Comparison of PCR, culturing and Pap smear microscopy for accurate diagnosis of genital Actinomyces

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Members of the genus Actinomyces, Gram-positive, non-spore-forming anaerobic bacteria, are normal inhabitants of the mucosal surfaces of the oral, gastrointestinal and genital tracts. Identification of these bacteria using conventional methods is generally difficult because of their complex transport and growth requirements and their fastidious and slow-growing nature. However, in recent years, the advancement of molecular techniques has provided much improved identification and differentiation of closely related Actinomyces species. The aim of the present study was to evaluate the efficacy of the PCR technique in the diagnosis of genital Actinomyces in comparison with culturing and Papanicolaou (Pap) smear microscopy. Multiple sampling was conducted from 200 women using smear microscopy, culturing and PCR. Cyto-brushes were smeared on glass slides and stained using the routine Pap technique. Culturing was performed from a sterile swab, and Actinomyces were determined using the BBL Crystal ANR ID kit. PCR was performed from a second swab, and the Actinomyces type was determined using type-specific primers designed in our laboratory. Only one vaginal fluid sample (0.5%) revealed Actinomyces-like organisms on Pap smear examination. Actinomyces were detected in nine samples (4.5%) using the BBL Crystal ANR ID kit. Using PCR, eight samples (4%) were found positive for Actinomyces. No specimens that gave positive results by Pap smear microscopy and culturing could be confirmed by PCR. Pap smear microscopy and culturing were both found to have zero sensitivity for Actinomyces. PCR appears to be a sensitive and reliable diagnostic method for the detection of Actinomyces, which are difficult to cultivate from genital samples. PCR can be used for diagnostic confirmation in cases diagnosed by conventional methods, to prevent false-positive results.

INTRODUCTION

Actinomyces is a genus of Gram-positive bacteria that are anaerobic, pleomorphic or filamentous, non-spore-forming, non-motile and non-acid-fast; they have DNA with a G+C content of 55–71 mol% (Hall et al., 2001; Kaszuba et al., 2008; Joshi et al., 2010; Fu & Tsai, 2010). Actinomyces species are normal inhabitants of the mucosal surfaces of the oral, gastrointestinal and genital tracts (Carrillo et al., 2010), and do not penetrate intact mucosae under normal conditions. However, neoplasia, diabetes mellitus, or the presence of a foreign body are predisposing factors that disrupt mucosae. When mucosal injury has occurred, these opportunistic organisms can be pathogenic and cause classical actinomycosis. Actinomyces species grow grossly and slowly by breaching anatomical boundaries, forming abscesses and sinus tracts filled with many neutrophils and surrounded by dense fibrotic tissue (Carrillo et al., 2010; Fitzhugh & Heller, 2008; Fiorino, 1996). Lesions become firm as they enlarge and may form fistulas between organs or to the skin (Fiorino, 1996).

Identification of Actinomyces species is generally challenging and unreliable (Fu & Tsai, 2010). Both clinical and laboratory findings are non-specific (Wang et al., 2007; Quercia et al., 2006) and clinical presentations are commonly confused with neoplasia (Fu & Tsai, 2010); actinomycosis has consequently been described as ‘the most misdiagnosed disease’ (Van Dellen, 2010). In most cases, diagnosis has either been missed or delayed until
surgery (Fu & Tsai, 2010; Ertan et al., 2005), and the preoperative diagnosis rate is very low (<10%) (Choi et al., 2009).

Among diagnostic techniques for identifying genital Actinomyces, the Papanicolaou (Pap) test lacks specificity and sensitivity (Westhoff, 2007). Sulphur granules observed in histopathological sections are highly suggestive of actinomycosis; however these granules can also occur in cases of nocardiosis and botryomycosis (Xia & Baumgartner, 2003). Culturing is considered a gold standard for identifying the presence of these bacteria, but Actinomyces species are fastidious and slow-growing, and sensitive to most antibiotics; these characteristics make the organisms difficult to cultivate and identify using routine specimen handling and culturing techniques (Wang et al., 2007; Westhoff, 2007; Tamer et al., 2006; Collins et al., 2000). Furthermore, conventional culturing methods are time-consuming (2–3 weeks) and may yield ambiguous results (Xia & Baumgartner, 2003). Techniques such as Pap smear microscopy, culturing and Gram staining are thus unsatisfactory for diagnosis of genital actinomycosis. In recent years, however, molecular techniques including PCR and 16S rRNA gene sequencing have resulted in much improved identification and differentiation of closely related species in oral samples (Xia & Baumgartner, 2003; Collins et al., 2000). However, to our knowledge, there have been no reports of the use of molecular techniques for detection of Actinomyces in genital samples. Thus, we sought to evaluate the efficacy of PCR techniques in the detection of Actinomyces in genital samples, in comparison with culturing and Pap smear microscopy.

METHODS

Actinomyces strains. The following dried standard Actinomyces strains were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ): Actinomyces viscosus (DSM 43327), Actinomyces meyeri (DSM 20733), Actinomyces odontolyticus (DSM 19120) and Actinomyces naeslundii (DSM 3013). The details of these strains are summarized in Table 1. Actinomyces iraei was kindly provided b Dr Takuichi Sato, Division of Oral Ecology and Biochemistry, Tohoku University Graduate School of Dentistry, Sendai, Japan.

Study population. A total of 200 women were seen in the outpatient clinic of the Department of Gynaecology and Obstetrics, Hacettepe University, Ankara, Turkey. Pregnant women were not included in the study. The study subjects comprised Turkish women with varied gynaecological complaints such as vaginal discharge, itching, urinary pain, abdominal pain and myoma. The patients were between 19 and 78 years of age, with the average being 40.1 years. Nineteen (9.5%) of the 200 women had used an intrauterine contraceptive device.

Clinical specimens. Cervico-vaginal fluid samples were collected from the 200 patients using a cyto-brush for diagnosis by Pap smear microscopy, and using sterile swabs for diagnosis by culturing and 16S rRNA analysis.

Pap staining. Cervico-vaginal samples were taken from each patient using a cyto-brush and fixed with 96% alcohol without air-drying. They were stained using the routine Pap technique and examined promptly by an experienced cytologist.

Bacterial culturing and identification of the Actinomyces species. Sterile swabs were transferred into plastic tubes consisting of medium in an anaerobic atmosphere (Anaerobic Culturette Collection and Transport System). They were delivered promptly to the Microbiology Laboratory, and the Actinomyces species were identified by both classical methods (culturing and Gram staining) and the BBL Crystal ANR ID kit (Becton Dickinson), including seven Actinomyces and related species (Actinomyces bovis, Actinomyces iraei, Actinomyces meyeri, Actinomyces naeslundii, Actinomyces odontolyticus, Actinomyces viscosus and Actinomyces pyogenes). After removal from the transport container, specimens on swabs were inoculated onto Schaedler 5% sheep blood agar (Becton Dickinson), and incubated in an anaerobic jar using AnaeroGen kits (Oxoid) or GENbox (bioMérieux) for up to 3 weeks. After incubation, developing colonies were examined microscopically to assess cell morphology, and Gram-positive colonies with the typical appearance of Actinomyces were classified as ‘possible’. Afterwards, these were subcultured on both Schaedler agar and Columbia agar. If growth occurred on Schaedler agar, but not on Columbia agar, the organisms were identified using the BBL Crystal ANR ID kit. The organisms were also checked for catalase and nitrate activity. Production of catalase was tested with 15% hydrogen peroxide and that of indole was tested with a spot test with 1% p-dimethylamino-n-cinnamaldehyde.

BBL Crystal ANR ID kit. Pure cultures of ‘possible’ colonies were incubated for 48 h. They were then harvested aseptically with cotton-tipped applicator swabs and suspended in water with 4.5% NaCl to a turbidity equivalent to the McFarland no. 4 standard. Each inoculum

Table 1. Standard Actinomyces strains used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Other collection no.</th>
<th>Isolated from</th>
<th>Cultivation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces viscosus</td>
<td>ATCC 15987</td>
<td>Periodontal disease in hamsters</td>
<td>Microaerophilic-anaerobic, 37°C</td>
</tr>
<tr>
<td>Actinomyces meyeri</td>
<td>ATCC 35568, CIP 103148</td>
<td>Human purulent pleurisy</td>
<td>Anaerobic, 37°C</td>
</tr>
<tr>
<td>Actinomyces odontolyticus</td>
<td>ATCC 17929, CCUG 20536, DSM 43760, JCM 14871, NCTC 9935</td>
<td>Dental caries, deep carious lesions around teeth</td>
<td>Microaerophilic-anaerobic, 37°C</td>
</tr>
<tr>
<td>Actinomyces naeslundii</td>
<td>NIH 279, WVU 45</td>
<td>Human sinus</td>
<td>Anaerobic, 37°C</td>
</tr>
</tbody>
</table>
tube was vortexed for 10–15 s and the contents were poured into a labelled panel base. The inoculum was gently rolled along the tracks of the base until all of the reaction wells were filled. A lid was aligned over each base and the inoculated panels were incubated in an incubator at 35 °C. After 4 h incubation, all panels were read visually with the BBL Crystal Panel Viewer and a 10-digit profile number was generated. This profile number and the results of the catalase, indole-spot and Gram staining tests were entered into a computer and interpreted using the BBL Crystal ID System Electronic Codebook.

Direct whole genomic DNA isolation. Cervico-vaginal samples collected from each patient with sterile swabs were placed in tubes containing Tris/EDTA buffer and transported to the Cytology Laboratory. Before DNA isolation, tubes with swabs were vigorously vortexed for 30 s in order to ensure disaggregation of all bacteria into the buffer. The swabs were then aseptically removed and the bacterial suspension was pelleted by centrifugation for 3 min at 10,000 r.p.m. The pellet was resuspended in 100 μl lysing solution from the A101 DNA Extraction Kit (Metis Biotechnology). Subsequently, total bacterial genomic DNA was isolated, following the protocol recommended by the manufacturer. Confirmation of DNA (3 μl per sample) was done by electrophoresis on a 0.8% agarose gel, viewed with an ultraviolet light box.

Primer design. The sequences of the 16S rRNA subunits for A. israelii (GenBank accession no. M33912), A. viscosus (X82453), A. naeslundii (M33911), A. meyeri (X82451) and A. odontolyticus (M33910) were downloaded from GenBank and aligned using the BioEdit Sequence Alignment Program (Ibis Biosciences). Variable regions of the highly homologous sequences were used to synthesize a forward organism-specific primer for each of the five organisms tested. The same reverse primer was used for these organisms. We thus used a total of six primers (one reverse and five forward) specific to the 16S rRNA of A. israelii, A. viscosus, A. meyeri, A. naeslundii and A. odontolyticus. A pair of universal primers was also used for the order Actinomyceales, which includes the genus Actinomyces, to eliminate other organisms found in the vaginal flora (Xia & Baumgartner, 2003). Specific primer sequences and the length of expected PCR products are shown in Table 2.

Specificity and sensitivity of newly designed primers. We tested the specificity of each primer pair designed in our laboratory to amplify 16S rRNA genes of A. israelii, A. meyeri, A. viscosus, A. naeslundii and A. odontolyticus against several most closely related species from the same order by an on-line BLAST search (National Center for Biotechnology Information). Although a separate PCR was not run for this purpose, we showed that our species-specific primer pairs did not amplify 16S rRNA genes of other members of the Actinomyceales such as Mobiluncus, Microbacterium and Arcanobacterium (see also Discussion). Thus, it is apparent that each primer pair is specific to each Actinomyces species tested.

The sensitivity of each primer pair was also tested against reference strains of Actinomyces species and no cross-reactivity was observed (Fig. 1). Also, positive controls with all reference strains of Actinomyces yielded the expected size of amplicon for each organism tested. Amplifications conducted with DNA from the reference A. viscosus strain yielded the predicted 785 bp fragment. Detection by PCR with primers specific to A. meyeri, A. odontolyticus, A. israelii and A. naeslundii produced amplification of the expected 519 bp, 353 bp, 339 bp and 103 bp products, respectively. No bands were observed in negative controls (Fig. 1).

PCR procedure. PCRs containing universal primer pairs for the order Actinomyceales were run for each of the 200 clinical samples. Only samples containing a band of the expected size (675 bp) for Actinomyceales were subjected to separate amplifications containing primer pairs for each Actinomyces species. A PCR assay was performed in a total volume of 50 μl containing 10× PCR buffer, 200 μM dNTPs, 4 μl MgCl₂, 10 pmol each of forward and reverse primers, 1 U TaqDNA polymerase and 10 μl extracted DNA template. The PCR reaction was conducted in a thermal cycler using the following parameters: initial denaturation for 10 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 90 s at 72 °C. After the main cycle programme was complete, an additional extension step was added of 10 min at 72 °C. PCR products were electrophoresed on a 1% agarose gel containing ethidium bromide, viewed on an ultraviolet light box, and photographed. Amplicon size was analysed by comparison with a ladder (GX174 DNA HaeIII digest). A positive control (DSMZ type strain DNA) and a negative control (distilled water) were included in each PCR experiment.

RESULTS

Light microscopic examination of Pap-stained cervico-vaginal smears

For cytological examination, Actinomyces-like organisms (ALOs) were defined as dense, basophilic, central aggregations surrounded by radially oriented, filament-like structures

<table>
<thead>
<tr>
<th>DNA template</th>
<th>Primer pair (5’–3’)</th>
<th>Base position (amplicon length, bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. meyeri</td>
<td>TCTGCGATTACTAGCGACTCC</td>
<td>818–1337 (519)</td>
</tr>
<tr>
<td></td>
<td>CACCGCCTGTGTTTCTGOG</td>
<td></td>
</tr>
<tr>
<td>A. viscosus</td>
<td>TCTGCGATTACTAGCGACTCC</td>
<td>538–1323 (785)</td>
</tr>
<tr>
<td></td>
<td>TCTGCGATTACTAGCGACTCC</td>
<td></td>
</tr>
<tr>
<td>A. odontolyticus</td>
<td>TCTGCGATTACTAGCGACTCC</td>
<td>888–1241 (353)</td>
</tr>
<tr>
<td>A. israelii</td>
<td>TCTGCGATTACTAGCGACTCC</td>
<td>783–1122 (339)</td>
</tr>
<tr>
<td>A. naeslundii</td>
<td>TCTGCGATTACTAGCGACTCC</td>
<td>1157–1260 (103)</td>
</tr>
<tr>
<td>Actinomyceales</td>
<td>GGCTTGGCGTCGTCGCCTC</td>
<td>632–1306 (675)</td>
</tr>
<tr>
<td></td>
<td>GCCATGACGGTGACGGCG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCTCCGACGGTGAGGG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GCTTAAAGGAGGAGGAGG</td>
<td></td>
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<td></td>
<td>GCTTAAAGGAGGAGGAGG</td>
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</table>

Table 2. PCR primers used in this study
From this examination, ALOs were detected in only one (0.5%) of the 200 smears.

Microbiological investigation of cervico-vaginal specimens

The Actinomyces were identified by both classical methods (culturing and Gram staining) and using the BBL Crystal ANR ID System (Becton Dickinson). Gram-positive branching bacilli seen in Gram-stained smears and dry and rough colonies on Schaedler agar plates were examined with the BBL Crystal ANR ID kit, and only nine of 200 samples (4.5%) were diagnosed as Actinomyces. Of these nine samples, six were positive for A. viscosus, two for A. meyeri and one for A. israelii.

DNA isolation and PCR

DNA extracted from a total of 200 cervico-vaginal samples and 16 clinical samples reacted positively with the universal pair of primers used for the first PCR assay, suggesting the presence of Actinomycetales in all 200 cervico-vaginal samples examined. When the PCRs with the species-specific primers were evaluated for only these 16 examined samples, five were positive for A. meyeri, two for A. viscosus (Fig. 3), and one for A. israelii. A. odontolyticus and A. naeslundii were not detected in any of the cervico-vaginal material. The detection rate of A. meyeri was higher than for the other two species.

Comparison of methods

A comparison of the results of the differing methods is shown in Table 3. As each positive PCR sample gave a strong reaction, and positive and negative controls were included in each experiment, it is not likely that PCR results were inaccurate. Thus, PCR was used as a reference standard to calculate the sensitivity and specificity of culturing and Pap smear microscopy.

There was no agreement between results obtained by culturing and PCR: no sample was positive by both culturing and PCR, but 183 samples were negative by both culturing and PCR. Eight samples were negative by culturing and positive by PCR. Nine samples (4.5%) were positive by culturing and negative by PCR (Table 3). The sensitivity and specificity of culturing versus PCR were thus 0% and 95.3% for Actinomyces.

PCR showed positive test results for eight specimens which were not positive by Pap smear microscopy. The sensitivity of Pap smear microscopy compared with PCR was 0%. The specificity of Pap smear microscopy was 99.5% as there was only one case with positive Pap smear microscopy and negative PCR result.
DISCUSSION

Our study evaluated diagnostic methods for genital Actinomyces, including Pap smear microscopy, culturing and PCR. The results of these methods were not concordant. There may be several reasons for their disagreement; however, we are of the opinion that the main reason was our use of different cervico-vaginal specimens for each method. The cytological samples were collected using cyto-brushes, whereas specimens for culturing and PCR were obtained from sterile swabs. In other words, the tested samples were different.

To identify and differentiate Actinomyces to species level directly from clinical samples, we used two PCR methods. These included a PCR with a pair of universal primers targeting the 16S rRNA gene of the Actinomycetales, which includes the genus Actinomyces, and another PCR with species-specific primers (Table 2) not previously used to our knowledge. The second PCR method targeted the variable regions of the 16S rRNA genes of five different Actinomyces species. The primers used are able to successfully differentiate the five species. The 16S rRNA gene is highly conserved within and among species of the same genus, and is thus considered the new standard for classification and identification of bacteria (Lau et al., 2004; Elsayed et al., 2006). Furthermore, PCR has been recommended as a reliable method for the differentiation of species that are difficult to cultivate. We can confirm that the 16S rRNA PCR technique is useful for identifying and differentiating Actinomyces species directly from clinical samples. Previous workers have used different molecular methodologies to identify and differentiate Actinomyces species from oral samples or type strains, after anaerobic cultivation, including PCR-RFLP, chromosomal DNA fingerprinting, 16S rRNA gene sequencing and oligonucleotide–DNA hybridization using universal primers or oligonucleotide probes (Hall et al., 2001; Johnson et al., 1990; Sato et al., 1998; Jauh-Hsun et al., 1999; Ruby et al., 2002; Tang et al., 2003, 2004). To our knowledge, ours is the first study of the detection of genital Actinomyces directly and accurately from clinical samples using PCR with species-specific primers.

Sixteen of 200 clinical samples (8%) reacted positively with the universal primer pair designed for the order Actinomycetales, suggesting the presence of members of this order. Subsequent PCR with species-specific primers gave positive reactions in eight of these 16 samples (4%), which were positive for at least one of three Actinomyces species, namely A. meyeri (2.5%), A. viscosus (1%) and A. israelii (0.5%). No A. odontolyticus or A. naeslundii were detected in any cervico-vaginal sample. These results show that PCR

Table 3. Comparison of culturing and Pap smear microscopy with PCR for the detection of Actinomyces in vaginal fluid samples from 200 women

<table>
<thead>
<tr>
<th>PCR results</th>
<th>Culturing results</th>
<th>Pap smear microscopy results</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Positive (n=9)</td>
<td>Negative (n=191)</td>
<td></td>
</tr>
<tr>
<td>Positive (n=8)</td>
<td>0 (0%)∗</td>
<td>8 (100%)</td>
<td></td>
</tr>
<tr>
<td>Negative (n=192)</td>
<td>9 (4.7%)</td>
<td>183 (95.3%)†</td>
<td></td>
</tr>
<tr>
<td>Total (n=200)</td>
<td>9 (4.5%)</td>
<td>191 (95.5%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PCR results</th>
<th>Culturing results</th>
<th>Pap smear microscopy results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=1)</td>
<td>Negative (n=199)</td>
<td></td>
</tr>
<tr>
<td>Positive (n=8)</td>
<td>0 (0%)∗</td>
<td>8 (100%)</td>
<td></td>
</tr>
<tr>
<td>Negative (n=192)</td>
<td>1 (0.5%)</td>
<td>191 (99.5%)†</td>
<td></td>
</tr>
<tr>
<td>Total (n=200)</td>
<td>1 (0.5%)</td>
<td>199 (99.5%)</td>
<td>200 (100%)†</td>
</tr>
</tbody>
</table>

∗The sensitivity of culturing or Pap smear microscopy as compared to PCR.
†The specificity of culturing or Pap smear microscopy as compared to PCR.

Fig. 3. PCR survey of clinical samples that reacted positively with the pair of universal primers for the order Actinomycetales. Lane 1, DNA ladder. Lanes 2–9 were amplified using A. viscosus-specific primers. Lane 2 contained distilled water as negative control. Lane 3 contained positive control DNA of A. viscosus. Lanes 4 and 6 clearly demonstrated the presence of A. viscosus.
targeting the 16S rRNA region can be used to accurately detect *Actinomyces* species in genital tract samples. The detection rates of *A. meyeri* and *A. viscosus* were higher than those of *A. israelii* in female genital tracts. This finding is not consistent with previous studies that have shown *A. israelii* to be the most common *Actinomyces* species in the female genital tract (Carrillo et al., 2010; Wang et al., 2007).

Among 16 samples positive for *Actinomyctaeles*, *Actinomyces* species were detected in eight samples; the remainder were found to be members of other genera of *Actinomyctaeles* using gene sequencing by Macrogen. These genera were *Mobiluncus* (*n=2*), *Microbacterium* (*n=2*) and *Arcanobacterium* (*n=1*). Three samples were identified as uncultured bacteria. Since the order *Actinomyctaeles* includes *Mobiluncus*, *Microbacterium* and *Arcanobacterium*, we were able to confirm that the universal primer pair designed by Xia & Baumgartner (2003) was specific to the order *Actinomyctaeles*. The uncultured bacteria may be hitherto unknown among the phylogenetic radiation of *Actinomyces* and related genera. This sequencing result also proved the specificity of our species-specific primer pairs. Although *Mobiluncus*, *Microbacterium* and *Arcanobacterium*, which are members of the order *Actinomyctaeles*, were present in the samples, primer pairs did not recognize and thus amplify their 16S rRNA genes. If the primer pairs were not specific to *Actinomyces* species, a band would be observed in agarose gel images of samples containing *Mobiluncus*, *Microbacterium* or *Arcanobacterium*.

For microbiological examination, we employed traditional culturing techniques and subsequent identification using a BBL Crystal ANR ID kit to identify the *Actinomyces* from vaginal fluid samples to the species level. Anaerobic culturing was done in all 200 cases, and nine *Actinomyces* were isolated using this kit. However, this result could not be confirmed by PCR. It is not likely that PCR results were inaccurate since each positive PCR sample gave a strong reaction, and positive and negative controls were included in each experiment. Thus, it is considered that the nine samples that cultured positive, but were PCR negative, might indicate false positive results by culturing. The false positivity in the culturing may be due to the BBL Crystal ANR ID kit, which is one of the commercial biochemical systems used widely in the phenotypic differentiation of anaerobic bacteria in clinical microbiology laboratories. Although the BBL Crystal ANR ID System is considered a useful and standardized method for the identification of classical *Actinomyces*, recently some investigators have reported that this system needs regular updating of databases in order to prevent erroneous identifications and offer better accuracy (Blairon et al., 2010). Our results supported this possibility. On the other hand, positive results by PCR in eight samples that were negative by culturing might be explained by the greater sensitivity of PCR compared with culturing rather than by false positivity. This finding would represent patients in whom the diagnosis of *Actinomyces* would have been missed by culturing techniques. One reason for this difference may be related to the possible effects of transporting specimens to the laboratory. During culturing, including sample collection, transportation, inoculation and incubation, some *Actinomyces* species may die, as they are all fastidious anaerobic bacteria. Also, the growth of *Actinomyces* species could be inhibited by the presence of other organisms, and thus affect their ability to grow in culture. DNA from dead organisms can still be detected by PCR. The sensitivity and specificity of culturing versus PCR were found to be 0% and 95.3%, respectively. We thus suggest that PCR may be useful in diagnostic confirmation in cases diagnosed by conventional methods, and that clinical microbiologists should confirm results obtained using the BBL Crystal ANR ID system with more sensitive methods such as PCR.

Pap staining is the widely used, economical and practical method for the early detection of genital infectious agents such as *Trichomonas vaginalis*, *Actinomyces* species and *Candida*. However, Pap smears do not give definitive identification of *Actinomyces* species and the term ‘*Actinomyces*-like organisms’ (ALOs) is thus preferred. According to some studies, the Pap test lacks specificity and sensitivity in the diagnosis of genital *Actinomyces* (Westhoff, 2007). We found that the sensitivity of Pap smear microscopy compared with PCR was 0% for *Actinomyces* and the specificity was 99.5%; there was only one case with positive Pap smear microscopy and a negative PCR result. This is consistent with results published by other investigators. *Actinomyces* species are coccobacilli. These microorganisms are seen as groups in Pap smears, and not individually. Since there are also other coccobacilli in the vaginal flora, it is suggested that Pap smear microscopy is not appropriate for differential diagnosis of *Actinomyces* infections. If ALOs are seen in Pap smears, we recommended that the diagnosis be confirmed by PCR.

A PCR method has several potential advantages in diagnosis of pelvic *Actinomyces*. PCR is highly sensitive and can be optimized to show very low numbers of bacteria. This sensitivity is very useful for identifying *Actinomyces* species since these organisms are present in low numbers in healthy females and are not easily cultivated because of their fastidious and slow-growing nature (Tamer et al., 2006). Another feature of PCR is that it quantifies DNA rather than viable organisms. This is also a potential advantage because it can efficiently detect all *Actinomyces*, either dead or alive (Schwebke & Lawing, 2001; Stellrecht et al., 2004). Like PCR, the Pap test is not dependent on viable organisms, but it is impossible to determine which *Actinomyces* species are present with this test (Kaya et al., 2009). Culturing methods cannot detect non-viable bacteria, and cluster formation of *Actinomyces* is temporary. Rapidity is another advantage of PCR (results can be obtained in less than 2 days) compared with culturing (results in 3–4 weeks) (Petrikkos et al., 2007). Thus, the diagnostic value of PCR is considered to be.
significant when *Actinomyces* species are expected to be present in vaginal specimens.

In conclusion, conventional methods of diagnosis such as Pap smears and cultivating have low sensitivity. However, PCR analysis, without the need for anaerobic incubation, appears to be a sensitive, specific and highly discriminatory diagnostic and typing method for identification of *Actinomyces* species of clinical origin. Advantages of this simple and rapid method compared with culturing are the ability to detect low concentrations of organisms in a short time and to detect all organisms, either dead or alive. Further application of this method, using the species-specific primers we have designed, may confirm its usefulness. PCR methods may replace culturing for the early and accurate detection of genital *Actinomyces* from vaginal samples. Thus, it is to be hoped that the number of surgeries will be significantly reduced.

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**REFERENCES**


