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Evaluation of the multiplex PCR combined with capillary electrophoresis technique for detecting pathogenic bacteria and antibiotic resistance genes in bone infections

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Abstract

Objective Orthopedic wound infection is a difficult problem in the clinic. Accurate and rapid microbiological test results are essential for case management, antibiotic therapy, and infection control.

Methods We retrospectively evaluated 1285 specimens (puncture fluid, catheter, secretions, joint fluid, lavage fluid, extraction fluid, blood culture, drainage fluid, cerebrospinal fluid, bone, prosthesis tissue, etc.) from 739 patients who received orthopedic diagnosis and treatment, using routine conventional method (RCM)s as a reference method to evaluate the performance of multiplex PCR combined with capillary electrophoresis (mPCR-CE) for detecting pathogens and antibiotic resistance genes associated with bone infection.

Results Among the 1285 samples analyzed, 1045 samples were consistent with the results of the RCM, with an agreement rate of 81.32%. Among the 155 inconsistent results, 13 (1.01%) were mPCR-CE negative but RCM positive, 142 (11.05%) was mPCR-CE positive but RCM negative. Compared with RCM, mPCR-CE demonstrated positive percentage and negative percentage agreement values of 65.37% and 98.35%, respectively. Moreover, the detection rate of multidrug-resistant bacteria by the mPCR-CE method was generally better than that by the RCM method. The detection rate of methicillin-resistant *Staphylococcus aureus* (MRSA) by the mPCR-CE method is relatively high. The traditional drug-sensitive culture method is more inclined to detect extended-spectrum β-lactamases (ESBLs). The mPCR-CE method has obvious advantages in terms of timeliness.

Conclusion This study revealed that mPCR-CE is a new and effective diagnostic method that can significantly reduce the identification time of bacterial identification and drug resistance, and has the potential to improve the management of orthopedic infections.

Keywords Bone infections, Multiplex PCR combined with capillary electrophoresis, Multidrug-resistant organisms

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Introduction

The infection of bones after trauma or surgery is complex, with a long course, high recurrence rate, and high risk of treatment failure and bone nonunion. Some cases may result in serious sequelae, requiring expensive long-term treatment, which poses a challenging issue in orthopedics [1–3]. Accurate and timely pathogen diagnosis is crucial for the effective treatment of such diseases. Traditional pathogen culture methods are time-consuming, and affected by various factors that impact sample quality and, therefore, the accuracy of culture results, with the majority of pathogens being unculturable. Additionally, the detection of mixed infections and unknown pathogenic microorganisms presents a barrier to traditional testing methods [4, 5]. Symptoms caused by different pathogens are often similar and difficult to differentiate. The overuse of antibiotics increases the risk of bacterial resistance, economic burdens and environmental pollution. The lack of rapid and effective microbiological evidence may be a significant factor leading to the overuse of antibiotics [6]. Hence, the development of new testing methods that meet clinical needs is urgently needed. Comprehensive, rapid, and accurate pathogen detection has become a clinical imperative.

Multiplex PCR combined with capillary electrophoresis (mPCR-CE) is a powerful tool covering viruses, bacteria, fungi, parasites, etc. Compared with routine conventional methods (RCM)s based on culture and antigen detection, syndromic testing via mPCR-CE offers many advantages [7, 8]. Owing to the nature of nucleic acid amplification, mPCR-CE demonstrates high sensitivity and specificity in detection; expanding the detection range by simultaneously detecting common and relatively uncommon pathogens helps reduce missed diagnoses and enhances the detection of polymicrobial infections. Rapid reporting of results (instrument times of 1–2 h or less), and the detection of pathogens and some antibiotic resistance genes facilitate early optimization of treatment plans. Its main applications include rapid testing of positive blood cultures, multiplex detection of respiratory pathogens, and multiplex detection of pathogens related to central nervous system infections [9–11]. This study aimed to evaluate the performance of mPCR-CE technology in detecting pathogenic bacteria and antibiotic resistance genes in bone infections compared with that of RCM.

Materials and methods

Research objects

The present study retrospectively analyzed specimens from patients who received orthopedic diagnosis and treatment at the Department of Orthopedics, Affiliated Honghui Hospital of Xi'an Jiaotong University, between May 1st, 2023 and November 30th, 2023. The specimens included puncture fluid, catheters, secretions, joint fluid, lavage fluid, exudate fluid, blood cultures, drainage fluid, cerebrospinal fluid (CSF), bone samples and periprosthetic tissues. A total of 13,189 specimens were tested via mPCR-CE technology, and 12,710 specimens were tested via traditional bacterial culture techniques. Both methods were used to test samples from 905 patients (1486 specimens). After analysis and exclusion of contaminated samples (35 cases), insufficient sample volume (29 cases), and repeated strains from the same patient at the same site (137 cases), a final total of 739 patients (1285 samples) were included in this study. Among these patients, for 548 patients, only one sample was collected; for 191 patients, multiple samples were collected with different types/sites/time periods. The basic information and underlying disease information of the patients are shown in Table 1.

Diagnosis criteria

Patients who met the criteria according to the WS/T 312–2023 "Standard for healthcare associated infection surveillance" (2023 edition) issued by the Ministry of Health of the People's Republic of China Health Industry, were diagnosed with infection.

The diagnosis criteria for multidrug-resistant organism (MDRO)s are bacteria that are resistant to three or more commonly used antibiotics that are usually sensitive, and the same strain of MDRO is continuously cultured positive three consecutive times. The MDRO strains included in this study for detection were methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase-producing Enterobacteriaceae (such as *Escherichia coli* and *Klebsiella pneumoniae*), multidrug-resistant *Pseudomonas aeruginosa*, and multidrug-resistant *Acinetobacter baumannii*.

Quality control

RCMs: The standard strains used including *Escherichia coli* ATCC44102, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC BAA-977, *Klebsiella pneumoniae* ATCC@BAA-1706TM, *Corynebacterium diphtheriae* ATCC13812, *Streptococcus pneumoniae* ATCC6305, *Acinetobacter baumannii* NCTC13304, *Staphylococcus epidermidis* CMCC26069, *Enterococcus faecalis* ATCC33186, *Candida albicans* ATCC14053, etc., were purchased from Wenzhou Kangtai Biotechnology Co., Ltd. Antimicrobial susceptibility testing included gram-positive cocci susceptibility testing via GP-AST cards and gram-negative rods via GN-AST cards. All testing procedures were automatically completed by identification instruments, and the results were interpreted following EUCAST recommendations (http://www.eucast.org/).

Table 1 Detection targets of mPCR-CE

Type of target	Organism(s) or gene
45 Bacteria	*Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Moraxella catarrhalis*, *Serratia marcescens*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Proteus vulgaris*, *Proteus Mirabilis*, *Streptococcus pyogenes*, *Enterobacter aerogenes*, *Alcaligenes faecalis*, *Acinetobacter nosocomialis*, *Acinetobacter calcoaceticus*, *Acinetobacter Pittii*, *Klebsiella oxytoca*, *Proteus Penneri*, *Staphylococcus hominis*, *Citrobacter koseri*, *Staphylococcus warneri*, *Staphylococcus hemolyticus*, *Staphylococcus lugdunensis*, *Citrobacter braakii*, *Citrobacter freundii*, *Finegoldia magna*, *Parvimonas micra*, *Peptostreptococcus anaerobius*, *Kingella kingae*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*, *Clostridium perfringens*, *Morganella morganii*, *Legionella pneumophila*, *Bordetella pertussis*
7 Fungi	*Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Cryptococcus neoformans*
3 Atypic strains	*Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*
9 Antimicrobial resistance genes	MRSA genes (mecA) Carbapenemases (blaKPC, blaIMP, blaVIM, blaOXA-23, blaOXA-48 like, blaNDM) ESBL (blaCTX-M) mcr-1

mPCR-CE was performed in strict accordance with the manufacturer's instructions. Negative and positive quality control products were randomly placed in each batch to be extracted and amplified simultaneously with patient samples. The Yang plasmid and internal standard Yang plasmid were synthetic plasmids containing specific gene sequences of target strains and internal reference gene sequences (GAPDH internal control genes). The card holder was selected with a high-resolution cartridge kit, and an appropriate alignment marker was selected for detection and analysis.

The periprosthetic tissues were preprocessed via ultrasonic grinding and optimized with multiple culture media, and all procedures were carried out in accordance with relevant guidelines and regulations.

Instruments and reagents

RCMs: automatic microbial analysis system (bioMérieux VITEK 2™ compact), automatic bacterial/mycobacterium culture monitoring system (bioMérieux BACT/ALERT 3D™).

mPCR-CE: Genesy 96 T Gene Amplification Thermocycler (Tianlong Biotech Co., Ltd.), Qsep400 Automated Capillary Electrophoresis System (LightDx Biotech Co., Ltd. (Jiangsu)), BSD-DY-1 Automatic capillary electrophoresis apparatus (Capgemini Biotechnology Co., Ltd.), BGNA-32P Automated Nucleic Acid Extractor (PerkinElmer Life Sciences), Nucleic Acid Extraction using Magnetic Bead Technology Nucleic Acid Extraction Kits (Chongqing Pasteur Biotechnology Co., Ltd., Cat. No.: T07-100 (lower respiratory tract fungi, central nervous system pathogens, bacteria), T09-100 (resistant genes)), amplification reagents include Bacterial Identification Reagent Kit, Lower Respiratory Tract Bacteria Identification Reagent Kit, Atypical Bacteria Infection Identification Reagent Kit, Bacterial Resistance Gene Identification Reagent Kit, Lower Respiratory Tract Fungi Infection Identification Reagent Kit, Central Nervous System Pathogen Identification Reagent Kit, Joint/Soft Tissue Infection Bacteria Identification Reagent Kit (Chongqing Meiji Tongyan Biotechnology Co., Ltd.).

A total of 45 types of bacteria, 7 types of fungi, 3 types of atypical bacteria, 9 types of drug resistance genes and their corresponding strains were detected (Table 1).

Statistical analysis

The results of mPCR-CE detection were compared with the RCM results to evaluate the true positive (TP), true negative (TN), false positive (FP), and false negative (FN) rates, and to calculate the positive predictive value (PPA, TP/(TP+FN)) and negative predictive value (NPA, TN/(TN+FP)). The positive predictive value [PPV, 100∗(TP/(TP+FP))], negative predictive value [NPV, 100∗(TN/(TN+FN))], and diagnostic coincidence rate were calculated to evaluate the agreement between the two methods. When the same bacteria were detected as true positive by both methods and no pathogen was detected by both methods, they were considered true negative. In the first analysis, conventional techniques were defined as reference criteria, meaning that microorganism identified only by mPCR-CE and not by conventional techniques were considered FP; conversely, target identified by conventional methods but not by mPCR-CE were considered FN.

Results

Basic patient data

The basic information of the patients is shown in Table 2.

Table 2 Patient demographics

Characteristic	Cases (*N* = 739)
Age (years)	
Median (range)	57 (28–78)
3–25 [n, (%)]	175 (23.7)
26–50 [n, (%)]	265 (35.9)
51–79 [n, (%)]	299 (40.5)
Gender [n (%)]	
Male	478 (64.7)
Female	261 (35.3)
Weight (kg)	70 (50—84)
Height (cm)	158 (105—180)
Comorbidities [n (%)]	
None	396 (53.6)
HIV	3 (0.4)
Hypertension	97 (13.1)
Diabetes	78 (10.6)
Chronic kidney disease	24 (3.2)
Coronary heart disease	36 (4.9)
Bronchiectasis	10 (1.4)
Syphilis	9 (1.2)
Hepatitis B	70 (9.5)
Others	16 (2.2)
Diagnostic category, n (%)	
Trauma (femoral fracture,Fracture of tibia and fibula,Humeral fracture, radius fracture, toes fractures etc.)	259 (35.0)
Spine (slipped disk, cervical vertebra fracture, thoracic vertebrae fracture, lumbar vertebral fracture etc.)	197 (26.7)
Arthrosis (total knee arthroplasty, femoral head necrosis, Anterior cruciate ligament reconstruction of knee joint etc.)	188 (25.4)
Others (Osteoporosis, ankylosing spondylitis, rheumatoid arthritis, etc.)	95 (12.9)

Data were presented as median (range) or n, number of cases (percentage). N, total number of cases

Strains and main pathogenic bacteria detected by mPCR-CE and RCM

Using the mPCR-CE method, a total of 4,736 positive samples were detected out of 13,189 samples, resulting in a detection rate of 35.91%. With the RCM method, out of the 12,710 samples tested, 3,796 positive results were obtained, with a detection rate of 29.87%. Both methods demonstrated the highest detection rates for aerobic gram-negative rods (mPCR-CE, 50.06%; RCM, 48.65%), with *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* being the most common strains observed. This was followed by aerobic gram-positive bacteria (mPCR-CE, 41.05%; RCM, 44.89%), fungi (mPCR-CE, 7.37%; RCM, 3.5%) and anaerobic bacteria (mPCR-CE, 1.10%, RCM, 0.95%) (Fig. 1).

Overall concordance in the retrospective study arm (orthopedic specimen dominated)

Among the 1285 samples analyzed together, 1045 samples were consistent with the results of pathogen identification (81.32%). Among these, 777 (60.47%) mPCR-CE negative results were considered consistent, 13 (1.01%) samples were mPCR-CE negative but culture positive, and 142 (11.05%) samples were mPCR-CE positive but culture negative. Compared with RCM (culture method), mPCR-CE showed 65.37% positive predictive value and 98.35% negative predictive value. The sensitivity was 95.37% (268/281), and the specificity was 84.55% (777/919). At the same time, only one strain was detected

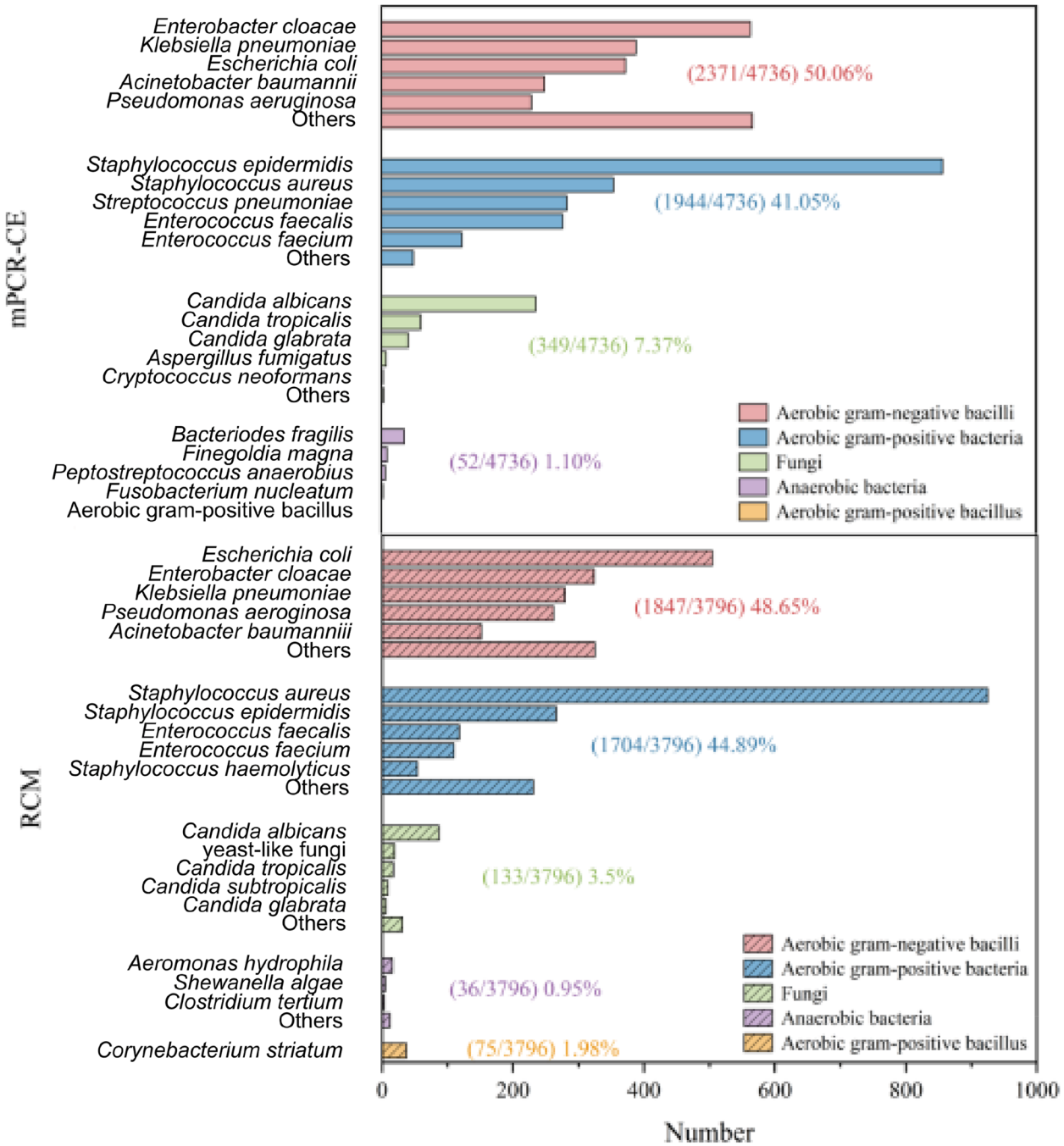


Fig. 1 Strains and main pathogenic bacteria detected by mPCR-CE and RCM. Abbreviations: mPCR-CE, multiplex PCR combined with capillary electrophoresis; RCM, routine conventional method

by the culture method in 80 samples, while multiple strains were detected by the mPCR-CE method. Five samples with different strains were detected by the two methods (Table 3).

Concordance and discordance analysis between mPCR and culture results

In different sample types, the mPCR-CE method had a much higher percentage of positive bacteria than the RCM method. This advantage is especially evident in sterile body fluids. The detection rate of cerebrospinal

Table 3 Comparison of the results of mPCR-CE and RCM in the retrospective study arm (orthopedic samples dominated)

Results type		Prospective study (*N* = 1285) n (%)
Concordant results		1045 (81.32)
	mPCR-CE and RCM negative	777 (60.47)
	mPCR-CE and RCM positive	268 (20.86)
Discordant results		155 (12.06)
	mPCR-CE positive, RCM negative	142 (11.05)
	mPCR-CE negative, RCM positive	13 (1.01)
Partially concordant results	mPCR-CE (multiple bacteria), RCM (one bacteria)	80 (6.23)
	Inconsistent strains	5 (0.39)

Data were presented as n, number of cases and percentage. N, total number of cases

fluid increased from 5.56% by RCM to 22.22% by mPCR-CE. The detection rate of joint fluid with mPCR-CE was approximately 1.75 times higher than that with the RCM method (Fig. 2A). Among the 240 inconsistent results, 80 samples (6.23%) had at least one or more type of bacteria detected by mPCR-CE as shown in Figure (Fig. 2B) (including *Escherichia coli* (Eco), *Kelbseilla pneumonia* (kpn), *Propidium monoazide* (PMA), *Enterobacter cloacae* (Ecl), *Stenotrophomonas maltophilia* (SMA), *Staphylococcus aureus* (SAU), *Klebsiella oxytoca* (Kox)), compared to one type of bacteria detected by culture method alone. Five positively identified strains were shown in Fig. 2C. These samples were mainly originated from secretions such as those shown in Fig. 2A, and the detected bacterial species did not tend toward skin commensal flora such as *Staphylococcus epidermidis.* Five samples showed inconsistency in bacterial species; however, no regular pattern was observed, suggesting that this may be due to sampling from different locations.

Distribution of drug resistance

Drug resistance testing was performed on 268 samples that yielded consistent positive results via both mPCR-CE and RCM methods. The results are shown in Table 4. The mPCR-CE method detected 9 types of drug resistance genes. The RCM method detected 5 main types of drug resistance mechanisms. The drug resistance detection rate of the mPCR-CE method was significantly higher than that of the RCM method (54.85% vs. 19.4%), with advantages, mainly reflected in the detection of MRSA, ESBLs, and CRE, while there was little difference in other drug resistance rates. The high detection rate of 54.85% by the mPCR-CE method is due to its sensitivity, possibly because some samples had more than one type of drug resistance gene detected.

Comparison of multidrug resistance genes detected in different departments (mPCR-CE)

In terms of distribution among different orthopedic fields, the highest isolation rate for MDROs was found in the Bone Microsurgical Repair Department, with 156 cases (19.21%), followed by the Hand Surgery Center, with 114 cases (14.04%). The lowest MDRO detection rate was observed in the Peripheral Vascular Department with only 4 cases (0.49%). Among these MDROs, MRSA was widely present in various departments, especially in the Hand Surgery Center, with a proportion as high as 11.33%. ESBL-positive *Escherichia coli* were mainly distributed in the Bone Microsurgical Repair Department, Trauma Department, and Surgery Department. Overall, under the joint control efforts of all medical staff members, the MDRO detection rates across different departments were satisfactory at our hospital. MRSA was primarily originated from secretion samples. The slightly higher MDRO detection rate in the Traditional Chinese Medicine Orthopedics Department might be attributed to relatively longer hospital stays for patients receiving such treatment. Since a single sample might contain more than one type of MDROs, it was possible that departments with fewer samples sent for testing could have a higher positivity rate (Fig. 3).

Trends of multidrug resistance genes in different months

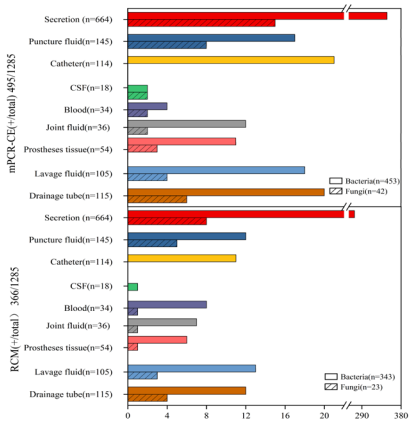
The trends of multidrug resistance genes between May and November 2023 is shown in Fig. 4.

Case analysis

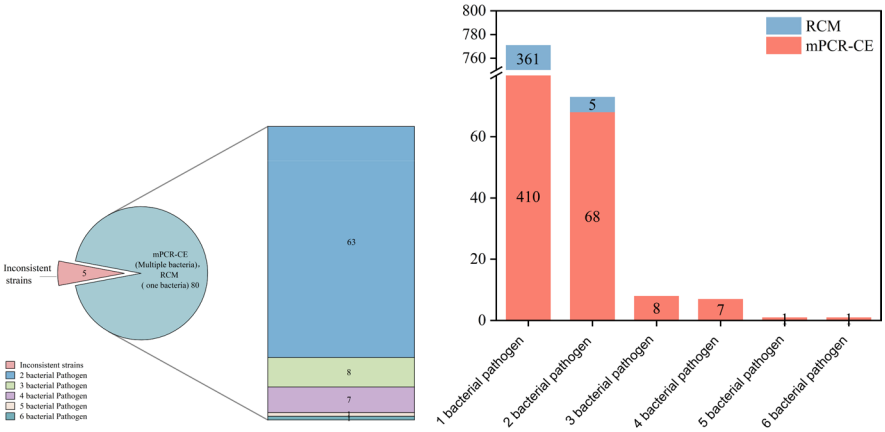
Case A

A 72-year-old female patient underwent right knee joint replacement surgery for “arthritis” in October 2022 at an external hospital. The procedure went smoothly, and the patient recovered well and was discharged. In August

A. Detection rates of different specimen types



B. Inconsistency analysis



C. Electrophoretic images of some identified strains detected by mPCR-CE

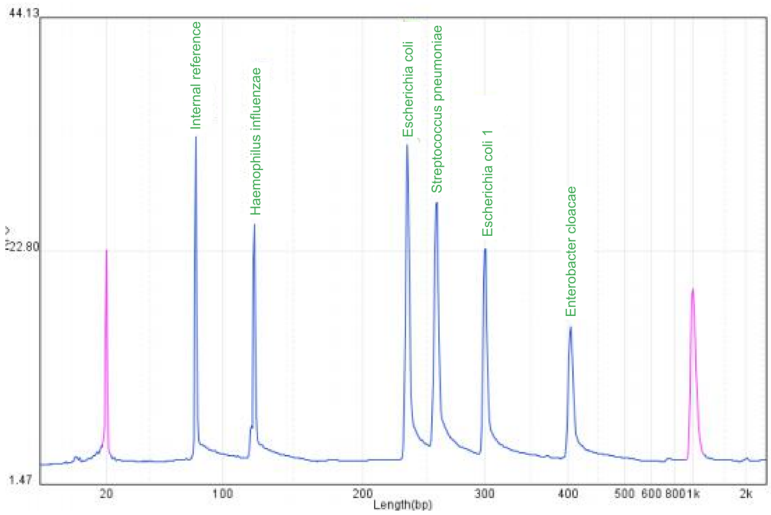


Fig. 2 Concordance and discordance analysis between mPCR-CE and RCM culture results. Abbreviations: mPCR-CE, multiplex PCR combined with capillary electrophoresis; RCM, routine conventional method

Table 4 Comparison of drug-resistant bacteria detected by the two methods

MDROs		mPCR-CE (*N* = 268)		RCM (*N* = 268)	
	Drug resistance gene	Positive number n (%)	Total drug resistance rate n (%)	Positive number n (%)	Total drug resistance rate n(%)
ESBLs	**CTX-M***	28 (10.45)	147 (54.85)	19 (7.09)	52 (19.4)
MRSA	**MecA***	78 (29.10)		13 (4.85)	
CRE	**KPC***	4 (1.49)		5 (1.87)	
	OXA-23*	16 (5.97)		7 (2.61)	
	OXA-48 like*	1 (0.37)		3 (1.12)	
	IMP*	3 (1.12)		1 (0.37)	
	VIM*	3 (1.12)		1 (0.37)	
	NDM*	13 (4.85)		1 (0.37)	
	mcr-1*	1 (0.37)			
VRE				1 (0.37)	
VRSA				1 (0.37)	

Data were presented as n, number of cases and percentage. N, total number of cases

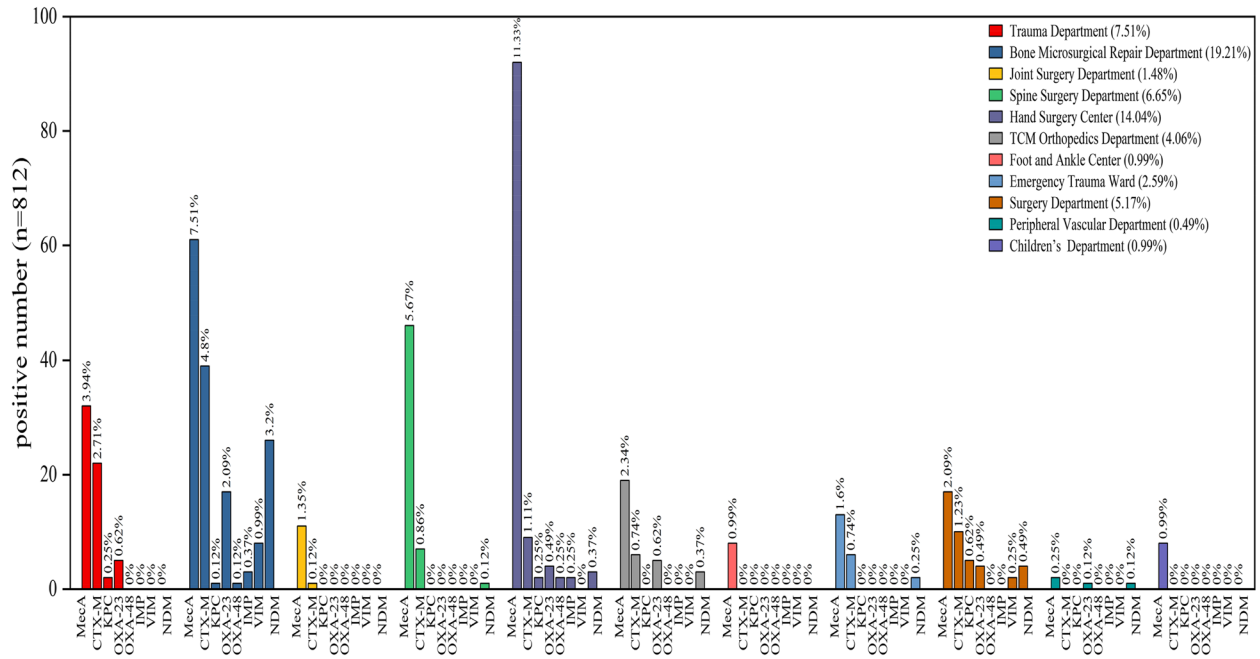


Fig. 3 Comparison of multidrug resistance genes detected in different departments

2023, a soft mass appeared on the right knee, without purulent discharge. The patient sought medical attention at a local hospital and received symptomatic treatment, such as anti-inflammatory medication, pain relief, and intravenous antibiotics, but there was no improvement. Therefore, the patient came to our hospital for further diagnosis and treatment in October 2023. The patient presented with redness, swelling, and elevated skin temperature in the local area around the right knee, with positive tenderness upon palpation. The range of motion of the right knee joint was 90°-0°-0°. The patient was diagnosed with "infection of the right knee joint prosthesis" and was admitted to the hospital. Routine blood, C-reactive protein (CRP), ESR, general bacterial culture and identification (joint fluid), and pathogen and antibiotic resistance gene testing (joint fluid) were performed. Both the CRP level and ESR were elevated. The mPCR-CE results after 6 h revealed infection with *Aspergillus fumigatus* (Fig. 5). Combined with X-ray and other auxiliary examinations, the patient was treated

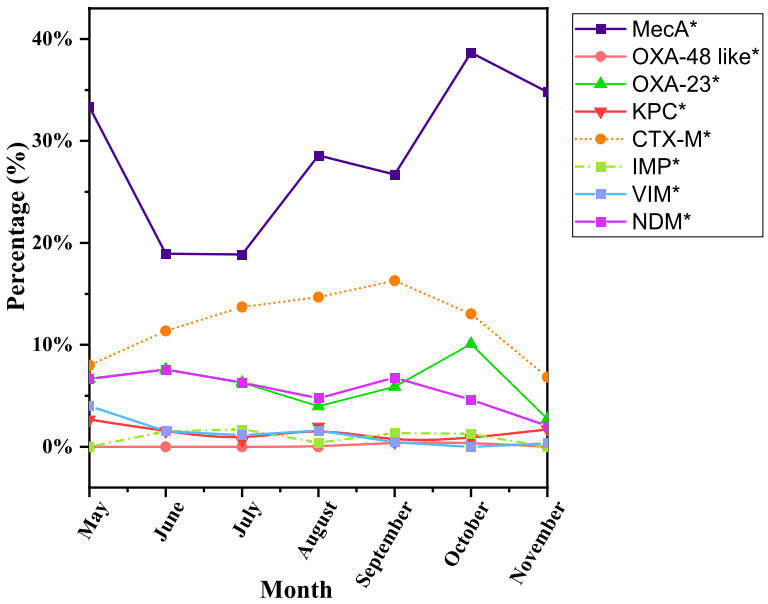


Fig. 4 Trends in resistance genes between May and November 2023

with amphotericin B and vancomycin. Joint revision surgery was also considered, and vancomycin was added to the bone cement. However, owing to the use of antibiotics before admission, the bacterial culture was negative after 48 h. After surgery, the patient continued to receive symptomatic treatment, such as pain relief, anti-inflammatory medication, and anticoagulants. After 15 days, the pathogen test was negative, and the patient's condition improved, so the patient was discharged. Therefore, for patients undergoing antibiotic treatment, the advantages of mPCR-CE are reflected not only in the timeliness but also in the detection rate, which is higher than that of traditional culture methods (RCMs).

Case B

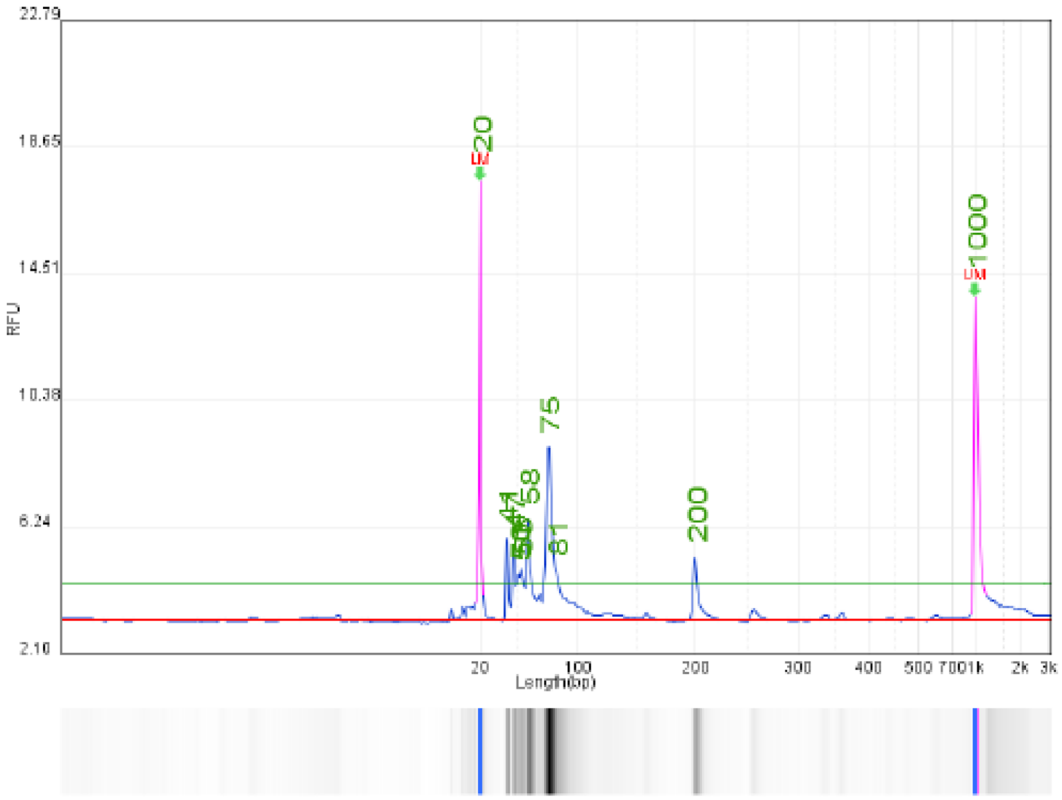
An 81-year-old male patient was diagnosed with chronic obstructive pulmonary disease 10 years ago. Since then, he has recurrent symptoms every autumn and winter. One day ago, after catching a cold, the patient developed a cough, yellow–green sputum, excessive sputum production, fever, chest tightness, and shortness of breath, and was admitted to hospital. After admission, routine blood, C-reactive protein (CRP), and electrolyte tests, as well as pathogen and antibiotic resistance gene testing (sputum) and general culture and identification (sputum) were performed. The routine blood test results showed elevated white blood cell counts, elevated CRP levels, and decreased lymphocyte counts. Considering the possibility of bacterial infection, empirical treatment with imported cefoperazone/sulbactam was administered. The mPCR-CE test results after 6 h revealed *Bordetella pertussis* (Fig. 6). The clinical staff promptly took protective measures, such as respiratory tract infection isolation, to prevent nosocomial infection. The antibiotic was switched to azithromycin. However, 24 h later, the general culture and identification (sputum) report revealed normal flora. mPCR-CE not only reduced empirical antibiotic use but also prevented nosocomial infections in the shortest possible time.

Discussion

Orthopedic inpatients often require invasive procedures, surgical treatment, or implantation, and wound infection is a challenging problem in clinical practice, which may also lead to patients developing acute osteomyelitis, resulting in partial loss of function or disability, posing a serious threat to patients' physical health and causing a great economic burden to patients and their families. Studies have reported incidence rates ranging from 1.64% to 4.85% for hospital-acquired infections in orthopedic hospitals [12]. Surgical site infections are the most common orthopedic hospital infections, which is consistent with the findings of Bath et al. [13]. In recent years, broad-spectrum antibiotics have been widely used in China, leading to significant changes in the distribution and resistance of pathogens. Therefore, understanding and quickly detecting pathogens and resistance to orthopedic wound infections will help reduce the infection rate in orthopedic patients [14].

This study evaluated the performance of mPCR-CE technology in the detection of 45 bacterial species, 7 fungi, 3 atypical bacteria, and 9 antibiotic resistance

Result:	20231013-2_85-X3_S3G05_R1_CH2.bopx		
Test Time:	2023-Oct-13 18:29(UTC+08)	Instrument ID:	0039008334910009
Operator:		Software & Firmware Version:	3.4.3 & 5
Method:	M-4-10-06-300	Cartridge & Run Number:	S1-O-4CH-230719-140 & 95
Sample Name:		Manufacture & Expiration Date:	2023-Jul-19 & 2024-Jan-15
Sample ID:	85-X3	Alignment Marker:	20-1K (20 bp & 1K bp)



No	Time (sec.)	RFU	PeakArea	bp	Concn. (ng/μl)	PeakStart (sec.)	PeakEnd (sec.)
1	103.96	14.31	192,203	20	N/A	102.76	104.60
2	110.36	2.63	43,339	41	0.16	109.92	111.28
3	112.04	2.45	45,417	47	0.16	111.44	112.64
4	113.04	1.47	23,050	50	0.08	112.64	113.28
5	113.36	1.44	11,295	51	0.04	113.28	113.56
6	113.92	1.63	49,491	53	0.17	113.56	114.96
7	115.52	3.27	98,585	58	0.32	114.96	117.56
8	120.72	5.61	244,911	75	0.69	119.20	122.60
9	122.68	1.61	73,667	81	0.21	122.60	125.76
10	156.92	2.05	72,245	200	0.15	155.76	158.80
11	226.32	10.52	360,087	1,000	N/A	225.48	229.28

Fig. 5 Capillary electrophoresis of *Aspergillus fumigatus* (Instrument model: Qsep400)

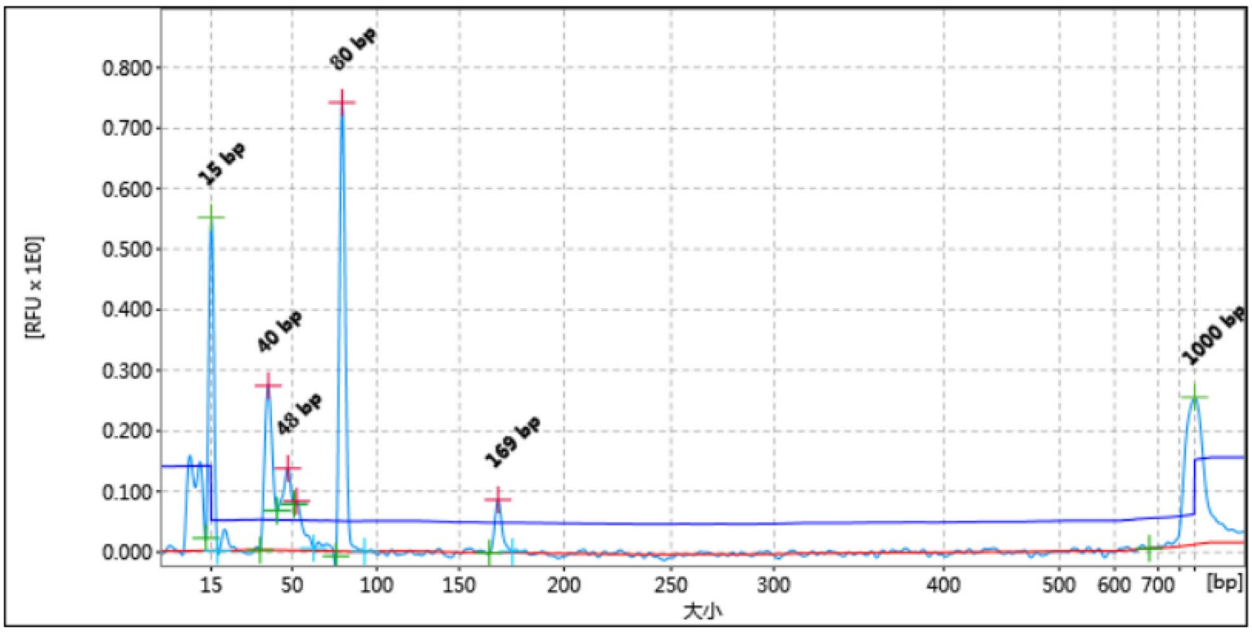


Fig. 6 Capillary electrophoresis of *Bordetella pertussis* (Instrument model:BSD-DY-1)

genes. Compared with traditional culture methods, the positive detection rate of mPCR-CE was 35.91%, while the rate of the culture method (RCM) was 29.87%. The strains detected by the two methods were aerobic gram-negative bacilli, aerobic gram-positive bacteria, fungi and anaerobic bacteria, in order. Except for aerobic gram-positive cocci, RCM had a slightly higher detection rate than mPCR-CE for the other species. The probable reason was that the bacteria detected by RCM were not included in the mPCR-CE detection spectrum (saprophytic Staphylococcus, viridans Streptococcus, coagulase-negative Staphylococcus, etc.). With the widespread use of broad-spectrum antibiotics, immunosuppressants, glucocorticoids, and invasive diagnostic and therapeutic techniques, infections caused by *Staphylococcus* have been increasing and have become important pathogens in orthopedics. They can cause infections in wounds, urinary tract, and bloodstream, especially when the wound loses the protective barrier of the skin, resulting in direct contact with *Staphylococcus* on human skin, which can easily cause *Staphylococcus* infection [15]. This study suggested that among the top 5 common gram-positive bacteria, 2 were Staphylococcus species, with *Staphylococcus aureus* accounting for the highest proportion at 25.92%, making it the most important pathogenic bacterium in orthopedic hospital infections, and the main pathogen of surgical site infections in orthopedics [16]. Notably, enterococcus accounted for a large proportion of the bacteria identified via both methods (mPCR-CE, 8.45%; RCM, 6.01%). Most of them were *Enterococcus faecalis*. *Enterococcus* is an important opportunistic pathogen in hospital-acquired infections [17]. The detection rate of fungi has increased in recent years, and a paper from the Lancet revealed that more than 6.55 million people worldwide are threatened by fungal diseases each year. More than 3.75 million patients died, of which 2.55 million deaths were directly attributable to mycosis [18]. On October 25, 2022, the World Health Organization (WHO) issued a list of priority fungal pathogens for the first time, calling on governments and researchers around the world to make major efforts to strengthen the response to fungal infections and enhance global capabilities in response to fungal infections and antifungal resistance [19]. The main laboratory testing methods for fungal infections in clinical practice are traditional microscopy, culture identification, and serological methods, but the time taken is long, and the positive rate is poor. The fungal detection rate of the mPCR-CE method involved in this experiment was approximately twice that of RCM (mPCR-CE, 7.37%; RCM, 3.5%). Although mPCR-CE fails to solve the problem that filamentous fungi cannot be tested for drug susceptibility via the RCM method [20], not all bacteria can undergo in vitro susceptibility testing. PCR detection can help further identify pathogens in sterile tissues of diagnosed patients. PCR methods have good sensitivity and specificity and can be recommended as microbiological evidence for assisting in the diagnosis of invasive fungal disease (IFD)s [21, 22]. This is well demonstrated in our case A study. The patient had joint prosthesis infection caused by

Aspergillus fumigatus, and the mPCR-CE method could accurately and rapidly identify the pathogen. Treatment with amphotericin B helped to shorten the patient's hospital stay and reduce medical expenses.

In this study, the detection rate of anaerobic bacteria was low (mPCR-CE, 1.10%; RCM, 0.95%). Owing to the high requirements of anaerobic bacteria for culture conditions, the detection rate of mPCR-CE was significantly higher than that of RCM. In addition, the detection rate of atypical bacteria (*Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Bordetella pertussis*) was higher than that of culture methods, but these bacteria were all detected in respiratory samples, so they were not specifically listed in this article. Owing to the limitations of the bacteria detected, RCM detected 1.98% aerobic gram-positive rods, which cannot be compared with mPCR-CE. Additionally, although mPCR-CE covers 55 bacterial species, further improvements, such as in aerobic gram-positive rods, are needed.

In the study, mPCR-CE showed better detection value than RCM in sterile body fluids such as cerebrospinal fluid, joint fluid, and prosthetic tissue. The detection rate of cerebrospinal fluid increased from 5.56% with RCM to 22.22% with mPCR-CE. The detection rate of joint fluid with mPCR-CE was approximately 1.75 times that of RCM. Joint fluid is an excellent specimen for diagnosing joint infections, but the culture positivity rate is low, especially after empirical antibiotic treatment. The mPCR-CE combination panel for joint infections effectively helps diagnose purulent infections of natural joints. The reason for the high positive rate of blood culture specimens in RCM culture is due to the limitation of mPCR-CE reagent sensitivity. False-negative results may occur due to the need of conversion to positive for microbial culture that needed to be tested when detecting bloodstream infections via mPCR-CE method, limited detection spectrum, or presence of substances that inhibit PCR reactions in the specimens. It is also possible that the samples used for routine culture were contaminated, resulting in false-positive cultures.

For samples with positive results in both single and multiple bacterial cultures, 348 out of 366 samples (95.08%) showed positive results in mPCR-CE for the same bacteria. The probable reason for the 13 samples (1.01%) being mPCR-CE negative and positive in culture is limitation of mPCR-CE sensitivity. The high rate of mPCR-CE positive, culture negative results (11.05%) is probably due to the use of antibiotics by most patients when seeking medical attention. In this study, mPCR-CE also detected *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *Bordetella pertussis*. mPCR-CE can detect not only typical cultivable microorganisms but also various fastidious bacteria, nonviable bacteria, and atypical bacteria in terms of morphology and biochemical reactions, including those microorganisms that have been treated with antibiotics, as long as there are undamaged fragments. Compared with traditional culture methods, molecular methods are more likely to be affected by specimen transportation or storage conditions. For samples from the respiratory tract, it is challenging to differentiate between colonizing bacteria, contaminating bacteria, and pathogenic bacteria, especially for intubated patients. Both mPCR-CE and RCM face the same difficulties.

mPCR-CE can also detect 9 types of antibiotic resistance genes (such as blaKPC, blaNDM, blaVIM, and blaOXA-48), and there is a strong correlation between the mPCR-CE results for gram-negative bacteria and the genotypes and phenotypes of the corresponding isolates. In cases where multiple resistance genes are detected in a sample, we believe that they can, to some extent, help prevent the abuse of antibiotics. The rapid detection of resistance genes by mPCR-CE can provide clues to the presence of potentially resistant microorganisms, indicating relevant resistance mechanisms. This helps alleviate the problem of long identification times in traditional antimicrobial susceptibility testing methods, leading to more precise treatment. However, there are limitations as well. The absence of resistance genes does not necessarily indicate sensitivity to antibiotics, as there may be resistance mechanisms not detected by mPCR-CE (such as resistance genes of enterococci), and discrepancies between some resistance genes and phenotypes. Therefore, mPCR-CE can only serve as an auxiliary diagnostic method and cannot replace traditional antimicrobial susceptibility testing. Owing to the development of resistance to multiple commonly used antibacterial drugs and the lack of effective antibacterial agents, infections caused by MDROs often lead to increased mortality rates and treatment costs, as well as a relatively poor prognosis, posing challenges for clinical treatment. During the research period from May to November 2023, the slightly higher detection rate of bone microorganisms with MDROs may be due to an increased number of secretion samples, and because a sample may contain more than one type of MDRO, leading to a potentially higher positivity rate in departments with fewer samples submitted for testing. Overall, the detection rate of MDROs in various departments of our hospital is relatively satisfactory. This is closely related to the strict national control of antibiotic abuse in recent years, which requires hospitals to reduce the rate of antibiotic use, as well as the emphasis placed by the hospital's infection management department and related medical staff on the management of MDROs. Notably, the detection rate of mecA ranks highest across different months and shows an increasing

trend. Clinical physicians need to strengthen continuous monitoring of patients during hospitalization, increase the rate of submitting different samples for patients with signs of infection, facilitate early diagnosis, tailor the use of antibiotics on the basis of drug susceptibility test results, regularly enforce strict management systems and standards for antibiotic use, periodically investigate and analyze the rationality of antibiotic use, and take timely measures to address any issues. Targeted monitoring is beneficial for the early detection of trends in infection clusters and outbreaks, allowing timely control. In addition, molecular detection of resistance genes may change the management mode of patients and potentially improve outcomes, such as reducing the duration of antibiotic treatment, shortening the average length of hospital stay, and lowering mortality rates. The combination of culture and molecular assays may therefore provide a better gold standard than culture alone [23].

The reduction in hospital stays due to the use of mPCR-CE is almost entirely attributed to faster reporting of microbiological test results. The advantage of mPCR-CE in terms of efficiency is evident, with the time from extracting sample DNA to completing bacterial identification through specific amplification taking approximately 4–6 h, which is significantly shorter than the average positive reporting time of conventional cultures (77.88 ± 15.53 h). This advantage is especially pronounced in sterile body fluids. Unfortunately, owing to limitations in sensitivity, blood samples need to be cultured and converted to positive before identification.

In summary, a total of 358 MDRO strains were isolated in our study, with a detection rate of 25.7%. Among them, MRSA was the most common, followed by ESBL-positive *E. coli*. The Bone Microsurgical Repair Department and Hand Surgery Center had the highest number of MDRO isolates, which requires more attention. The resistance patterns of these MDROs indicate that antibiotic resistance is still a serious problem. Notably, doctors must be aware of the risk of multidrug-resistant bacterial infections. Open wounds, central nervous system injuries, and patients over the age of 60 are more prone to multidrug-resistant infections. Clinicians should pay more attention to these patients and actively prevent and control MDRO infections.

However, there are several limitations in this study. This was a single-center study and did not compare the MDRO resistance situation with that of other departments or other hospitals. This study analyzed only the resistance of the top 5 MDROs infection and lacked comprehensiveness. In future studies, we recommend analyzing the resistance in different subspecialties to make the results more relevant. Although the mPCR-CE method used in this study has high sensitivity, it cannot quantify the detected bacterial species or determine the dominant strains, which is also a challenge for molecular detection. The coverage of mPCR-CE detection is not ideal, especially for the detection of biofilm pathogens on difficult-to-cultivate prostheses in orthopedics or clinically suspected bone tuberculosis, which needs to update the target microorganisms to meet the needs in orthopedic infection.

In conclusion, early detection of pathogens and proper antibiotic selection are crucial for the treatment of infections in orthopedic patients. mPCR-CE can serve as a screening test, as its high sensitivity and short detection time are highly important for the rational clinical use of antibiotics, particularly in cases of polymicrobial infections or empirical therapy. Although mPCR-CE cannot distinguish between colonization and infection, it provides clinicians with more valuable information for treatment decisions.

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Authors' contributions
JYL and YY conceived and designed the study. WF, ZHH, MFW, and WKH contributed to data collection and experimental implementation. YNZ, YL, YZY and HPZ contributed to the data analysis and data interpretation. YZY and HPZ contributed to writing the report and revising the report. All authors read and approved the final version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All patients were informed and signed informed consent forms voluntarily. This study was approved by the ethics committee of Honghui Hospital, Xi'an Jiaotong University and complied with the guidelines outlined in the declaration of Helsinki were followed. Written consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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