

Post Lumbar Fixation Spondylodiscitis: Prevalence and Microbiological Profile

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Abstract

Background: Spondylodiscitis refers to spinal infections, including vertebral osteomyelitis, spondylitis, and discitis. Its incidence is rising due to invasive spinal interventions among other reasons. Postoperative spondylodiscitis (POSD) often results from poor aseptic techniques or hematogenous dissemination, with incidence rates varying widely. Culture negativity in spondylodiscitis complicates diagnosis and treatment plans. This study evaluates culture-positive versus culture-negative cases and reflects on the prevalence of POSD among patients underwent spinal surgery. **Methods:** This retrospective cohort study, conducted at Beni Suef University Hospital (2022-2024), included 20 postoperative spondylodiscitis (POSD) patients divided into culture-positive and culture-negative groups. Data extracted included demographics, clinical symptoms, surgical details, and microbiological, laboratory, and radiological findings. Outcomes such as recovery, hospital stay, and treatment were analyzed. Statistical methods included chi-square, Fisher's exact test, t-tests, logistic regression, and survival analysis to compare groups and identify factors associated with culture positivity. Ethical approval was obtained, and patient confidentiality was ensured through anonymized data. The primary goal was to assess the prevalence of POSD among spine surgical patients and draw comparisons between culture-positive and culture-negative cases. **Results:** This study analyzed 20 cases of POSD among 288 lumbar fixation surgeries (6.94% prevalence). The average patient age was 45.4 years, with a male to female ratio of 1:1.5. Nearly half had diabetes, and 80% had multiple spinal levels affected. Symptoms typically began 16 days post-surgery and included wound discharge (70%) and back pain (45%). Elevated CRP and ESR levels were observed. Half of the cultures showed no growth; others revealed pathogens like *S. aureus* (15%) and MRSA (15%). BMI was the sole significant factor associated with culture positivity, doubling the odds of culture-positive POSD (OR = 2.003). Culture-positive cases required earlier second surgeries

than culture-negative ones, highlighting the role of obesity and microbial culture results in treatment outcomes. **Conclusion:** Our study revealed a 6.94% prevalence of POSD in lumbar fixation surgery patients, with BMI as the only identified predictor for culture-positive cases. Culture-negative POSD showed better surgical outcomes, highlighting diagnostic challenges and the need for methods to improve microbiological detection and evidence-based management strategies.

Keywords

Postoperative Spondylodiscitis, Culture Negative Spondylodiscitis, Culture Positive Spondylodiscitis, Lumbar Fixation, Postoperative Spinal Infections

1. Introduction

Spondylodiscitis encompasses various forms of spinal infections, including vertebral osteomyelitis, spondylitis, and discitis [1]. The incidence of spondylodiscitis is rising progressively, largely due to the increasing prevalence of invasive spinal interventions as well as the growing population of immunocompromised patients [2]. Other causes include hematogenous spread from other tissues, namely spontaneous spondylodiscitis [3] [4].

The majority of patients with spondylodiscitis present with pain, while fever is the presentation in around 60% of cases. A high erythrocyte-sedimentation rate (ESR) and C-reactive protein (CRP) levels are commonly observed; however, the total leucocytic count (TLC) is only elevated in half of the cases [5] [6]. The imaging modality of choice in spondylodiscitis is magnetic resonance imaging (MRI), a method known for its sensitivity to soft tissues, providing critical insights into neural involvement and paraspinal infectious foci [7].

Pyogenic spondylodiscitis is a bacterial infection of the vertebral bodies, intervertebral disc spaces, and related structures [8]. Staphylococcal species are the most frequently implicated pathogens [9] [10]. However, microbiological cultures often yield negative results, complicating diagnosis and raising the issue of culture-negative spondylodiscitis, for which treatment guidelines are yet to be elucidated [11]-[13].

Postoperative spondylodiscitis, a subset of postoperative spinal infections (PSIs), occurs when microorganisms invade the vertebrae, intervertebral structures, and surrounding soft tissues. This could potentially result from inadequate aseptic techniques or due to hematogenous spread from sources such as the gastrointestinal or genitourinary tracts [14]. The prevalence of postoperative spondylodiscitis is hard to narrow down based on the currently available literature, which offers such a wide range (0.5% - 18.8%); however, it is broadly recognized that the incidence of PSIs increases with more invasive procedures, as well as the use of instrumentation during spinal surgeries [15]-[17].

While several studies have explored differences in treatment outcomes with

culture-negative discitis in comparison to culture-positive discitis, they did not expound on the risk factors associated with culture positivity, and most hypotheses regarding the reason for culture negativity in spondylodiscitis remain largely presumptuous. Additionally, most study designs are retrospective, with missing data on patients leading to the exclusion of a large number of patients with post-operative spondylodiscitis [12] [18]-[20].

In the following study, we aim to assess the proportion of culture-positive versus culture-negative cases among patients with clinically suspected POSD, touching upon the prevalence of POSD among all patients who underwent spinal surgery throughout the study duration.

2. Methods

During reporting this manuscript, we adhered to the items of the STROBE checklist [21]. This retrospective cohort study was conducted at the Department of Neurosurgery, Beni Suef University Hospital (Beni Suef, Egypt), from June 2022 to June 2024, based on secondary data, and following the approval of the Beni Suef scientific and ethics committee. It included 20 patients divided into two groups: 10 culture-positive and 10 culture-negative cases with postoperative (POSD). 8 of the subjects were males and 12 were females. The age range of participants was between 20 - 64 years. Data was extracted from medical records and databases. We ensured patient confidentiality by anonymizing data.

The primary aim was to assess the proportion of culture-positive versus culture-negative cases among patients with clinically suspected POSD. Secondly, we compared the demographic, clinical, and radiological features of the two groups, as well as their treatment outcomes (e.g. hospital stay, recovery, need for additional surgery), and reflected on the prevalence of POSD in all patients who underwent spinal surgery during the study.

Inclusion criteria

1) All patients underwent posterolateral lumbar fixation, other lytic or degenerative and developed POSD.

Exclusion criteria

- 1) Cases with previous lumbar surgery.
- 2) Cases with previous spinal infection.
- 3) Other cases of lumbar interbody fusion (PLIF, TLIF).
- 4) Patients with incomplete records for culture results.

Data extracted from medical records included demographic data collection, which included age, gender, and comorbidities (e.g. diabetes, obesity, smoking). Surgical data included the type of lumbar fixation performed, the use of instrumentation, and the duration of surgery. Clinical data was also extracted, namely symptoms (back pain, fever, and muscle spasms), in addition to the onset and duration of symptoms post-surgery.

All patients received an antibiotic regimen: intravenous 3rd generation cephalosporin (Cefotaxime) within 1 hour before skin incision and then every 12 hours

for five days postoperative.

Laboratory data consisted of inflammatory markers such as CRP, ESR, and WBC count. Microbiological data included culture results, with the identification of organisms from positive cultures and their antibiotic sensitivity.

Culture sample is obtained either from wound swab, discharge or surgical site intra operative.

Once sample was taken, we shifted to another regimen of antibiotics (vancomycin and meropenem) till culture results appear.

Radiological data was obtained in the form of CT or MR imaging with findings suggestive of POSD as in **Figure 1**.

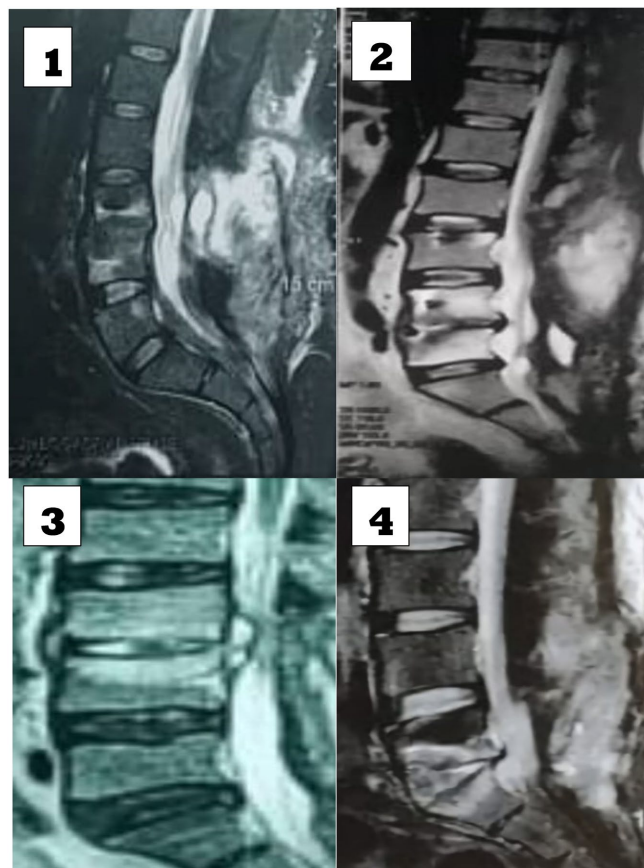


Figure 1. Showing sagittal T2 MRI lumbosacral spine 4 cases. Case 1: showing culture negative L3-4 POSD; Case 2: showing E-coli culture positive L4-5 POSD; Case 3: showing S-aureus culture positive L3-4 POSD with epidural abscess; Case 4: showing culture negative L5-S1 POSD.

Outcome data was documented, underlining the treatment provided in the form of antibiotics or surgical debridement, as well as the length of hospital stay and the outcomes of recovery.

3. Definitions

Culture-Positive POSD: Growth of microorganisms from biopsy, surgical site, or

blood cultures.

Culture-Negative POSD: No microorganism growth despite clinical and imaging findings consistent with POSD.

4. Statistical Analysis

We calculated the frequencies and percentages for categorical variables. Use means (\pm SD) for continuous variables. We used chi-square and Fisher's exact tests for categorical variables. We used t-tests (for parametric) continuous variables. We performed logistic regression to identify factors associated with culture positivity (e.g., elevated CRP, ESR, imaging findings). We did survival analysis for timing to second surgery between culture positive and culture negative cases with Log Rank significance assessment between both categories. P-value was considered significant at less than 0.05.

Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine, Beni-Suef University, Egypt for the retrospective review of patient records. We ensured patient privacy by strictly keeping personal data undisclosed.

5. Results

Table 1 provides a comprehensive summary of the demographic, clinical, and microbiological characteristics of the 20 patients included in the study. Among 288 cases who underwent lumbar fixation during the study period (2022-2024), we included 20 patients who experienced POSD. The prevalence of POSD during the study period was calculated to be 6.94%. The mean age of the patients was 45.4 years, with a broad age range from 20 to 64 years, indicating a diverse age distribution within the sample. There was a female preponderance in our sample with a male to female ratio off 1:1.5. The average BMI was 28.3 kg/m², with a median of 27.5 kg/m², indicating that most patients were overweight or had mild obesity. Notably, nearly half of the patients (45%) had diabetes mellitus. The data also show that 80% of the patients had affection at multiple spinal levels. The median time from surgery to the onset of symptoms was 16 days, though some had delayed symptoms up to 90 days after surgery. The most frequent symptoms reported were wound discharge (70%) and low back pain (45%).

Table 1. Descriptive data.

Items	Values (no = 20)
Age	
mean \pm SD	45.4 \pm 12.1
Median (IQR)	49 (20 - 64)
Sex	
Male	8 40.0
Female	12 60.0

Continued

BMI	
mean \pm SD	28.3 \pm 6.5
Median (IQR)	27.5 (18 - 40)
DM	
	9 45.0
Multiple level affection	16 80.0
Operative time (minute)	
mean \pm SD	179.5 \pm 46.5
Median (IQR)	185 (90 - 250)
Time from operation to complain (day)	
mean \pm SD	26.1 \pm 21.9
Median (IQR)	16 (7 - 90)
wound discharge	14 70.0
Low back pain	9 45.0
CRP	
mean \pm SD	51.7 \pm 20.1
Median (IQR)	47.5 (25 - 90)
ESR	
mean \pm SD	51.8 \pm 17.6
Median (IQR)	53 (23 - 85)
Organism of the culture	
	10 50.0
No growth	
S aureus	3 15.0
Streptococci	2 10.0
E coli	2 10.0
MRSA	3 15.0
Need second surgeries	14 70.0
Number of surgeries among who needed surgeries (no = 14)	
1	11 78.6
2	1 7.1
3	2 14.3

Laboratory results showed elevated CRP and ESR levels, with means of 51.7 mg/L and 51.8 mm/h, respectively, which are consistent with inflammatory responses seen in infections. The culture results were varied, with half of the patients (50%) showing no microbial growth, while others tested positive for *Staphylococcus aureus* (15%), *Streptococcus* species (10%), *Escherichia coli* (10%), and *Methicillin-resistant Staphylococcus aureus* (15%). Finally, the need for a second surgery was observed in 70% of the patients, with most requiring only one additional surgical intervention.

50% of patients with clinically were culture positive as suspected discitis as shown in **Figure 2**.

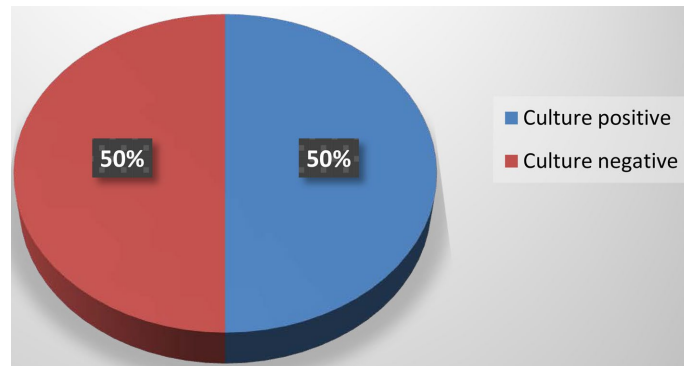


Figure 2. Prevalence of culture positivity among patients with clinically suspected discitis.

The univariate analysis in **Table 2** reveals that among the factors assessed, only BMI showed a statistically significant association with culture positivity in patients with clinically suspected postoperative spondylodiscitis (POSD). Culture-positive patients had a higher mean BMI (31.5) compared to culture-negative patients (25.1), with a p-value of 0.024, suggesting that obesity may increase the likelihood of culture-positive POSD. Other factors such as age, sex, diabetes mellitus, multiple level affection, and operative time did not show significant differences between the two groups, as indicated by p-values greater than 0.05.

Table 2. Univariate analysis for factors associated with culture positivity of postoperative spondylodiscitis among who clinically suspected.

Items	Culture negative (no = 10)	Culture positive (no = 10)	P-value
Age (mean \pm SD)	43.4 \pm 13.4	47.4 \pm 10.7	0.473
Sex			>0.999
Male	4 (40.0%)	4 (40.0%)	
Female	6 (60.0%)	6 (60.0%)	
BMI (mean \pm SD)	25.1 \pm 6.2	31.5 \pm 5.3	0.024*
DM			0.653 (FET)
No	6 (60.0%)	5 (50.0%)	
Yes	4 (40.0%)	5 (50.0%)	
Multiple level affection	2 (20.0%) 8 (80.0%)	2 (20.0%) 8 (80.0%)	>0.999
Operative time (minute) mean \pm SD	186 \pm 48.3	173.0 \pm 46.2	0.546

The binary logistic regression analysis in **Table 3** identifies BMI as the only significant factor associated with culture positivity in patients with clinically

suspected postoperative discitis (POSD). A higher BMI nearly doubles the odds of a positive culture result (OR = 2.003, $p = 0.048$), suggesting that obesity may play a critical role in the risk of infection. Other factors, including age, sex, diabetes mellitus, multiple affected levels, and operative time, did not show significant associations with culture positivity, as their p -values were above the threshold for statistical significance.

Table 3. Binary logistic regression analysis for factors associated with culture positivity.

Risk factors	P-value	OR	95% C.I. for OR	
			Lower	Upper
Age (years)	0.878	1.017	0.818	1.265
Female sex	0.712	2.009	0.050	81.373
BMI (kg/m ²)	0.048	2.003	1.007	3.984
DM	0.080	0.002	0.000	2.155
Multiple affected levels	0.282	0.010	0.000	44.136
operative time [min]	0.765	0.990	0.928	1.056

Table 4. Association between culture positivity and clinical presentation and outcomes.

Items	Culture negative (no = 10)	Culture positive (no = 10)	P-value
wound discharge			0.628
no	4 (40.0%)	2 (20.0%)	
yes	6 (60.0%)	8 (80.0%)	
Low back pain			0.370
No	4 (40.0%)	7 (70.0%)	
Yes	6 (60.0%)	3 (30.0%)	
CRP (mean \pm SD)	56 \pm 18.4	47.4 \pm 21.7	0.353
ESR (mean \pm SD)	47.7 \pm 17.1	56 \pm 18.1	0.305
Need second surgeries			0.141 (FET)
No	5 (50.0%)	1 (10.0%)	
Yes	5 (50.0%)	9 (90.0%)	
Number of surgeries among who needed surgeries (no = 14)			0.346
1	5 (100.0%)	6 (66.7%)	
2	0 (0.0%)	1 (11.1%)	
3	0 (0.0%)	2 (22.2%)	

Table 4 highlights several clinical presentations and outcomes in relation to culture positivity in patients with clinically suspected postoperative spondylodiscitis (POSD), but none of the factors showed statistically significant associations. While

culture-positive patients exhibited a higher incidence of wound discharge (80% vs. 60%) and a greater need for second surgeries (90% vs. 50%), these differences were not significant ($p = 0.628$ and $p = 0.141$, respectively). Similarly, there were no significant differences in the prevalence of low back pain, CRP, or ESR levels between the two groups. Additionally, the number of surgeries required for those needing a second procedure did not differ significantly ($p = 0.346$).

Table 5 presents the survival analysis comparing the time to second surgery between culture-positive and culture-negative cases of clinically suspected post-operative spondylodiscitis (POSD). The mean time to second surgery for culture-negative cases was significantly longer at 72.375 days (95% CI: 62.769 - 81.980), compared to 22.800 days for culture-positive cases (95% CI: 11.238 - 34.362). The median time to second surgery was also longer for culture-negative cases (70 days, 95% CI: 56.382 - 83.618) compared to culture-positive cases (15 days, 95% CI: 7.409 - 22.591).

Table 5. Survival analysis for timing to second surgery between culture positive and culture negative cases.

Culture	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Negative	72.375	4.901	62.769	81.980	70.000	6.948	56.382	83.618
Positive	22.800	5.899	11.238	34.362	15.000	3.873	7.409	22.591

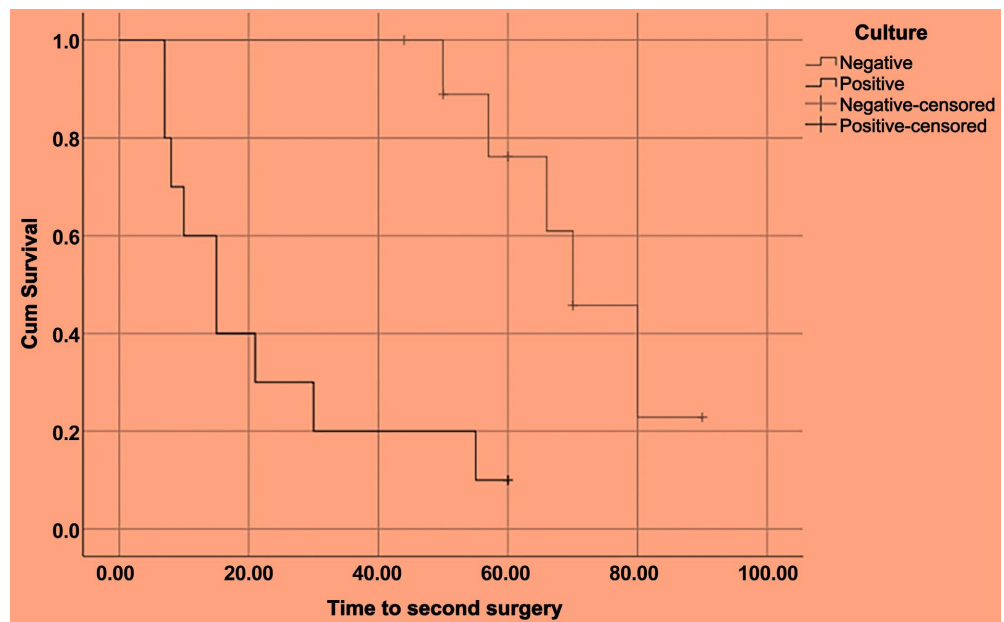


Figure 3. Showing the time to the second surgery between the two groups.

These findings suggest that culture-negative patients generally experienced a delayed need for second surgery, while culture-positive patients required surgical intervention more quickly. This trend is visually represented in **Figure 3**,

showing a clear distinction in the time to the second surgery between the two groups.

6. Discussion

POSD is an infection of the vertebral plates following discectomy, it can either be superficial or deep and can be localized to the intervertebral space or spread to the surrounding tissues causing spondylitis and epidural abscess [18]. While staphylococci are broadly known to be the most common cause of spondylodiscitis [9] [10], culture-negative spondylodiscitis has been on the rise with no clear guidelines to elucidate appropriate antibiotic treatment [19] [22].

Our aim in this retrospective cohort study was to assess the proportion of culture-positive versus culture-negative cases among those with clinically suspected POSD. We also estimated the prevalence of POSD among all patients who underwent spinal surgery within the timeframe of the study.

In our present study, we investigated the medical records of 288 patients who underwent spinal surgeries over a period of two years, and we deduced that the prevalence of POSD was 6.94%. We evaluated and divided our patients according to the results of their cultures into culture-negative POSD (50%), and those who had microbial growths on their cultures were classified as culture-positive POSD (50%). We noted that 15% of culture-positive cases had staphylococcus aureus growth, 10% had streptococcus spp., 10% had Escherichia coli, and 15% had Methicillin-resistant Staphylococcus aureus (MRSA). Lee *et al.* reported in their retrospective study that among a sample of 175 patients with spondylodiscitis, 83 patients were culture-positive, while 89 patients were culture-negative [19]. Singh *et al.* inferred that in a sample of 854 patients who underwent discectomy during their study period which lasted 5 years, 31 patients had POSD (prevalence = 3.62%), and in those with POSD, microbiological cultures from patients who did not respond to antibiotics were taken, and staph. Aureus was identified in only two patients, while the rest of the patients had negative biopsy cultures, and all patients had negative blood culture [18]. Li *et al.* reported that in a sample of 31 patients with POSD, 17 cases were culture-negative and only 14 had positive cultures. They further elaborated that among the 14 cases with positive cultures, 7 yielded staph. Aureus, while 2 cases yielded E. coli, and 2 cases had growth of S. epidermidis, Klebsiella was found in 2 cases, and pseudomonas cepacia in one case [20]. Bintachitt *et al.* relayed that among their subjects, 28 of those with a positive culture (n = 56), had staphylococci of different species growing on their cultures, most notably coagulase-positive staph. Aureus [23].

We addressed the possible variables that might have influenced culture results, and we found that only the BMI showed a statistically significant association with culture positivity, as those with a positive culture had a substantially higher BMI than those with culture-negative POSD (31.5 vs. 25.1; p = 0.024). This finding suggested that obese patients are at a higher risk of developing culture-positive POSD. Other factors such as age, sex, DM, multiple level affection, and operative

time, were analyzed but resulted in no statistically considerable association with culture positivity ($p > 0.05$). Furthermore, we conducted a binary logistic regression analysis to further investigate the influence of several factors such as age, female sex, BMI, DM, multiple level affection, and operative time, and among those only the BMI demonstrated a positive correlation with culture positivity with double the odds of having culture-positive POSD and this was deemed statistically significant (2.003 OR, 95% CI: 1.007:3.984; $p = 0.048$). In agreement with our findings, Bintachitt *et al.* reported that there were no considerable differences between their two groups, culture-positive and culture-negative, in terms of age, sex, number of affected vertebrae ($p = 0.116$, $p = 0.739$, $p = 0.129$). However, they did not touch upon the BMI of their studied patients [23]. Singh *et al.* reported that 22.5% of a sample of patients with POSD were morbidly obese, with a BMI of >35 [18]. Obesity is a broadly recognized risk factor for infections as it has been shown to alter the immune system and increase soft tissue burdens leading to a heightened risk of infection [24]. Bhagat *et al.* contrasted our findings as they found that patients with culture-negative spondylodiscitis are more likely to be diabetic, establishing a significant association between diabetes and culture-negativity ($p = 0.02$). While this was at odds with our findings, it is worth noting that their sample was relatively smaller than ours (10 patients in total), and only 6 of those patients had undergone discectomy previously [11]. Kim *et al.* reported in agreement with our findings, highlighting a lack of correlation between culture positivity and age or gender ($p = 0.462$, $p = 0.626$); however, their results were at odds with ours regarding the association with diabetes, as they showed that DM was significantly associated with culture-positivity in patients with spondylodiscitis ($p = 0.028$) [12].

Our data regarding the association between culture results and clinical and surgical data revealed no significant differences between the two groups regarding wound discharge, back pain, the need for more surgeries, or the number of secondary surgeries required ($p > 0.05$). Moreover, laboratory data, ESR, CRP, and WBC count, showed no significant correlation with culture results. Although CRP was slightly higher in those with a negative culture, the difference was not statistically significant (56 vs. 47.4; $p = 0.353$). In accordance with our findings, Bintachitt *et al.* inferred that there were no considerable differences between the culture-positive and culture-negative cases regarding pain, fever, or neurological compromise, neither were there any significant variance in terms of their ESR and CRP ($p = 0.363$, $p = 0.233$) [23]. Reports by Dai *et al.* were at odds with our findings as they reported that those with culture-positive spondylodiscitis had the significantly higher fever ($p = 0.005$), higher ESR (77.78 vs. 38.75; $p < 0.001$), higher CRP (67.62 vs. 21.373; $p < 0.001$), and a higher WBC count (8.49 vs. 6.63; $p < 0.001$) [25].

Lastly, we performed a survival analysis comparing the time to the second surgery between those who had a positive culture and those with culture-negative POSD. We noted that the mean time to second surgery for culture-negative cases

was significantly longer (72.375 days; 95% CI: 62.769 - 81.980) compared to those with culture-positive POSD (22.800 days; 95% CI: 11.238 - 34.362). Moreover, we reported that the median time to the second surgery was also longer for culture-negative cases (70 days; 95% CI: 56.382 - 83.618) as opposed to culture-positive cases (15 days; 95% CI: 7.409 - 22.591). These findings highlight the better surgical outcome with culture-negative POSD with delayed need for a second surgical intervention with culture-negativity. Dai *et al.* reported that among the factors that were associated with relapse, culture-positivity was not one ($p = 0.307$). Furthermore they showed that patients with culture-positive and culture-negative results did not differ in terms of relapse rate ($p = 0.127$). However, the duration of hospital stay was significantly longer for those with positive culture ($p = 0.002$) [25]. Lee *et al.* concluded that the clinical outcome of patients with culture-negative spondylodiscitis was notably better than those with a positive culture, as 35% with a negative culture had excellent scores on Macnab's criteria, while only 1% of those with culture positivity had excellent scores [26]. Pola *et al.* indicated that culture negativity was associated with a worse outcome in spondylodiscitis (0.41 OR; $p = 0.02$), which they confirmed with a multivariate analysis (0.26 AOR; $p = 0.04$) [27]. While others, such as Kim *et al.* showed that relapses and the need for repeated intervention were lower in culture-negative spondylodiscitis; however, these findings were not statistically significant ($p = 0.157$) [12].

7. Study Strengths and Limitations

Limitations

Culture-negative cases may represent false negatives due to prior antibiotic use. Retrospective nature may result in missing or incomplete data.

Small sample size.

Strengths

- 1) Focused Analysis: Targets patients with suspected POSD, allowing for direct comparison of culture-positive and culture-negative cases.
- 2) Clinical Relevance: Provides insights into the differences in clinical and imaging findings, aiding in diagnosing and managing culture-negative cases.
- 3) Data-Driven Outcomes: Facilitates understanding of risk factors and outcomes associated with culture positivity.

8. Conclusion

In conclusion, our study put forth data regarding the prevalence and microbiological profiles of POSD among patients undergoing spinal surgery. We observed a 6.94% prevalence of POSD, with an equal distribution between culture-positive and culture-negative cases. Staphylococcal species, particularly *Staphylococcus aureus* and MRSA, were the most common pathogens identified in culture-positive cases. Among the clinical and demographic factors analyzed, BMI emerged as the only statistically significant predictor of culture positivity, suggesting that obese patients are at greater risk of developing culture-positive POSD. Our survival

analysis revealed that patients with culture-negative POSD had a significantly longer time to secondary surgical intervention, indicating potentially better surgical outcomes. To our knowledge, this is the first report that included a survival analysis on POSD in this context. We did not note any significant differences regarding the clinical features or the laboratory parameters between patients with culture-positive POSD and those with negative cultures, amplifying diagnostic challenges. Our findings underlined the critical need for standardized diagnostic and therapeutic approaches for culture-negative POSD. Future research should focus on refining microbiological and molecular techniques to optimize pathogen detection in those where routine cultures do not yield any growths in order to guide management plans.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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