrug-resistant mycobacterium strains (Fig. 5). Isolates clustered in a group of bacteremic patients obtained from Temirtau Hospital in 2019 had similar spectra in those patients who were first diagnosed with TB, with preserved sensitivity to broad-spectrum antibiotics and anti-TB drugs (Fig. 6).

The picture provided shows *Klebsiella pneumoniae* mass spectra obtained from patients with open TB in Karaganda. The intensity of the red colour in the picture corresponds to the probability of matching peaks in the samples, showing the degree of similarity between the samples themselves. This study focuses on the interpretation of antimicrobial susceptibility results of isolated *K. pneumoniae* strains by comparing drug resistance clusters. Particular attention was paid to the identification of a cluster of strains showing resistance to the fourth generation of cephalosporins, cefepime. No other similarities were found (Fig. 7). This result emphasises the importance of cefepime resistance in the context of tuberculosis patients in Karaganda and possibly influences the spread of *K. pneumoniae* in this setting. It is important to understand that the accuracy of identification and typing depended on the quality of the mass spectra database as well as the condition of the sample and the technical aspects of the procedure. Two isolates of *K. pneumoniae* resistant to ciprofloxacin were isolated from patients newly diagnosed with tuberculosis (Fig. 8). These *K. pneumoniae* strains were also resistant to antimicrobials from other groups.

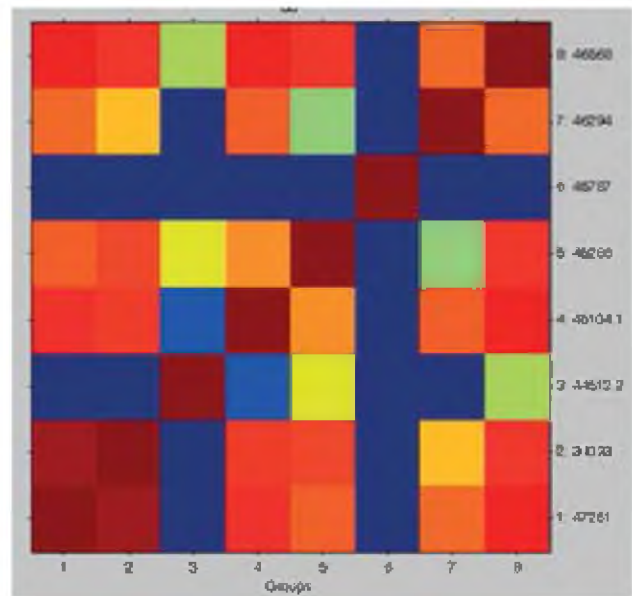
As a result of this study, the presented clusters from the experimental sample of isolates were investigated, structured, and described. By performing spectral analysis



**Fig. 6.** Mass spectra of *K. pneumoniae* obtained from patients with open tuberculosis, city of Temirtau  
 Note: the more intense the red colour, the more likely the peaks of the compared samples, i.e., the samples themselves, will match.



**Fig. 7.** Mass spectra of cepipime-resistant *K. pneumoniae*  
 Note: the more intense the red colour, the more likely the peaks of the matched samples, i.e., the samples themselves, will match.



**Fig. 8.** Mass spectra of ciprofloxacin-resistant *K. pneumoniae*  
 Note: the more intense the red colour, the more likely the peaks of the matched samples, i.e., the samples themselves, will match.

of the selected strains and analysing the data obtained, it was possible to experimentally and analytically prove the relatedness of the strains obtained from individuals who served their sentences in the selected isolates between 2015 and 2018, and to determine that the clusters that were isolated in these years and the clusters of strains directly related to the antimicrobial resistance phenotypes of the antimicrobials presented in the publication were almost identical, which confirms the purpose of this study. No correlation was obtained for the other antimicrobials presented above.

The results of a study conducted using MALDI-TOF MS (matrix assisted laser desorption and ion time-of-flight analysis in a mass spectrometer) provided valuable findings. Using MALDI-TOF MS, identification of microorganisms, including *Klebsiella pneumoniae*, in patients with tuberculosis was performed, but the study could not provide all information about the epidemiology of *K. pneumoniae* based on data, sources, and experiments. This method allowed for the identification of microorganisms to concrete species and strains. Analysis of microorganisms using MALDI-TOF MS also revealed similarities in antibiotic sensitivity. This allowed the identification of groups of microorganisms that showed comparable levels of resistance or sensitivity to antibacterial drugs. These results provided important information on the association between *Klebsiella pneumoniae* and tuberculosis in patients presenting to the hospital in Karaganda. Analysis of microorganisms using MALDI-TOF MS helped to identify the characteristics and resistance of these organisms, which may be key to further investigation and treatment of patients.

The study showed that *K. pneumoniae* subspecies isolated from patients with pulmonary tuberculosis may

have different genetic characteristics such as pathogenic genes and antibiotic resistance. This may have critical implications for the treatment of infections caused by these subspecies. Analysis of *K. pneumoniae* subspecies has revealed epidemiological links between infections and tuberculosis. This may be useful information for infection control in clinical settings. Nevertheless, the evaluation of sub-species typing by MALDI-TOF MS method on the example of *K. pneumoniae* isolated from patients with pulmonary tuberculosis in Karaganda oblast indicates the universality of isolates, which may indicate the out-of-hospital nature of these strains. The results of the MALDI-TOF MS study are a major step in understanding the biological and medical aspects of microorganisms and their relationship with pathologies, and they can serve as a basis for future research and clinical practice. The study presented above highlights the need for additional research to identify the causes of resistance and impact on treatment, as well as to develop treatment strategies and prevention of associated infections.

## DISCUSSION

MALDI-TOF MS is widely used in medical research for diagnosing infections, determining protein signatures in cancer samples, and for other applications. It is also used in chemistry to analyse organic compounds and in pharmaceuticals to control the quality of drugs. MALDI-TOF MS is highly sensitive and accurate, allows the analysis of a large range of molecule masses and promotes high throughput as the analysis usually takes place within seconds [18]. Nevertheless, in the context of the current study, the MALDI-TOF method had limited opportunities for in-depth investigation. There are reference methods for

molecular typing of bacterial isolates such as multilocus sequence typing – MLST, pulsed-field gel electrophoresis – PFGE and whole-genome sequencing (WGS) [19, 20]. These typing approaches provide high capacity but are still labour-intensive, technically challenging, and expensive procedures [21]. Prompt identification of clinically relevant clones is essential for infection control measures. Fast and reliable methods are required that can be easily applied in a routine workflow, without increasing financial and time costs, and without the need for additional staff [22]. Identification of microorganisms by MALDI-TOF mass spectrometry is now being actively pursued worldwide. In many microbiological laboratories, mass spectrometry (MALDI-TOF MS) has already become a standard tool for pathogen detection in clinical practice. With the continuous addition of libraries and improvements in microbial/fungal identification protocols, MALDI-TOF identification is becoming a simple, affordable, reliable, and easy to perform method without the need for additional reagents. This is not the sole application of MALDI-TOF in modern microbiological diagnostics; it is actively used for epidemiological typing of clinically significant microorganisms, identification of clonal clusters of nosocomial pathogens correlating with different genotypes [23]. Notably, the method is cost-effective and easy to use, sometimes comparable to DNA-based typing methods [24].

One of the key findings of the current study was to prove the relatedness between strains isolated during the specified period, and to confirm the association between these strains and the antibiotic resistance phenotypes for the specific antimicrobials presented in this publication. This indicates the significant role of these strains in the spread of antibiotic resistance and may be useful in developing infection control strategies. However, the study did not find a correlation between other antimicrobials, which may indicate variable dynamics of antibiotic resistance in distinct strains of *K. pneumoniae*. A recent study by L. Hleba et al. [25] investigated methods for detection of KPC-producing *Klebsiella pneumoniae* using MALDI-TOF MS. These bacteria are dangerous, antibiotic resistant, and cause infections with high mortality rates. The study evaluates three different methods for detecting KPC-producing bacteria. Based on the results, it was found that the third method based on protein fingerprinting is fast and relatively simple but requires prior verification using other methods. Detection of KPC-producing *Klebsiella pneumoniae* using MALDI-TOF provides rapid and accurate results, which may help reduce mortality and morbidity in the hospital environment when used in diagnostic situations.

A recent review by M.Y. Ashfaq et al. [26] on the application of MALDI-TOF mass spectrometry for the identification of bacteria in the environment emphasised the importance of investigating various bacteria that play a role in ecosystems and perform various functions such as participation in nutrient cycling, biodegradation of toxic substances. Conventional molecular methods for bacterial identification are time-consuming and expensive, and therefore MALDI-TOF mass spectrometry has attracted attention and is used for routine

bacterial identification. A review of the article looked at the application of MALDI-TOF MS in various environmental studies such as ecology, food microbiology, environmental biotechnology, agriculture, and plant science, and showed how this method is applied to identify bacteria performing a variety of functions in the environment. The researchers noted that further studies should focus on the relationship between the phylogenetic affiliation of bacteria and their concrete functions or metabolic characteristics, which will help to accelerate the development of environmental research and save time and money. At the same time, the current study leaves some questions unanswered and does not provide a complete picture of the epidemiology of *K. pneumoniae*. Wang et al. investigated the rapid detection of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) using machine learning and MALDI-TOF MS technology. The study developed machine learning models that successfully classify carbapenem-resistant *Klebsiella pneumoniae* strains with high accuracy. The use of machine learning in combination with MALDI-TOF MS allows rapid and inexpensive detection of carbapenem-resistant bacteria, which can be an alternative to existing detection methods [27].

The current study investigated the resistance of bacteria to cephalosporins. A study by C.M. Wilhelm et al. [28] evaluated the combined effects of the antibiotics aztreonam and ceftazidime/avibactam on the bacterium *Klebsiella pneumoniae* using the MALDI-TOF MS method. The paper discussed the problems of resistance of this bacterium to carbapenems and suggested the use of combination therapy with aztreonam and ceftazidime/avibactam. The researchers developed a method using MALDI-TOF MS (SynMALDI) to quickly determine whether synergy exists between aztreonam and ceftazidime/avibactam in vitro. They tested this method on *Klebsiella pneumoniae* isolates with certain genes and experimented with antibiotics. The results showed that the combination of aztreonam and ceftazidime/avibactam exhibited synergy as presented in the time-kill curve experiments. In addition, the SynMALDI method confirmed the presence of synergy in all isolates tested. As a result, the researchers suggested that synergy between aztreonam and ceftazidime/avibactam could be rapidly identified using MALDI-TOF MS, which could improve patient treatment.

*Klebsiella pneumoniae* is a bacterium that causes infections and actively develops antibiotic resistance mechanisms. It can acquire genes encoding  $\beta$ -lactamases and antibiotic efflux pumps. Mutations in various proteins may also contribute to antibiotic resistance. Biofilms also play a role in this process. Combination therapy is used to treat resistant strains, but it is important to properly manage antibiotic consumption in hospitals to reduce antibiotic resistance [29]. The study yielded important results that have significant implications for understanding the epidemiology and relatedness of *K. pneumoniae* strains isolated from individuals who served sentences between 2015 and 2018. These results are of interest in terms of antibiotic resistance and diversity of these strains, the study of which has become increasingly relevant in recent times. Thus, the study by

X. Jin et al. [30] describes a case of infection caused by a dangerous strain of *Klebsiella pneumoniae* (hv-CRKP), which showed a high capacity to evolve and develop resistance to the antibiotics tigecycline and polymyxin during the treatment of the patient. Eleven strains were identified in the patient, of which seven were highly virulent, three were resistant to tigecycline and four to polymyxin. The study also revealed similarities between the strains in the blaKPC-2 gene on a large plasmid. The strains belonged to the hypervirulent type ST11-KL64. Using Nanopore technology, several plasmids were discovered, including those responsible for tigecycline and polymyxin resistance. All this indicates the high risk of ST11-KL64 strain in the treatment of infections and its ability to rapidly develop resistance to antibiotics during treatment.

Thus, this study and earlier work emphasise the need for further research and provides a basis for a better understanding of the diversity of *K. pneumoniae*, its antibiotic resistance and epidemiology, as well as the applicability of various laboratory methods, including MALDI-TOF MS.

## CONCLUSIONS

The current study evaluated sub-species typing using MALDI-TOF technology on the example of *K. pneumoniae* isolated from patients with pulmonary tuberculosis in Karaganda region. Based on the totality of facts, sources and experimental results, it can be logically and reasonably concluded that the study did not provide a complete picture of the epidemiology of *K. pneumoniae*. Further experiments in this area should be conducted in the light

of the data and experience gained, responding to the lack of data by increasing sampling and processing more complete anamnestic data for sub-species typing of all strains submitted to MALDI-TOF.

By repeating the study under these conditions and adhering to the experimental experience obtained, it was possible to obtain well-defined clusters associated with appropriately defined phenotypes from different phenotypic, geographical, and clinical features, developing a pattern between them. In this study, the MALDI-TOF MS method had limited ability to investigate the epidemiology of *K. pneumoniae* in depth and even though initially effective, shows much less accurate and meaningful results. This confirms that MALDI-TOF is more likely to detect phenotypic differences between isolates of the same species than intrinsic genetic variation. In general, the evaluation of sub-species typing by MALDI-TOF method on the example of *K. pneumoniae* isolated from patients with pulmonary tuberculosis in Karaganda region showed the universality of isolates, indicating their out-of-hospital nature, which can underlie further research in this particular area, in the treatment, detection, and study of tuberculosis and other bacterial diseases.

The current study demonstrates the difference in the researched groups in a limited sample. Findings from large-scale studies involving larger numbers of patients will yield even more accurate results. It is also necessary to continue work on the development and improvement of methods for sub-species typing and in-depth research of *K. pneumoniae* epidemiology.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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