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Microbiology of Bartholin's Gland Abscess in Japan

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This study was conducted to determine the current epidemiology concerning the causative organisms for Bartholin's gland abscess in Japan. Microbiological examination of 224 cases showed positive results in 219 cases and negative results in 5 cases. Of all of the bacterial isolates, 307 and 118 were aerobes and anaerobes, respectively. The most frequently isolated bacterium was *Escherichia coli*. Of the anaerobes, the most frequently isolated organism was *Bacteroides* species, followed by *Prevotella* species. The organisms related to respiratory infectious diseases, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, including resistant bacteria, were sometimes involved between 2000 and 2004.

Bartholinitis is one of the most common infections in gynecologic fields (28). In acute Bartholin's gland abscess, incision and drainage are considered the primary treatment (4, 18). However, antimicrobial agents are frequently administered in the treatment in addition to surgical procedures, and an antimicrobial chemotherapeutic regimen is usually chosen based on empirical knowledge of clinical doctors.

Previous studies in 1960s and 1970s on the bacteriology of Bartholin's gland abscess had emphasized the significance of gonococcus (12, 19, 27). It has been reported to be involved in approximately one-third or more cases (12, 19, 27). Anaerobic bacteria have also been reported to be often involved (3, 14). *Chlamydia trachomatis* has been identified in Bartholin's gland abscess (2, 5, 20). However, there are only a few reports on *C. trachomatis* causing bartholinitis (2, 8), and the incidence of chlamydial bartholinitis has not been thoroughly studied.

To our knowledge, there have been no reports on the current microbiology of Bartholin's gland abscess in the last decade. The aim of this study was to determine which microbes are currently the most common pathogens in Bartholin's gland abscesses so that the antimicrobial regimens could be correctly directed against the most liable pathogens even before definite identification of the causative organisms. This is the most current report on clinical microbiology for the causative organisms of Bartholin's gland abscesses.

A total of 224 women who came to Gifu University Hospital, Gihoku General Hospital, Matsunami General Hospital, or Gifu Municipal Hospital from July 2000 to June 2004 and were diagnosed as Bartholin's gland abscess were subjects for this study. Institutional Review Board approval in the Life Science Research Center, Gifu University, was obtained for this study.

The samples were taken and studied according to general principles of diagnostic laboratory methods. Briefly, the surface of the abscess was thoroughly cleansed with povidone-iodine and 70% alcohol. The content of the abscess was aspirated percutaneously or at the time of incision of the abscess with a needle attached to a plastic syringe of 0.5 to 20 ml.

Immediately after collection of the specimen, 0.05 ml of the specimen was suspended in 5 ml of the anaerobic buffer. The composition of the anaerobic buffer was as follows: KH_2PO_4, 4.0 g; Na_2HPO_4, 6.0 g; L-cysteine·HCl·H_2O, 1.0 g; Tween 80 (Sigma, St. Louis, Mo.), 1.0 g; agar, 1.0 g; distilled water, 1,000 ml/pH 7.2.

Staphylococcus selective agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and MacConkey agar (Becton Dickinson and Company, Cockeysville, MD) were used for aerobic culture. Sheep blood agar (Nissui) and chocolate agar (Nissui) were used for carbon dioxide culture. As for *Gardnerella vaginalis*, a medium designated HBT (human blood-bilayer-Tween) and developed by Totten et al. (24) was used for carbon dioxide culture.

For anaerobic culture, brucella HK (hemin, vitamin K_1) RS (rabbit, sheep) blood agar (Kyokuto Pharmaceutical Co., Ltd., Tokyo, Japan) was used as a nonselective medium; β-phenylethylalcohol (PEA) brucella HK blood agar (Kyokuto), paromomycin/vancomycin brucella HK blood agar (Kyokuto), and *Bacteroides* bile esuculin agar (Kyokuto) were used as selective media.

Sabouraud dextrose agar (Becton Dickinson) was used for yeast culture. Aerobic culture was done at 37°C for 2 days, carbon dioxide culture in 5% carbon dioxide in air at 37°C for 3 days, anaerobic culture in GasPak Pouch (Mitsubishi Gas Chemical Co., Tokyo, Japan) at 37°C for 7 days, and fungal culture at 37°C for 7 days.

The presence of *C. trachomatis* was determined by a PCR method, using a commercially available kit (Roche Amplicor *Chlamydia trachomatis*; Roche, Branchburg, NJ) containing an internal control.

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Among aerobes, gram-positive and catalase-positive cocci were identified by an Api STAPH identification system (bioMerieux SA, Marcy l'Etoile, France). Gram-positive and catalase-negative cocci were identified by an Api STREP identification system (bioMerieux SA, Marcy l'Etoile, France), and gram-negative rods were identified by Enterotube II (Becton Dickinson) or OxiFerm Tube II (Becton Dickinson). *Haemophilus* species, *Neisseria* species, and *G. vaginalis* were identified by Rap ID NH (Innovative Diagnostic System, Inc., Atlanta, GA). Anaerobic bacteria were identified by Rap ID ANA System II (Innovative Diagnostic System, Inc., Atlanta, GA).

Yeasts were identified by Api AUXANOGRAM (bioMerieux). For *Streptococcus pneumoniae*, the *lytA* gene (7) encoding the autolysin enzyme specific to *S. pneumoniae* was amplified simultaneously with the three penicillin binding protein (PBP) genes to confirm that isolates were *S. pneumoniae*. Oligonucleotide primers for detection of the three PBP genes were designed to amplify proportions of the normal *pbp1a* (25), *pbp2x* (1), and *pbp2b* (6) genes detected only in susceptible strains. PCR cycling conditions consisted of 35 cycles at 94°C for 15 s, 53°C for 15 s, and 72°C for 15 s. Amplified DNA fragments were analyzed by electrophoresis on a 3% agarose gel. In cases in which DNA was not amplified, we recognized that the strain tested had a mutant gene.

For *Haemophilus influenzae*, PCR was carried out for *H. influenzae* isolates by using five sets of primers reported previously (9): P6 primers to amplify the *p6* gene, which encodes the P6 membrane protein specific for *H. influenzae* (16); TEM-1 primers to amplify a part of the $bla_{\text{TEM-1}}$ gene (23); PBP3-S primers to identify an Asn-526→Lys amino acid substitution in the *ftsI* gene (9); PBP3-BLN primers to identify Asn-526→Lys and Ser-385→Thr amino acid substitutions in the *ftsI* gene (9); and serotype b primers to amplify a portion of the gene encoding the serotype b capsule (26). PCR cycling conditions were 35 cycles at 94°C for 15 s, 53°C for 15 s, and 72°C for 15 s.

Microbiological examination of 224 cases showed positive results in 219 cases and negative results in only 5 cases. Of all bacterial isolates, 307 and 118 were aerobes and anaerobes, respectively (Table 1). *Candida albicans* was identified in only one case. *C. trachomatis* was also detected in only one case. Positive growth of polymicrobial organisms was recorded in 129 cases. On the average, 1.91 bacterial strains/case were recorded, with total numbers ranging from 1 to 7, although over 30 different bacterial species were found (Table 1).

In 88 cases, only one microbial strain was recovered. Results of tests for aerobic bacteria alone were positive in 84 cases, while results of tests for anaerobic bacteria alone were positive in 4 cases (Table 2). Polymicrobial infections by aerobes and anaerobes were found in 115 cases (51.3%).

Most of bacteria isolated could be regarded as being opportunistic pathogens. The most frequently isolated organism was *Escherichia coli*. The sexually transmitted pathogens *Neisseria gonorrhoeae* and *C. trachomatis* were not found in significant numbers. Of the anaerobes, the most frequent microbe was *Bacteroides* species, followed by *Prevotella* species.

Based on the PCR results, strains tested were classified into six groups according to genotype as follows: (i) strains with three normal *pbp* genes (penicillin-susceptible *S. pneumoniae* [PSSP]); (ii) strains with an abnormal *pbp2x* gene (penicillin-intermediate *S. pneumoniae* [PISP]); (iii) strains with an abnormal *pbp2b* gene ([PISP]); (iv) strains with abnormal *pbp2x* and *pbp2b* genes ([PISP]); (v) strains with abnormal *pbp1a* and *pbp2x* genes (PISP); and (vi) strains with three abnormal genes, *pbp1a*, *pbp2x*, and *pbp2b* (penicillin-resistant *S. pneumoniae* ([PRSP]) (25). Strains tested were classified according to genotype as follows: PSSP ($n = 2$, 25.0%), PISP ($n = 5$, 62.5%), and PRSP ($n = 1$, 12.5%).

On the basis of the PCR results, all *H. influenzae* strains tested could be placed in one of six classes (10): non-beta-

TABLE 1. Bacterial findings in 224 Bartholin's gland abscess cases of 224 patients

Organism	No. of strains
Aerobes	307
Gram positive	178
Staphylococcus aureus	12
Staphylococcus epidermidis	16
Staphylococcus species	40
Streptococcus agalactiae	20
Streptococcus pneumoniae	8
Streptococcus milleri	6
Streptococcus species	32
Enterococcus faecalis	20
Enterococcus species	6
Micrococcus species	2
Gardnerella vaginalis	2
Others	12
Gram negative	129
Escherichia coli	78
Proteus group	8
Haemophilus influenzae	8
Klebsiella pneumoniae	5
Klebsiella species	2
Neisseria gonorrhoeae	4
Neisseria species	1
Others	23
Anaerobes	118
Gram positive	36
Peptostreptococcus anaerobius	5
Finegoldia magna	14
Micromonas micros	7
Propionibacterium species	2
Others	8
Gram negative	82
Bacteroides fragilis	9
Bacteroides species	21
Prevotella species	28
Fusobacterium species	5
Porphyromonas species	6
Veillonella species	2
Others	11
Chlamydia	1
Chlamydia trachomatis	1
Yeast	1
Candida albicans	1
Total	427

TABLE 2. Patterns of infection in 224 Bartholin's gland abscess cases of 224 patients

Pattern of infection	No. of cases
None	5
1 aerobe	84
1 anaerobe	4
1 *Candida* sp.	1
1 *Chlamydia* sp.	1
2 aerobes	14
1 aerobe + 1 anaerobe	60
2 aerobes + 1 anaerobe	43
3 aerobes + 1 anaerobe	5
4 aerobes + 1 anaerobe	3
4 aerobes + 2 anaerobe	3
5 aerobes + 2 anaerobe	1
Total	224

lactamase-producing, ampicillin-susceptible (BLNAS) strains, which lack all resistance genes; beta-lactamase-producing, ampicillin-resistant (BLPAR) strains; non-beta-lactamase-producing, ampicillin-resistant strains, which show low-level resistance associated with a substitution of Lys-526 or His-517 in *ftsI* (low-BLNAR); BLNAR strains; beta-lactamase-producing and amoxicillin-clavulanic acid-resistant strains, which have the $bla_{\text{TEM-1}}$ gene and *ftsI* with the same substitution as the low-BLNAR strains (BLPACR I); and BLPACR II strains, which have the $bla_{\text{TEM-1}}$ gene and *ftsI* with the same substitution as the BLNAR strains. The prevalence of each class among eight strains of *H. influenzae* isolated from Bartholin's gland abscesses was as follows: three BLNAS strains, one BLPAR strain, two low-BLNAR strains, one BLNAR strain, and 1 BLPACR I strain.

Bartholin's gland abscess was mainly caused by opportunistic bacteria in this study. *E. coli* was the most frequently isolated bacteria causing Bartholin's gland abscess in this study, as in the previous studies (3, 14). In addition to its role as the major cause of urinary tract infections, *E. coli* has been reported as an important cause of various infections in the female genital tract, including bartholinitis (28). According to the previous studies with the same sample size as this study (12, 19, 27), *N. gonorrhoeae* has been one of the main causative organisms for Bartholin's gland abscess. However, the incidence of gonococcal infection had decreased in Japan since the 1970s (17) whereas it increased after the 1990s because of drug-resistant strains, etc. Therefore, it is not surprising that the gonococcus is no longer a major pathogen in Bartholin's gland abscess in this population. In contrast, although the incidence of *C. trachomatis* has increased during the last decade (15), our study shows that *C. trachomatis* is of no special significance in causing Bartholin's gland abscess. *C. trachomatis* should be respected as a rare cause of bartholinitis, and it is likely that the Bartholin's gland is not the primary site for chlamydial infection.

Polymicrobial abscesses caused by aerobes and anaerobes were detected with high frequency (51.3%). Anaerobes would be derived from vaginal flora and might strengthen the pathogenicity of aerobes.

Compared with the previous study results (3, 14, 22), the isolation rates of *S. pneumoniae* and *H. influenzae* from Bartholin's gland abscesses were high in this study. Orogenital contact as a sexual activity has been common in normal Japanese women (15). The increasing rate of isolation for respiratory tract-associated infectious organisms, such as *S. pneumoniae* and *H. influenzae*, might be strongly associated with this tendency. Interestingly, the resistance patterns for *S. pneumoniae* and *H. influenzae* were almost identical in Bartholin's gland abscesses and respiratory tract infections (10, 25).

The recommended treatment of Bartholin's gland abscess is incision and drainage (3). Opinions on the benefit of including antimicrobial agents in the treatment are somewhat discordant (11). In our study, a considerable number of Bartholin's gland abscess cases were caused by bacteria regarded as being opportunistic pathogens. However, there have been some documented cases of septic shock arising from bartholinitis (13, 21). It would seem advisable to include antimicrobial agents to avoid the spread of infection in the treatment of Bartholin's gland abscess in addition to surgical procedures, especially in patients presenting with systemic symptoms.

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