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Coagulase-negative staphylococci are the most common bacteria found in cultures from the deep portions of hidradenitis suppurativa lesions, as obtained by carbon dioxide laser surgery

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Summary The significance of bacterial findings in hidradenitis suppurativa (HS) is controversial. Interpretation of the results of bacteriological examinations from the surface of HS lesions is obscured by the possible contamination of resident skin bacteria. Bacteriological analysis of aspirates from deeper parts of HS is liable to show low sensitivity. We used a carbon dioxide (CO_2) laser method to evaporate the diseased tissue level by level from the surface downwards, allowing concurrent sampling of bacteriological cultures from each level and thereby minimizing contamination with bacteria from the level above. In this study, 22 women and three men with a mean age of 35·3 years and a mean HS duration of 10·6 years were treated with this CO_2 laser surgical method. Aerobic and anaerobic cultures from superficial and deep levels were taken during surgery. The regions treated were axillary in eight and perineal in 17 cases. Bacterial cultures were positive for one or more specimens from at least one level in all cases and from deep levels in all but three cases. Sixteen different species or subspecies were found. *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) were the species most frequently found. *Peptostreptococcus* species and *Propionibacterium acnes* were not uncommon. *S. aureus* was detected in a total of 14 cases, six of which were from the deep levels. *S. aureus* was the sole bacterium isolated in two deep cultures. CNS were found in 21 patients and 16 of these isolates were from the deep levels. In nine of the 16 deep samples CNS were the only bacteria detected. These findings motivate a re-evaluation of the significance of bacteria in the progress of HS and in particular they suggest that CNS are true pathogens. It is known that foreign bodies aggravate the virulence of the CNS in surgical implants, and an environment which resembles that produced by a foreign body, as found in chronic HS tissue, serves to intensify the pathogenic properties of CNS in HS.

Key words: bacteriological analysis, coagulase-negative staphylococci, CO_2 laser, hidradenitis suppurativa.

Hidradenitis suppurativa (HS) is a chronic relapsing disease of unknown aetiology, but comedonal occlusion obstructing the outflow from the follicles in apocrine gland-bearing areas with subsequent inflammation is believed to initiate the disease process. Whether this initial inflammatory change is due to a bacterial infection or is secondary to factors similar to those involved in acne formation is not known. In later stages of the disease, bacterial infection seems to be a risk factor for the destructive scarring and extension of the HS lesions, and once sinuses have formed the risk of secondary infection is obvious. Negative cultures from superficial parts of the HS lesion on routine bacteriological examination are frequent, and the role of bacteria in the pathogenesis of HS is not yet clearly defined. When bacteria are found, they are considered by some as overgrowth or contaminants from skin bacterial residents or occasionally as a result of secondary infection in an otherwise sterile inflammatory process.

In most studies of HS, samples for bacteriological culture are collected from the surface of the lesions.[1–6] With this technique, contamination from the resident bacterial population obscures the interpretation of the culture result. Recently, Jemec *et al.* circumvented the

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problem by introducing a method for bacteriological sampling from the deeper parts of HS by an aspiration technique.[7] The cultures were negative in half of the cases.

Our study was undertaken to characterize a representative bacterial milieu in the deeper parts of HS. In an attempt to get a sufficient bacterial yield for culture and to minimize interference from resident skin bacteria, we used a technique for the treatment of HS aiming at complete carbon dioxide (CO_2) laser ablation of diseased tissue and preservation of healthy tissue.[8] The diseased tissue of HS was exposed during the CO_2 laser surgery and the ablation of the overlying skin gave optimal access to material for bacteriological culture from the deeper parts of HS. Bacteriological samples were taken from three different depths in the HS tissue, separated by heat destruction.

Materials and methods

The diagnosis of HS was based on history and clinical presentation at the initial consultation. All of the patients either had one or more active suppurating lesions and had had three to 12 recurrent episodes of abscesses during the 12 months prior to the consultation, or had continuous suppurating lesions involving the axillary and perineal (perianal, genital, inguinal and pubic) areas. All patients were stage II cases according to clinical staging as adapted from Hurley,[9] i.e. they had recurrent abscesses with tract formation and cicatrization and single or multiple, widely separated lesions. The patients were otherwise healthy.

The lesions causing the most trouble for each individual patient as regards recurrence, discharge, inflammation and manifest abscesses were selected for treatment and bacteriological culture. The study was approved by the ethical committee of the Karolinska Institute at the Huddinge Hospital.

Anaesthesia and surgical procedure

Following cleansing with 0·05 mg/mL chlorhexidine solution, the area was anaesthetized with injections of lignocaine 1·0 mg/mL (Xylocaine®, Astra, Södertälje, Sweden). The solution was injected around and not

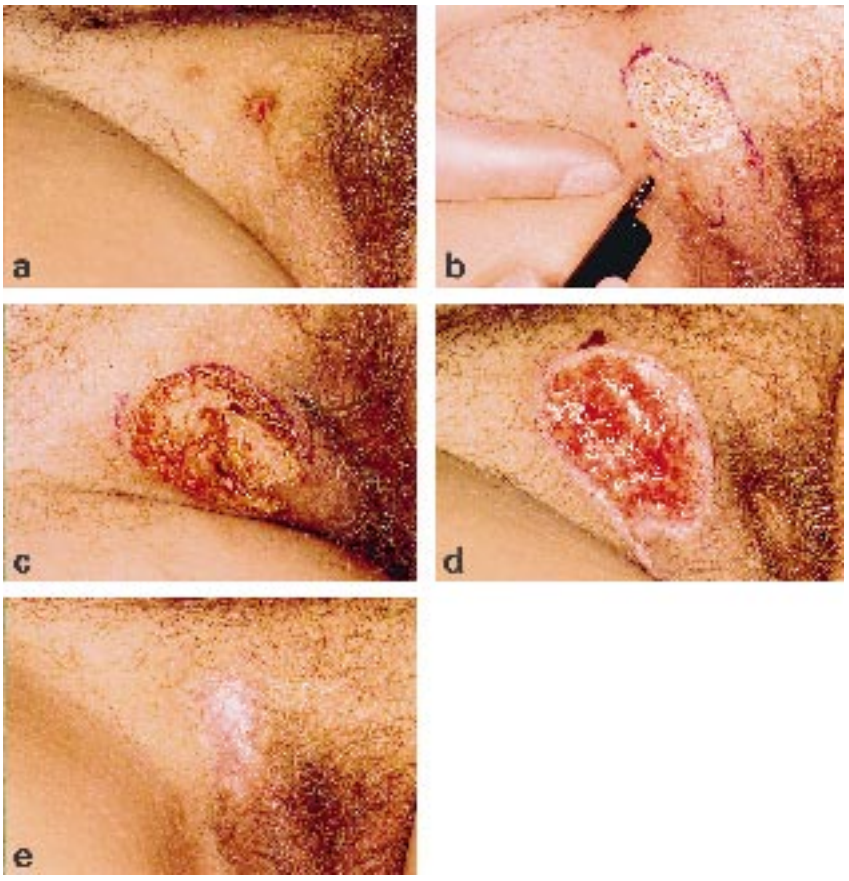

Figure 1. A 42-year-old woman (a) before, (b) at level 1 and (c) at level 3 during carbon dioxide laser vaporization surgery of hidradenitis suppurativa of the groin. The appearance (d) 2 weeks and (e) 3 months after surgery left to heal by secondary intention.

Table 1. Results from aerobic bacteriological cultures performed at three different levels of the hidradenitis suppurativa lesions in 25 patients. For the different bacterial species the total number of findings at each level is given to the left and the number of patients with positive cultures to the right

Species	No. of positive findings			No. of patients with positive findings	
	Level 1	Level 2	Level 3	Levels 1 + 2 + 3 (cumulative numbers for all levels)	Levels 2 + 3 (cumulative numbers for the deeper two levels)
Staphylococcus aureus	12	4	5	14	6
Coagulase-negative staphylococci	17	12	12	21	16
Enterococci	2	2	1	3	2
Group B haemolytic streptococci	2	1	0	2	1
Group C haemolytic streptococci	0	1	1	1	1
Bacillus cereus	0	1	0	1	1
Diphtherioids	3	0	0	3	0
Enterobacteriacae	1	1	1	2	1

directly into the affected area to avoid direct contact with infected tissue. A Sharplan 1030® laser (Laser Industries Ltd, Tel Aviv, Israel) was used, operating at 30 W with a manually controlled handpiece as described previously.[8] The selected area was ablated in one initial level with the laser beam by passing it rapidly over the tissue using a 'paintbrush' technique. The treated area could easily be evaluated, and, if necessary, retreated with subsequent ablations. The vaporization procedure was repeated downwards and outwards until fresh yellow fat tissue was exposed at the bottom with macroscopically normal margins laterally, with no remaining dense or discoloured tissue (Fig. 1).

Bacteriological sampling

The bacteriological analysis was performed from HS lesions at three different levels: first, at the skin surface (0–4 mm); second, at a depth of ≈ 6 mm (6–10 mm); and third, at 12 mm (12–16 mm) below the epidermis. The levels are named level 1 (superficial), level 2 (middle) and level 3 (deep) (Tables 1 and 2). Each level was ≈ 6 mm deep, sufficient to get deeper than the biopsy defect of the former level. At each level, two tissue specimens each 3–4 mm deep and 4 mm wide were collected by a punch biopsy technique followed by two bacteriological samplings using sterile cotton swabs. One of the specimens was placed in a sterile tube, the other in a tube filled with oxygen-free gas, and the two swabs were placed in two tubes containing Stuart's medium.[10] The specimens were immediately transferred to the Department of Bacteriology.

Bacteriological methods

For each of the three skin layers, procedures according to Figure 2 were performed. The aerobic streptococci were characterized by the agglutination test (Phadebact, Boule Diagnostics AB, Huddinge, Sweden), staphylococci by the coagulase (Difco Laboratories,

Table 2. Results from anaerobic bacteriological cultures performed at three different levels of the hidradenitis suppurativa lesions in 25 patients. For the different bacterial species the total number of findings at each level is given to the left and the number of patients with positive cultures to the right

Species	No. of positive findings			No. of patients with positive findings	
	Level 1	Level 2	Level 3	Levels 1 + 2 + 3 (cumulative numbers for all levels)	Levels 2 + 3 (cumulative numbers for the deeper two levels)
Peptostreptococcus species	7	5	3	9	7
Microaerophilic streptococci	1	0	0	1	0
Propionibacterium acnes	3	2	4	7	5
Lactobacillus species	0	0	2	2	2
Bacteroides fragilis	0	1	0	1	1
Other *Bacteroides* species	2	2	2	3	3
Prevotella species	0	1	2	3	3

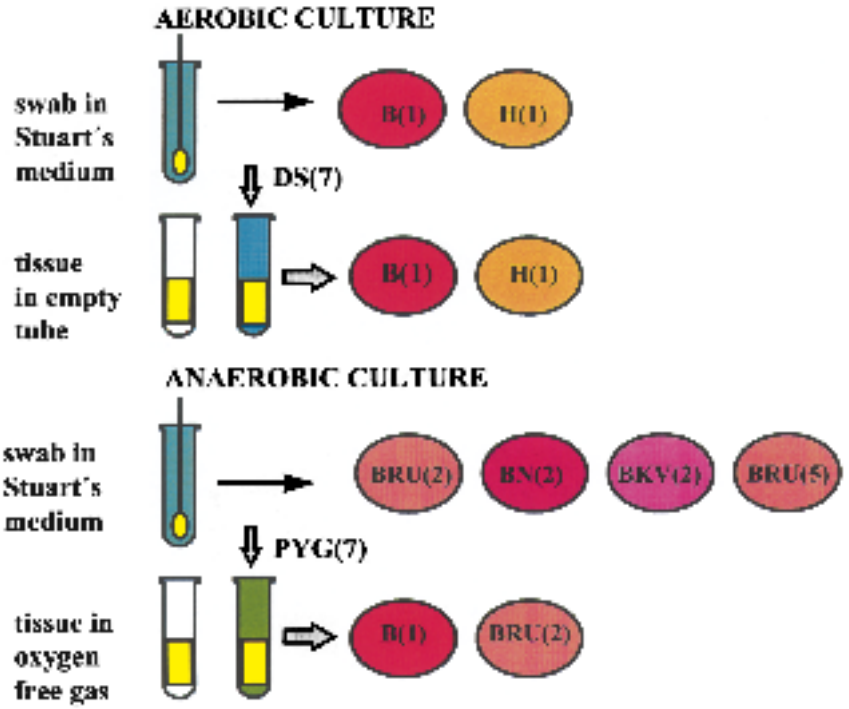

Figure 2. Material from three layers of a hidradenitis suppurativa lesion was received for culture. For each layer the procedures as shown in the picture were performed. For detection of aerobic bacteria, blood agar plates (B) and chocolate agar plates (H) were inoculated from a swab as well as from dextrose broth (DS) to which a piece of tissue had been added and incubated for 7 days. For anaerobic culture, plates containing Brucella agar (BRU)[11] with or without kanamycin and vancomycin (BKV)[11] or neomycin (BN)[11] were inoculated from a swab or from broth with peptone-yeast-glucose (PYG)[11] to which a piece of tissue had been added. The plates were incubated for 1, 2 or 5 days. The figures within parenthesis denote the duration of incubation. Chocolate agar plates were incubated in a 5% CO_2 atmosphere. All incubations were carried out at 37 °C.

Detroit, MI, U.S.A.) test and aerobic Gram-negative bacteria by API 20 (bio Mérieux, Lyon, France).

For the anaerobic bacteria, antimicrobial discs containing vancomycin 5 μg (Biodisc, Stockholm, Sweden), kanamycin 1000 μg (Rusco Diagnostica, Taastrup, Denmark) and colistin 10 μg (Biodisc) were used to confirm the Gram reaction and for preliminary characterization.[11] Indole and esculin tests, as well as fermentation of the carbohydrates rhamnose, trehalose, sucrose, glucose, lactose, arabinose, xylose and cellobiose with bromothymol blue as indicator, were performed in microtitre plates as previously described.[2,12] Growth in the presence of bile was tested with bile discs (Rosco Diagnostica, Taastrup, Denmark). A catalase test was done as previously described.[11] Gas–liquid chromatography,[11] a nitrate disc (Rosco Diagnostica) reduction test,[11] RapID Ana (Biodisc) and API 20A (Trio Lab, Malmö, Sweden) were performed occasionally.

Results

Twenty-five patients, 22 women and three men, mean age 35·3 years (range 19–49), were studied. The mean duration of disease was 10·6 years (range 1–26). The treated anatomical regions were axillary in eight and inguinal or perineal in 17 cases. All three men included had axillary lesions.

All patients were culture-positive from at least some level (Tables 1 and 2). Two superficial cultures were negative, three cultures were negative at both of the deep levels and three cultures were negative at one of the two deep levels. In nine cases, findings from the deep level were similar to those from the superficial ones. In 18 cases, cultures that were positive from at least one of the deep levels included bacteria found at the superficial level, and in half of these cases there were no additional bacteria found at the superficial level. In findings from 13 deep cultures there were one or more bacteria that were not simultaneously found at the superficial level in the same patient.

Staphylococcus aureus and coagulase-negative staphylococci (CNS) were the species most frequently found (Table 1). They could be detected at all levels but were most frequent at the superficial level. On the other hand, the anaerobic bacteria were, as expected, most often found at the two deeper levels (Table 2). Staphylococci were found in 23 of 23 positive cultures of superficial samples and in 20 of 22 deep culture-positive samples. *S. aureus* was detected in 14 patients and was found at the deep levels in six of these cases. *S. aureus* was the sole bacterium isolated in two deep cultures. CNS were found in 21 patients and in 16 samples from the deep levels. In nine of the 16 deep samples CNS were the sole detectable bacteria. After the staphylococci the

anaerobic *Peptostreptococcus* species and *Propionibacterium acnes* were most common, found in nine (seven at the deep levels) and seven lesions (five at the deep levels), respectively (Table 2). Twelve other bacterial species were isolated, each of them found in three or fewer lesions. The aerobic bacteria were found at deep levels in 28 of 47 (60%) positive cultures and the anaerobic bacteria in 21 of 26 (81%) positive cultures. The distribution of positive cultures from all three levels is given in Tables 1 and 2.

Discussion

In the disease course of HS the bacterial involvement is sometimes considered as intermittent or lacking, depending on the result from bacteriological studies. The pathogenic role of the bacteria is under debate. We used a CO_2 laser surgical method, by which we took advantage of the CO_2 laser capability of heat sterilization.[13,14] As the deep levels reached during laser surgery were separated by vaporization of the overlying layer and at a level deeper than that of normal hair follicles and ducts of sebaceous glands, and as the bacteria were vaporized together with several millimetres of the skin tissues, we could assume them to be separate compartments as regards their bacteriology.

Our bacteriological findings show that bacterial growth is frequent in the deeper parts of HS even when this level is separated from the superficial commensal skin bacteria which in our study are represented by isolates from level 1 (Tables 1 and 2). All but three of the 25 cases were positive in one or both of the deep cultures. The most commonly isolated bacteria were CNS, which were found in 64% of the deep bacterial cultures, and *S. aureus,* in 24%.

After the *Staphylococcus* species the most commonly cultured bacteria were the anaerobic *Peptostreptococcus* species and *Propionibacterium acnes* which were, respectively, found in seven and five of the deep levels in the lesions. Twelve other different bacterial species were isolated, each of them found in three or fewer lesions. The aerobic bacteria were found at deep levels in 28 of 47 (60%) positive cultures and the anaerobic bacteria in 21 of 26 (81%) positive cultures. The anaerobic bacteria, as expected, were most often found in the two deeper levels. *S. aureus* is a recognized pathogen, but the clinical significance of CNS is unclear, as they generally are considered to be non-pathogenic and a part of the normal skin flora. However, recent studies have suggested that CNS, under certain conditions, can cause invasive disease.[15]

As CNS are often found as the sole bacteria in the deep portion of the lesions it is important to elucidate their pathogenic properties in HS. Recent studies have shown that the pathogenic potential of the CNS varies according to species. The pathogenic potential of *S. haemolyticus* and *S. saprophyticus* has been established, but other species, such as *S. hominis*, *S. xylosus* and *S. simulans*, are rarely pathogenic. On the other hand, *S. lugdunensis*, *S. schleiferi* and *S. caprae* are now considered as emerging pathogens.[15] Recently, it has been suggested that sweat duct occlusion from periodic acid-Schiff (PAS)-positive material could be caused by *S. epidermidis* producing a PAS-positive extracellular polysaccharide substance.[16] Such strains were capable of inducing miliaria under experimental conditions.[17] It can be speculated that a similar mechanism is of pathogenic importance in HS.

The earliest inflammatory event in HS is a rupture of the follicular epithelium, followed by the spilling of foreign body material such as corneocytes, bacteria, sebum products and hair into the dermis. The dumping of foreign products initiates an inflammatory response to cause a foreign body granuloma. Epithelial strands form sinuses in this inflammatory tissue. Secondary bacterial colonization in this milieu can intensify the chronic inflammation.[18] CNS are predominantly associated with hospital-acquired infections occurring on intravascular catheters[19] and prosthetic devices[20] where the presence of a foreign body enhances the pathogenic properties of CNS. It is for this reason attractive to suggest that the milieu in HS can provide a comparable situation and thus enhance the pathogenic properties of CNS.

In most studies, cultures sampled from the surface of HS lesions have been the rule.[1–6] With this technique there are obvious problems with contamination from skin bacterial flora and for that reason the CNS found were not considered as pathogens. Accordingly, negative cultures or findings of CNS motivated further searches for other pathogens such as anaerobic bacteria.[5] The method used in these previous reports also excludes the possibility of culturing from lesions that do not open to the skin surface. In contrast, the method presented here allows bacterial sample collection from deep parts of the HS lesion, which is an advantage as closed but active lesions are common in HS.

Deep needle aspiration has been used to obtain bacteriological cultures from the deeper parts of HS inflammatory tissue. With this method the interference from skin commensal bacteria was avoided. In a study

by Jemec *et al.*[7] using this method, *S. aureus* and CNS were the bacteria most commonly found and a few cultures of anaerobic bacteria were positive. Fifty-one per cent of the cultures were considered sterile. The relatively large portion of negative cultures found by Jemec *et al.* as compared with our results, could be explained by the uncertainty of locating the infected part of HS with an aspiration technique. In 1988, Highet *et al.* found *Streptococcus milleri* to be the most frequently isolated bacterium from HS.[2] We have not been able to reproduce that result. However, we found other *Streptococcus* and *Peptostreptococcus* species fairly frequently.

In conclusion, our results indicate that bacterial infection is common and sterile inflammatory lesions are exceptional in HS. The infections with aerobic and/or anaerobic bacteria are probably secondary in a structurally abnormal tissue. The frequent findings of CNS in the deeper parts of HS suggest that they have a pathogenic significance. In future studies, speciation of the CNS might shed more light on their pathogenic potential. The presumed destructive properties of these otherwise harmless members of the skin commensal flora could be secondary to mechanisms similar to the ones involved in the foreign body situation.

Acknowledgments

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