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REVIEW

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The association between low virulence organisms in different locations and intervertebral disc structural failure: A meta-analysis and systematic review

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Abstract

Many factors may trigger intervertebral disc (IVD) structural failure (intervertebral disc degeneration (IDD) and endplate changes), including inflammation, infection, dysbiosis, and the downstream effects of chemical factors. Of these, microbial diversity in the IVD and elsewhere in the body has been considered as one of the potential reasons for disc structural failure. The exact relationships between microbial colonization and IVD structural failure are not well understood. This meta-analysis aimed to investigate the impact of microbial colonization and its location (such as skin, IVD, muscle, soft tissues, and blood) on IVD structural failure and corresponding low back pain (LBP) if any. We searched four online databases for potential studies. The potential relationships between microbial colonization in different sample sources (such as skin, IVD, muscle, soft tissues, and blood) and IDD and endplate change were considered as primary outcomes. Odds ratio (OR) and 95% confidence intervals (CI) for direct comparisons were reported. Grading of Recommendations Assessment, Development and Evaluation (GRADE) scale was used to assess the quality of evidence. Twenty-five cohort studies met the selection criteria. Overall pooled prevalence of microbial colonization in 2419 patients with LBP was 33.2% (23.6%–43.6%). The pooled prevalence of microbial colonization in 2901 samples was 29.6% (21.0%–38.9%). Compared with the patients without endplate change, the patients with endplate changes had higher rates of microbial colonization of disc (OR = 2.83; 95% CI = 1.93–4.14; I^2 = 37.6%; p = 0.108). The primary pathogen was *Cutibacterium acnes* which was present in 22.2% of cases (95% CI = 13.3%–32.5%; I^2 = 96.6%; p = 0.000). This meta-analysis and systematic review found low-quality grade evidence for an association between microbial colonization of disc with endplate changes. The primary pathogen was *C. acnes*. Due to lack of enough high-quality studies and methodological limitations of this review, further studies are required to improve our understanding of the potential relationships and mechanisms of microbiota, dysbiosis, IVD colonization and IVD structural failure.

KEYWORDS
back Pain, disc degeneration, meta-analysis, microbiome, microbiome dysbiosis, systematic review

1 | INTRODUCTION

Low back pain (LBP) is the most common cause of disability worldwide leading to loss of productivity along with psychological stress.[1,2] Within the vast spectra of LBP, disc structural failures such as intervertebral disc (IVD) degeneration (IDD) and endplate changes have been considered as the main reasons.[3] A major concern around IVD structural failure (IDD and endplate change) is the presence of chemical factors or cues that lead to inflammation. However, understanding the exact cause and nature of such inflammation is still unclear. One of the putative triggers for inflammation has been hypothesized to be infection of the IVDs by low virulence and anaerobic bacteria.[4,5] Growing evidence exists that demonstrates that bacterial microbiome may be crucial to the pathogenesis of IVD structural failure,[6–11] particularly with respect to *Cutibacterium acnes*. Although several studies on animal models and clinical research have reported a significant relationship between low virulence organisms and pathogenesis of IDD and endplate change, the existence and their impact on etiology and pathways of these infections have been fiercely debated in recent years.[12–17]

Intriguingly, alterations of the microbiome composition in the mouth and gastrointestinal system are associated with a variety of chronic diseases, such as gut inflammation disorders, autoimmune diseases, chronic kidney disease, cardiometabolic diseases, neurological and respiratory diseases, mental health disorders, and osteoarthritis.[5,18–22] In this direction, our previous review discusses the concept of the gut-bone axis, gut-bone marrow axis, and gut-disc axis based on different sources of samples to explain the relationship between bacterial microbiome and disc structural failure/LBP.[23] The review has summarized the potential mechanisms and discusses microbiome dysbiosis's possible influence on IDD and LBP. However, ambiguity still exists for the posited mechanisms. We assume that this knowledge gap may be closed by yet-undiscovered axes of gut-end organ influence based on alternate microbiome sources, or by linking known dysbiosis states to IVD structural failure and LBP. The imperative to distinguish and define terms such as "microbial colonization," "infection," "microbiome dysbiosis," etc. (Table 1) for a better understanding of the microbiome research in LBP is of the essence. Furthermore, an understanding of the various techniques and their objectives used for identification of different or certain types of bacteria in a sample needs emphasis (Table 1).

Here, we performed a systematic review and meta-analysis (1) to investigate the impact of microbial colonization on IVD structural

TABLE 1 Summary of the terms related to the understanding of Microbiome in Back Pain

Terms	Description
Microbiome	Collection of microbial genomes within a system and can include viruses, bacteria, fungi, etc.
Microbiota	Ecological community of microorganisms within a system.
Microbial colonization	Colonization means that the organism can be found in or on the body, but it is not causing any symptoms or disease. Microbial colonization of the human body in early life is essential for the development of host immunity and metabolism.
Bacterial microbiome	Collection of different species of bacteria within a microbiome
Infection	Growth of bacteria or other microorganisms in the body causing damage to the body.
Microbiome dysbiosis	Dysbiosis is often defined as an "imbalance" in the gut microbial community that is associated with the disease. This imbalance could be due to the gain or loss of community members or changes in the relative abundance of microbes. This primarily focuses on changes in the taxonomic makeup of the microbial community and functions associated with individual members or subsets of members in the community. Characterizing dysbiosis has traditionally relied on taxonomic catalogs of gut microbes generated in different individuals at single time points using 16S or 18S rRNA sequencing. More recent efforts have attempted to catalog not the microbial species, but rather the microbial genes in the gut using shotgun metagenomics. The taxonomic characterization or genetic makeup of the community is then used to infer its functions based on data in the literature from studies using reference microbial strains. Usually, these model microbes are studied as single organisms, and often in vitro, in order to generate functional or their impact data.
Microbial culture	Identification of bacteria using selective media based on characteristics such as shape, size, and the types of dyes it absorbed.
16s rRNA sequencing (Targeted Amplicon Sequencing)	This is a PCR-dependent technique where DNA is amplified using a specific primer targeting a gene yielding only taxonomic information
16s RNA sequencing (Shotgun metagenomics)	This is a PCR-independent technique specifically targeting one or two regions of the 16S gene, with regions V1–V2 and V3–V4 being the most frequently used. In contrast to targeted amplicon methods, this technique produces relative abundance information and functional annotation for all genes detected in the sample

failure; (2) to review and assess the current evidence on the potential relationships between microbial colonization in different locations and IVD structural failure; (3) to identify the potentially involved microorganisms and their effect on LBP if any.

2 | METHODS

2.1 | Search strategy

Based on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, four online databases such as EMBASE, MEDLINE, Web of Science, and Cochrane Central Register of Controlled Trials (CCRCT) were searched for all relevant studies published in English inception through to October 2021.[24] The search included the following terms: "low back pain," "radicular pain," "disc degeneration," "endplate change," "bacterial infection," "antibiotics," "*Propionibacterium acnes*," "*Cutibacterium acnes*," and "pathophysiology" (Table A1). The reference lists from reviews and potentially relevant studies were evaluated. The protocol of this review is registered on PROSPERO (International Prospective Register of Systematic Reviews number, CRD42021258703).

2.2 | Inclusion criteria

Eligible studies for the review included:

1. Studies including adult patients (age > 18 years) with spinal origin pain (such as LBP, and/or radicular pain) and microbiology cultures or tests performed on at least one sample such as skin, IVD material, muscle, soft tissue, or blood.
2. Randomized clinical trials (RCT) and observational studies.

2.3 | Exclusion criteria

1. Studies not reporting the prevalence of microbial infection on at least one sample such as skin, IVD material, muscle, soft tissue, or blood.
2. Case reports, reviews, editorials, expert opinions, and conference reports;
3. In vitro biomechanical studies, animal studies, and computational modeling studies.

2.4 | Types of outcomes measures

Primary outcomes:

1. Pain intensity scores for spinal origin pain such as the Visual Analogue Score (VAS) or Numerical Rating Score (NRS).
2. Modic changes as seen on magnetic resonance imaging (MRI) scans as a marker of IDD and endplate changes.[25] IDD was defined as the presence of at least one of the following: disc bulge or disc herniation, annular tear, Pfirrmann score of nucleus pulposus degeneration ≥3,[26] endplate change based on Modic classification, or Schmorl's node(s).[25,27–30]
3. Laboratory results on the microbial culturing or testing of least on at least one sample such as skin, IVD material, muscle, soft tissue, or blood. Testing includes microbial culture, metagenomics sequencing, proteomics, and 16S sequencing (such as 16S rDNA and 16S rRNA).

Secondary outcomes: Disability questionnaires such as Oswestry Disability Index (ODI), Roland-Morris Questionnaire (RMQ), mental health scores (such as Short Form-36 [SF-36]), and adverse event reporting.

2.5 | Selection of studies

Two reviewers independently screened all studies following eligibility criteria, including titles and abstracts screening in initial search results, reference lists, and full-text screening. A third reviewer was consulted to resolve areas of disagreement during article screening.

2.6 | Data extraction

Two independent reviewers extracted the following data:

1. Relevant publication information such as author, publication year, country
2. Study design
3. Participant demographics; enrolled participants, gender, age, body mass index (BMI)
4. Pathology specifics: type of pain, location of spinal pathology, diagnosis
5. Interventions; surgical technique, surgical level
6. Samples; source, number, organism, and laboratory methods on the microbiology culturing or testing
7. Outcomes; pain intensity scores, disc degeneration data, and laboratory results on the microbiology culturing or testing, disability scores, mental health scores, and adverse events
8. Funding sources

There was no restriction on included laboratory methods for microbial samples such as culturing or testing, culture types (aerobic or anaerobic), sequencing method (metagenomics sequencing or 16 S sequencing), or liquid chromatography/tandem mass spectrometry.

2.7 | Risk of bias within trials

Version 2 of the Cochrane Tool for Assessing Risk of Bias in Randomized Trials (RoB 2)[31] and Newcastle-Ottawa Scale (NOS)[32] were used

to assess the risk of bias in included RCTs and observational studies, respectively. Seven or more stars on the NOS score were regarded as high-quality. Interobserver reliability of risk of bias was examined. The third observer was consulted to resolve the controversial scores.

2.8 | Statistical analysis

Calculation of pooled mean infection rate was done by the summation of total infection events divided by the total number of patients included in the studies reporting that specific infection. For the available main variables, a meta-analysis of proportions was conducted. I^2 tests were used for assessing the heterogeneity. Fixed-effects model and random-effects model were used for $I^2 < 50\%$ and $I^2 > 50\%$, respectively. Sensitivity and subgroup analysis were performed for assessing the impact of heterogeneity. The pooled estimates of odds ratio (OR) and 95% confidence intervals (CI) for direct comparisons were reported. Forest plots were used for displaying the meta-analysis results. The Begg-Mazumdar test was used for evaluating the risk of publication bias. The statistical significance was set at 5% ($\alpha = 0.05$). STATA software (release 15, StataCorp LLC, TX) was used for the statistical analysis.

This meta-analysis and systematic review have been reported according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and AMSTAR (Assessing the methodological quality of systematic reviews) Guidelines.[33–35]

2.9 | Evaluating the quality of evidence

Grading of Recommendations Assessment, Development and Evaluation (GRADE) scale was used to rate evidence quality as high, moderate, low, or very low[36] (Table A2). The evidence from observational studies was rated as low quality.

3 | RESULTS

3.1 | Study selection

The literature search is illustrated in the PRISMA flow diagram (Figure 1). Twenty-five prospective observational studies met the selection criteria.[6,8,9,15,37–57]

3.2 | Study characteristics

A total of 2419 symptomatic patients with LBP and/or radicular pain were found in the eligible studies. The mean age of patients was 46.2 years, and the population was 43.3% female. Twenty-five studies reported the culture/16S sequencing/next-generation sequencing outcomes of patients with lumbar disc herniation or/and lumbar disc degeneration,[6,8,9,15,37–57] including eight studies with radiological IDD[6,8,41,42,45,47,48,51] and fifteen studies with Modic changes.[8,9,15,39,41,42,45–48,50,52–55] All studies had microbial testing performed on the IVD samples, including one study with microbial culture of the skin,[50] five studies with microbial culture of the muscle and soft tissue,[38,41,46,50,57] and one study with microbial culture of blood.[6] Only 2/25 studies reported results from 16S sequencing using shotgun metagenomics.[45,54] Ten studies were conducted on both microbial cultures and 16S sequencing using targeted amplicon sequencing.[9,41,43,46,47,49,50,52,55,56] The study characteristics of all included studies are presented in Table 2.

3.3 | Quality assessment

During full-text screening and risk of bias analysis, overall interobserver reliability was good (Table 3). A complete agreement was reached on all items during the first consensus meeting. All observational studies were ranked as high quality due to more than seven stars in NOS assessment (Table 4).[6,8,9,15,37–57]

3.4 | Impact of microbial colonization on disc structural failure

Rajasekaran et al.[11] reported the presence of the gut/skin/spine microbiome axis using 16S rRNA sequencing and posited this as evidence as part of the etiology of the development of disc structural failure. This evidence included demonstrating 58 overlapping bacterial species between IVDs and gut, and the presence of 29 overlapping bacterial species between IVDs and the skin.

3.5 | Relationship between microbial colonization in different locations and disc structural failure

3.5.1 | Outcomes for microbiology cultures and 16S sequencing

Among all included studies and 2374 patients, the pooled prevalence of microbial colonization was 33.2% (95% CI = 23.6%–43.6%, $I^2 = 96\%$, $p = 0.000$) (Figure 2). From 2901 samples in different locations (such as skin samples, IVD samples, muscle or soft tissue, and blood), the pooled prevalence of microbial colonization was 29.6% (95% CI = 21.0%–38.9%, $I^2 = 96.3\%$, $p = 0.000$) (Figure 3).

Based on different sources of samples for subgroup analysis, the pooled prevalence of microbial colonization from blood, IVD, muscle, muscle and skin, and IVD & muscle was 30.7% (95% CI = 23.2%–39.1%; $I^2 = -$; $p = -$), 30.3% (95% CI = 20.5%–41.1%; $I^2 = 96.4\%$; $p = 0.000$), 10.7% (95% CI = 3.2%–21.4%; $I^2 = -$; $p = -$), 81.7% (95% CI = 69.6%–90.5%; $I^2 = -$; $p = -$), and 26.1% (95% CI = 17.3%–36.6%; $I^2 = -$; $p = -$) respectively (Figure 3A).

Based on different testing methods for subgroup analysis, the pooled prevalence of microbial colonization by using ELISA, microbial

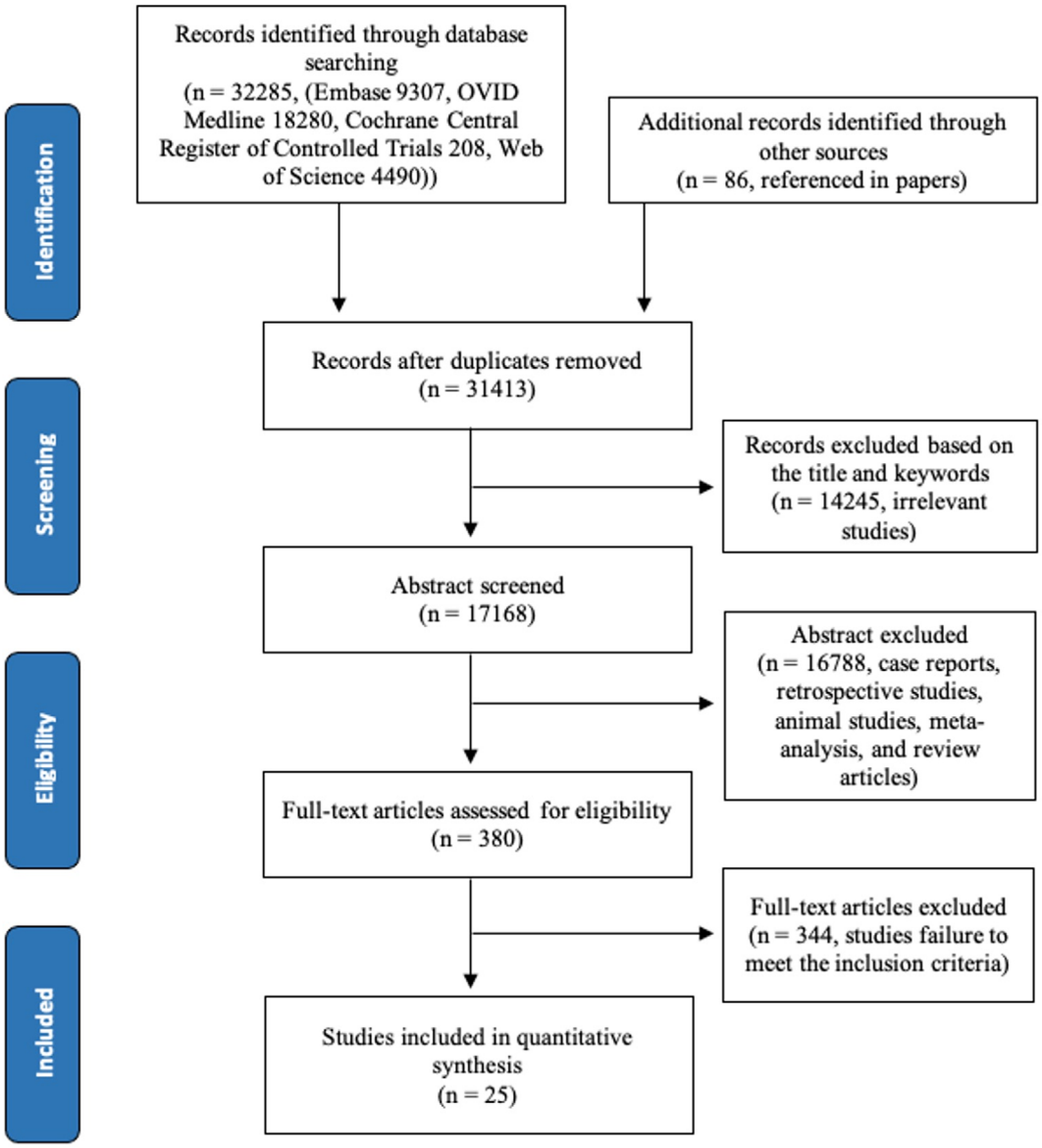

FIGURE 1 Flow chart showing the procedure and results of the literature search in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.

culture, microbial culture & 16S sequencing, 16S sequencing & LC–MS/MS, and 16S sequencing was 30.7% (95% CI = 23.2%–39.1%; I^2 = –; p = –), 20.3% (95% CI = 10.2%–32.8%; I^2 = 96.5%; p = 0.000), 32.6% (95% CI = 20.8%–45.7%; I^2 = 94.8%; p = 0.000), 81.8% (95% CI = 59.7%–94.8%; I^2 = –; p = –), and 90.0% (95% CI = 68.3%–98.8%; I^2 = –; p = –) respectively (Figure 3B).

3.5.2 | Comparison between patients with and without IDD

The prevalence of microbial colonization of disc in patients with and without IDD was compared using data from 204 patients.[6] In this group, no significant difference in the prevalence of microbial colonization of disc could be established (patients with IDD vs. without IDD 35.2% (62/176) vs. 7.1% (2/28); OR = 0.25; 95% CI = 0.04–1.62; I^2 = 35.1%; p = 0.214) (Figure A1).

3.5.3 | Comparison between patients with and without endplate change

Based on 10 studies,[8,9,15,41,42,46,48,50,52,53] a significant difference in the prevalence of microbial colonization of disc between the patients with and without endplate changes (OR = 2.83; 95% CI = 1.93–4.14; I^2 = 37.6%; p = 0.108) was observed (Figure 4). The quality of evidence was rated as very low due to the heterogeneity of results and lack of blinding in estimates. Based on Modic classification for endplate change for subgroup analysis, no significant difference in the prevalence of microbial colonization of disc between the patients with

TABLE 2 Summary of demographic, clinical, and radiological characteristics, and culture results of patients

	Population					Outcome			Radiological data		Sample						
Author year	No. of patient	No. of infected patient	Diagnosis	No. of female	Age	Back pain	Leg pain	Neck pain	IDD	Modic change	Type	No. of sample	No. of infected (culture)	Organism	No. of infected (16S sequencing)	Organism	Method of organism detection	Study design
Stirling 2001	140	43 (31%)	Lumbar disc herniation			Y	Y		Y		Blood	140	43 (31%)	Raised IgG to lipid S (gm +)			ELISA, lipid S antigen	Prospective cohort
	36	19 (53%)									Intervertebral disc	36	19 (53%)	*P. acnes* (84%)			Microbiology cultures	
Ben-Galim 2006	30	2 (6.7%)	Lumbar disc herniation	12	46.4	Y	Y				Intervertebral disc	120	4 (3.3%)	CoNS			Microbiology cultures	Prospective cohort
Carricajo 2007	54	2 (3.7%)	Lumbar disc herniation	22	44.8	Y	Y				Intervertebral disc	54	4 (7.4%)	*P. acnes*			Microbiology cultures	Prospective cohort
	54	12 (22.2%)									Ligamentum + muscle	54	12 (22.2%)	*P. acnes*			Microbiology cultures	
Wedderkopp 2009	24	2 (8.3%)	Modic type I change of endplate	14	43	Y				Y	Intervertebral disc	24	2 (8.3%)	*Staphylococcus epidermidis*, CoNS			Microbiology cultures	Prospective cohort
Agarwal 2011	52	10 (19.2%)	Lumbar disc herniation	24	43.9	Y	Y				Intervertebral disc	52	10 (19.2%)	7 *P. acnes*			Microbiology cultures	Prospective cohort
Arndt 2012	83	40 (48.2%)	Lumbar disc degeneration	49	41.2	Y	Y		Y	Y	Intervertebral disc	83	40 (48.2%)	18 *P. acnes*, 16 CoNS			Microbiology cultures	Prospective cohort
Albert 2013	61	28 (44%)	Lumbar disc herniation	16	46.4	Y	Y			Y	Intervertebral disc	61	28 (44%)	26 anaerobic bacteria (24 *P. acnes*), 4 aerobic bacteria			Microbiology cultures + PCR 16S sequencing	Prospective cohort
Zhou 2015	46	9 (19.6%)	Lumbar disc herniation	21	54.7	Y	Y		Y	Y	Intervertebral disc	46	9 (19.6%)		11 (23.9%, 2 in muscle)	*P. acnes*	Microbiology cultures + PCR 16S sequencing	Prospective cohort
	46	3 (6.5%)									Muscle	46	3 (6.5%)				Microbiology cultures	
Capoor 2016	290	130 (44.8%)	Lumbar disc herniation	119	47	Y	Y				Intervertebral disc	290	130 (44.8%)	115 *P. acnes*, 31 CoNS, 8 alpha-hemolytic streptococci	39 (11%)	*P. acnes* abundant	Microbiology cultures + PCR 16S sequencing	prospective cross-sectional
Li 2016	22	4 (18.2%)	Lumbar disc herniation	9	49.1	Y	Y			Y	Intervertebral disc	30	4 (13.3%)	2 CoNS, 1 particle chain bacterium, 1 *Staphylococcus epidermidis*			Microbiology cultures	Prospective cohort
Rigal 2016	313	6 (1.9%)	Lumbar disc herniation	127	47.6	y			Y	Y	Intervertebral disc	385	6 (1.6%)	2 *P. acnes*, 2 *Staphylococcus epidermidis*, 1 *Citrobacter freundii*, 1 *Saccharopolyspora hirsuta*			Microbiology cultures	Prospective cohort
Aghazadeh 2017	120	60 (50%)	Lumbar disc herniation	51	43.15	Y	Y			Y	Intervertebral disc	120	60 (50%)	56 Anaerobic bacteria, 12 aerobic bacteria	46 (38.3%)	46 P. acnes	Microbiology cultures + PCR 16S sequencing	Prospective cohort
	120	8 (6.7%)									Muscle	120	8 (6.7%)					

TABLE 2 (Continued)

	Population					Outcome			Radiological data		Sample							
Author year	No. of patient	No. of infected patient	Diagnosis	No. of female	Age	Back pain	Leg pain	Neck pain	IDD	Modic change	Type	No. of sample	No. of infected (culture)	Organism	No. of infected (16S sequencing)	Organism	Method of organism detection	Study design
Capoor 2017	368	162 (44%)	Lumbar disc herniation	146	49.3	Y	Y				Intervertebral disc	368	162 (44%)	119 *P. acnes*, 42 non-*P. acnes*				Prospective cohort
Rajasekaran 2017	22	18 (81.8%)	Lumbar disc herniation + disc degeneration	7	40				Y	Y	Intervertebral disc	22			18 (81.8%)	18 *P. acnes*	Metagenomics 16S sequencing + LC–MS/MS	Prospective cohort
Tang 2018	80	26 (32.5%)	Lumbar disc herniation	45	51	Y	Y		Y	Y	Intervertebral disc	80	26 (32.5%)	21 *P. acnes*, 5 CoNS			Microbiology cultures	Prospective cohort
Yuan 2018	76	23 (30.3%)	Lumbar disc herniation	37	55.3	Y	Y		Y	Y	Intervertebral disc	76	23 (30.3%)	20 *P. acnes*, 3 unidentified bacteria	20	20 *P. acnes*	Microbiology cultures + PCR 16S sequencing	Prospective cohort
Ahmed-Yahia 2019	45		Lumbar disc degeneration	27	46.6	Y				Y	Intervertebral disc	77	12 (15.6%)	10 *C. acnes*, 1 *Staphylococcus epidermidis*, 1 CA	6 (7.8%)	4 Staphylococcus, 1 CA, 1 Streptococcus	Microbiology cultures + PCR 16S sequencing	Prospective case control
Fritzell 2019	40	34 (85%)	Lumbar disc herniation	30	33.6	Y				Y	Intervertebral disc	40	34 (85%)	29 *C. acnes* (1 only in intervertebral disc)	1	1 *C. acnes*	Microbiology cultures + PCR 16S sequencing	Prospective cohort
	20	17 (85%)	Scoliosis								Skin + muscle	20	17 (85%)	14 *C. acnes* (1 only in intervertebral disc)	1	1 *C. acnes*	Microbiology cultures	
Rao 2019	88	23 (26.1%)	Lumbar disc degeneration				Y				Intervertebral disc + muscle	88	23 (26.1%)				Microbiology cultures	Prospective cohort
Salehpour 2019	120	60 (50%)	Lumbar disc herniation	51	43.15	Y	Y				Intervertebral disc	120	60 (50%)	Microorganisms	46 (38.3%)	43 *P. acnes*	Microbiology cultures + PCR 16S sequencing	Prospective cohort
Tang 2019	176	33 (18.8%)	Lumbar disc degeneration	78	51.69	Y			Y		Intervertebral disc	176	33 (18.8%)	31 *P. acnes*, 2 CoNS			Microbiology cultures	Prospective cross-sectional
Drago 2020	39	7 (15.4)	Lumbar disc degeneration	17	52.9	Y				Y	Intervertebral disc	39	7 (15.4)	4 *P. acnes*, 1 *Bacillus* spp., 1 *Lactobacillus* spp., 1 *Staphy hominis*			Microbiology cultures	Prospective cohort
Najafi 2020	37	23 (62.2%)	Lumbar disc herniation		43.6	Y	Y			Y	Intervertebral disc	37	23 (62.2%)	23 *P. acnes*	37	23 (62.2%)	Microbiology cultures + PCR 16S sequencing	Prospective cross-sectional
Singh 2020	20	18 (90%)	Lumbar disc degeneration			Y				Y	Intervertebral disc				20	18 (90%)	PCR 16S sequencing	Prospective cohort
Astur 2021	17	0	Lumbar disc herniation	5	42.8	Y	Y				Intervertebral disc	17	0				Microbiology cultures + metagenomics 16S sequencing	Prospective cross-sectional

Abbreviations: CA, *Cutibacterium avidum*; *C. acnes*, *Cutibacterium acnes*; CoNS, coagulase-negative staphylococci; ELISA, enzyme-linked immunosorbent assay; IDD, intervertebral disc degeneration; LC–MS/MS, liquid chromatography–tandem mass spectrometry; No., number; *P. acnes*, *Propionibacterium acnes*; Y, yes.

normal endplate and type I/II Modic changes was seen (Figures A2 and A3). The large magnitude of effect upgraded the low-quality evidence from the cohort studies to moderate-quality. However, the heterogeneity in the reported data downgraded the quality rating to low for the statistically significant difference between the patients with and without endplate change for the prevalence of microbial colonization of disc. There was no publication bias regarding the funnel plot of included trials.

TABLE 3 Full text screening and risk of bias agreement

Agreement for full text screening	
	Examiners (1&2)
N (articles)	380
% agreement	90%
κ [CI (95%)]	0.87 [0.84–0.92]
Agreement for risk of bias	
N (questions)	200
% agreement	88%
κ [CI (95%)]	0.84 [0.78–0.89]

Note: κ = Kappa coefficient, CI = confidence intervals, *N* = number of questions.
Based-on number of questions asked per section * 25 articles selected.

3.6 | Involved organism

Previous studies have reported *Cutibacterium acnes* as the primarily involved pathogen in disc samples. This was confirmed based on the results of 23 studies[6,8,9,15,37–53,55,56]; there was evidence of *Cutibacterium acnes* in 22.2% of patients with lumbar disc herniation or/and lumbar disc degeneration (95% CI = 13.3%–32.5%; I^2 = 96.6%; p = 0.000) (Table 2 and Figure A4).

3.7 | Relationship between microbial colonization and clinical outcome

Zhou et al. (2015) reported that the rate of intervertebral discs containing the 16S rDNA gene was 25% in patients with sciatica and 9%

TABLE 4 Assessment of the methodological quality of observational studies according to the Newcastle-Ottawa Scale (NOS)

Author	Year	Country	Study type	Selection (/4)	Comparability (/2)	Outcome/ exposure (/3)	Total score (/9)
Stirling	2001	United Kingdom	Cohort	3	1	3	7
Ben-Galim	2006	Israel	Cohort	3	2	3	8
Carricajo	2007	France	Cohort	2	2	3	7
Wedderkopp	2009	Denmark	Cohort	3	2	3	8
Agarwal	2011	United States of America	Cohort	3	2	3	8
Arndt	2012	France	Cohort	3	2	3	8
Albert	2013	Denmark	Cohort	3	2	3	8
Zhou	2015	China	Cohort	3	2	3	8
Capoor	2016	United States of America	Cross-sectional	3	2	3	8
Li	2016	China	Cohort	3	2	3	8
Rigal	2016	France	Cohort	3	2	3	8
Aghazadeh	2017	Iran	Cohort	4	2	3	9
Capoor	2017	United States of America	Cohort	3	2	3	8
Rajasekaran	2017	India	Cohort	4	2	3	9
Tang	2018	China	Cohort	3	2	4	9
Yuan	2018	China	Cohort	4	2	3	9
Ahmed-Yahia	2019	France	Case control	3	2	3	8
Fritzell	2019	Sweden	Cohort	3	2	4	9
Rao	2019	Australia	Cohort	3	2	4	9
Salehpour	2019	Iran	Cohort	3	2	3	8
Tang	2019	China	Case control	3	2	4	9
Drago	2020	Italy	Cohort	3	2	2	7
Najafi	2020	Iran	Case control	3	2	3	8
Singh	2020	India	Cohort	3	2	2	7
Astur	2021	Brazil	Case control	3	2	4	9

Note: A study awarded seven or more stars was regarded as a high-quality study.

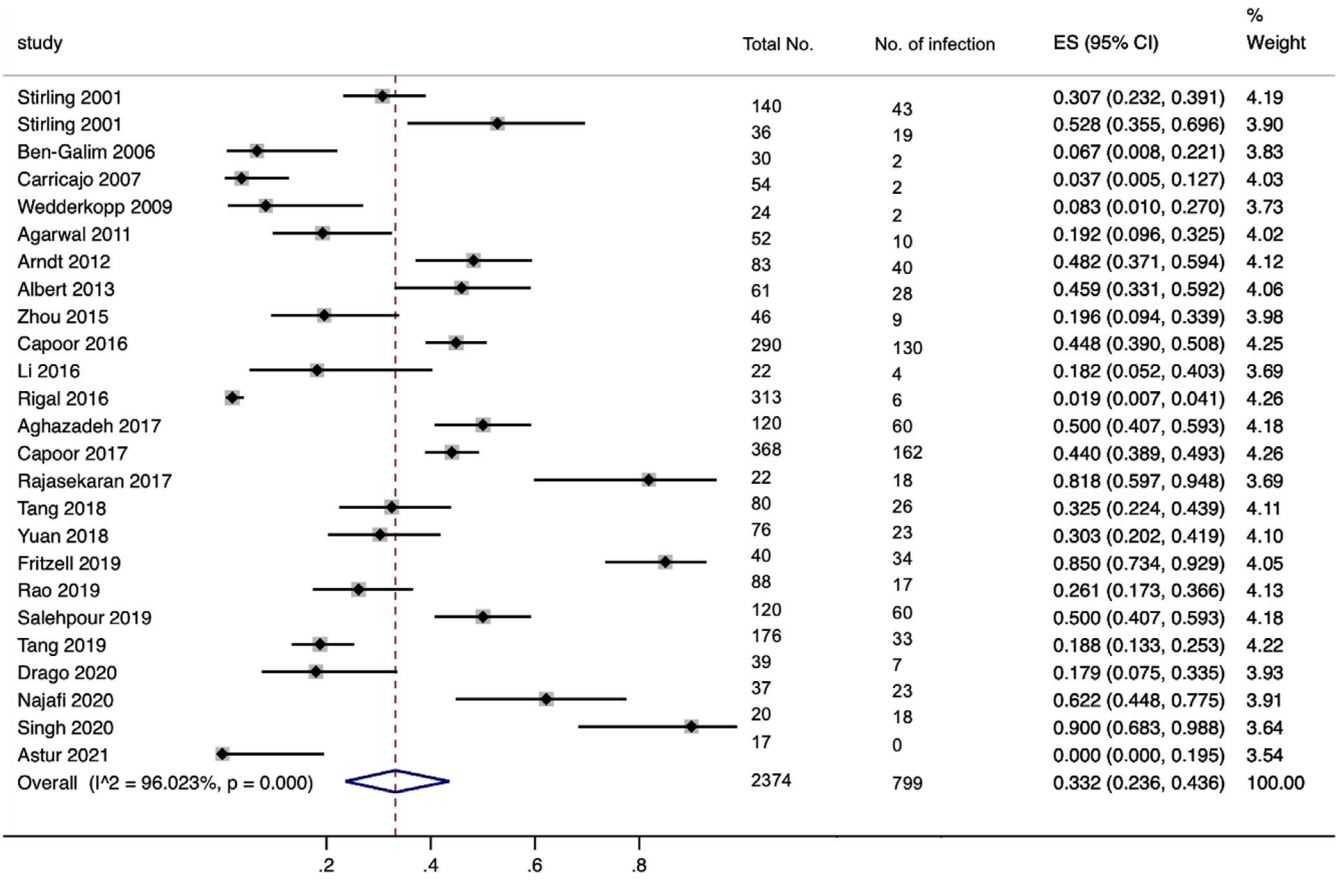

FIGURE 2 Forest plot for pooled proportion of bacterial microbiome in lumbar spine population. ES and CI indicate effect size and confidence interval, respectively.

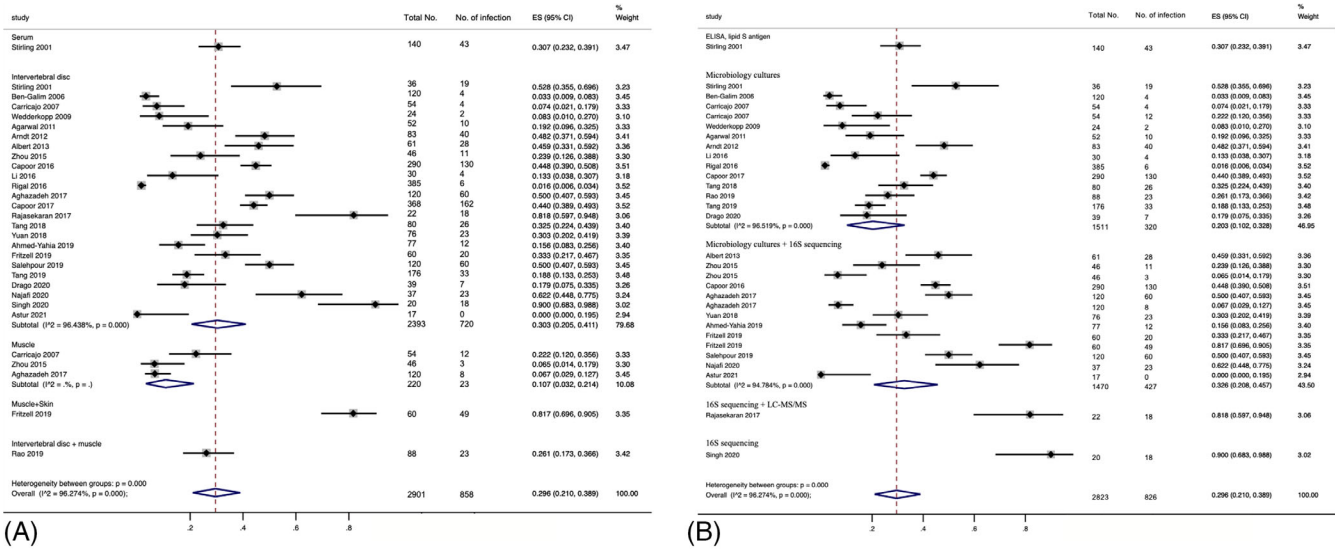

FIGURE 3 Forest plot for pooled proportion of bacterial microbiome in different source of samples (e.g., skin sample, intervertebral disc sample, muscle, and blood) from lumbar spine participants in (A). Forest plot for pooled proportion of bacterial microbiome by different testing method for samples from lumbar spine participants in (B). ES and CI indicate effect size and confidence interval, respectively. ELISA, enzyme-linked immunosorbent assay; LC–MS/MS, liquid chromatography–tandem mass spectrometry

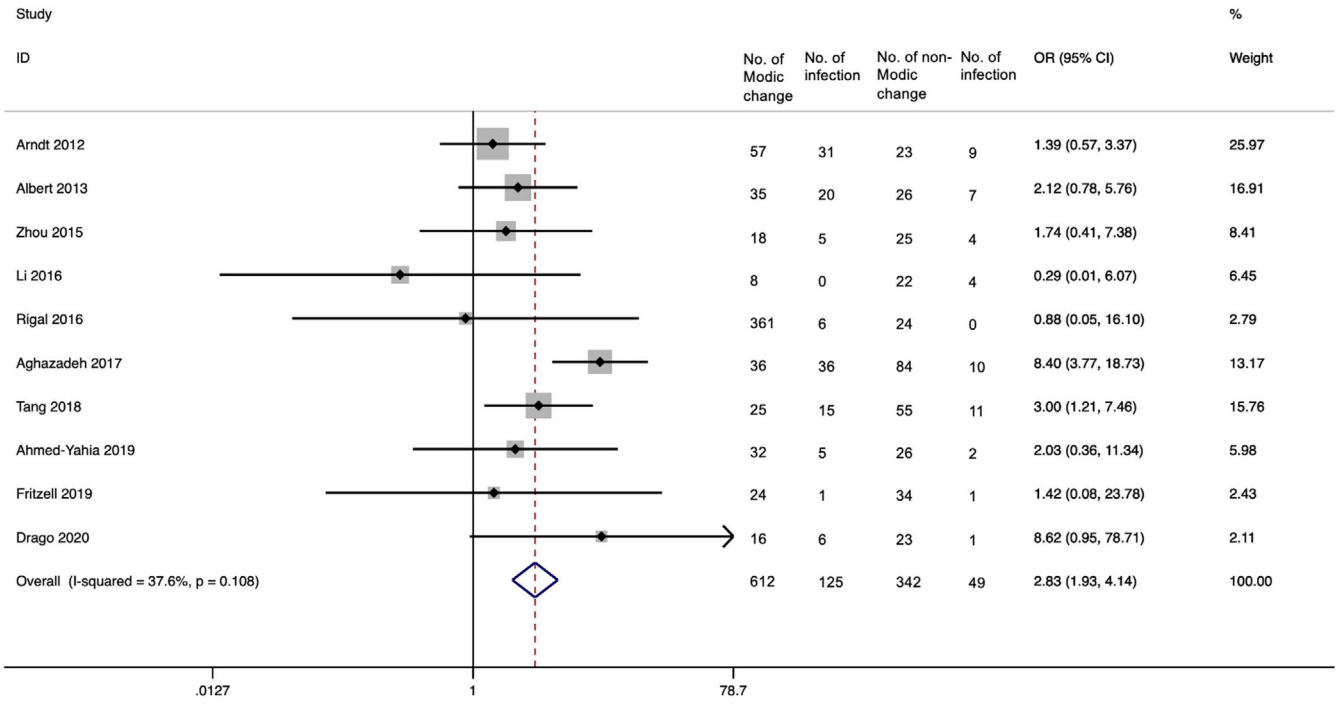

FIGURE 4 Forest plot for pooled proportion of bacterial microbiome in patients with versus without endplate change. OR and CI indicate odd ratio and confidence interval, respectively.

in patients without sciatica, although statistical significance was not reached.[41] Tang et al. (2018) showed that there was no significant association between microbial colonization of IVDs and patients' scores for VAS LBP and leg pain.[48] Najafi et al. (2020) also demonstrated that there was no significant difference between patients with positive and negative bacterial cultures and their corresponding VAS or level of disability based on ODI scores.[55]

4 | DISCUSSION

This is the first meta-analysis and systematic review addressing the impact of microbial colonization in different sampling and anatomical locations with respect to disc degeneration and endplate changes. We identified 2419 symptomatic patients in total with LBP and/or radicular pain from 25 eligible studies. The prevalence rate of infection was affected by the different sources of samples. This study also suggests that there is an association between microbial colonization of disc and the endplate changes.

4.1 | Gut-disc axis

A comparative study showed that microbial colonization in patients with healthy IVD differed from those with degenerative IVD and herniated IVD.[11] This study also showed the presence of 58 overlapping bacterial species between IVDs and gut, and 29 overlapping bacterial species between IVDs and skin using 16S rRNA sequencing. This bacterial landscape suggests that the IVD microbiome may have an interplay with the gut microbiome; the hypothesis is that the gut microbiome infiltrates the IVD environment and plays an important role in the development of IDD. The theory that microbiome dysbiosis may be a key cause of inflammation and IDD requires future validation in adequately powered, prospective registries/studies.

A recent review by Li et al. (2022) listed three potential mechanisms for the establishment of the gut-disc axis[23]:

1. The translocation of bacteria through the gut epithelial barrier into IVDs.
2. Bacterial regulatory action of the mucosal and systemic immune system.
3. Regulation of nutrient absorption and metabolite formation at the gut epithelium level.

4.2 | Contamination/colonization or Infection

The human microbiome has been considered an important component in regulating human health and disease states. 16S rRNA sequencing is the most widely used technique for analysis of microbiome diversity and can be used to characterize microbiota from patients with IVD disease. Previously published reviews strongly suggest that microbiome dysbiosis may play a role in the pathogenesis of IVD structural failure and in regulation and management of spinal pain.[23] Microbiome dysbiosis is an imbalance among different microbiota which can be affected by various factors including medical interventions,

diseases, diet, and the genetic makeup of the host. Dysbiosis disturbs the IVD microorganisms' diversity by reducing the richness of bacterial communities. Some published studies have hypothesized that contamination could be one of the potential reasons for causing the similar positive culture between disc material and skin flora.[38,57,58] In order to reduce the false positive rate from possible sampling contamination, samples should be obtained under strict sterile protocols and quarantined into sampling areas prior to being processed in a high-quality laboratory. In our review, we observed that the prevalence of microbial colonization was altered depending on the source of samples. The pooled incidence of bacterial presence in the lumbar spine was greater for the muscle and skin samples than the blood samples, which was in turn higher than the IVD samples. These findings, which demonstrate a higher rate of contaminant skin organisms for sampled areas that are in close proximity to the skin, suggesting a degree of confounding effect by sampling contamination.

4.3 | Relationship between microbial colonization and IDD

Although IDD is multifactorial, low virulence or anaerobic bacteria may be cofactors in uncontrolled low-grade inflammation in IDD.[13,59] The mechano-immunological and infectious pathways that lead to IDD all theoretically accelerate tissue damage in the disc. Previous published systematic reviews and observational studies suggest a higher prevalence of low virulence organisms in patients with disc disease.[6,13,50,59] However, the currently available evidence supporting the potential association between low virulence organisms and IDD is inadequate. The lack of control groups in eligible studies makes it challenging to draw conclusions on the association between low virulence organisms and IDD from this meta-analysis.

4.4 | Relationship between microbial colonization and endplate change

Endplate change as a common finding in patients with IDD was first classified by Modic in 1988 based on the signal change in MRI scans.[60] The change has a high specificity for IDD, discogenic LBP, and sciatica. Previous studies provided evidence to support the presence of inflammation in association with mechanical insults as contributors to the development of endplate change.[61] Interestingly, at present only a few studies have demonstrated a significant relationship between low virulence organisms and the presence of endplate change.[48,53] However, the influence of microbial colonization on endplate changes remains undocumented. Our meta-analysis and systematic review for the first time report a significant association between microbial colonization of disc and endplate change, which is in favor of a causative hypothesis.[48,53,62] Our meta-analysis also provides evidence that *Cutibacterium acnes* is the primary pathogen in the disc of patients with lumbar disc herniation or/and lumbar disc degeneration, which corroborates with published evidence in the field.[13,62]

Although an association between microbial colonization of disc and endplate change was found in our study, there is still ambiguity on the association between microbial colonization of disc and different classifications of endplate change. The wide variation in the classification of endplate change, insufficient data on definition of contamination, and differing sample sources between different studies make the endeavor of drawing conclusions from this meta-analysis and systematic review a difficult one. The findings of this meta-analysis and systematic review should be interpreted with some perspective as there was a relatively small sample size of available studies. Future RCTs with a large number of participants are warranted to investigate the role of microbiome dysbiosis in the pathogenesis of symptomatic IDD and endplate change.

4.5 | Microbial colonization and clinical outcome

In theory, microbiome dysbiosis and host responses to the microbiota could cause pathological bone development and involution, which may lead to IDD/endplate changes and LBP. The invasion of low virulence organisms into the IVDs dysregulates the local inflammatory response, which stimulates the secretion of inflammatory cytokines, and induces proinflammatory phenotypes of immune cells. Due to increased innervation of the degenerative IVDs, these cascade responses lead to pain amplification and the transmission of pain signals to peripheral afferent nerve fibers located in the dorsal root ganglia (DRG) and brain.[63]

In this study, we did not find a linear correlation between microbial colonization, LBP, and disability. One potential reason is that the studies we captured omitted patients who did not have clinical data on back pain available, therefore reducing our available sample size for analysis. Furthermore, a complementary review will be conducted a systematic review of the use of antibiotics for LBP.

4.6 | Limitations

Several methodological issues should be considered. First, the lack of RCTs may have reduced the statistical robustness. Second, due to lacking the benefit of randomization for the comparisons of trial-level characteristics, a meta-analysis of proportions only describes the observational rates across trials which cannot provide evidence for supporting the causal interpretation of findings. Third, variations in techniques used to identify microbes are a significant limitation of microbiome research in the field of LBP. Microbial cultures only provide quantitative results and do not provide functional annotations. Targeted 16s amplicon sequencing, on the other hand, would identify the bacterial load of the specific gene only as opposed to shotgun metagenomics which qualities all bacterial species. Therefore, it is unjust to compare all these methodologies on the same scale. This study design heterogeneity may have affected the type of low virulence organisms detected and their absolute quantity. Also, high-end imaging techniques should be supplemented with future studies for

validating the presence of specific bacterial types in a sample. Fourth, there is heterogeneity in the eligible studies due to wide variation in the definition of sampling contamination. Fifth, pharmacological therapy as a trial treatment for low virulence organisms would be preferable for future research. Finally, there is a lack of a standardized structured protocol to conduct the sample collection, storage, delivery, and testing in included studies. Guidelines for collecting and measuring different samples and better standardization of the definition of contamination are needed.

5 | CONCLUSION

This study demonstrates the prevalence of microbial colonization in different tissue and blood samples in patients with disc structural failure. There seems to be a significant association between microbial colonization of disc and endplate changes. Currently, there is insufficient evidence to implicate low virulence organisms as a cause of IDD, LBP, and disability. Standardizing sample collection, standardizing testing methods, and having a consensus on the definition of sampling contamination, with consistent reporting of multicenter registry studies will allow more meaningful analysis in the future.

AUTHOR CONTRIBUTIONS

The authors of this paper all participated in the study design. All authors have read and approved this version of the article, and due care has been taken to ensure the integrity of the work. The material of this article is original research, and no part of this paper has been previously published. The material has also not been submitted for publication elsewhere while under consideration. No conflict of interest exists in the submission of this manuscript. All authors have the appropriate permissions and rights to the reported data.

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CONFLICT OF INTEREST

Ashish Diwan is an Editorial Board member of JOR Spine and a co-author of this article. To minimize bias, they were excluded from all editorial decision-making related to the acceptance of this article for publication. [Correction added on 22 June 2023, after first online publication: Conflict of Interest statement was revised]

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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REFERENCES

1. Hartvigsen J, Hancock MJ, Kongsted A, et al. What low back pain is and why we need to pay attention. *Lancet*. 2018;*391*(10137):2356-2367. doi:10.1016/s0140-6736(18)30480-x
2. Hurwitz EL, Randhawa K, Yu H, Cote P, Haldeman S. The global spine care initiative: a summary of the global burden of low back and neck pain studies. *Eur Spine J*. 2018;*27*(suppl 6):796-801. doi:10.1007/s00586-017-5432-9
3. Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? *Spine (Phila PA 1976)*. 2006;*31*(18):2151-2161. doi:10.1097/01.brs.0000231761.73859.2c
4. Capoor MN, Birkenmaier C, Wang JC, et al. A review of microscopy-based evidence for the association of *Propionibacterium acnes* biofilms in degenerative disc disease and other diseased human tissue. *Eur Spine J*. 2019;*28*(12):2951-2971. doi:10.1007/s00586-019-06086-y
5. Boer CG, Radjabzadeh D, Medina-Gomez C, et al. Intestinal microbiome composition and its relation to joint pain and inflammation. *Nat Commun*. 2019;*10*(1):4881. doi:10.1038/s41467-019-12873-4
6. Stirling A, Worthington T, Rafiq M, Lambert PA, Elliott TSJ. Association between sciatica and *Propionibacterium acnes*. *Lancet*. 2001;*357*(9273):2024-2025. doi:10.1016/s0140-6736(00)05109-6
7. Ohtori S, Inoue G, Miyagi M, Takahashi K. Pathomechanisms of discogenic low back pain in humans and animal models. *Spine J*. 2015;*15*(6):1347-1355. doi:10.1016/j.spinee.2013.07.490
8. Arndt J, Charles YP, Koebel C, Bogorin I, Steib JP. Bacteriology of degenerated lumbar intervertebral disks. *J Spinal Disord Tech*. 2012;*25*(7):E211-E216. doi:10.1097/BSD.0b013e318269851a
9. Albert HB, Lambert P, Rollason J, et al. Does nuclear tissue infected with bacteria following disc herniations lead to Modic changes in the adjacent vertebrae? *Eur Spine J*. 2013;*22*(4):690-696. doi:10.1007/s00586-013-2674-z
10. Kapoor SG, Huff J, Cohen SP. Systematic review of the incidence of discitis after cervical discography. *Spine J*. 2010;*10*(8):739-745.
11. Rajasekaran S, Soundararajan DCR, Tangavel C, et al. Human intervertebral discs harbour a unique microbiome and dysbiosis determines health and disease. *Eur Spine J*. 2020;*29*(7):1621-1640. doi:10.1007/s00586-020-06446-z
12. Manniche C, O'Neill S. New insights link low-virulent disc infections to the etiology of severe disc degeneration and Modic changes. *Future Sci OA*. 2019;*5*(5):FSO389. doi:10.2144/fsoa-2019-0022
13. Ganko R, Rao PJ, Phan K, Mobbs RJ. Can bacterial infection by low virulent organisms be a plausible cause for symptomatic disc degeneration? A systematic review. *Spine (Phila PA 1976)*. 2015;*40*(10):E587-E592. doi:10.1097/BRS.0000000000000832
14. Chen Z, Zheng Y, Yuan Y, et al. Modic changes and disc degeneration caused by inoculation of *Propionibacterium acnes* inside intervertebral discs of rabbits: a pilot study. *Biomed Res Int*. 2016;*2016*:9612437.
15. Li B, Dong Z, Wu Y, et al. Association between lumbar disc degeneration and *Propionibacterium acnes* infection: clinical research and preliminary exploration of animal experiment. *Spine*. 2016;*41*(13):E764-E769.
16. Lin Y, Jiao Y, Yuan Y, et al. *Propionibacterium acnes* induces intervertebral disc degeneration by promoting nucleus pulposus cell

apoptosis via the TLR2/JNK/mitochondrial-mediated pathway article. *Emerg Microbes Infect*. 2018;7(1):1.

17. Tang G, Han X, Lin Z, et al. *Propionibacterium acnes* accelerates intervertebral disc degeneration by inducing pyroptosis of nucleus pulposus cells via the ROS-NLRP3 pathway. *Oxid Med Cell Longev*. 2021; *2021*:4657014.
18. Biver E, Berenbaum F, Valdes AM, et al. Gut microbiota and osteoarthritis management: an expert consensus of the European society for clinical and economic aspects of osteoporosis, osteoarthritis and musculoskeletal diseases (ESCEO). *Ageing Res Rev*. 2019;*55*:100946. doi:10.1016/j.arr.2019.100946
19. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med*. 2015;*21*(8):895-905. doi:10.1038/nm.3914
20. Asquith M, Sternes PR, Costello ME, et al. HLA alleles associated with risk of ankylosing spondylitis and rheumatoid arthritis influence the gut microbiome. *Arthritis Rheumatol*. 2019;*71*(10):1642-1650. doi:10.1002/art.40917
21. Tajik N, Frech M, Schulz O, et al. Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat Commun*. 2020;*11*(1):1995. doi:10.1038/s41467-020-15831-7
22. Vijay A, Valdes AM. Role of the gut microbiome in chronic diseases: a narrative review. *Eur J Clin Nutr*. 2022;*76*(4):489-501. doi:10.1038/s41430-021-00991-6
23. Li W, Lai K, Chopra N, Zheng Z, Das A, Diwan AD. Gut-disc axis: a cause of intervertebral disc degeneration and low back pain? *Eur Spine J*. 2022;*31*(4):917-925. doi:10.1007/s00586-022-07152-8
24. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;*6*(7):e1000097. doi:10.1371/journal.pmed.1000097
25. Zhang YH, Zhao CQ, Jiang LS, Chen XD, Dai LY. Modic changes: a systematic review of the literature. *Eur Spine J*. 2008;*17*(10):1289-1299. doi:10.1007/s00586-008-0758-y
26. Sharma A, Lancaster S, Bagade S, Hildebolt C. Early pattern of degenerative changes in individual components of intervertebral discs in stressed and nonstressed segments of lumbar spine: an in vivo magnetic resonance imaging study. *Spine (Phila PA 1976)*. 2014;*39*(13):1084-1090. doi:10.1097/BRS.0000000000000265
27. Suthar P, Patel R, Mehta C, Patel N. MRI evaluation of lumbar disc degenerative disease. *J Clin Diagn Res*. 2015;*9*(4):TC04-TC09. doi:10.7860/JCDR/2015/11927.5761
28. Taher F, Essig D, Lebl DR, et al. Lumbar degenerative disc disease: current and future concepts of diagnosis and management. *Adv Orthop*. 2012;*2012*:970752. doi:10.1155/2012/970752
29. Urrutia J, Besa P, Campos M, et al. The Pfirrmann classification of lumbar intervertebral disc degeneration: an independent inter- and intra-observer agreement assessment. *Eur Spine J*. 2016;*25*(9):2728-2733. doi:10.1007/s00586-016-4438-z
30. Williams FM, Manek NJ, Sambrook PN, Spector TD, Macgregor AJ. Schmorl's nodes: common, highly heritable, and related to lumbar disc disease. *Arthritis Rheum*. 2007;*57*(5):855-860. doi:10.1002/art.22789
31. Sterne JAC, Savovic J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;*366*:l4898. doi:10.1136/bmj.l4898
32. Wells GA, Shea B, O'Connell D, et al. *The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses*. 2011.
33. Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of reporting of meta-analyses. *Lancet*. 1999;*354*(9193):1896-1900. doi:10.1016/s0140-6736(99)04149-5
34. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA*. 2000; *283*(15):2008-2012. doi:10.1001/jama.283.15.2008
35. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Int J Surg*. 2021;*88*:105906. doi:10.1016/j.ijsu.2021.105906
36. Balshem H, Helfand M, Schunemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol*. 2011;*64*(4):401-406. doi:10.1016/j.jclinepi.2010.07.015
37. Ben-Galim P, Rand N, Giladi M, et al. Association between sciatica and microbial infection: true infection or culture contamination? *Spine (Phila PA 1976)*. 2006;*31*(21):2507-2509. doi:10.1097/01.brs.0000238657.13263.b2
38. Carricajo A, Nuti C, Aubert E, et al. Propionibacterium acnes contamination in lumbar disc surgery. *J Hosp Infect*. 2007;*66*(3):275-277. doi:10.1016/j.jhin.2007.04.007
39. Wedderkopp N, Thomsen K, Manniche C, Kolmos HJ, Secher Jensen T, Leboeuf YC. No evidence for presence of bacteria in modic type I changes. *Acta Radiol (Stockholm, Sweden: 1987)*. 2009;*50*(1):65-70.
40. Agarwal V, Golish SR, Alamin TF. Bacteriologic culture of excised intervertebral disc from immunocompetent patients undergoing single level primary lumbar microdiscectomy. *J Spinal Disord Tech*. 2011; *24*(6):397-400. doi:10.1097/BSD.0b013e3182019f3a
41. Zhou Z, Chen Z, Zheng Y, et al. Relationship between annular tear and presence of *Propionibacterium acnes* in lumbar intervertebral disc. *Eur Spine J*. 2015;*24*(11):2496-2502. doi:10.1007/s00586-015-4180-y
42. Rigal J, Thelen T, Byrne F, et al. Prospective study using anterior approach did not show association between Modic 1 changes and low grade infection in lumbar spine. *Eur Spine J*. 2016;*25*(4):1000-1005. doi:10.1007/s00586-016-4396-5
43. Capoor MN, Ruzicka F, Machackova T, et al. Prevalence of *Propionibacterium acnes* in intervertebral discs of patients undergoing lumbar microdiscectomy: a prospective cross-sectional study. *PLoS One*. 2016;*11*(8):e0161676. doi:10.1371/journal.pone.0161676
44. Capoor MN, Ruzicka F, Schmitz JE, et al. Propionibacterium acnes biofilm is present in intervertebral discs of patients undergoing microdiscectomy. *PLoS One*. 2017;*12*(4):e0174518. doi:10.1371/journal.pone.0174518
45. Rajasekaran S, Tangavel C, Aiyer SN, et al. ISSLS prize in clinical science 2017: is infection the possible initiator of disc disease? An insight from proteomic analysis. *Eur Spine J*. 2017;*26*(5):1384-1400. doi:10.1007/s00586-017-4972-3
46. Aghazadeh J, Salehpour F, Ziaeii E, et al. Modic changes in the adjacent vertebrae due to disc material infection with *Propionibacterium acnes* in patients with lumbar disc herniation. *Eur Spine J*. 2017; *26*(12):3129-3134.
47. Yuan Y, Chen Y, Zhou Z, et al. Association between chronic inflammation and latent infection of *Propionibacterium acnes* in non-pyogenic degenerated intervertebral discs: a pilot study. *Eur Spine J*. 2018; *27*(10):2506-2517. doi:10.1007/s00586-017-5363-5
48. Tang G, Wang Z, Chen J, Zhang Z, Qian H, Chen Y. Latent infection of low-virulence anaerobic bacteria in degenerated lumbar intervertebral discs. *BMC Musculoskelet Disord*. 2018;*19*(1):445. doi:10.1186/s12891-018-2373-3
49. Salehpour F, Aghazadeh J, Mirzaei F, Ziaeii E, Alavi SAN. *Propionibacterium acnes* infection in disc material and different antibiotic susceptibility in patients with lumbar disc herniation. *Int J Spine Surg*. 2019; *13*(2):146-152. doi:10.14444/6019
50. Fritzell P, Welinder-Olsson C, Jonsson B, et al. Bacteria: back pain, leg pain and Modic sign-a surgical multicentre comparative study. *Eur Spine J*. 2019;*28*(12):2981-2989. doi:10.1007/s00586-019-06164-1
51. Tang G, Chen Y, Chen J, Wang Z, Jiang W. Higher proportion of low-virulence anaerobic bacterial infection in young patients with

intervertebral disc herniation. *Exp Ther Med*. 2019;*18*(4):3085-3089. doi:10.3892/etm.2019.7910
52. Ahmed-Yahia S, Decousser JW, Flouzat-Lachaniette CH, et al. Is the discopathy associated with Modic changes an infectious process? Results from a prospective monocenter study. *PLoS One*. 2019;*14*(8): e0221030. doi:10.1371/journal.pone.0221030
53. Drago L, Romano CL, Cecchinato R, et al. Are modic type 2 disc changes associated with low-grade infections? A pilot study. *J Neurosurg Sci*. 2020;*64*(3):243-246. doi:10.23736/S0390-5616.17.03997-2
54. Singh S, Siddhlingeswara GI, Rai A, Iyer RD, Sharma D, Surana R. Correlation between Modic changes and bacterial infection: a causative study. *Int J Spine Surg*. 2020;*14*(5):832-837. doi:10.14444/7118
55. Najafi S, Mahmoudi P, Bassampour SA, Shekarchi B, Soleimani M, Mohammadimehr M. Molecular detection of propionibacterium acnes in biopsy samples of intervertebral disc with modic changes in patients undergoing herniated disc surgery. *Iran J Microbiol*. 2020; *12*(6):516-521.
56. Astur N, Maciel BFB, Doi AM, et al. Next generation sequencing (NGS) to determine microbiome of herniated intervertebral disc. *Spine J*. 2021;*22*:389-398. doi:10.1016/j.spinee.2021.09.005
57. Rao PJ, Maharaj M, Tang K, et al. Degenerate-disc infection study with contaminant control (DISC): application of a proposed histological scoring system. *J Clin Neurosci*. 2019;*66*:87-91. doi:10.1016/j.jocn.2019.05.016
58. Rao PJ, Maharaj M, Chau C, et al. Degenerate-disc infection study with contaminant control (DISC): a multicenter prospective case-control trial. *Spine J*. 2020;*20*(10):1544-1553. doi:10.1016/j.spinee.2020.03.013
59. Sadowska A, Touli E, Hitzl W, et al. Inflammaging in cervical and lumbar degenerated intervertebral discs: analysis of proinflammatory cytokine and TRP channel expression. *Eur Spine J*. 2018;*27*(3):564-577. doi:10.1007/s00586-017-5360-8
60. Modic MT, Steinberg PM, Ross JS, Masaryk TJ, Carter JR. Degenerative disk disease: assessment of changes in vertebral body marrow with MR imaging. *Radiology*. 1988;*166*(1 Pt 1):193-199. doi:10.1148/radiology.166.1.3336678
61. Albert HB, Sorensen JS, Christensen BS, Manniche C. Antibiotic treatment in patients with chronic low back pain and vertebral bone edema (Modic type 1 changes): a double-blind randomized clinical controlled trial of efficacy. *Eur Spine J*. 2013;*22*(4):697-707. doi:10.1007/s00586-013-2675-y
62. Iyer S, Louie PK, Nolte MT, Phillips FM. The relationship between low-grade infection and degenerative disk disease: a review of basic science and clinical data. *J Am Acad Orthop Surg*. 2019;*27*(14):509-518. doi:10.5435/JAAOS-D-18-00257
63. Lyu FJ, Cui H, Pan H, et al. Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions. *Bone Res*. 2021;*9*(1):7. doi:10.1038/s41413-020-00125-x

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APPENDIX A

Tables A1 and A2

TABLE A1 Search strategy

1. low back pain OR back pain OR lumbago OR back ache OR spinal stenosis OR canal stenosis OR lumbar stenosis OR lateral stenosis OR foramina stenosis OR neurogenic claudication OR radicular pain OR radiculopathy OR radicular pain OR spondylolisthesis OR spondylosis OR sciatica OR intervertebral disc displacement OR referred pain OR spinal nerve roots OR neurologic signs OR radiate pain OR radiate symptoms OR paresthesia OR numbness OR neck pain OR cervical pain OR neck ach OR neck disability OR disc degeneration OR Modic changes OR endplate changes
2. bacterial infection OR Propionibacterium acnes OR pathophysiology OR antibiotics OR antibiotics OR cefazolin OR archaea OR fungi OR protists OR algae OR microbiome OR microbiota OR bacteriome OR archaeoma OR mycobiome OR virome
3. 1 and 2

Filter: Human, English, Clinical study

TABLE A2 Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for rating the quality of estimates of treatment effect

GRADE assessment
Ratings **High quality** (⊕⊕⊕⊕)—We are very confident that the true effect lies close to that of the estimate of the effect **Moderate quality** (⊕⊕⊕O)—We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different **Low quality** (⊕⊕OO)—Our confidence in the effect estimates is limited: The true effect may be substantially different from the estimate of the effect **Very low quality** (⊕OOO)—We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect
Down rating • The quality rating may be rated down by −1 (serious concern) or − 2 (very serious concern) for the following reasons ○ Risk of bias (such as failure to conceal random allocation or blind participants in randomized controlled trials or failure to adequately control for confounding in observational studies) ○ Inconsistency (such as heterogeneity of estimates of effects across trials) ○ Indirectness (such as surrogate outcomes, study populations or interventions that differ from those of interest, or intransitivity) ○ Imprecision (for example, 95% confidence intervals are wide and include or are close to null effect) ○ Publication bias
Up rating • Rating up is typically applied only to observational studies; the most common reason is for a large or very large effect seen over a short period of time and altering a clear downward trajectory

Note: In the GRADE approach, RCTs start as high quality evidence and cohort studies as low quality evidence.

Figures A1–A4

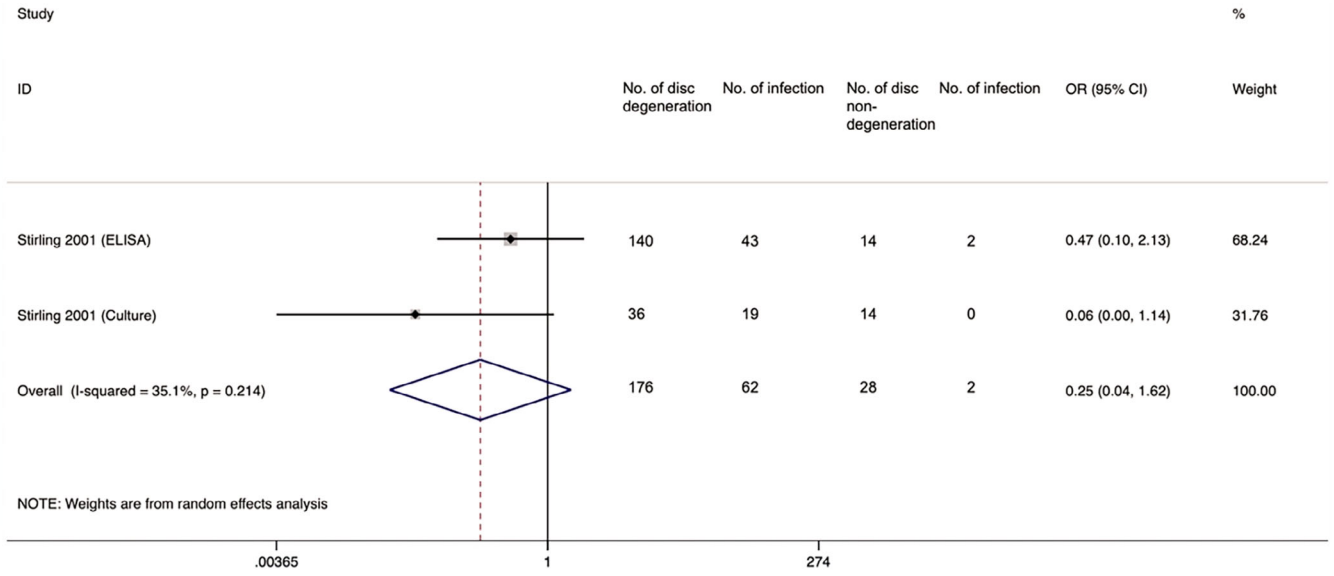

FIGURE A1 Forest plot for disc infection prevalence in patients with versus without intervertebral disc degeneration. Subgroup analysis based on different location of spine. OR and CI indicate odd ratio and confidence interval, respectively. ELISA, enzyme-linked immunosorbent assay

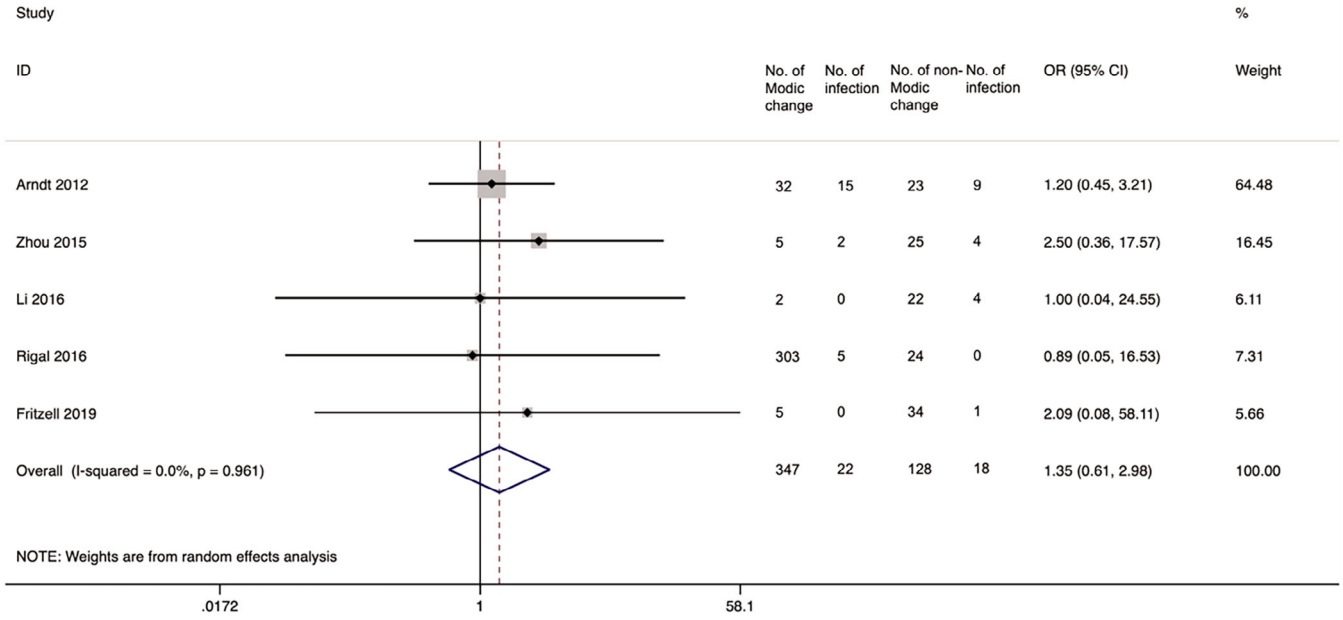

FIGURE A2 Forest plot of disc infection prevalence in patients with type I Modic change versus without endplate change. Subgroup analysis based on different location of spine. OR and CI indicate odd ratio and confidence interval, respectively.

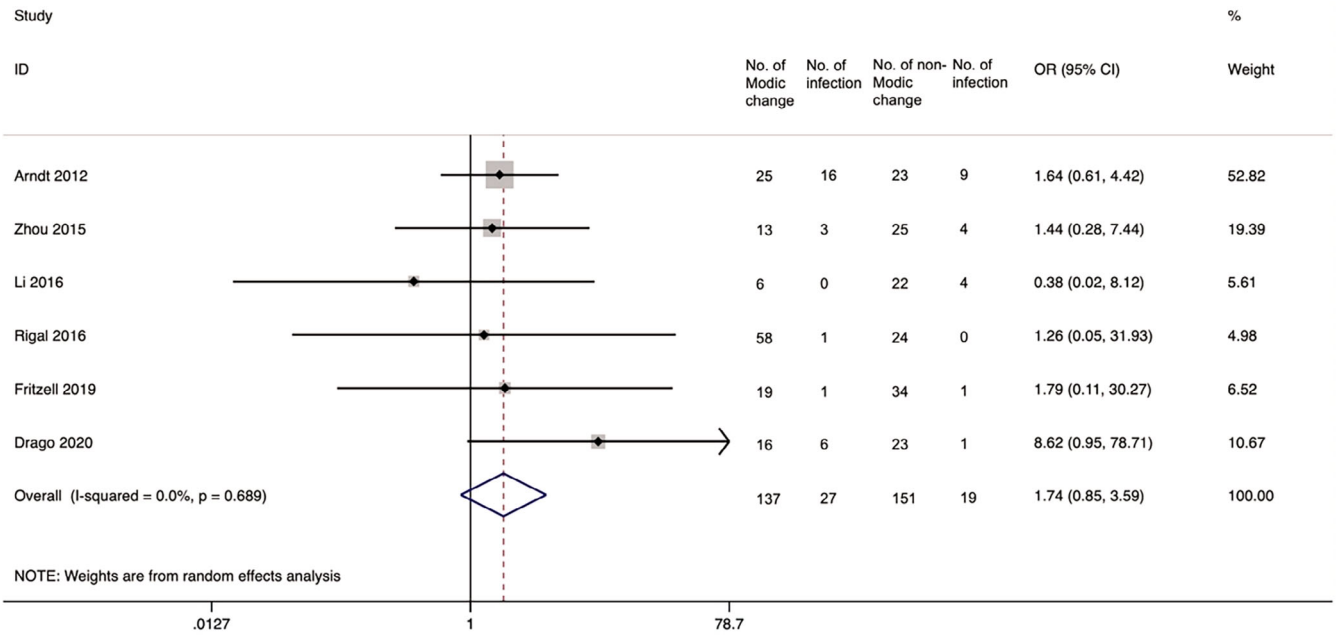

FIGURE A3 Forest plot of disc infection prevalence in patients with type II Modic change versus without endplate change. Subgroup analysis based on different location of spine. OR and CI indicate odd ratio and confidence interval, respectively.

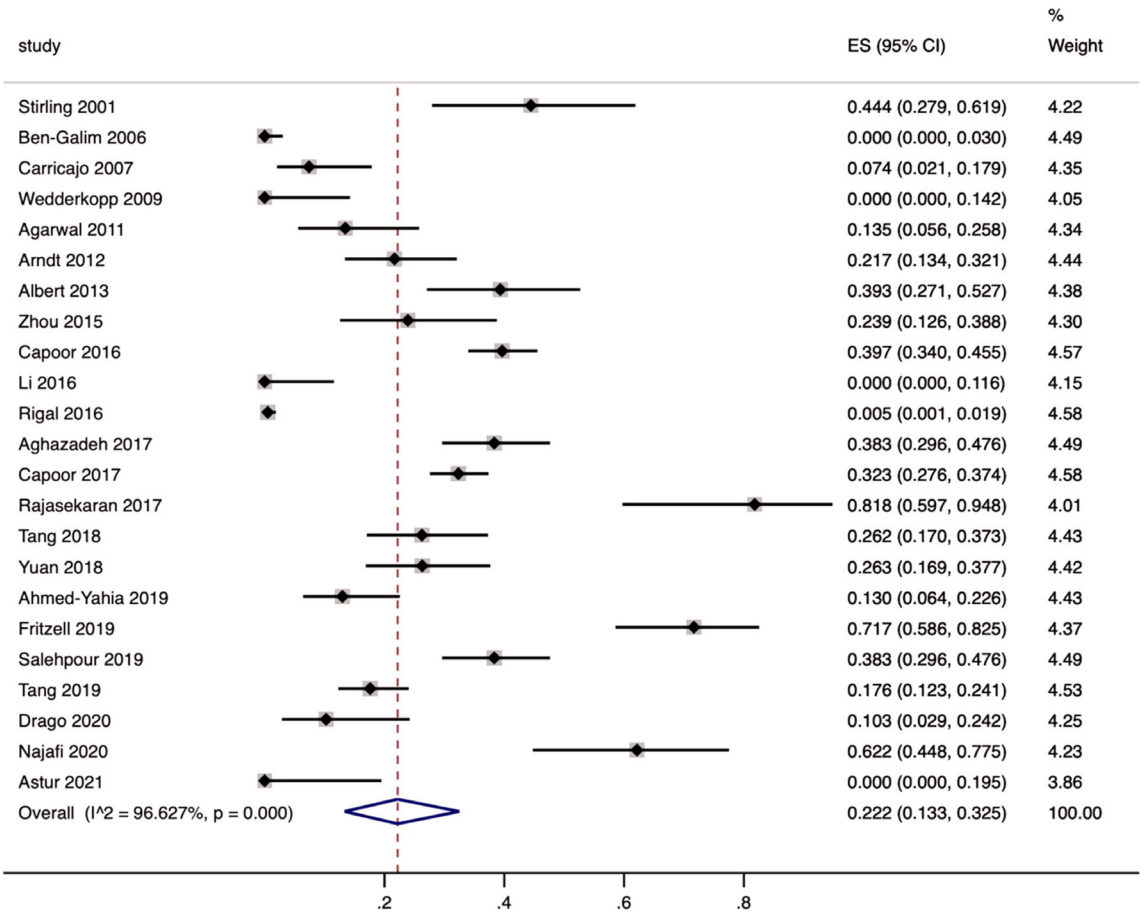

FIGURE A4 Forest plot for pooled proportion of infected discs having evidence of Propionibacterium acnes in lumbar spine. ES and CI indicate effect size and confidence interval, respectively.