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Clinical characteristics of patients with granulomatous lobular mastitis associated with Corynebacterium parakroppenstedtii infection and drug sensitivity analysis of the isolated strains

Yifei Zeng[1,2], Mengjie Wang[1,2], Xiang Gao[3], Dongxiao Zhang[1*], Na Fu[1], Wenjie Zhao[1] and Qiao Huang[1]

Abstract

Background It is presently considered that Corynebacterium especially Corynebacterium kroppenstedtii (CK) infection, is one of the important causes of granulomatous lobular mastitis (GLM). However, the pathogen of mastitis in the past two years has been identified as a newly discovered Corynebacterium. But it is unclear whether the pathogen associated with the occurrence of GLM is also this bacterium.

Methods GLM female patients with positive bacterial culture in pus specimens from February 2023 to February 2024 who were identified as CK infection by mass spectrometer were selected as the research objects in this study, and the clinical isolates were identified by 16S rDNA sequencing technology to identify the specific pathogen of GLM-related bacterial infection. Subsequently, the clinical characteristics of the patients were compared with those of GLM patients without bacterial infection during the same period, to explore the effect of this particular type of Corynebacterium infection on disease development in GLM patients. Finally, we tested the minimum inhibitory concentration (MIC) values of antibiotics when inhibiting these separation strains in vitro through the E-Test experiment, to evaluate their medicine sensitivity.

Results A total of 31 GLM patients initially diagnosed with Corynebacterium kroppenstedtii (CK) infection via MALDI-TOF MS were enrolled in the study. However, subsequent 16S rDNA sequencing revealed that 28 isolates (90.32%) were actually identified as the newly recognized Corynebacterium parakroppenstedtii (CPK). This discovery challenges the conventional belief that CK is the primary pathogen of GLM, suggesting instead that CPK is the predominant pathogen associated with GLM bacterial infections. Comparative analysis of the clinical characteristics between the two groups revealed a significantly higher recurrence rate among CPK-infected GLM patients compared to those without CPK infection, along with elevated prolactin levels ($P < 0.05$). The sensitivity test results indicated high sensitivity of the isolates to vancomycin, linezolid, and rifampicin.

*Correspondence:
Dongxiao Zhang
morningdong@163.com

Full list of author information is available at the end of the article

Conclusion In conclusion, this study highlights that Corynebacterium kroppenstedtii strains isolated from GLM specimens were Corynebacterium parakroppenstedtii, serving as the primary pathogen closely linked to GLM's occurrence. CPK infection significantly increases the risk of recurrence in GLM patients, with elevated prolactin levels potentially playing a pivotal role in this process. In clinical antimicrobial treatment, antimicrobials other than penicillin and ciprofloxacin may be empirically administered when sensitivity test results are inconclusive.

Keywords Granulomatous lobular mastitis, Corynebacterium Parakroppenstedtii, Pathogens, Bacterial infection, Antimicrobial sensitivity, Corynebacterium kroppenstedtii, Recurrence

Introduction

Granulomatous lobular mastitis (GLM), also known as idiopathic granulomatous mastitis, is a chronic inflammatory disease that occurs in the mammary gland. In the early stage, patients typically present with breast lumps accompanied by redness and pain. With the development of the disease, abscesses can form, eventually rupturing and leading to the formation of fistulas and sinuses, causing obvious alteration in breast shape. GLM is a complicated condition characterized by a high recurrence rate and is often subject to clinical misdiagnosis and mistreatment [1, 2]. In recent years, the incidence of GLM has gradually increased, seriously affecting both the physical and mental health of patients.

Many studies in the past have investigated the correlation between bacterial infection, especially Corynebacterium infection, and the onset of GLM [3–6]. These studies identified clinical isolates by various methods such as 16s rRNA sequencing, Quantitative Real-time PCR (qPCR), Sanger sequencing, next-generation sequencing (NGS), and other methods. Previous results mostly support Corynebacterium kroppenstedtii (CK) as the main pathogen relevant to GLM.

However, with the development of sequencing technology, the studies in recent two years have revealed that the CK strains previously separate from mastitis specimens have been identified as other pathogens, such as Corynebacterium parakroppenstedtii. This raises questions about whether CK strains isolated from GLM patients may also represent other pathogens. Is it possible that the pathogen closely related to GLM is not CK but rather another pathogen? Furthermore, the clinical characteristics of GLM associated with this pathogen remain unclear, with no existing research on these issues at present. Additionally, the optimal antimicrobial treatment options for this pathogen have yet to be elucidated. Therefore, this study aimed to explore whether the specific type of Corynebacterium closely linked to the pathogenesis of GLM is CK or a newly identified pathogen, using 16S rDNA sequencing technology. The clinical characteristics of GLM female patients with and without bacterial infection were compared to explore the role of this pathogen in GLM pathogenesis. Finally, the antimicrobial susceptibility test was employed to evaluate the effect of drugs against the identified pathogens and provide corresponding antibiotic treatment strategies. The study endeavors to provide etiological evidence for GLM and identify clinical features following bacterial infection.

Materials

Origin of isolated strains

All clinically isolated strains were obtained from the pus specimens collected from female GLM patients admitted to Beijing Hospital of Traditional Chinese Medicine, Capital Medical University from February 2023 to February 2024. These strains were subsequently isolated and cultured on Columbia blood agar plates.

Inclusion criteria: (1) Patients from whom pus specimens yielding strains identified as Corynebacterium kroppenstedtii by mass spectrometry; (2) Patients diagnosed as granulomatous lobular mastitis by puncture biopsy or histological diagnosis after operation; (3) Isolates that were well-preserved and capable of successful resuscitation; (4) Patients with complete clinical information and who signed informed consent.

Exclusion criteria: (1) Strains showing evidence of contamination with other microorganisms after resuscitation; (2) Repeated isolates obtained from the same patient; (3) Patients diagnosed with other inflammatory diseases; (4) Patients with other bacterial infections at the same time.

Instruments and materials

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Zhuhai Meihua, China); Turbidity comparator (DensiCHEK Plus, bioMerieux, France); Columbia blood agar plate (BNFUTURE, China); the cation-adjusted Mueller-Hinton broth (Solarbio, China) (Lot: No.A526R031); Tween 80 (Bioruler, China), et al.

MIC Test Strip of E-test: Ceftriaxone (CRO), Ciprofloxacin (CIP), Linezolid (LNZ), Penicillin (PEN), Vancomycin (VAN), Moxifloxacin (MXF), Rifampicin (RIF) (Lionfllchem, Italy) (Lot: 070822014; 101122020; 060721006; 091522030; 052323008; 111022008; 020123016).

Methods

Strain identification

MALDI-TOF MS

The pus specimens were inoculated on blood plates and cultured in a 35℃, 5% CO_2 incubator for 72 h until small and scattered grayish-white colonies appeared. Once colony growth stabilized, a single colony was transferred to a new blood plate and further incubated for 48 h. Subsequently, the colony was selected for identification by MALDI-TOF MS mass spectrometry.

Gene sequence identification

16S rDNA sequencing technology was used for sequencing and identification of clinical isolates (Beijing Tianyi Huiyuan Life Science & Technology Inc. China). Bacterial 16S rDNA gene primers 27F: 5 '-AGAGTTTGATC CTGGCTCAG-3', 1492R: 5 '-CTACGGCTACCTTGT TACGA-3' were used to amplify the 16SrDNA of each clinical strain. Purified PCR products of each strain were obtained and DNA sequencing was performed using the ABI3730-XL sequencing instrument. The obtained sequencing results were imported to the NCBI GenBank website for homology comparison with known sequences to identify bacterial species.

Clinical data collection

The clinical information of female GLM patients with obtained isolates included visit time, age, onset time, strain isolation time, personal history, medical history, treatment history, and auxiliary examination results. In addition, GLM female patients with negative bacterial culture results were included in a 1:1 ratio. Finally, Microsoft Office Excel 2019 was used to input and organize the clinical information.

Antimicrobial susceptibilities tests

Bacterial colonies in the logarithmic growth phase were selected by an aseptic inoculation ring and prepared into a concentration of 0.5 McFarland turbidity units (about 5×10^6 CFU/mL) in 0.9% sterile normal saline. Before use, blood plates were incubated in a 35℃ environment for 15 min to ensure surface dryness. According to the Clinical and Laboratory Standards Institute (CLSI) recommended standard medicine-sensitive experiment procedure, a cotton swab was dipped into the prepared bacterial suspension and evenly spread onto a blood agar plate. The test strip was carefully attached to the plate's surface and then cultured in a 5% CO_2 incubator at 35℃ for 18 to 24 h. After the culture was completed, test results were observed and interpreted. The drug concentration corresponding to the inhibition zone and the test strip's incisure was recorded as the minimum inhibitory concentration (MIC) value of the antibiotic. The test results were interpreted according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

The flow chart of this study is as follows (Fig. 1).

Statistical analysis

IBM SPSS Statistics 27.0 software (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis. Measurement data were expressed as mean ± standard deviation (mean ± SD) and compared using a t-test. Count data was summarized as frequency and percentage, and univariate comparisons were conducted using the χ^2 test.

Results

Genotypic identification of 31 clinical isolates

A total of 31 GLM female patients with CK infection identified by MALDI-TOF MS were included in this study. The resuscitated isolates were cultured on blood plates for 24 h, yielding very small scattered grayish-white colonies with a diameter < 1 mm. After incubation for 48 h, slightly larger grayish-white colonies were observed, still with a diameter < 1 mm. After the colony smears were selected, gram staining revealed short rod-shaped and disordered microorganisms under microscopic observation (Figs. 2 and 3). The mass spectrum peak of the isolates is shown in Fig. 4.

According to the 16S rDNA sequencing results of the 31 clinical isolates, 28 strains (90.32%) were identified as Corynebacterium parakroppenstedtii, with similarities exceeding 99.00%. In addition, the other three isolates were matched as Corynebacterium pseudokroppenstedtii, Corynebacterium tuberculostearicum, and Corynebacterium amycolatum (Table 1).

Clinical characteristics analysis of 28 GLM patients Associated with CPK infection

A total of 28 GLM female patients identified by sequencing as having CPK infection were included in the clinical characteristics study. At the ratio of 1:1, 28 female GLM patients with negative bacterial cultures were randomly matched as controls. We compared the personal history, clinical characteristics, and laboratory indicators of the two groups to explore the role of CPK infection in the occurrence and development of GLM. A comparison of the clinical characteristics revealed that 28.57% of CPK-infected patients had a history of mastitis, and the recurrence rate was significantly higher than that of the control group (7.14%) ($P<0.05$). Additionally, the incidence of breast blunt trauma in the CPK infection group was significantly lower than that in the control group ($P<0.05$). However, there were no significant differences between the two groups in age, time of onset, gestation history, lactation history, and the maximum range of the breast mass ($P>0.05$) (Table 2).

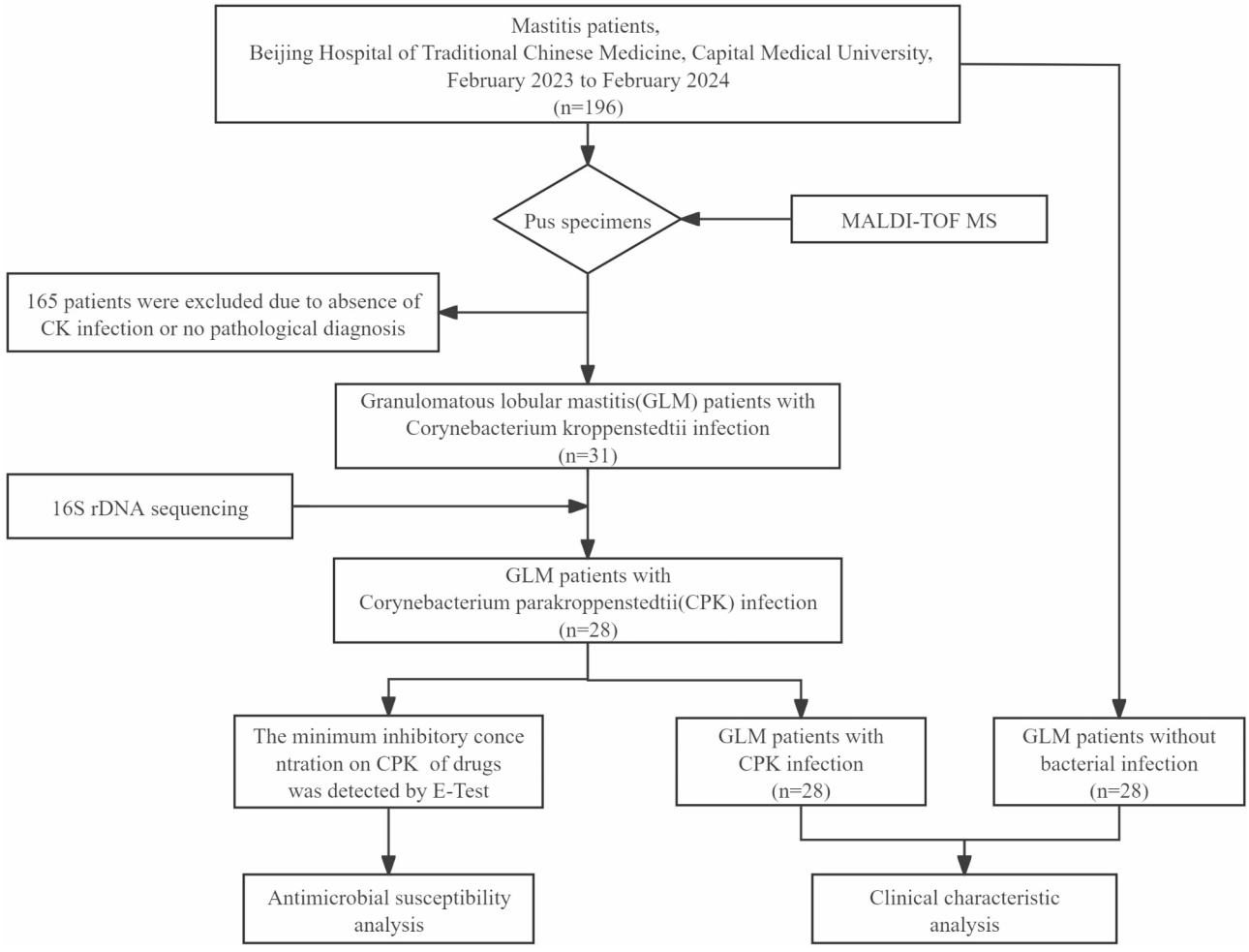

Fig. 1 Flow chart of the study

When comparing the routine laboratory examination indexes between the two groups, the results indicated no significant differences in white blood cell count, neutrophil percentage and count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) between the two groups ($P>0.05$). However, while most prolactin levels in both groups fell within the normal range, the CPK infection group exhibited a significantly higher prolactin level compared to the control group ($P<0.05$). (Table 3)

Antimicrobial susceptibilities analysis of clinical isolates

The antimicrobial sensitivity test showed that all 28 CPK strains were sensitive to Vancomycin (VAN), Rifampicin (RIF), and Linezolid (LNZ), with a majority (82.14%) showing sensitivity to Ceftriaxone (CRO). Additionally, 57.14% of strains were sensitive to Moxifloxacin (MXF) and 25.00% to Penicillin (PEN). However, all CPK isolates displayed resistance or intermediate susceptibility to Ciprofloxacin (CIP). (Table 4; Fig. 5)

Discussion

In recent years, the incidence of GLM has been steadily increasing, causing considerable distress to affected patients. However, the precise causative factors of GLM remain elusive. The detection rate of Corynebacterium kroppenstedtii has significantly risen in breast inflammatory disease, underscoring the importance of bacterial infection as a contributing factor to mastitis morbidity. More and more scholars have recognized the important role of CK infection in the occurrence and progression of GLM [7–9]. Nevertheless, with the ascension of sequencing identification technology, a recent study unveiled a significant revelation: CK, traditionally viewed as the culprit in mastitis specimens, bears a remarkably close genetic relationship to another microbial strain, Corynebacterium parakroppenstedtii (CPK). This discovery is a major subversion of the previous understanding of mastitis etiology. Although CK has been reclassified as CPK in some mastitis specimens, no research has explored whether pathogens previously identified as CK in granulomatous mastitis are also CPK or other microbial

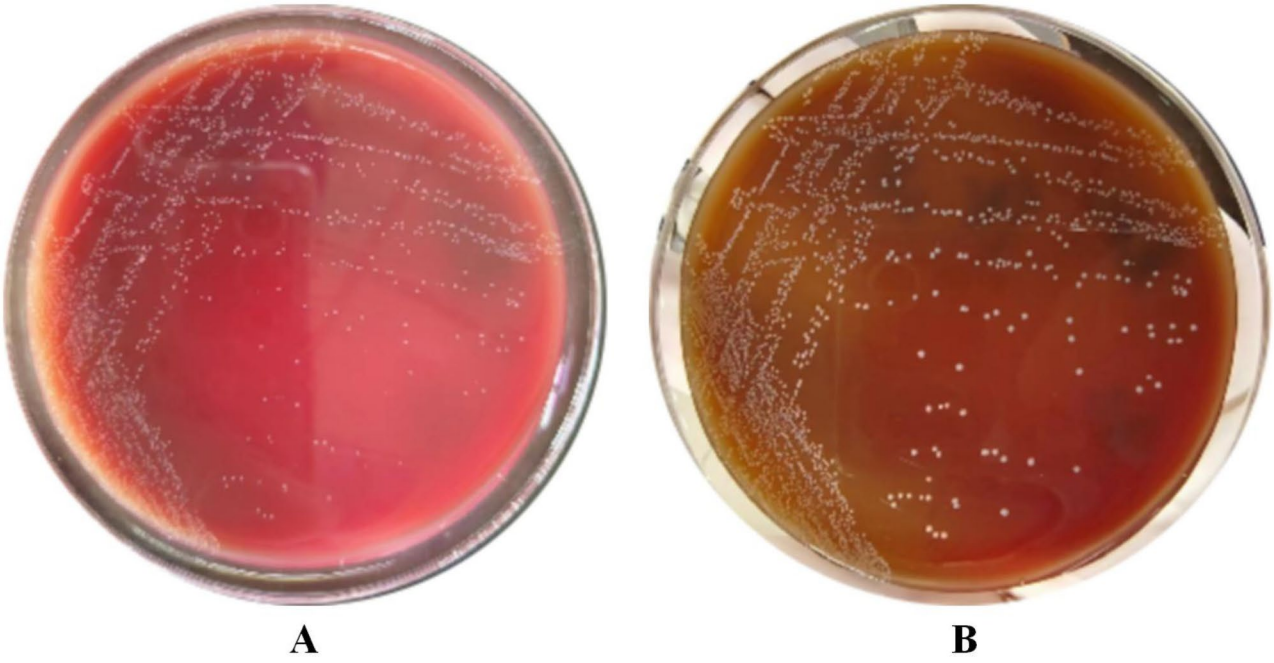

Fig. 2 Morphological observation of clinical isolates after 24 h (**A**) and 48 h (**B**) of in vitro incubation

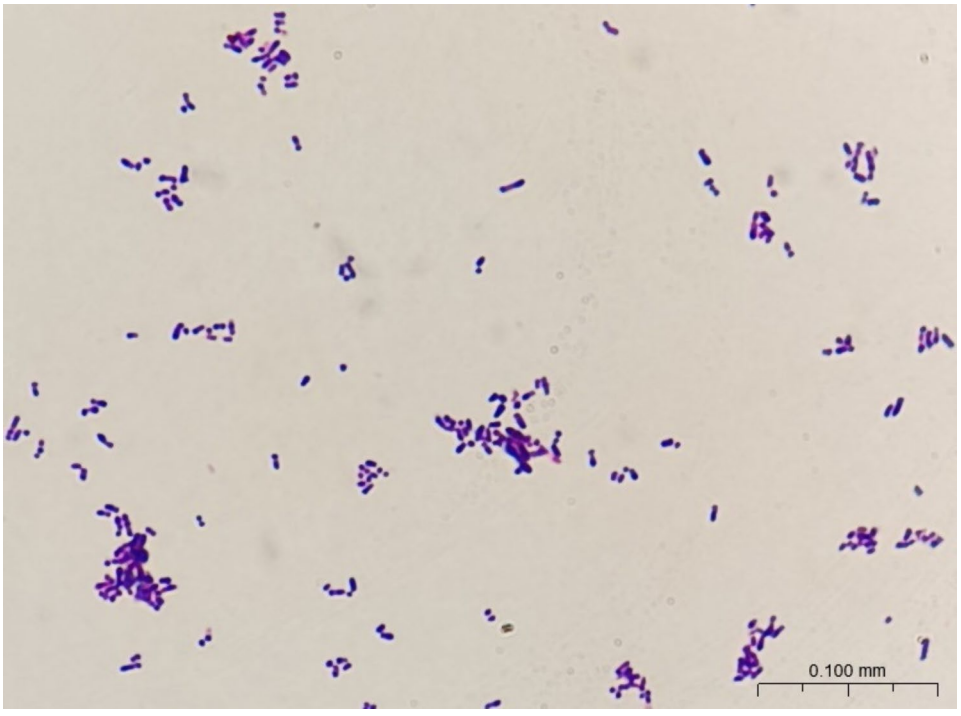

Fig. 3 Microscopic observation of clinical isolates (Gram stain, 1000×)

strains. Consequently, the clinical characteristics of GLM patients associated with this new pathogen and the sensitivity of isolates to antibiotics remain poorly studied. In this pioneering study, we identified the CPK strains from GLM patients by sequencing technology for the first time, thereby revealing that the primary disease-causing pathogens in GLM are mainly Corynebacterium parakroppenstedtii. Building upon this discovery, we meticulously analyzed the clinical characteristics of GLM patients associated with CPK infection and conducted antibiotic sensitivity tests on these clinical isolates. Our findings aim to provide valuable insights for clinical

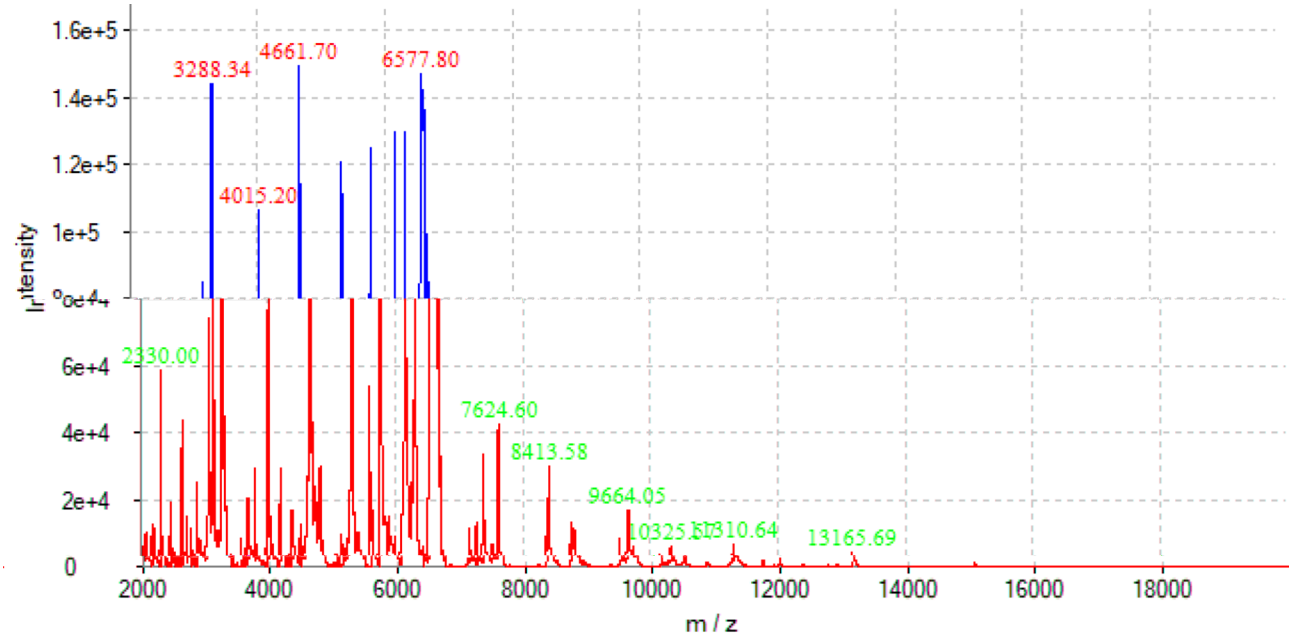

Fig. 4 Mass spectrogram of Corynebacterium kroppenstedtii clinical isolates identified by MALDI-TOF MS

diagnosis and guide prudent antibiotic use in the management of GLM.

A large number of studies have supported the strong correlation between CK infection and the pathogenesis of GLM. In a 2022 study, our research group analyzed clinical samples from 44 GLM patients by 16S rDNA technique, confirming the pivotal role of Corynebacterium, especially CK, in the pathogenesis of GLM [5]. With the improvement of microbial identification and the development of sequencing technology, in a 2022 study, Corynebacterium kroppenstedtii-like isolates obtained from pus and tissue specimens of 27 mastitis patients were analyzed using, phenotypic characterization, MALDI-TOF MS, and genome sequencing methods such as 16S rRNA, rpoB, and fusA respectively [10]. Phylogenetic analyses led to the final identification of two major clusters closely related to CK, which were named Corynebacterium parakroppenstedtii and Corynebacterium pseudokroppenstedtii. This study challenged the traditional view, suggesting that these two new Corynebacterium spp. microorganisms may be the true pathogens associated with mastitis development, rather than CK. Subsequently, in 2024, another study [11] identified one breast specimen isolate as Corynebacterium parakroppenstedtii through sequencing of its 16S rRNA genome, further supporting the view that the strain CPK, closely related to CK, may be the pathogen linked to mastitis development. In addition, this study included genome sequences of 28 CK isolates associated with mastitis from the NCBI database, revealing that 24 of the 28 isolates were all highly matched to CPK, while the remaining 4 were Corynebacterium pseudokroppenstedtii by re-clustering analysis. (Table 5)

The above study highlighted the challenge in distinguishing between strains due to their high similarity in 16S rRNA genes, posing CK and CPK are highly similar and difficult to distinguish by MAIDI-TOF MS technique [10]. Therefore, this raised the question of whether the CK strains previously identified in GLM were indeed CPK or another CK-like strain. Given the lack of clarity regarding the correlation between CK and CPK identified in GLM, 31 GLM isolates identified as CK were included in our present study, and the isolates were identified by genome sequencing using 16S rDNA technology. Comparative analysis of the sequencing results with gene sequences in the NCBI database suggested that among the 31 isolates, 28 were highly matched to Corynebacterium parakroppenstedtii, while the remaining three were Corynebacterium pseudokroppenstedtii, Corynebacterium tuberculostearicum, and Corynebacterium amycolatum, respectively. Thus, our sequencing results align with the conclusions in the aforementioned papers relevant to CPK, underscoring Corynebacterium parakroppenstedtii, as a rod-shaped bacillus with high similarity to CK, may indeed be the pathogen intricately associated with the onset and progression of mastitis.

Based on the limited studies reported so far, CPK shares a strikingly high sequence similarity with the CK gene within the Corynebacterium genus. Notably, current mass spectrometry techniques cannot distinguish CPK from CK. Moreover, CPK and CK are also highly similar in growth conditions and colony morphology.

Table 1 Results of 16S rDNA sequencing of 31 clinical isolates

No.	Total Score	E value	Similarity	GenBank Accession No.	Scientific Name
1	2481	0.00	99.705	MW819649.1	Corynebacterium parakroppenstedtii
2	2497	0.00	99.78	OR794029.1	Corynebacterium parakroppenstedtii
3	2562	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
4	2483	0.00	100.00	OR794048.1	Corynebacterium parakroppenstedtii
5	2529	0.00	100.00	OR794036.1	Corynebacterium parakroppenstedtii
6	2534	0.00	99.855	OR794108.1	Corynebacterium parakroppenstedtii
7	2566	0.00	99.928	OR794108.1	Corynebacterium parakroppenstedtii
8	2566	0.00	99.928	OR794108.1	Corynebacterium parakroppenstedtii
9	2553	0.00	100.00	OR794036.1	Corynebacterium parakroppenstedtii
10	2272	0.00	99.919	MN175944.1	Corynebacterium tuberculostearicum
11	2549	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
12	2449	0.00	99.925	OR794108.1	Corynebacterium parakroppenstedtii
13	2446	0.00	99.119	OR794029.1	Corynebacterium parakroppenstedtii
14	2538	0.00	99.855	OR794108.1	Corynebacterium parakroppenstedtii
15	2507	0.00	99.926	OR794029.1	Corynebacterium parakroppenstedtii
16	2542	0.00	99.712	OR794108.1	Corynebacterium parakroppenstedtii
17	2566	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
18	2420	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
19	2394	0.00	99.77	OR794108.1	Corynebacterium parakroppenstedtii
20	2549	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
21	2401	0.00	99.847	OR794108.1	Corynebacterium parakroppenstedtii
22	2495	0.00	100.00	OR794107.1	Corynebacterium parakroppenstedtii
23	2549	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
24	2416	0.00	99.848	OR794108.1	Corynebacterium parakroppenstedtii
25	2453	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
26	2486	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
27	2453	0.00	100.00	OR794108.1	Corynebacterium parakroppenstedtii
28	2551	0.00	100.00	OR793958.1	Corynebacterium pseudokroppenstedtii
29	1783	0.00	99.094	OR794108.1	Corynebacterium parakroppenstedtii
30	2326	0.00	99.454	OR794108.1	Corynebacterium parakroppenstedtii
31	2538	0.00	99.855	CP120206.1	Corynebacterium amycolatum

Therefore, gene sequencing techniques, including both partial sequence sequencing and whole genome sequencing, maybe the primary methods to identify CPK at present.

After clarifying the correlation between CPK infection and GLM, we noted that the clinical characteristics of GLM patients with concomitant CPK infection have also not been reported previously. To provide a basis for clinical diagnosis and differential diagnosis, we compared the clinical characteristics differences between GLM patients with CPK infection and those without bacterial infection during the same period. According to the literature we searched internationally, the clinical characteristics of GLM patients infected with CPK have not been previously reported. Statistical analysis revealed a significant correlation between CPK infection and the increased recurrence rate in GLM patients. It is well known that the recurrence rate of GLM is high in clinical, ranging from 15.4 to 24.8% according to existing literature [12–14], placing it amongst the most recurrent breast diseases. Remarkably, our study disclosed that the recurrence rate of GLM patients with CPK infection was 28.57%, significantly higher than the 7.14% recurrence rate in patients without bacterial infection. Therefore, we hypothesized that CPK infection is an important factor contributing to the heightened risk of recurrence in GLM patients. A retrospective study in 2022 compared the detection rate of CK in patients with acute and chronic breast abscesses, revealing a detection rate of 32% (10/31) in chronic abscesses and only 1% (1/104; $P<0.01$) in acute abscesses [15]. This suggests a close association between bacterial infection and recurrence, long-term chronic infections, potentially serving as the most important cause of recurrence in mastitis patients.

Blunt breast trauma was also found as an important predisposing factor for the development of non-CPK-infected GLM in this study [16]. This pathogenesis is mainly based on the fact that acute breast tissue injury can lead to mammary duct rupture and subsequently trigger a localized inflammatory immune response in the breast [17]. Breach of the mammary ducts induces a type IV allergy, and the resultant swelling from aggregation

Table 2 Comparison of personal history and clinical characteristics between the two groups

Characteristics		CPK infection group [n (%)] (*n* = 28)	No bacterial infection group [n (%)] (*n* = 28)	*P* value
Age, [year, (Mean ± SD)]		33.00 ± 3.70	33.00 ± 4.80	0.803
Time of onset, [months, (Mean ± SD)]		5.11 ± 8.32	3.46 ± 5.16	0.378
Gestation history	Yes	26 (92.86)	24 (85.71)	0.388
	No	2 (7.14)	4 (14.29)	
Lactation history	Yes	15 (53.57)	22 (78.57)	0.831
	No	4 (14.29)	5 (17.86)	
Lactation time [months, (Mean ± SD)]		13.3 ± 11.31	10.89 ± 7.86	0.336
Time after the last delivery, [Years, (Mean ± SD)]		4.00 ± 1.75	5.57 ± 3.74	0.128
History of GLM	Yes	8 (28.57)	2 (7.14)	0.036
	No	20 (71.43)	26 (92.86)	
Blunt breast trauma history	Yes	2 (7.14)	11 (39.39)	0.004
	No	26 (92.86)	17 (60.71)	
History of hyperprolactinemia	Yes	5 (17.86)	2 (7.14)	0.225
	No	23 (82.14)	26 (92.86)	
Ipsilateral axillary lymph node enlargement	Yes	19 (67.86)	16 (57.14)	0.091
	No	5 (17.86)	12 (42.86)	
Maximum range of the mass, [cm^2, (Mean ± SD)]		13.27 ± 12.09	12.91 ± 10.49	0.908

Table 3 Comparison of laboratory examination indexes between the two groups

Characteristics	CPK infection group [n(%)], (*n* = 28)	No bacterial infection group [n(%)], (*n* = 28)	*P* value
Leucocyte count	9.73 ± 2.50	9.59 ± 3.09	0.861
The percentage of the neutrophils	72.43 ± 8.13	71.20 ± 7.50	0.575
Neutrophil count	7.12 ± 2.16	6.99 ± 2.81	0.850
Erythrocyte sedimentation rate	35.13 ± 18.80	34.80 ± 28.63	0.965
C-reactive protein	22.02 ± 26.07	13.90 ± 15.07	0.216
prolactin	22.09 ± 21.57	13.14 ± 6.90	0.049

of antigen-antibody complexes may be one of the main pathogenic mechanisms of non-CPK-infected GLM. In contrast, morbidity in CPK-infected individuals is more often associated with bacterial infection. In addition, we found that prolactin levels were significantly higher in the CPK-infected group than in patients without bacterial infection. Previous research has highlighted that increased intraductal secretions due to hyperprolactinemia can increase the risk of GLM after bacterial infection [18]. This finding suggests that elevated prolactin levels may increase the risk of CPK-induced mastitis, ultimately leading to a higher incidence of mastitis in affected patients.

For CPK, the main pathogen identified in GLM, there has been no previous report on its related antibiotic susceptibility profile, which limits the treatment options for this type of disease. Consequently, we conducted an antimicrobial drug sensitivity experiment based on the above study. The results of E-test experiments showed that all 28 CPK isolates were highly sensitive to VAN, LNZ, and RIF; partially sensitive to MXF and CRO; and mostly resistant to PEN and CIP.

Table 4 Antimicrobial sensitivity of the clinical CPK isolates

Antibiotics	MIC breakpoints* (mg/L)	MIC (mean ± SD) (mg/L)	CPK (*n* = 28)		
			S [*n*(%)]	I [*n*(%)]	*R* [*n*(%)]
Vancomycin	S, ≤ 2; R, >2	1.036 ± 0.324	28 (100.00)	0 (0.00)	0 (0.00)
Linezolid	S, ≤ 2; R, >2	0.285 ± 0.094	28 (100.00)	0 (0.00)	0 (0.00)
Rifampicin	S, ≤ 0.06; *R* > 0.06	0.004 ± 0.009	28 (100.00)	0 (0.00)	0 (0.00)
Ceftriaxone	S, ≤ 1; R, >2	0.764 ± 0.743	23 (82.14)	1 (3.57)	4 (14.29)
Moxifloxacin	S, ≤ 0.5; R, > 0.5	0.751 ± 0.999	16 (57.14)	0 (0.00)	12 (42.86)
Penicillin G	S, ≤ 0.125; R, > 0.125	0.349 ± 0.285	7 (25.00)	0 (0.00)	21 (75.00)
Ciprofloxacin	S, ≤ 0.001; R, > 0.5	3.624 ± 9.552	0 (0.00)	15 (53.57)	13 (46.43)

S, Susceptible, standard dosing regimen; I, Susceptible, increased exposure; R, Resistant

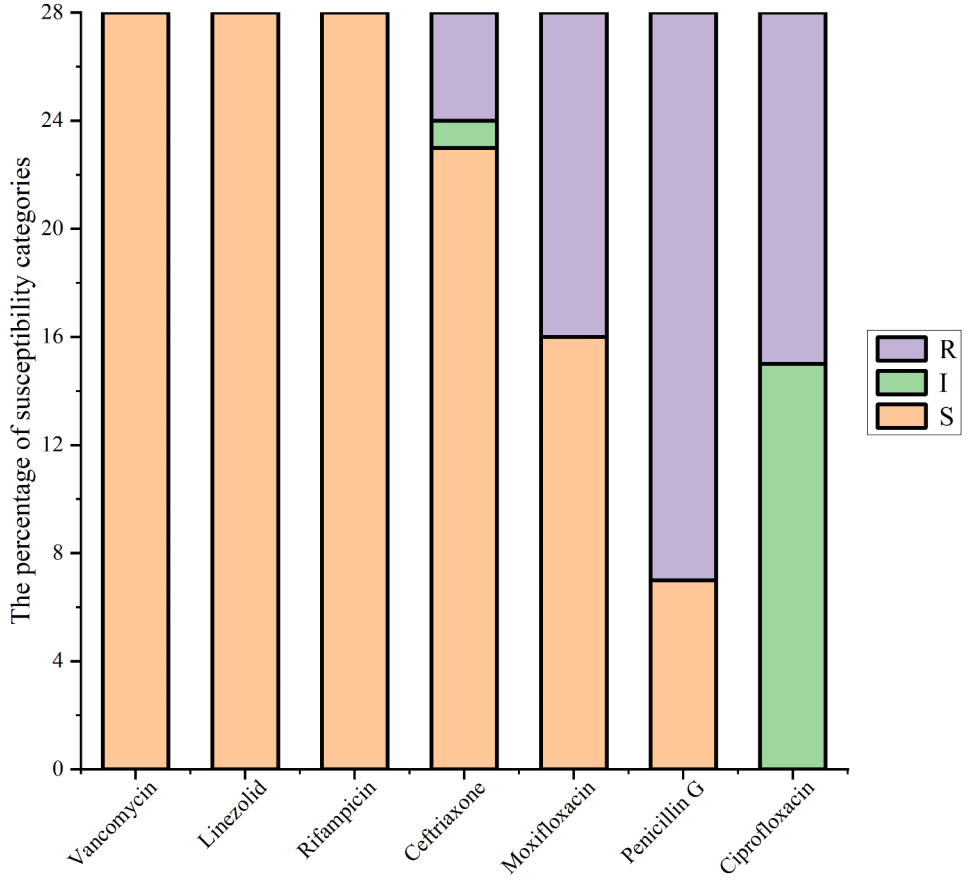

Fig. 5 Antimicrobial sensitivity of the clinical CPK isolates (S, Susceptible, standard dosing regimen; I, Susceptible, increased exposure; R, Resistant.)

Table 5 Literaturevieww ofreporteddcasessrelevantt to Corynebacterium parakroppenstedtii before

No.	Author, year	Ethnicity	Cases	Specimen source	diagnosis	Identification methods	Other strains identified	CPK Ratio
1	Qiang, et al. [10] 2022	Chinese	23	Pus, tissue, secretion, puncture fluid	9 granulomatous lobular mastitis, 1 suppurative mastitis, 13 mastitis	MAIDI-TOF MS, 16S rRNA, rpoB, fusA	Corynebacterium pseudokroppenstedti (4 cases)	85.19% (23/27)
2	Ying, et al. [11] 2024	Chinese	25 (24 cases from NCBI)	breast specimen	mastitis	16S rRNA, rpoB, fusA	Corynebacterium pseudokroppenstedtii (4 cases)	86.21% (25/29)

Current studies predominantly consider that CK, highly similar to CPK, is resistant to β-lactam antibiotics and sensitive to other types of antibiotics [19–21], aligning with our results. Therefore, non-β-lactam antibiotics should be prioritized in the clinical antimicrobial treatment of GLM patients with CPK infection. Notably, the results of our susceptibilities analysis showed the superiority of RIF for the antimicrobial effects of the isolates. RIF is a semi-synthetic broad-spectrum antimicrobial agent and is a first-line antituberculosis agent with antimicrobial activity against a wide range of pathogenic microorganisms. A previous prospective study [22] included 30 GLM patients and confirmed the definitive clinical efficacy of RIF treatment across all disease stages. Therefore, the rational application of RIF may exert significant clinical efficacy in the selection of antimicrobial treatment regimens for mastitis patients [23]. In addition, no clinical studies have been reported on the application of VAN or LNZ for the treatment of GLM. However, previously reported small sample studies indicated 100% susceptibility of CK clinical isolates to VAN and LNZ [24], consistent with our findings. These results provide valuable insights and a basis for the selection of clinical antibiotics.

Nonetheless, this study still has some limitations. Our genomic-level observations of CPK strains hinder a comprehensive understanding of the underlying mechanism by which CPK exerts its pathogenic effects and influences the development of GLM. Therefore, the intrinsic link between CPK and the pathogenesis, along with associated mechanisms should continue to be explored. Continued investigation will be crucial for unraveling the intricate mechanisms underlying CPK's role in the onset and progression of GLM. In addition, given that our study results show that antibacterial drugs have significant in vitro inhibition effect on CPK strains, the clinical studies on the treatment of GLM with topical antibiotics should be further improved in future.

Conclusion

In summary, our study identified the primary pathogen in GLM pathogenicity is predominantly Corynebacterium parakroppenstedtii by genome sequencing of clinical isolates from GLM patients, diverging from the previous assumption of Corynebacterium kroppenstedtii. This strain is highly similar to Corynebacterium kroppenstedtii and is difficult to distinguish by mass spectrometry. Furthermore, our comparative analysis of clinical characteristics suggests that Corynebacterium parakroppenstedtii infection significantly elevates the recurrence risk in GLM patients and increased prolactin levels potentially play an important role in this inflammatory immune response. In addition, a history of breast trauma may be an independent factor in the development of GLM patients without bacterial infection. Finally, in the clinical antimicrobial therapy for GLM patients with Corynebacterium parakroppenstedtii infection, vancomycin, linezolid, and rifampicin exhibit robust drug susceptibility, followed by moxifloxacin and ceftriaxone, whereas penicillin and ciprofloxacin are mostly resistant. These findings provide critical lessons for the clinical selection of antibiotics.

Author contributions

DZ and NF designed the research. YZ, MW and XG performed the research. WZ and QH reviewed the paper. YZ and DZ wrote the paper. NF revised the paper. All authors contributed to the article and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, and the ethics batch number is 2023BL-20-01.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Galactophore, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing 100010, China
[2]Beijing University of Chinese Medicine, Beijing 100029, China
[3]Department of Clinical Laboratory Medicine, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing 100010, China

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