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Optimal Incubation Time for Synovial-Fluid Cultures in Acute and Chronic Periprosthetic Joint Infection: A Retrospective Cohort Study

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Abstract

Background

Standard 14-day incubation protocols for periprosthetic joint infections (PJIs) lack specificity.The optimal culture duration for distinct acute and chronic PJI remains undefined. This study aimed to define the microbial time-to-positivity (TTP) and distribution in different PJI categories to evaluate the feasibility of a stratified culture strategy.

Methods

This retrospective study analysed the medical records of patients from 1 June 2016 to 1 July 2024. Patients who met the Musculoskeletal Infection Society (MSIS) criteria and had positive intraoperative synovial-fluid cultures were classified into Early acute PJI, Late acute PJI, and Late chronic PJI based on clinical symptoms. The microbial spectrum and TTP of synovial-fluid specimens were recorded. The microbial profiles and detection rates of different PJI subtypes were compared across different time stages.

Results

We included 181 patients (mean age 65 years). Among them, 41 cases were Early acute PJI; 35 cases, Late acute PJI; and 105 Late chronic PJI. All pathogens recovered within 12 days. In Early acute PJI and Late acute PJI, 90.3 and 94.3% of pathogens, respectively, grew by day 7, whereas 96.2% of Late chronic PJI isolates required up to 10 days. The rate of new microbial detection decreased sharply after day 5 of incubation. After 7 days, delayed microbial growth was predominantly seen in the Late chronic PJI cohort. Previous antibiotic exposure did not significantly affect the growth of microorganisms in acute PJI (OR = 2.52, 95% CI 0.43–14.65; P = 0.31).

Conclusion

Previous use of antibiotics prolongs the incubation time of microorganisms but has a limited effect on the incubation time of microorganisms in acute PJI. A 7-day incubation period is sufficient to reliably detect pathogen in acute PJI. For chronic PJI, considering the delayed growth of some pathogen, at least 10 days of culture are required.

Introduction

Periprosthetic joint infection (PJI) is a leading cause of revision arthroplasty, requiring surgical intervention with removal of infected tissues and components, and reimplantation of a new prosthesis [1, 2]. Accurate diagnosis of PJI is the prerequisite for treatment. Although the 2018 International

Consensus Meeting (ICM) addressed the number of specimens necessary for PJI diagnosis, it did not specify the recommended duration for pathogen culture [3]. Furthermore, timely microbiological diagnosis is critical for optimal clinical outcomes, as it guides the physician in formulating a targeted antibiotic regimen [3].

Low-virulence pathogens such as coagulase-negative staphylococci, characterised by slow growth, predominate in PJI, and extending culture duration, significantly increases the pathogen culture positivity rate [4–6]. Consequently, extending the culture incubation time to 14–21 days has become routine practice at some centres [4–7]. However, recent evidence presents conflicting findings, demonstrating that prolonged incubation does not enhance microorganism identification but may instead increase the risk of false-positive results due to contaminating bacteria [8–11].

Theoretically, pathogens causing acute PJI typically exhibit rapid proliferation, facilitating swift identification through standard culture methods. Conversely, organisms in chronic PJI often exist in a quiescent state within established biofilms. These pathogens are shed intermittently, demanding prolonged culture for detection [4, 10–12]. Despite these recognized differences in microbial behaviour, no consensus on culture duration tailored to PJI subtypes has been established, and evidence-based guidelines are lacking.

Therefore, this study aimed to compare the distribution of microbial species in the intraoperative synovial fluid of patients with acute and chronic PJI at different latent periods, aiming to determine the optimal incubation time for both acute and chronic PJI. We hypothesised that acute PJI demonstrates a shorter culture incubation period than chronic PJI, and that a prolonged 14-day incubation may not be required for the diagnosis of many acute infections.

Material and methods

Study population

Institutional Review Board approval of this study was obtained. This single-centre retrospective study included patients with PJI who underwent revision joint surgery between June 2016 and June 2024, and who had positive intraoperative joint fluid cultures. All cases were diagnosed according to the MSIS criteria (Appendix). Patients were stratified into three PJI subtypes based on the timing and nature of symptom onset: Early acute PJI, with infectious signs emerging ≤ 3 months postoperatively; Late acute PJI, with acute symptom onset > 3 months postoperatively and a diagnosis made < 4 weeks from initial symptoms; and Late chronic PJI, with an insidious onset or symptoms persisting for ≥ 4 weeks, occurring > 3 months postoperatively [13–15].

A total of 181 patients met the inclusion criteria, comprising 87 hip and 94 knee PJIs. The cohort was stratified into 41 Early acute PJI, 35 Late acute PJI, and 105 Late chronic PJI cases (Table 1). A retrospective review of electronic medical records was conducted to extract patient data. Demographic variables included age, sex, and body mass index (BMI). Clinical variables included the affected joint (hip

or knee), laterality, and history of antibiotic therapy prior to sample collection. Microbiological data were also collected, including the number of culture-positive specimens, the specific microorganisms isolated, and the time-to-positivity (TTP) for each positive culture. Any discrepant or ambiguous results were re-evaluated by a senior microbiologist to resolve inconsistencies; unresolved cases were excluded from the analysis.

Table 1
Patient demographic characteristics

Characteristic	Total (N = 181)	Early acute(n = 41)	Late acute (n = 35)	Late chronic(n = 105)	*P*-value
Age (years)	65.5 (51-71.75)	69.48 ± 8.78	65.5 (60–74)	63.5 (52–71)	*P* = 0.003[a]
Sex (n, %)					*P* = 0.189[b]
Women	100 (55.2%)	23 (56.1%)	24 (68.6%)	53 (50.5%)	
Men	81 (44.8%)	18 (43.9%)	11 (31.4%)	52 (49.5%)	
BMI (kg/m^2)	25 (22–28)	25(20.25-28)	26 ± 4.16	25.14 ± 4.04	*P* = 0.51[a]
Laterality (n, %)					*P* = 0.629[b]
Left	90 (49.7%)	23 (56.1%)	16(45.7%)	51 (48.6%)	
Right	91 (50.3%)	18 (43.9%)	19(54.3%)	54 (51.4%)	
Operative treated joint (n, %)					*P* = 0.855[b]
Hip	87 (48.1%)	19 (46.3%)	16(45.7%)	52 (49.5%)	
Knee	94 (51.9%)	22 (53.7%)	19(54.3%)	53 (50.5%)	
ASA (n, %)					*P* = 0.023[c]
I	2 (1.1%)		2 (5.7%)		
II	99 (53.7%)	18 (43.9%)	15 (42.9%)	66 (62.9%)	
III	77 (42.5%)	22 (53.7%)	18 (51.4%)	37 (35.2%)	
IV	3 (1.7%)	1 (2.4%)		2 (1.9%)	
Sinus Tract (n, %)					*P* = 0.529[b]

BMI, body mass index; ASA, American Society of Anesthesiologists; TTP, time to positive; Early acute PJI, within 3 months after the index arthroplasty; Late acute PJI, infection within 4 weeks from occurrence of symptoms (but > 3 months after the index arthroplasty); Late chronic PJI, > 4 weeks after symptom onset (but > 3 months after the index arthroplasty). [a]Kruskal–Wallis H test; [b]Chi-square test; [c]Fisher–Freeman–Halton exact test.

Characteristic	Total (N = 181)	Early acute(n = 41)	Late acute (n = 35)	Late chronic(n = 105)	*P*-value
Yes	68 (37.6%)	18 (43.9%)	11 (31.4%)	39 (37.1%)	
No	113 (62.4%)	23 (56.1%)	24 (68.6%)	66 (62.9%)	
Antibiotic therapy, ≤ 4 weeks before admission	62 (34.3%)	19 (46.3%)	16 (45.7%)	27 (25.7%)	*P* = 0.017

BMI, body mass index; ASA, American Society of Anesthesiologists; TTP, time to positive; Early acute PJI, within 3 months after the index arthroplasty; Late acute PJI, infection within 4 weeks from occurrence of symptoms (but > 3 months after the index arthroplasty); Late chronic PJI, > 4 weeks after symptom onset (but > 3 months after the index arthroplasty). [a]Kruskal–Wallis H test; [b]Chi-square test; [c]Fisher–Freeman–Halton exact test.

Culture sampling technique

During revision arthroplasty, synovial fluid was aseptically aspirated and directly inoculated into both aerobic and anaerobic blood culture bottles (Bact/ALERT FA Plus and FN Plus; bioMérieux). The inoculated bottles were immediately transported to the microbiology laboratory and loaded into a BACT/ALERT 3D 120 automated culture system (bioMérieux) within 2 hours of collection. Bottles were incubated for a total of 14 days or until flagged positive by the system. Upon a positive signal, an aliquot was immediately removed for Gram staining and was sub-cultured onto both Columbia blood agar and MacConkey agar plates. The agar plates were incubated at 35°C in an atmosphere of 5–10% CO_2 and were inspected for growth at 24 and 48 hours. Bacterial colonies from the agar plates were identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) with a VITEK MS system (bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing was subsequently performed and interpreted according to the prevailing Clinical and Laboratory Standards Institute guidelines. Cultures demonstrating no growth on subculture plates after 48 hours of incubation were finalised as negative. Prophylactic antibiotics were administered before all surgical incisions [16, 17].

Data analyses

Statistical analysis was performed using IBM SPSS Statistics (version 29, Chicago, IL) software. Continuous data are presented as mean and standard deviation or median and interquartile range (IQR). Categorical data are expressed as numbers and percentages. Non-parametric tests were used to compare continuous and categorical variables among groups. The Kruskal–Wallis test was used for comparisons involving more than two groups. The Chi-squared test, Fisher's exact test, or Fisher-Freeman-Halton exact test was used to compare categorical variables. To investigate the association

between prior antibiotic therapy and delayed microbial growth, we performed a univariate logistic regression analysis. A P-value < 0.05 was considered statistically significant.

Results

Overall pathogen distribution and time to detection in PJI

Overall, among the 181 pathogenic bacteria, 88.7% were detected within 7 days, and all were detected within 12 days (Fig. 3). Coagulase-negative staphylococci (CoNS, n = 39) was the most common isolated pathogen, followed by methicillin-resistant *Staphylococcus epidermidis* (MRSE, n = 34) and methicillin-sensitive *staphylococcus aureus* (MSSA, n = 32) (Table 2). There was no significant difference in the distribution of CoNS (p = 0.45). MSSA and slow-growing bacteria was the most common in Late chronic PJI (p < 0.05) (Table VI). All *Enterobacteriaceae*, *Streptococcus*, and Enterococcus were detected within 5 days (Table 2, Fig. 1). A total of five cases of fungus were detected.

Table 2
Overall distribution of major pathogens in all PJI positive cultures and their detection across different time intervals

Pathogen	No. of Positive Cultures (No. of Strains Identified) from Synovial Fluid/Incubation Period Days					
	D0–3 (87, 48.07%)	D4–5 (59, 32.60%)	D6–7 (15, 8.29%)	D8–10 (15, 8.29%)	D11–12 (5, 2.76%)	TOTAL (181, 100%)
MSSA	18	10	1	1	2	32
CoNS*	20	14	2	2	1	39
MRSE	15	8	7	3	1	34
Enterobacteriaceae	10	3				13
Klebsiella	2	1				3
Enterococcus	3	4				7
Streptococcus	3	8		1		12
Brucella	3	3	2	1		9
Fungus	1	2	1	1		5
Corynebacterium	1	1		1	1	4
MRSA	5	3	1			9
Pseudomonas	3	1		1		5
Baumanii	1					1
Bacillus	2					2
Burkholderia		1				1
Cutibacterium acnes			1	2		3
Kocuria varians				1		1
Nocardia				1		1

MSSA, methicillin-sensitive Staphylococcus aureus; CoNS, coagulase-negative Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis. *CoNS (Staphylococcus epidermidis, Staphylococcus lugdunensis, Staphylococcus hominis, Staphylococcus caprae, Staphylococcus capitis, Staphylococcus simulans, Staphylococcus haemolyticus)

Pathogen distribution at different incubation time intervals

Early period (days 0–3)

In the early incubation period, 48% of microorganisms were detected. In Early acute PJI, 56% of the pathogenic bacteria were detected, with CoNS (n = 8), MRSE (n = 5), and Enterobacteriaceae (n = 5) being the predominant types. In the Late acute PJI cohort, most organisms were fast-growing MSSA (n = 7) and Enterobacteriaceae (n = 3). The microorganisms in the Late chronic PJI cohort were dominated by common species such as con (n = 10), MSSA (n = 10), and MRSE (n = 8) (Table 3,4,5; Fig. 2, 3).

Table 3
Microbial distribution across time intervals by Early acute PJI

Pathogen	No. of Positive Cultures (No. of Strains Identified) from Synovial Fluid/Incubation Period Days					
	D0–3 (23, 56.10%)	D4–5 (10, 24.39%)	D6–7 (4, 9.76%)	D8–10 (3, 7.32%)	D11–12 (1, 2.44%)	TOTAL (41, 100%)
MSSA	1					1
CoNS*	8		1			9
MRSE	5	3	2	1	1	12
Enterobacteriaceae	5					5
Klebsiella		1				1
Enterococcus	1	1				2
Streptococcus		1				1
Brucella		1				1
Fungus	1	1				2
Corynebacterium				1		1
MRSA	2	1	1			4
Burkholderia		1				1
Nocardia				1		1

MSSA, methicillin- sensitive Staphylococcus aureus; CoNS, coagulase-negative Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis.

*CoNS (Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus simulans, Staphylococcus haemolyticus)

Table 4
Microbial distribution across time intervals by Late acute PJI

Pathogen	No. of Positive Cultures (No. of Strains Identified) from Synovial Fluid/Incubation Period Days					
	D0–3 (19, 54.29%)	D4–5 (11, 31.43%)	D6–7 (3, 8.57%)	D8–10 (2, 5.71%)	D11–12	TOTAL (35, 100%)
MSSA	7	1				8
CoNS*	2	2		1		5
MRSE	2	1	1	1		5
Enterobacteriaceae	3	3				6
Klebsiella	1					1
Enterococcus	1					1
Streptococcus	2	3				5
Brucella			2			2
Fungus		1				1
Baumanii	1					1

MSSA, methicillin-sensitive Staphylococcus aureus; CoNS, coagulase-negative Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis.

*CoNS (Staphylococcus epidermidis, Staphylococcus lugdunensis)

Table 5
Microbial distribution across time intervals by Late chronic PJI

Pathogen	No. of Positive Cultures (No. of Strains Identified) from Synovial Fluid/Incubation Period Days					
	D0-3 (45,42.86%)	D4-5 (38,36.19%)	D6-7 (8,7.62%)	D8-10 (10,9.52%)	D11-12 (4,3.81%)	TOTAL (105, 100%)
MSSA	10	9	1	1	2	23
CoNS*	10	12	1	1	1	25
MRSE	8	4	4	1		17
Enterobacteriaceae	2					2
Klebsiella	1					1
Enterococcus	1	3				4
Streptococcus	1	4		1		6
Brucella	3	2		1		6
Fungus			1	1		2
Corynebacterium	1	1			1	3
MRSA	3	2				5
Pseudomonas	3	1		1		5
Bacillus	2					2
Cutibacterium acnes			1	2		3
Kocuria varians				1		1

MSSA, methicillin-sensitive Staphylococcus aureus; CoNS, coagulase-negative Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; MRSE,

methicillin-resistant Staphylococcus epidermidis.

*CoNS (Staphylococcus epidermidis, Staphylococcus lugdunensis, Staphylococcus hominis, Staphylococcus caprae, Staphylococcus capitis)

Table 6
The distribution differences of the main microorganisms

Pathogen	Early acute PJI (N = 41)	Late acute PJI (N = 35)	Late chronic PJI (N = 105)	χ^2 value	P-value
MSSA	1 (2.4%)	8 (22.9%)	23 (21.90%)	8.16	0.01
MRSA	4 (9.76%)	0 (0.0%)	5 (4.8%)	3.82	0.15
CoNS	9 (22.0%)	5 (14.3%)	25 (23.8%)	1.58	0.45
MRSE	12 (29.3%)	5 (14.3%)	17 (16.2%)	5.97	0.05
Enterobacter	5 (12.2%)	6 (17.1%)	2 (1.9%)	11.23	0.003
Streptococcus	1 (2.4%)	5 (14.3%)	6 (5.7%)	5.92	0.05
P. aeruginosa	0 (0.0%)	0 (0.0%)	5 (4.8%)	6.89	0.03
Slow-growing bacteria*	2 (4.9%)	0 (0.0%)	9 (8.6%)	8.83	0.01
Other Pathogens	7 (17.07%)	6 (17.1%)	13 (12.38%)	1.03	0.60

MSSA, methicillin- sensitive Staphylococcus aureus; CoNS, coagulase-negative; Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis. *Cutibacterium acnes, Corynebacterium, Nocardia, Bacillus, Kocuria varians

Middle period (days 4–7)

During this period, 40.9% of microorganisms were detected. The detection rate of microorganisms significantly decreased after 5 days (32.6% to 8.29%, $\chi^2 = 32.88$, $P < 0.001$). In Early acute PJI, MRSE (n = 5) remained the predominant microorganism. In Late acute PJI, Enterobacteriaceae (n = 3) and Streptococcus (n = 3) were the main microorganisms detected. In Late chronic PJI, CoNS (n = 13), MSSA (n = 10), and MRSE (n = 8) remained the main pathogenic bacteria detected (Table 3,4,5; Fig. 2, 3).

Late-stage period (days 8–12)

During this period, 11.04% of the pathogenic bacteria were detected, 70% of these pathogenic bacteria originated from Late chronic PJI, and most were slow-growing bacteria. Acute PJI was detected in only six cases (3 MRSE, 1 CoNS, 1 Corynebacterium, 1 Nocardia) (Table 3,4,5; Fig. 2, 3).

Microbial abundance changes

In acute PJI (Figs. 2A, 2B), species richness was highest during the first 5 days of incubation. After day 5 the profile narrowed rapidly; by days 10–12 almost all cultures yielded a single organism, indicating most of them grew during the early stage.

Chronic PJI displayed a different pattern (Fig. 2C). Diversity declined up to day 7, then rebounded between day 8 and day 10, before falling again after day 11. Compared with acute PJI, the differences were significant ($\chi^2 = 7.23$, P = 0.03, Fisher's exact). This rebound suggests that several organisms begin to proliferate only with prolonged incubation.

Previous antibiotic treatment

A significant association was found between preoperative antibiotic therapy and the type of PJI (p = 0.017). Patients presenting with acute PJI were substantially more likely to have received recent antibiotics (56.5%) compared to those with chronic PJI (43.5%). Furthermore, the use of antibiotics prior to admission was found to be significantly associated with the delay in microbial growth (OR = 2.64, 95% confidence interval [CI]: 1.03–6.76; P = 0.044). However, after conducting subgroup analysis, no significant findings were observed in acute PJI (OR = 2.52, 95% CI: 0.43–14.65; P = 0.305) (Table 7).

Table 7
Univariate logistic regression analysis of prior antibiotic therapy as a risk factor for delayed microbial growth (> 7 days)

Antibiotic therapy, ≤ 4 weeks before admission	**Univariate logistic analysis**				
	β	**SE**	**Z**	**P-value**	**OR (95%CI)**
PJI					
NO					1.00
YES	0.97	0.48	2.02	**0.044**	2.64 (1.03 ~ 6.76)
Acute PJI (Early & Late acute)					
NO					1.00
YES	0.92	0.90	1.03	0.305	2.52 (0.43 ~ 14.65)
Chronic PJI					
NO					1.00
YES	1.27	0.59	2.14	**0.032**	3.55 (1.11 ~ 11.32)
SE, standard error; OR, odds ratio; CI, confidence interval; PJI, periprosthetic joint infection;					

Discussion

The distinct microbial profiles observed within the first 72 hours of culture support distinct pathogenic pathways of each PJI subtype. Early acute PJI is usually caused by direct intraoperative contamination, and its microbial spectrum is typically dominated by commensal bacteria on the skin, especially CoNS [1, 2]. In contrast, Late acute PJI, which is primarily due to hematogenous dissemination, exhibits a broader

microbial spectrum with a significantly higher proportion of *Staphylococcus aureus* and *Streptococcus* spp, consistent with reports identifying them as common pathogens in hematogenous PJI [18–20]. These pathogens rapidly proliferate in the synovial fluid in a planktonic state, leading to high bacterial loads and severe acute inflammatory responses in the host. In Late chronic PJI, although *Staphylococcus aureus* and *CoNS* still dominate, multiple factors such as biofilm-mediated protection and host immune factors jointly contribute to the complex phenomenon of bacterial species diversity [1, 2, 21, 22]. Our findings, based on the dominant, rapidly growing isolates recovered within the first 3 days of culture, provide robust support for this framework, implicating these organisms as the primary causative pathogens in their respective PJI subtypes.

Clinical decisions on incubation duration for PJI should be tailored to both the suspected pathogen and the infection subtype. This study reaffirms that a 7-day incubation period sufficiently detects most microorganisms in synovial fluid, consistent with previous reports [11]. We further observed that gram-negative bacilli, particularly *Enterobacteriaceae*, consistently recovered within 5 days [24, 25]. This may be attributed to their unique cell wall structure, which facilitate rapid proliferation in the host environment, thereby enabling earlier crossing of the diagnostic threshold [23]. In contrast, fungal detection presents greater complexity. While optimal incubation times range from 7 to 14 days [25, 26], previous studies lacked PJI subtype stratification. Our analysis provides crucial clarification on this point. In acute PJI, fungal pathogens were detected within 5 days, aligning with Morreel et al. [15]. In contrast, all instances in our cohort in which extended incubation (> 5 days) was required for fungal recovery occurred in patients with chronic PJI. This pattern is consistent with the typically insidious process of chronic fungal infections. For patients with chronic PJI who have risk factors, a high degree of suspicion for fungal aetiology should be maintained, especially considering that approximately 20% of fungal PJI are polymicrobial [27–29].

We identified days 6–7 as a critical inflection point in microbial culture. During this window, the yield of new pathogens declined markedly (from 32.6% to 8.3%), coinciding with a shift from highly virulent, fast-growers, to low-virulence, slow-growing organisms. Importantly, this transition manifested differently across PJI subtypes. In acute PJI, the microbial profile had already stabilised before day 7, with minimal new pathogen emergence thereafter. In contrast, chronic PJI demonstrated continued microbial detection beyond this timepoint, with a substantial proportion of isolates emerging after day 7. While several studies have questioned the cost-effectiveness of extending incubation beyond 7 days [8, 30], others have shown that late-growing isolates, particularly in chronic infections, may alter antimicrobial therapy selection [4, 31]. We, therefore, recommend selective extension of the incubation period for suspected chronic PJI, where late-growing microorganisms may significantly impact treatment decisions.

Prior antibiotic therapy was associated with a delay in microbial detection only in chronic infections, even though it was administered more frequently in acute cases, where no comparable delay was observed. Prior antibiotic therapy can reduce the sensitivity of microbial culture [32]. However, Esteban et al. did not observe such a difference in their research [30]. We observed that the growth of chronic PJI

pathogenic bacteria is more susceptible to the influence of prior antibiotic therapy. Although the proportion of prior antibiotic therapy in acute PJI is higher, the effect is not significant. There are several potential explanations for this difference. First, acute PJI is usually treated with a short course of therapy, which can partially inhibit the planktonic bacteria, but due to the vigorous metabolism of the bacterial community, the bacteria can still rapidly multiply and reach the detection threshold. Second, in chronic PJI, bacteria are encased within a mature biofilm, leading to a dormant state. This enables them to be resistant to antibiotics [1, 2, 21, 22]. Therefore, even with long-term antibiotic treatments in the past, these pathogens could not be eradicated [21–23]. Antibiotics eliminate the active planktonic bacteria, while the dormant subgroups persist [35, 36]. When cultured outside the body, these “persistent” cells require a longer incubation period before they can be detected [33, 34, 36]. Therefore, growth beyond day 7 may reflect the dormant pathogens within the biofilm; the antibiotic exposure itself cannot fully explain this phenomenon. Since our study only included synovial-fluid samples, further validation of the conclusions is still needed in terms of sample types.

Our study has some limitations. As a retrospective analysis, our study is subject to selection and information bias. Furthermore, the absence of a prospective 7-day control group precludes direct comparison of diagnostic yields, necessitating validation through randomised controlled trials. Finally, although phenotypic classification criteria for PJI have been established, in clinical practice, the boundary between acute and chronic infections is sometimes blurred and overlapping cases may exist. Future research should integrate molecular diagnostics with traditional culture to enhance detection efficiency while reducing time to diagnosis.

In conclusion, based on synovial fluid cultures, an incubation period of 7 days is sufficient to detect most pathogenic bacteria in acute PJI, whereas the incubation period for chronic PJI should be extended to at least 10 days. The previous use of antibiotics did not affect the microbial incubation time for acute PJI. This finding needs to be confirmed in a larger prospective cohort. Whether these time ranges are applicable to the peri-prosthetic tissue and the ultrasound fluid requires further study.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of First Affiliated Hospital of Xinjiang Medical University (**220120-15**).The requirement for obtaining written informed consent from participants was waived by this Institutional Review Board (IRB) due to the retrospective nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author contributions

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Xiaobin Guo: Data curation, Methodology, Investigation.

Xiaogang Zhang:Data curation, Methodology, Investigation.

Li Cao: Writing - review & editing, Conceptualization, Resources, Supervision.

Availability of data and materials

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

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Figures

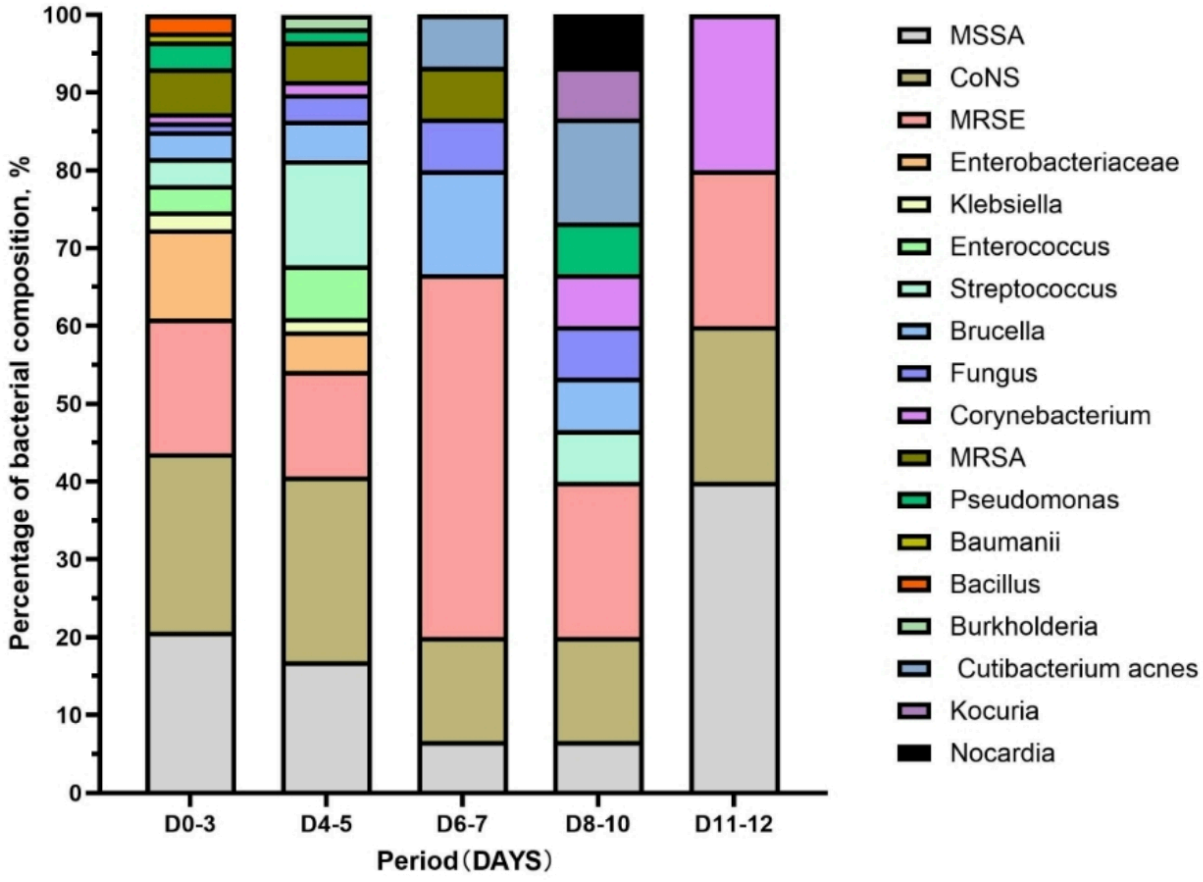


Figure 1

Stacked bar chart depicting the percentage composition of various pathogens over the five cultivation periods. MSSA, methicillin- sensitive Staphylococcus aureus; CoNS, coagulase-negative; Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis.

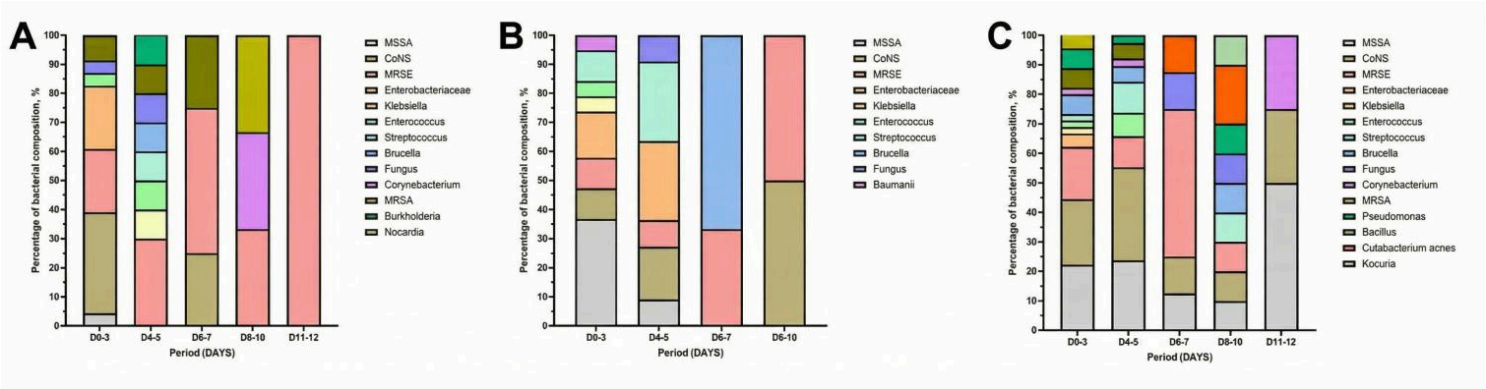


Figure 2

A. Stacked bar chart depicting the percentage composition of various pathogens during the five culture periods in the Early acute PJI. **B.** Stacked bar chart depicting the percentage composition of various pathogens during the four culture periods in the Late acute PJI. **C.** Stacked bar chart depicting the percentage composition of various pathogens during the five culture periods of Late chronic PJI. MSSA, methicillin- sensitive Staphylococcus aureus; CoNS, coagulase-negative; Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis.

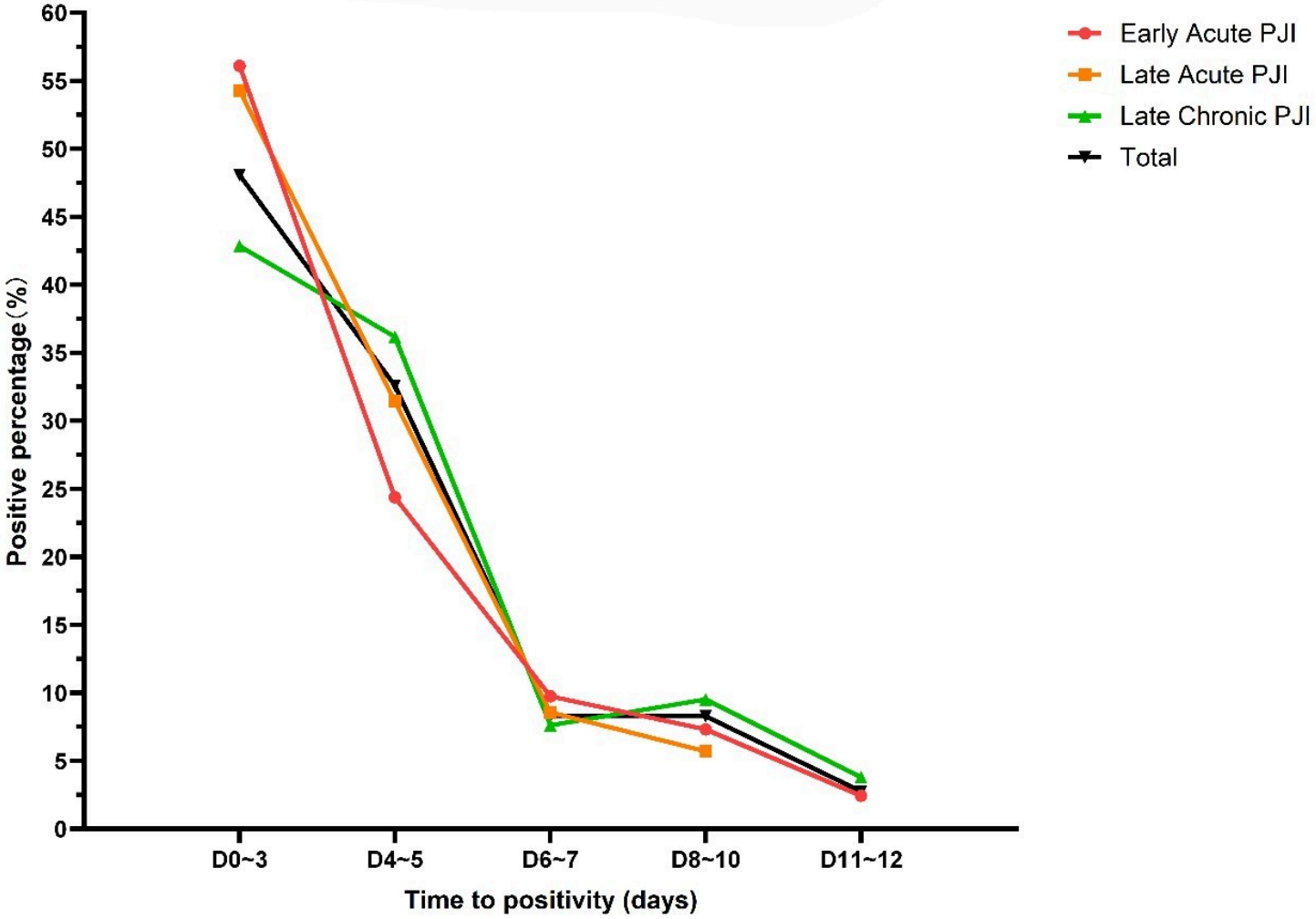


Figure 3

Line graph showing the percentage of positive cultures over five time periods. Different coloured lines represent different classifications.

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