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# Isolation of *Bacteroides ureolyticus* (*B corrodens*) from clinical infections

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SUMMARY The introduction of an improved anaerobic system resulted in the isolation of *Bacteroides ureolyticus* (*B corrodens*) in numbers that suggested a pathogenic role from many more clinical specimens. During a three-year period *B ureolyticus* was isolated from 103 fairly superficial necrotic or gangrenous lesions all of which showed evidence of active infection. These included 27 perineal or genital infections, 15 perianal abscesses, 15 other soft tissue infections such as pilonidal abscesses and infected sebaceous cysts and 16 ulcers or gangrenous lesions of the lower limb. *B ureolyticus* was rarely isolated in pure culture but was usually one of the predominant organisms; the other organisms were mostly anaerobes and the combination of *B ureolyticus* with anaerobic Gram-positive cocci was particularly noticeable. The isolation and identification of *B ureolyticus* is not difficult but depends upon a reliable anaerobic system and the incubation of primary cultures for at least 72 h.

Improvements in anaerobic techniques have shown that Gram-negative anaerobic non-sporing bacilli of the family Bacteroidaceae are implicated in a wide variety of infections. Appropriate media, reliable anaerobiosis and the recognition of the need for prolonged incubation of anaerobic cultures have not only resulted in the isolation of anaerobes from more infections but also in the isolation of some of the more demanding species.

*Bacteroides ureolyticus* is one of these more demanding species in the non-fermentative group of *Bacteroides*. Gram-negative bacilli that produced "corroding" colonies were first described in buccal abscesses by Eiken[1] who called them *B corrodens*, but there was confusion among early workers because facultative and strictly anaerobic strains were not distinguished. Jackson and Goodman[2] assigned the facultative strains to a separate genus as *Eikenella corrodens*, reserving the name *B corrodens* for the strict anaerobes. Subsequently they suggested that the name *B ureolyticus* should replace *B corrodens* to avoid confusion[3] and this is now generally approved. "Corroding bacilli" have been isolated from a variety of clinical specimens, usually mixed with anaerobes or facultative species, but few reports have distinguished between the facultative and anaerobic "corroding" strains. However, Labbé *et al*[4] isolated *B ureolyticus* (anaerobic *B corrodens*) from 61% of specimens from anal abscesses and from 6% of vaginal swabs.

After improving our routine anaerobic system three years ago, we began to isolate *B ureolyticus* in numbers that suggested the possibility of a pathogenic role from many more specimens. Our experience of this organism in clinical specimens is reported here.

## Material and methods

### SOURCE OF SPECIMENS AND STRAINS

The Bacteriology Laboratory of the Royal Hallamshire Hospital and a subsidiary laboratory at the Jessop Hospital for Women serve all the adult hospitals of the Southern District of the Sheffield Area Health Authority (Teaching). All specimens of pus or exudates and wound swabs submitted to the laboratories are examined by aerobic and anaerobic methods (see below). Strains of Gram-negative anaerobic bacilli that are regarded as significant isolates and justify reporting to the clinicians are submitted to the Department of Medical Microbiology, University Medical School, Sheffield, for identification. All strains of *B ureolyticus* reported as significant isolates during the past three years are included in this report.

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ANAEROBIC METHODS

Specimens for anaerobic culture are processed on the open bench. They are seeded on plates of prereduced blood agar base No 2 (Oxoid) with 7% defibrinated horse blood (Oxoid), and, when appropriate, lysed blood agar with menadione 1 μg/ml and kanamycin 75 μg/ml.[5] The plates are incubated in BTL (Baird and Tatlock Ltd, Romford, RM1 1HA) or Whitley (Don Whitley Scientific, 4 Wellington Crescent, Shipley, W Yorks, BD18 3PH) enamelled anaerobic jars equipped with 5 g catalyst sachets; the jars are evacuated to a negative pressure of 25 mbar and refilled with an atmosphere of 80% $N_2$:10% $H_2$:10% $CO_2$ (BOC Ltd, Special Gases, Deer Park Road, London, SW19 3UF). A culture of *Pseudomonas aeruginosa* on nutrient agar is included in each jar as a control. The plates are examined after 24 h and then immediately replaced in an anaerobic jar and reincubated for a further 48 h.

IDENTIFICATION OF BACTEROIDES SPP.

Significant isolates of Gram-negative anaerobic bacilli are identified by a combined set of tolerance, antibiotic disc resistance, biochemical and fermentation tests.[6 7]

## Results

IDENTIFICATION OF B UREOLYTICUS

Obligate anaerobic strains that produced the characteristic "pitting" or "corroding" colonies and were sensitive to metronidazole were provisionally identified as *B ureolyticus*; in Gram-stained smears they were slender, delicate and pale-staining bacilli. They were tolerant of Victoria blue 4R but inhibited by gentian violet and sodium taurocholate in tolerance tests, and in antibiotic disc resistance tests they were sensitive to neomycin (1000 μg), kanamycin (1000 μg) and penicillin (2 U) but many strains were resistant to rifampicin (15 μg) and most others showed reduced sensitivity. They did not produce indole, hydrolyse aesculin or ferment any carbohydrates, and their identity could be confirmed by positive tests for oxidase, the reduction of nitrate and the production of urease.

*B ureolyticus* could be grown on blood agar but was rarely recognised on the initial inspection after incubation for 24 h. The typical colonies from which it derives its name, could be seen only after incubation for several days. The "corroding" or "pitting" effect represents a true erosion and depression of the surface of the medium around the raised centre of the colony which appears to be surrounded by a pit or halo, but it also represents the spreading edge of the colony which extends as a thin film of growth across the agar; with prolonged incubation *B ureolyticus* can swarm across the entire plate.

A total of 103 strains of *B ureolyticus* were isolated from specimens from individual infections in numbers that suggested that they might be significant pathogens.

SOURCE OF B UREOLYTICUS STRAINS

The 103 isolates of *B ureolyticus* were from fairly superficial necrotic or gangrenous lesions. The most common sites of infection were the perineal and perianal areas with 27 isolates from the perineum and genitalia and 15 from perianal abscesses. Fourteen of the perineal or genital isolates were from men and 13 from women; they included cases of necrotic cellulitis, abscesses and gangrene of the scrotum, penis or vulva and a post-partum uterine infection. Some infections were complications of surgery but others were primary infections such as balanitis or Bartholin's abscesses. A further 15 isolates were from other soft tissue abscesses: eight were from pilonidal abscesses, six from infected sebaceous cysts, including three axillary abscesses, and one was from the pustular lesions of acne conglobata.

The fourth major group of isolates were 16 strains from ulcers or gangrenous lesions of the lower limb in patients with peripheral vascular disease, many of whom were diabetics. All were quite severe and progressive lesions in which an anaerobic infection was contributing to the continuing destruction of highly compromised tissues.

The remaining isolates were from a variety of sites but all the infections showed the common features of tissue necrosis with or without abscess formation. Eight isolates were from oral abscesses; most were periapical or root-canal infections but two were submandibular abscesses, in one of which *B ureolyticus* and *Actinomyces israelii* were present together. There were only seven isolates from abdominal wounds, and only three of these were from wound infections after appendix or large bowel surgery, although one was a very severe synergistic gangrene of the abdominal wall that required extensive skin excision and grafting; two isolates were associated with umbilical hernias in obese women and two followed oesophagogastric surgery. The other 15 isolates included three from breast infections—one abscess, one infection after breast reduction and one after mastectomy—three from cases of chronic otitis media and two from nail-bed infections. There were single isolates from postauricular and chest wall abscesses, a laminectomy wound, an infected scalp-nose skin pedicle and an infected pressure sore. In one infection no details were obtained.

PRESENCE OF OTHER BACTERIA

*Bacteroides ureolyticus* was rarely isolated in pure

culture from these infections and was seldom the only potential pathogen present. It was the sole organism reported from only five of the specimens. However, the density of growth of *B ureolyticus* in the mixed cultures and its fairly consistent appearance in these groups of infections indicated that it might be important in the pathogenesis of the lesions. Moreover, in most cases the bacteria grown from these specimens were predominantly anaerobic; apart from such examples of contaminant skin flora as diphtheroid organisms and coagulase-negative staphylococci, aerobes and facultative species were not a regular feature of the microbial flora of these lesions.

The most impressive association was between *B ureolyticus* and anaerobic Gram-positive cocci. Anaerobic cocci were isolated from 57 of the lesions including most of the perianal (11/15) and pilonidal or sebaceous cyst infections (12/15), half of the perineal and genital lesions and half of the peripheral ulcers or gangrenous lesions of the limbs and the other soft tissue infections including three of the four breast infections. In many cases *B ureolyticus* and the anaerobic coccus were the only significant isolates. However, an anaerobic coccus was isolated from only one of the eight oral abscesses.

In addition to *B ureolyticus*, other members of the Bacteroidaceae were also found in many of the infections; 70 strains were isolated from 53 of the infections but there was a wide variety of species and they were often present in smaller numbers than *B ureolyticus* or the anaerobic cocci. Most strains belonged to the less common species; they included *B asaccharolyticus*, members of the *B melaninogenicus-B oralis* group and some *Fusobacterium* spp. *Bacteroides fragilis*, however, was isolated from only six specimens.

Aerobic and facultative species were isolated from the specimens infrequently and in small numbers. *Staphylococcus aureus* was isolated from 15 specimens; eight of these were from peripheral ulcers or gangrene, five from perineal lesions and one each from the scalp-nose skin pedicle and the laminectomy wound. There were 26 streptococci isolated from the specimens. Nine were $\beta$-haemolytic but only one belonged to Lancefield group A, two were group C, four group G and one from a vaginal infection was group B; the other streptococci were $\alpha$-haemolytic and non-haemolytic species of doubtful significance. Enterobacteriaceae were not a common component of the microbial flora of these lesions with a total of only nine isolates. Occasional strains of *Haemophilus*, *Neisseria*, *Actinobacillus*, *Actinomyces* and *Capnocytophaga* were isolated principally from oral lesions.

## Discussion

Our results show that *B ureolyticus* is commonly one of the predominant organisms in specimens from superficial necrotic or gangrenous lesions and abscesses and may be significant in the pathogenesis of such lesions. This supports earlier reports of the isolation of *B ureolyticus* (anaerobic *B corrodens*) from perianal abscesses and lesions of the female genital tract.[4 8 9] The presence of an organism does not mean that it is necessarily pathogenic. However, most previous descriptions have recorded the isolation of *B ureolyticus* from actively infected lesions, it has not been recognised as a common normal human commensal, and there is good circumstantial evidence for a pathogenic role in the present series. We isolated *B ureolyticus* from lesions where infection appeared to contribute to the tissue damage and the organisms isolated from these lesions were mostly anaerobes; few significant aerobic or facultative species were found and *B ureolyticus* was usually one of the predominant anaerobes. Moreover, *B ureolyticus* was rarely isolated as a surface contaminant from apparently uninfected lesions.

Anaerobic infections usually yield a mixture of organisms with several species contributing to the pathogenesis of the lesions; this is particularly true of those in which *B ureolyticus* is implicated.[4] Other potentially pathogenic species were isolated from all except five specimens and there was a particularly strong association between *B ureolyticus* and Gram-positive anaerobic cocci. The contribution of anaerobes to superficial gangrene and perineal, perianal or pilonidal lesions which provide the basis for this report has been recognised for many years and anaerobic cocci have been implicated in many of these infections, especially in association with other bacteria in conditions described as synergistic bacterial gangrene.[10] Synergy between *B ureolyticus* and anaerobic cocci may have been important in many of the lesions in the present series and this combination may be particularly significant. The role of anaerobes in varicose and decubitus ulcers is more controversial. Many clinicians consider that vascular insufficiency is the primary lesion and that bacteria have only a minor role, if any, in the pathogenesis of the lesions, but many clinical microbiologists consider that anaerobes are important pathogens in these conditions. Our results support those of the several groups of workers who have implicated *Bacteroides* spp in the continued tissue necrosis in such lesions[11–13] and of Jones *et al*[14] who found that ulcers healed more quickly when treated with metronidazole. We consider that the presence of *B ureolyticus* in specimens from superficial necrotic lesions and abscesses is a significant finding with

implications for the understanding and management of the clinical condition and this emphasises the need to apply a reliable anaerobic system to the examination of specimens from these lesions.

The infections from which *B ureolyticus* was isolated form a subgroup amongst anaerobic soft tissue infections. They are distinct from the commonest group of anaerobic infections which comprises the intra-abdominal abscesses, peritonitis and postoperative wound infections related to surgery or to pathology of the appendix and large intestine.[15] The commonest pathogen in these lesions and the commonest anaerobe isolated from clinical specimens is *B fragilis*[13 16 17] but this species was rarely present in the lesions where *B ureolyticus* was found and *B ureolyticus* was not found in the type of abdominal lesions dominated by *B fragilis*.

The results of our laboratory tests on the anaerobic "corroding bacilli" conform with the characteristics described for *B ureolyticus* (anaerobic *B corrodens*).[2-4] Our experience confirms that of Labbé *et al*,[4] that the growth and initial recognition of *B ureolyticus* is not difficult; all strains with typical "corroding" colonies which were sensitive to metronidazole were subsequently confirmed as *B ureolyticus*. However, recognition of *B ureolyticus* in primary cultures depends upon an efficient anaerobic system with prolonged incubation of primary plates for at least 72 h to allow the development of the characteristic colonies.

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