

Microbiological investigation of Bartholin's gland abscesses in urban women in Johannesburg

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Bartholin's gland abscess of the female genital tract is fairly common in South Africa. Relatively few studies on the aetiology of this condition had been conducted before 1992 and even fewer after this date. Early literature suggested that Neisseria gonorrhoeae was the main cause of Bartholin's gland abscesses but it was subsequently shown that the majority of these abscesses had a polymicrobial aetiology with anaerobic bacteria featuring prominently. The study described here was conducted in 1992 and was designed to determine the aetiology of Bartholin's gland abscess in Johannesburg women admitted to the Hillbrow Hospital and at the same time establish the prevalence of genital pathogens in the endocervical canal of these women. Well established techniques were used for the culture of gonococci, Chlamydia trachomatis, mycoplasmas and other aerobic and anaerobic bacteria from properly collected pus aspirates from abscesses and endocervical swabs. Potential pathogens were found in 21 out of 33 aspirates. N. gonorrhoeae was isolated from four abscesses, two of which were in pure culture, while gonococci were also recovered from five endocervical swabs. C. trachomatis could only be isolated from one endocervical swab but not from any of the abscesses. Anaerobic bacteria were recovered in mixed culture from abscesses of nine patients and were the most common organisms found in pus aspirates. Mycoplasmas, including ureaplasmas, featured prominently. Pyogenic streptococci and staphylococci, as well as Escherichia coli and other Gram-negative bacilli were also found in abscesses while the anaerobic bacterium Fusobacterium nucleatum and the aerobic bacillus Brevundimonas vesicularis were each isolated in pure culture from an abscess of two different patients. This study confirms the polymicrobial aetiology of Bartholin's gland abscesses while pyogenic anaerobic and aerobic bacteria, the gonococcus and mycoplasmas were the predominant pathogens.

Introduction

Bartholin's glands are located low-down in the superficial perineal pouch of the uro-genital triangle of females with their ducts opening on either side of the vaginal orifice into the space between the hymen and the labium minus. These glands develop embryonically from an outgrowth of the uro-genital sinus and are not normally palpable. Bartholin's glands may, however, become enlarged as a result of bartholinitis and/or abscess formation caused by invasive micro-organisms and when infected are extremely tender on palpation.

Abscess formation involving Bartholin's glands in women in South Africa is common (authors' unpublished observations) and may require emergency admission to hospital. It ranks with abortion, pelvic inflammatory disease, ectopic pregnancy and gynaecological malignancies as an entity that often requires urgent hospital management. Septic shock is

an uncommon life-threatening complication of this condition.¹ The main causative agents of this complication are *Streptococcus pyogenes* and *Escherichia coli*. The exotoxin of *S. pyogenes* mediates the streptococcal toxic shock syndrome while endotoxic shock is caused by the interaction of lipopolysaccharide from the outer membrane of the Gram-negative bacterial cell wall with the host's immune system. Traditionally *Neisseria gonorrhoeae* has been accepted as the most common aetiological agent causing Bartholin's gland abscess. Microbiological studies have, however, indicated that the aetiology is frequently polymicrobial with *Bacteroides* spp and *E. coli* the predominant organisms²⁻⁷ while *Chlamydia trachomatis* has also been implicated as a cause of bartholinitis.^{8,9}

Two studies conducted by the same group in Durban in the 1990s confirmed the polymicrobial aetiology of Bartholin's gland abscess and its association with other sexually transmitted infections (STIs)⁵, including HIV/AIDS.⁹ Apart from these studies, limited information is available on the aetiology of infection involving Bartholin's gland in women in Africa. It was therefore decided to conduct a study in Johannesburg to determine the frequency of genital pathogens in the endocervical canal of patients presenting with Bartholin's gland abscess/es, and at the same time investigate the aetiology of this condition.

Aspirated pus specimens were collected under suitable operating theatre conditions by a single operator (AP) and cultured aerobically and anaerobically for pyogenic pathogens, including indigenous vaginal micro-organisms. Although this study was conducted in 1992, the findings have

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not been published before. As very few articles on the aetiology of Bartholin's gland abscess are presently available in the literature⁶ and the findings are considered to be of sufficient interest, it was decided to present the study for publication at the present time.

Patients and methods

Patients

Fifty consecutive patients with Bartholin's gland abscess treated by one of us (AP) when admitted to the Gynaecology Department of Hillbrow Hospital in Johannesburg between January 1992 and November 1992 were entered into the study after informed consent was obtained.

Thirty-three patients who required surgical drainage on clinical grounds qualified for microbiological investigation. Seventeen of the 50 patients were excluded as the Bartholin's abscess was draining spontaneously before the patient's admission or the patient had received antibiotic treatment within the previous three weeks. In theatre, the patient was placed in a lithotomy position and, under general anaesthesia, the Bartholin gland area was cleaned with a synthetic phenolic plus detergent preparation (Savlon, Medico Suppliers) and a speculum examination performed to visualise the ectocervix which was cleaned with a dry swab.⁷

Methods

Swabs were taken from the endocervical canal for the microbiological isolation of *N. gonorrhoeae*, *C. trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis*, using thin calcium alginate swabs (Calgiswab:Medical Wire & Equipment, Wilshire, UK).

Pus was aspirated from the abscesses using a 20 ml syringe with a 14 gauge needle. Care was taken to avoid needle-tip contamination with vaginal secretions. A drop of pus was added to each of the selective transport media used respectively for the culture of ureaplasmas, mycoplasmas, chlamydiae and *N. gonorrhoeae*.

Laboratory procedures

Routine culture for the isolation of *N. gonorrhoeae* was carried out by culturing the pus on Modified New York Medium (Diagnostic Media Production, NHLS),¹⁰ for

chlamydiae in cycloheximide-treated McCoy cells (Highveld Biological, Johannesburg), for mycoplasmas in PPLO broth (Diagnostic Media Production, NHLS)¹¹ and ureaplasmas in the U-9 urease colour test medium (Diagnostic Media Production, NHLS).¹²

The remainder of the pus was sent in a sterile container for standard microbiological testing, including microscopy of Gram-stained smears and aerobic and anaerobic culture. Aerobic culture required the aspirated pus to be inoculated onto blood agar and chocolate agar and incubated at 37°C in a CO₂ atmosphere and onto MacConkey agar (Diagnostic Media Production, NHLS) which was incubated in an aerobic environment at 37°C for 24 hours. For anaerobic culture, the pus was inoculated onto a non-selective pre-reduced 10% horse blood agar plates, as well as plates containing amikacin 100 mg/L, and into Thioglycolate Broth (Oxoid Ltd, Basingstoke, UK). The inoculated media were incubated under anaerobic conditions for 24 and 48 hours, using a GasPack jar (BBL Microbiology Systems). Isolates were identified using standardised routine methods, including carbohydrate biochemical identification of *N. gonorrhoeae*, A2 agar for ureaplasma (Diagnostic Media Production, NHLS),¹² and gas-liquid chromatography for anaerobes. In addition, 10 ml of venous blood was collected from patients for performance of the macroscopic rapid plasma reagin (RPR) test for syphilis antibodies (Becton & Dickinson, Sparks, USA) and micro-immunofluorescence (MIF) testing for serological evidence of chlamydial infection.¹³

Results

Endocervical swabs

Endocervical specimens from 31 of the 33 patients included in the study were processed microbiologically. Twenty-two specimens yielded genital pathogens (Table 1). Five endocervical swabs were positive for *N. gonorrhoeae*, one as the sole pathogen and four together with genital mycoplasmas (*N. gonorrhoeae* was also isolated from the Bartholin gland abscess of four of the five patients whose endocervical swabs were positive for this organism). *C. trachomatis* was cultured from one endocervical swab while *M. hominis* and *U. urealyticum* were recovered from endocervical swabs of 14 and 10 patients, respectively (Table 1).

Table 1: Genital pathogens isolated from the endocervix and Bartholin gland abscesses of 33 patients

| Pathogen | Number of patients with single or multiple pathogens from: | | | | | |
|-----------------------|--|----------------|-------|------------------|-----------------------|-------|
| | Swab from endocervix ^a | | | Pus from abscess | | |
| | Single | Multiple | Total | Single | Multiple ^b | Total |
| <i>N. gonorrhoeae</i> | 1 | 4 ^c | 5 | 2 | 2 | 4 |
| <i>C. trachomatis</i> | 1 | 0 | 1 | 0 | 0 | 0 |
| <i>M. hominis</i> | 7 | 7 ^d | 14 | 1 | 7 | 8 |
| <i>U. urealyticum</i> | 2 | 8 ^e | 10 | 1 | 4 | 5 |

^a Two of 33 swabs were not processed in the laboratory

^b Multiple infections involved genital mycoplasmas and aerobic and anaerobic bacteria (See text and Table 2)

^c *N. gonorrhoeae* + *M. hominis* (3), *N. gonorrhoeae* + *M. hominis* + *U. urealyticum* (1).

^d *M. hominis* + *U. urealyticum* (7).

^e *U. urealyticum* + *M. hominis* + *N. gonorrhoeae* (1), *U. urealyticum* + *M. hominis* (7).

Table 2: Bacterial pathogens isolated from Bartholin abscesses in 33 patients

| Pathogens | Number of abscesses yielding single or multiple pathogens | | | Co-pathogens |
|----------------------------|---|----------------|-----------|---|
| | Single | Multiple | Total | |
| Aerobic bacteria | 7 | 6 | 13 | See below |
| <i>N. gonorrhoeae</i> | 2 | 2 | 4 | <i>M. hominis</i> (2), <i>U. urealyticum</i> (1) <i>Eubacterium</i> (1), <i>B. melanogenicus</i> (1) |
| Streptococcal | 2 ^a | 1 ^b | 3 | <i>Bacteroides distasonis</i> |
| Staphylococcal | 2 ^c | 0 | 2 | Nil |
| Gram-negative bacilli | 1 ^d | 3 ^e | 4 | <i>Peptostreptococcus</i> (2), <i>Bacteroides</i> spp (1) |
| Anaerobic bacteria | 0 | 12 | 12 | See below |
| Peptostreptococcal | 0 | 5 | 5 | <i>M. hominis</i> (3), <i>U. urealyticum</i> (3), <i>E. coli</i> (1), <i>P. mirabilis</i> (1), <i>Eubacterium</i> spp (1) |
| <i>Bacteroides</i> spp | 0 | 3 | 3 | <i>N. gonorrhoeae</i> (1), <i>S. milleri</i> (1), <i>A. lwoffii</i> (1), <i>M. hominis</i> (1), <i>U. urealyticum</i> (1), <i>Peptostreptococcus prevotii</i> (1) |
| <i>Eubacterium</i> spp | 0 | 3 | 3 | <i>N. gonorrhoeae</i> (1), <i>M. hominis</i> (3), <i>U. urealyticum</i> (1) |
| <i>F. nucleatum</i> | 1 | 0 | 1 | Nil |
| Genital mycoplasmas | 4 | 5 | 9 | See below |
| <i>M. hominis</i> | 2 | 6 | 8 | <i>U. urealyticum</i> (4), <i>N. gonorrhoeae</i> (1) <i>Peptostreptococcus</i> spp (4), <i>Eubacterium</i> spp (3) |
| <i>U. urealyticum</i> | 1 | 4 | 5 | <i>M. hominis</i> (4), <i>Peptostreptococcus</i> spp (4) <i>Bacteroides</i> spp (1), <i>Eubacterium</i> spp (1) |

^a *Streptococcus milleri* (1), Group B *Streptococcus* (1)

^b *S. milleri* + *B. distasonis*.

^c *Staphylococcus aureus* (1), *Staphylococcus epidermidis* (1).

^d *Brevundimonas vesicularis*.

^e *Escherichia coli* (1), *Proteus mirabilis* (1), *Acinetobacter lwoffii* (1).

Pus from abscesses

Potential pathogens were recovered from 21 of the 33 patients who required surgical drainage of their Bartholin's gland abscesses (Table 2). *N. gonorrhoeae* was isolated from abscesses of four patients, other aerobic bacteria from abscesses of nine patients while abscesses from 12 patients yielded anaerobic bacteria, all part of mixed infections comprising genital mycoplasmas (9) and aerobic bacteria (6), including two as co-pathogens with *N. gonorrhoeae*. Genital mycoplasmas were found in abscesses of nine patients, eight with *M. hominis* of which four shared *U. urealyticum* as co-pathogen while a fifth abscess yielded *U. urealyticum* as a single agent.

Apart from *N. gonorrhoeae*, other important aerobic bacteria with invasive potential and generally associated with abscess formation that were isolated from Bartholin's gland abscesses in this study, included *Streptococcus milleri* (2), *Staphylococcus aureus* (1), *Streptococcus* Group B (1) and *E. coli* and *Proteus mirabilis* (one each). Aerobic bacteria with lower invasiveness status but known to be capable of sepsis and abscess formation that were isolated from aspirated pus in this study were *Acinetobacter lwoffii* (1), *Staphylococcus epidermidis* (1) and *Brevundimonas vesicularis* (an unusual pathogen formerly known as *Pseudomonas vesicularis*) from one abscess.

Anaerobic bacteria isolated from abscesses of 12 patients comprised peptostreptococci from five abscesses and *Bacteroides* spp and *Eubacterium* spp from three each.

Fusobacterium nucleatum recovered from one abscess was the only anaerobic bacterium that was isolated in pure culture as a single pathogen from a Bartholin's gland abscess (Table 2). The three *Bacteroides* spp-positive abscesses were mixed with *N. gonorrhoeae*, *S. milleri* and *M. hominis* respectively and all exhibited a heavy growth of the anaerobic bacterium.

Five patients, including the *C. trachomatis* culture-positive case, had a strongly positive MIF test for *C. trachomatis* with a titre of =64 and three of the 33 patients were positive with the RPR test for syphilis.

Discussion

Few studies on the aetiology of Bartholin's gland abscess have been published over the last two decades. Tanaka *et al* (2005) reported a dearth of articles on this subject during the 10 years preceding 2005 and scrutiny of the literature showed that most of the information available relates to studies performed in the 1960s and 1970s.^{2,3,14,15} A polymicrobial aetiology involving pyogenic aerobic and anaerobic bacteria was a common finding but *N. gonorrhoeae* was the predominant aetiological agent in these studies. In the present study *N. gonorrhoeae* was isolated from four abscesses, two of which were in pure culture - a reflection of the high incidence of gonorrhoea in the study population at the time.¹⁶ Gonococcal infections were common in HIV-infected women in South Africa in the 1990s¹⁵ and an increasing trend in the incidence of male urethritis in South African miners was shown by Ye and his colleagues during this period.¹⁷ This coincided with the escalation of HIV infection in South Africa. In 1995 Hoosen *et al* recorded a high isolation rate of

N. gonorrhoeae from Bartholin's gland abscesses in HIV-seropositive women in Durban.⁹

Apart from *N. gonorrhoeae*, genital mycoplasmas also featured prominently as putative causes of abscesses in this study. In four patients, either *M. hominis* alone (three cases) or a mixture of *M. hominis* and *U. urealyticum* (one case) were recovered as exclusively mycoplasmal pathogens from abscesses, while in a fifth patient *U. urealyticum* was the only organism isolated. In another five patients these two genital pathogens were found in abscesses as co-pathogens, mainly with anaerobic bacteria.

C. trachomatis was not isolated from any of the abscesses, in contrast to previous reports implicating this organism as an uncommon cause of Bartholinitis.⁸ In the present study, as was the case in the Durban study performed during the same year, *C. trachomatis* was isolated from the endocervix of one patient with a Bartholin's gland abscess but not from the abscess of the patient involved. In contrast, Hoosen *et al* (1995) in a subsequent study did isolate *C. trachomatis* from three patients with Bartholin's gland abscesses but all three patients were HIV-infected.⁹

Other pathogens recognised as aetiological agents of Bartholin's gland abscesses cultured from abscesses in the present study were pyogenic streptococci (two *S. milleri*, one group B streptococcus) and staphylococci (*S. aureus* and *S. epidermidis*, one each), as well as the Gram-negative bacilli *E. coli*, *P. mirabilis*, *A. lowffii* and *B. vesicularis*.

As expected from previous studies, anaerobic bacteria, mostly *Bacteroides* spp *Peptostreptococcus* spp and *Eubacterium* spp were frequently isolated, in most instances as co-pathogens (nine of 10 abscesses). The fusobacterium, *F. nucleatum*, a known cause of necrotising mucosal lesions, was the only anaerobic pathogen in this study that was isolated in pure culture from a Bartholin's gland abscess.

The finding of *B. vesicularis* isolated in pure culture from one of the Bartholin's gland abscesses deserves special mention. This organism was previously known as *Pseudomonas vesicularis* but has subsequently been assigned to the *Brevundimonas* genus.¹⁸ It has been isolated from environmental tap water aerators and hospital sinks¹⁹ and has been rarely implicated in human infections.¹⁸ Five bacteraemic cases have been described in adults, four of which in immuno-compromised patients.²⁰⁻²² A further bacteraemic case in a child with sickle cell anaemia with presumed functional asplenia has been reported²³ as well as a case of septic arthritis in a previously healthy child.²⁴ *B. vesicularis* has also been recovered from other sites in a series of cultures collected by the Centers for Disease Control and Prevention (CDC) in Atlanta, USA, including seven isolates from the central nervous system and others from the eye, urine and wounds of patients. Important in the context of the present study, seven isolates from the CDC collection were from the cervix uteri of patients.²⁵

The lower isolation rate of pathogens achieved in the present study compared with others²⁻⁶ may be due to differences in technique, e.g. Tanaka *et al*⁶ diluted pus from abscesses 100-fold (0.05 ml pus aspirate in 5 ml anaerobic buffer) which may have minimised toxic effects in specimens while Lingham *et al*⁵ took samples from pus and abscess walls,

increasing chances of isolating true pathogens but also of contamination with vaginal flora. In the present study special care was taken to avoid such contamination. It is also possible that some of our patients received unrecorded antibiotic treatment prior to the aspiration of pus.

The present study confirms the polymicrobial aetiology of Bartholin's gland abscesses, the continued role of gonococcal infection, the frequent isolation of genital mycoplasmas, (suggesting an important role for them), as well as the established role of pyogenic aerobic and anaerobic bacteria in this condition. Notably absent in the present study were capnophilic bacteria, including *Streptococcus pneumoniae* and *Haemophilus influenzae*^{4,5} which are unusual but important causes of Bartholin's gland abscesses. *S. pneumoniae* is also a causative agent of primary peritonitis, another uncommon infection originating from the female genital tract.

Aetiological studies of localised infections may produce surprise encounters with unusual organisms. Such was the case where in a recent publication, *Brucella* organisms were implicated as a cause of Bartholin's gland abscess.²⁷ Also, studies involving sites colonised by indigenous microorganisms where polymicrobial aetiology features regularly can be rewarding as exemplified in the present study where the isolation of *B. vesicularis*, only recently recognised as an endogenous organism with pathogenic potential,^{22,25} was an unexpected but significant finding.

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