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*SYSTEMATIC REVIEW*THE ROLE OF BACTERIOLOGY IN THE DEVELOPMENT OF ANAL FISTULA: A SYSTEMATIC
REVIEW OF THE LITERATUREAuthors

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THE ROLE OF BACTERIOLOGY IN THE DEVELOPMENT OF ANAL FISTULA: A SYSTEMATIC REVIEW OF THE LITERATURE

ABSTRACT

BACKGROUND: A cryptoglandular anorectal abscess is a collection of purulent material close to anus and anal fistula development afterwards it is a very frequent outcome after its drainage. It has been considered that Escherichia coli and Bacteroides fragilis reside in gastrointestinal tract and can infect anal glands appearing involved in both entities. However, disparities in observational studies and recent scientific evidence, this consideration have to be revisited. Understanding the microbiology of anorectal abscess and anal fistulae is crucial for effective management and treatment.

OBJECTIVE: Analyse the specific presence and role of bacteria in the development of anal fistula.

DATA SOURCES: MEDLINE database via PubMed, EMBASE, COCHRANE library and Google Scholar.

STUDY SELECTION: Articles reporting microbiology of cryptoglandular anorectal abscess and anal fistula developed from anorectal abscess in humans.

INTERVENTIONS: Search and review of articles related to the aim of research and describe results.

MAIN OUTCOMES MEASURES: Evaluate presence of bacteriology on cultures of anorectal abscesses or in patients who developed anal fistula. Additional goals were identify characteristics of patients and assess clinical results according to bacteriology isolated.

RESULTS: A total of 22 articles for perianal abscess and 7 for anal fistula describing microbiologic outcomes were analysed for this review. Men were the most prevalent in both cases. *Escherichia coli* and *Bacteroides* genus were the most isolated microorganisms in abscess and fistulas. Although in the latest it seemed to be just a non-constant finding. *Bilophila wadsworthia* was a new isolated bacteria in abscesses and *Rothia* was the remarkable new one in fistulas regarding new molecular techniques.

LIMITATIONS: Heterogeneity between recruited studies without an appropriate analysis by groups regarding associated pathologies.

CONCLUSIONS: *Escherichia coli* and *Bacteroides* are intestinal commensal bacterias involved in perianal abscess that are not seem to be related to fistula formation. New insights about underlying pro-inflammatory triggers like bacterial remnants/products or own inflammation residues should be taken into account in order to explain the fistulization process.

PROSPERO NUMBER: CRD42024548060

THE ROLE OF BACTERIOLOGY IN THE DEVELOPMENT OF ANAL FISTULA: A SYSTEMATIC REVIEW OF THE LITERATURE

INTRODUCTION

An anorectal abscess is a collection of purulent material near the anus or rectum. It can drain through an opening in the perianal skin or rectal mucosa, potentially leading to a fistula. Most anorectal abscesses have a cryptoglandular origin (90% to 97%). Fistula formation is the primary complication after drainage.

Regarding the most common germs associated with anal abscesses, they include various bacteria such as *Escherichia coli*, *Bacteroides fragilis*, and *Streptococcus* species. These organisms can infect the anal glands and lead to abscess formation. *Escherichia coli* (*E. coli*) is a frequent culprit in anal abscesses. It normally resides in the intestines but can cause infection when it enters the anal glands or surrounding tissues. Additionally, *Bacteroides fragilis*, a common anaerobic bacterium found in the gastrointestinal tract can contribute to abscess formation. Various *Streptococcus* species, such as *Streptococcus milleri*, may also play a role in abscess development. Proper diagnosis and treatment are crucial to prevent complications.

Anal fistulae are abnormal tunnels or tracts that connect the anal canal or rectum to the skin near the anus. They typically arise from cryptoglandular infection of the anal glands, which lie within the intersphincteric space. Microorganisms involved in anal fistula development also include *Escherichia coli*, *Enterococcus* spp., and *Bacteroides fragilis*. Interestingly, chronic inflammation in anal fistulae does not seem to be solely due to excessive numbers of organisms or unusual types of organisms.

Understanding the microbiology of anorectal abscess and anal fistulae is crucial for effective management and treatment. However, there is an increasing new techniques for the study of the presence and the effect of bacteria in the development of anal fistula during follow-up of anorectal abscess. Therefore, the aim of this systematic review was to analyse the specific presence and role of bacteria in the development of anal fistula.

METHODS

A systematic review was performed following a detailed protocol according to the PRISMA checklist and it was registered in the PROSPERO registry under ID: CRD42024548060

Search strategy and information sources:

A literature search published was conducted using the MEDLINE database via PubMed, EMBASE, and COCHRANE library and a manual search from Google Scholar. The keywords combinations using the MeSH terms included anal abscess OR rectal abscess OR anorectal abscess OR perianal abscess OR anorectal sepsis OR anal fistula OR rectal fistula OR anorectal fistula OR perianal fistula AND bacteria OR microbiology OR culture.

Relevant articles from reviewed citations or a secondary articles that were identified, were also retrieved. Two reviewers' evaluated titles and abstracts of the articles focusing on bacteriology in anorectal abscess, anal fistula or development from anorectal abscess to anal fistula. A third reviewer solved any discrepancies about inclusion. Consequently, a full-text paper retrieve and its evaluation had been performed.

Inclusion and exclusion criteria:

Inclusion criteria: Articles reporting microbiology of cryptoglandular anorectal abscess and of anal fistula developed from this kind of anorectal abscesses in humans.

Exclusion criteria: Articles reporting solely cases without follow-up. Articles in a language other than English or Spanish were also excluded. We excluded studies made entirely in children and, in case of fistulas, we excluded studies in Crohn's patients.

Outcomes:

The primary endpoint of interest was to evaluate the presence of specific bacteriology on cultures of anorectal abscess or in patients who developed anal fistula. Additional secondary goals included identify characteristics of patients and assess clinical results according to bacteriology isolated. Articles and their information were classified in two of the endpoints: anorectal abscess or anal fistula development.

Quality Methodology assessment:

The quality of the retrieved human trials was assessed with a validated methodology quality score (MINCIR score) ¹. Two investigators completed the quality assessment independently and blinded to each other's result. Discrepancy between evaluations was solved by a third reviewer.

RESULTS

A total of 147 articles were found following the key words mentioned in order to recruit perianal abscess microbiology data. 34 abstracts of these was selected concerning the research target. ~~So, we excluded 1 article in German language, 1 in Chinese, 6 without the required data and 4 made in children.~~ There were 10 studies that included IBD patients, one of them with until 10% of cases which was the highest percentage included, so, these were also included considering it like a low rate of sample affectation. There were 3 studies including children but in a very low percentage (<10%), hence were included too.

For perianal fistula, the research yielded a total of 86 articles in which 14 abstracts were initially chosen. ~~There were 2 articles in Chinese, 1 without enough data and 4 studies made entirely with Cronh patients, so these were excluded.~~

Finally, a total of 22 articles for perianal abscess and 7 for anal fistula describing microbiologic outcomes were analysed for this revision. [Figure 1 y 2](#)

To get rates and frequencies we review the culture outcomes in each article regarding the total of cultured samples taken or total patients for simple cultures and regarding the total of isolated specimens for polymicrobial cultures or in genomic sequencing's cases.

Regarding the abscess's microbiology, 12 prospective serie of cases and 10 retrospective studies were collected. Men were the most prevalent part of the sample with high difference except in 1 article that women were the most of them ². 13 articles described

abscesses type being the most frequent the perianal location, follows by interesfinteric and ischiorrectal concerning Park's description. There were performed a conventional cultures in all reviewed studies, 5 of them used a simple culture identifying wide bacterial groups and another one added a genomic sequencing to the investigation.

[Table 1](#)

Enteric bacteria were widely described in all studies except in Narayanan's study who describe high percentages of skin flora cultured. *Escherichia coli* was the most isolated microorganism in 11 of 22 studies until 81% of isolated bacterias (Sheng-Fang et al) nearly followed by *Bacteroides* group that even was the most isolated bacteria over *Escherichia coli* in 5 studies up to 63% follows Sheng-Fang, specially *Bacteroides fragilis*. Another important findings were *Staphylococcus aureus* with a generally low proportion up to 32% in Abdul's study, *Streptococcus* groups from skin flora and *Peptostreptococcus*, generally lower than 20% of cultures.

Technological advantages using genomic sequencing was usefull in one study (Hong et al) in which *Bilophila wadsworthia* and *Bacteroides fragilis* appeared isolated in more patients (71% and 57% respectively) meanwhile, regarding the total amount of isolated specimens, *Escherichia coli* and *Bacteroides fragilis* were the most predominat bacterias (39% and 26% respectively) and *Bilophila wadsworthia* only 2%. As well as, the conventional culture made in their same patients yielded *Escherichia coli* as the most remarkable one (43%). In addition, there were 14 studies in which a percentage of no bacterial growth was descibed, being the Hong's study where it was reported up to 43% of negative conventional cultures despite their genomic sequencing results was positive in 100% of cases. [Table 1](#)

For perianal fistula's microbiology we recruited 7 prospective serie of cases studies with a wide proportion of male gender compared with women; and more frequently made with transinteric and interesinteric fistulas. Four studies performed conventional cultures for aerobic and anaerobic specimens with one of them using complementary rRNA sequencing (Van Onkelen et al). One study employed conventional cultures for aerobic specimens and PCR amplification for anaerobic's one, another one used fluorescence in situ hybridization (FISH) with a rRNA sequencing in evaluation of specific germs and the last one used a rDNA sequencing for quantify all possible bacteria specimens. Three studies reported percentages of no bacterial isolation as the Tozer's study which reported up to 94,4% through FISH technique and specific described sampling. Van Onkelen's study does not identified bacterial growth in 10% of conventional cultures too, but through rRNA sequencing these were negative in 90%.

[Table 2](#)

Escherichia coli was the most isolated germ in 4/7 studies and the only one in the Van Onkelen's study made with rRNA sequencing. Tozer et al used FISH technique with specific target to Bacteroides, Bifidobacteria, Escherichia coli and Faecalibacterium and confirmed its presence only in 5,6% of samples. Finally, in a recent study made for Yang et al following a rDNA sequencing, it was identified Acinetobacter guillouiae in more than 80% of isolated germs and important quantities of Streptococcus anginosus, Anaerococcus, Staphylococcus, Caulobacteraceae sp, Alloprevotella and Corynebacterium (more than 10% each one); similar to skin microbiome. Besides, it was found Rothia genus isolation more characteristic in fistula tract than in others cultured locations like anal canal, skin buttock, anal gland, feces and both inner and outer fistulous openings.

[Regarding quality evaluation, none of articles got an adequate MINCIR score for abscess and fitula studies \(<18 pts\). Table 1 y 2](#)

DISCUSSION

There were a broad date range articles showing differences in culture methods along time [\(Figure 2\), although the articles found could not reach an adequate quality¹ these were accepted as all available evidence for our purpose](#). More of ancient articles did not achieve the wide bacterial variety that could be isolated nowadays regarding molecular techniques availables [or, for example, through mass spectrometry methods like matrix-assisted laser desorption/ionization time-of-flight mass spectrometry \(MALDI-TOF MS\), identifying microorganisms by creating a spectro based on protein profile](#). There were more studies based on abscess microbiology compared with fistula all the time, so the microbiology of anorectal fistula was not well reported. We believe it was due in part to factibility of sampling, regarding that frank pus is a copious fluid compared to fistula content, that require an hystological culture or needs a specific techniques to achieve bacterial growth from the granulation tissue of its luminal surface ^{26, 27}.

In order to found the abscess-fistula association more articles were addressed to compare the bacteriology in each pathological case ⁴⁻⁷. The hypothesis, in general, was the anorectal sepsis coming from gut-derived bacterias, specially anaerobics infecting anal glands, would be responsables of fistulization and others skin-derived bacterias infecting apocrine glands in perianal zone would not be able to generate fistulas ^{2, 4-8, 10}.

Posterior studies had questioned this hypothesis based on cultures with skin and gut-derived bacterias both present in fistula and no-fistula patients like *Peptostreptococcus* genus, an anaerobic gut specimen, that was identified in a not low percentage of cultures ^{2, 6, 9, 10, 15, 23} and which was associated with no fistulization ¹⁰. Therefore, it had proposed a possible synergistic polymicrobial effect rather than a specific bacterial role ^{5, 9, 11, 12, 17}. However, the role of antibiotics was also signaled as an important intervening factor. The previous antibiotic use was other feature that could induce a bacterial selectivity reflected in cultures ^{7, 11}. Narayanan's study, for example, isolated more skin flora in cultures than gut-derived bacterias ¹⁷, although others dismissed the true antibiotic role overall ¹⁰. Certainly, an important thing to keep in mind is the contamination risk when sampling is performed ¹² or some conditions like the type of cultures available for specimens at the moment (aerobics, anaerobics) that would be another important reasons to avoid an exact inference about bacteria type related to fistula formation.

Anaerobes are predominant bacterial type in gastrointestinal tract where they outnumber aerobes in a ratio of 1000:1 ^{9, 15}. *Escherichia coli* and *Bacteroides* genus, both aerobic and anaerobic respectively, was most isolated bacteria in abscess and fistulas. Some of these bacterias could have the ability of form colonies and complex organised exogenous matrix called biofilm. The ability of many of these bacterias to produce biofilm mass is a new concept that could explain its difficult management, thus gut-bacterial colonization and biofilm formation would have a role in fistula development without the need of a specific virulent pathogen ^{26, 29}. Further studies could not demonstrate this hypothesis and propose that this colonization in fistulas is only a simple finding without meaning ^{24, 27, 28}. Therefore, there could be another pro-inflammatory factor underlying

to colonizing bacteria that leads to develop the granulation tissue found over its luminal surface and would act as fistulization trigger ^{24, 25}. Van Onkelen et al proposes that Peptidoglycan (a bacterial remnant) could begin this inflammation process as it was formulated for Crohn disease ²⁴. In the other hand, Jaiswal et al, proposes the matrix metalloproteinases (MMP3, MMP9) implicates also in Crohn disease, as tissue injury facilitating fistula formation, although its role was not demonstrate ²⁹.

Is true the treatment of perianal abscess classically was the derroofing/debridement with more or less variation in the technique, but the fact of take out the pus substance from the abscess cavity would be enough to heal, without necessity of antibiotics added except in some specific cases or patients conditions ^{13-16, 19, 22}. Thus, an added antibiotic therapy follows a decision according to practitioner criteria. Some studies point out the high sensitivity of these bacteria, to first line antibiotics. So, the abscess recurrence rates appears reduced when a postoperative antibiotic is used, although it had not shown statistical significance ¹⁶ and apparently is not related to microbiological results follow Bender et al ¹⁹. Although an inadequate antibiotic treatment could be associated to high rates to recurrence in complex perirectal abscesses ³¹. Therefore, regarding high percentage of abscesses treated in emergency room are frequently a punctual care episode and, in the study made by Seow-En et al, a big part of deferred culture results are not recorded in clinical records and when it happens the management was unchanged ¹⁶, so a pus culture seem to be not necessary for not complicated abscess and would mean an unnecessary use of resources ^{16, 32}. By the way, the fact to do pus cultures in all cases could induce to give an antibiotic course in order to avoid recurrence, bacteremia or fistula formation. Nevertheless, some of these complications could be also avoided through another tools. A recent study of Khan et al identified the

leucopenia is associated with an increased risk of pulmonary and haematologic complications, readmission, reoperation, discharge and death after incision and drainage of perianal abscess ^{22, 33}.

Another stage on microbiology is seen when all the quantitative molecular techniques as new genomic and DNA/RNA amplification are used. They have been able to reveal an increased complexity respect to flora composition compared to conventional culturing ^{23-25, 29, 30}. Through this method, Jian-Chen et al confirmed *Escherichia coli* and *Bacteroides fragilis* as predominant species in the perianal abscess, being the latter the most virulent. Interestingly, it was identified too *Biophila wadsworthia*, although low in abundance, it was the most frequently detected and, follow the author, it could be related to increase intestinal barrier defect, bile acid dysmetabolism, changes in microbiome functional profile and systemic inflammation ^{23, 34, 35} and could become a clue to understand the abscess-fistula process. Nevertheless, not all bacterias seen at the conventional culturing was identified by mNGS, maybe because a not complete lysis of bacteria walls during the samples processing ²³.

Not expected outcomes in fistula cases was obtained by Van Onkelen et al with rRNA sequencing and Tozer et al with FISH technique in chronic fistulas, and despite in Van Onkelen study the conventional cultures was positive they considered clinically insignificant ²⁴. Tozer et al, making use a careful sampling to avoid sample contamination, he does not achieve identify any important quantity of bacteria, so, they consider that bacteria is not realted to absceso-fistula process ²⁵. Latest study compared, through rDNA amplification, different sites cultures in patient with fistula and concluded the fistula microbiome could originate from perianal skin. Besides, they highlight *Rotia*

abundance (a periodontal disease-related microorganism) as the new non-studied bacteria that could be implicated in fistulization process³⁰.

Thus, antibiotics use should be chosen only in certain cases. It represents a very thin line regarding lately, have been proposed as option for avoid fistulization because its possible anti-inflammatory properties³⁶⁻³⁸. By the way, the antibiotic indication should therefore be guided by the microbiological examination of pus obtained with high recommendation regarding increased rates of drug resistance appeared to common antibiotics nowadays, so this aspect should be clarified by further prospective studies¹⁹. So, with the seeming limited role of bacterial microorganism in fistula pathology this is important shift to understanding of the aetiology and later the potential implications for treatment, specifically the antibiotic use²⁵.

Finally, is clear that *Escherichia coli* and *Bacteroides* are intestinal commensal bacterias involved in perianal abscess that are not seem to be related to fistula formation. Routine cultures should be taken in specific cases to avoid complications and choose an appropriate postoperative antibiotic course. New insights about underlying pro-inflammatory triggers should be taken into account in order to explain the fistulization process.

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FIGURE 1: Flowchart of the selected articles

FIGURE 2: Articles includes per year