Molecular analysis of bacterial flora associated with chronically inflamed maxillary sinuses

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Chronic maxillary sinusitis is a chronic inflammatory condition in which the role of microbial infection remains undefined. Bacteria have been isolated from chronically inflamed sinuses; however, their role in the chronicity of inflammation is unknown. The objective of this study was to determine whether bacteria are present in clinical samples from chronic maxillary sinusitis and to assess the diversity of the flora present. Washes and/or tissue samples from endoscopic sinus surgery on 11 patients with chronic maxillary sinusitis were subjected to PCR amplification of bacterial 16S rDNA using three universal primer pairs, followed by cloning and sequencing. The samples were also assessed for the presence of bacteria and fungi by conventional culture methods. Viable bacteria and/or bacterial 16S rDNA were detected from maxillary sinus samples of five of the 11 patients examined (45%). Three sinus samples were positive by both PCR and culture methods, one was positive only by PCR, and one only by culture. Thirteen bacterial species were identified: Abiotrophia defectiva, Enterococcus avium, Eubacterium sp., Granulicatella elegans, Neisseria sp., Prevotella sp., Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Stenotrophomonas maltophilia, Streptococcus gordonii, Streptococcus mitis/Streptococcus oralis and Staphylococcus sp. Fungi were not detected. In one patient Streptococcus mitis/Streptococcus oralis, and in another patient Pseudomonas aeruginosa, were detected from both the sinus and the oral cavity using species-specific PCR primers. These results suggest that both aerobic and anaerobic bacteria can be detected in nearly half of chronic maxillary sinusitis cases.

INTRODUCTION

The maxillary sinuses are a unique anatomical structure that varies in size and form, warms and moistens breathed air, resonates the voice and lightens the skull. The secretions of the maxillary sinus are cleared by cilia. Maxillary sinuses are considered well-established, but the definition of chronic maxillary sinusitis remains undefined. Bacteria have been isolated from chronically inflamed sinuses; however, their role in the chronicity of inflammation is unknown. The objective of this study was to determine whether bacteria are present in clinical samples from chronic maxillary sinusitis and to assess the diversity of the flora present. Washes and/or tissue samples from endoscopic sinus surgery on 11 patients with chronic maxillary sinusitis were subjected to PCR amplification of bacterial 16S rDNA using three universal primer pairs, followed by cloning and sequencing. The samples were also assessed for the presence of bacteria and fungi by conventional culture methods. Viable bacteria and/or bacterial 16S rDNA were detected from maxillary sinus samples of five of the 11 patients examined (45%). Three sinus samples were positive by both PCR and culture methods, one was positive only by PCR, and one only by culture. Thirteen bacterial species were identified: Abiotrophia defectiva, Enterococcus avium, Eubacterium sp., Granulicatella elegans, Neisseria sp., Prevotella sp., Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Stenotrophomonas maltophilia, Streptococcus gordonii, Streptococcus mitis/Streptococcus oralis and Staphylococcus sp. Fungi were not detected. In one patient Streptococcus mitis/Streptococcus oralis, and in another patient Pseudomonas aeruginosa, were detected from both the sinus and the oral cavity using species-specific PCR primers. These results suggest that both aerobic and anaerobic bacteria can be detected in nearly half of chronic maxillary sinusitis cases.
methods suggest that about 40% of clones in the oral cavity (Paster et al., 2001) and about 70% of clones in the gut and colon (Suau et al., 1999; Wilson & Blitchington, 1996) do not correspond to known organisms, and are therefore novel (and probably non-cultivable) species. A previous study found PCR to be more efficient than standard culture for the detection of aerobic bacteria in chronic rhinosinusitis; such bacteria were detected in 62% of samples tested by PCR, compared to 50% using culture techniques (Keech et al., 2000). However, definitive studies that describe the total bacterial flora of the chronically inflamed maxillary sinus using molecular methods are not yet available.

Oral infections and inflammatory diseases such as periodontitis may influence systemic health by contributing to underlying mechanisms responsible for chronic coronary heart disease (Beck et al., 1999), pregnancy complications (Offenbacher et al., 1998), diabetes (Grossi & Genco, 1998) and respiratory infections (Scannapieco, 1999). The oral cavity may also serve as a reservoir for infection of contiguous structures such as the sinuses or middle ear. Oral bacteria may ascend from the mouth through the middle meatus to the maxillary sinus, or through the Eustachian tube to the middle ear, causing infections. Dental conditions such as periodontitis or periapical granuloma have been associated with 40% of chronic maxillary sinusitis cases (Melen et al., 1986). Typically oral anaerobic bacteria such as Porphyromonas, Prevotella and Fusobacterium species can be detected by culture methods in infections of the middle ear (Brook et al., 2000), tonsils (Rajasuo et al., 1996) and sinuses (Le Moal et al., 1999). Aerobic species that normally reside in the oral cavity, such as viridans streptococci, have also been found in chronic sinusitis (Biel et al., 1998). Additionally, elevated serum levels of antibodies against F. nucleatum and Prevotella intermedia have been observed in patients with chronic maxillary sinusitis (Brook & Yocum, 1999).

The objective of this study was to determine whether bacteria were present in clinical samples from chronic maxillary sinusitis. We also attempted to determine if bacteria detected within the chronically inflamed maxillary sinus were simultaneously present in the oral cavity of the same patient. Using culture-independent methods, we also aimed to detect novel as-yet-uncultivable bacterial species in the chronically inflamed maxillary sinus.

### METHODS

**Subjects.** Eleven adult patients with a history of chronic maxillary sinusitis, who had been referred to an ear, nose and throat surgeon (J.M.B.) for endoscopic sinus surgery, were studied (Table 1). Each patient had at least 50% opacification of the sinus, as determined by lateral radiographical scans. There was no evidence of clinical disease of the gingiva or periodontal tissues, nor of endodontically involved teeth, as the cause of the sinus condition. Some of the subjects had received standard courses of antibiotic treatment, but none of them had received antibiotics for at least 2 weeks prior to sample collection. This protocol was approved by the State University of New York at Buffalo Human Studies Institutional Review Board, and informed consent was obtained from each subject enrolled in the study.

**Specimen collection.** Washes and/or tissue samples from maxillary sinuses were obtained during standard surgical treatment as follows: after sterilization of the nasal vestibule and inferior meatus with ethanol, a sterile 18 G trocar needle was introduced into the maxillary sinus. Tissue specimens were aspirated directly from the sinus, or the sinus was flushed with sterile saline and the fluid was removed with a sterile 10 cm3 syringe attached to the trocar. Oral swabs from the buccal marginal gingiva in the maxillary premolar regions of both sides of the mouth were also collected at the time of surgery. A portion of each maxillary sinus sample was sent to a clinical laboratory for microbiological culture, and the remaining samples were stored at −70°C until they were processed for PCR analysis.

**Microbiological culture.** Samples were transported to the clinical laboratory in brain heart infusion solution for routine cultivation of bacteria and fungi. Samples were cultured in thioglycollate broth and on blood, chocolate, MacConkey, Columbia CNA, Sabouraud and Mycosel (Becton Dickinson) agars. All media were maintained aerobically at 37°C in 5% CO2. Any growth in thioglycollate broth or on blood agar, chocolate, MacConkey, Columbia CNA, Sabouraud and Mycosel (Becton Dickinson) agars. All media were maintained aerobically at 37°C in 5% CO2. Any growth in thioglycollate broth or on blood agar, chocolate, MacConkey, Columbia CNA, Sabouraud and Mycosel (Becton Dickinson) agars. All media were maintained aerobically at 37°C in 5% CO2. Any growth in thioglycollate broth was subcultured to blood agar and incubated both aerobically and anaerobically. The lower limit of culture detection was approximately 100 c.f.u. (ml sample)−1.

**Specimen digestion and DNA extraction.** Tissue samples and oral swabs were suspended in sterile dH2O. Maxillary washes (at least 4 ml)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Co-morbidity</th>
<th>Antibiotic treatment</th>
<th>Antibiotics</th>
<th>Time without antibiotics prior to surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>58</td>
<td>None</td>
<td>Yes</td>
<td>Amoxicillin/clavulanate, clindamycin</td>
<td>2 months</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>63</td>
<td>None</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>36</td>
<td>None</td>
<td>Yes</td>
<td>Amoxicillin/clavulanate, cefprozil, levofloxacin</td>
<td>1 year</td>
</tr>
<tr>
<td>4</td>
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<td>32</td>
<td>Sarcoidosis</td>
<td>Yes</td>
<td>Levofloxacin</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>25</td>
<td>Cystic fibrosis</td>
<td>Yes</td>
<td>Amoxicillin/clavulanate</td>
<td>4 months</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>26</td>
<td>None</td>
<td>No</td>
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<tr>
<td>10</td>
<td>F</td>
<td>49</td>
<td>Asthma</td>
<td>No</td>
<td>Levofloxacin</td>
<td>7 months</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>40</td>
<td>None</td>
<td>Yes</td>
<td>Moxifloxacin</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>
were centrifuged at 10,000 g for 30 min and the resulting pellet was suspended in sterile dH2O. An equal volume of lysis buffer containing 400 μg proteinase K ml⁻¹, 2 % SDS (w/v), 100 mM Tris/HCl (pH 8.5) and 2 mM EDTA (Kroes et al., 1999) was added to all samples, which were then incubated at 55 °C overnight. DNA was extracted by phenol/ chloroform extraction and ethanol precipitation. Isolation of DNA from each sample was confirmed by agarose gel electrophoresis and ethidium bromide staining; the DNA was stored at −70 °C until use.

**PCR amplification of 16S rDNA.** PCR was performed in a reaction volume of 50 μl, consisting of 0·2 μl each dNTP (Invitrogen), 0·4 μl each primer, 5 μl 10× PCR buffer (Promega), 1·5 mM MgCl₂ and 1·0 U Taq DNA polymerase (Promega). Host primers used to amplify bacterial 16S rDNA were 27F (5'-AGAGTTTGATCCTGCTGAG-3') and 1492R (5'-GGTACCTTGGAGCTTGTAG-3') (Lane et al., 1985), 63F (5'-CGAGCCATTACAACTGAGTTC-3') and 1387R (5'-GCCGAGGTGTGATCCAGCTTC-3') (Garnier et al., 1998). PCR amplifications were performed using a thermal cycle (Perkin Elmer) in combination with a genomic DNA extraction kit (Wizard PCR Preps DNA purification system (Promega)). The purified amplificon was ligated into the vector pGEM-T (Promega) and transformed into E. coli DH5α competent cells (Invitrogen), according to the manufacturers’ instructions. From each sample, 100–200 ampicillin-resistant transformants, identified as white colonies on LB agar containing IPTG (Invitrogen) and X-Gal (Fisher Scientific), were included in each set of PCR amplifications. Chromosomal DNA from Escherichia coli was used as a positive control. PCR amplification was performed with 30 cycles as follows: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min. Amplification products were separated by 10% agarose gel electrophoresis and visualized under UV light after staining with ethidium bromide. To exclude false negative results caused by inhibitors in the sample, several dilutions of the original sample were used as a template, followed by a second PCR using the amplified product as a template. Each negative result was confirmed at least four times. In the literature, the lower detection limit for PCR has been estimated to be from 1 to 10 bacterial cells to more than 100 c.f.u. per PCR (Ahmet et al., 1997; Stoesser et al., 2000; Pseudomonas aeruginosa (5'-GGGCTGAACTAGGATCC-3') and 5'-CCACCTTCATGCTCTGCTGAGC-3') (Wilson et al., 1999) and Serratia marcescens (5'-GGGAAGGCTGTCGATGTTG-3') and 5'-GGGTAATTAGGGAAGAACATATGTG-3') (Wilson et al., 1999) were used in PCR amplifications as described in this study and the original publications. Representative clones were sequenced as positive controls in PCR amplifications.

**Cloning of amplified 16S rDNA.** The 16S rDNA products were purified using the Wizard PCR Preps DNA purification system (Promega). The purified amplificon was ligated into the vector pGEM-T (Promega) and transformed into E. coli DH5α competent cells (Invitrogen), according to the manufacturers’ instructions. From each sample, 100–200 ampicillin-resistant transformants, identified as white colonies on LB agar containing IPTG (Invitrogen) and X-Gal (Fishare Scientific) after overnight incubation at 37 °C for 3 days, were selected by colony hybridization. Cells from representative colonies were suspended in 50 μl dH₂O, boiled, and the supernatant was used as a template in PCR with primers M13 forward (5'-GTTACCTTGCATGAGCTGCAG-3') and M13 reverse (5'-GGAAAACAGCTATGACCATG-3') to amplify bacterial 16S rDNA in maxillary sinuses.

**Detection of bacterial species identified from maxillary sinuses in oral samples from the same patient.** For each patient, an attempt was made to detect the species obtained from the sinus within the oral sample obtained at the time of sinus surgery. Primers specific to Streptococcus oralis/Streptococcus mitis (5'-GTCAAGGTCTAGATGATG-3') and 5'-GTGATCCCTGACGTTACGCAG-3') (Garnder et al., 1997), Streptococcus gordonii (5'-TGATAGCTGCTACGTGC-3') and 5'-TACACCGGTGGAAGAAG-3') (Brown et al., 1999), Staphylococcus aureus (5'-TTTTAGGAAGAACATATGTGC-3') and 5'-CCACCTTCATGCTCTGCTGAGC-3') (Wilson et al., 1999) were used in PCR amplifications as described in this study and the original publications. Representative clones were sequenced as positive controls in PCR amplifications.

**RESULTS**

**Detection and identification of bacteria and bacterial 16S rDNA in maxillary sinuses.** Bacteria and/or bacterial 16S rDNA were detected in maxillary sinus samples from five of the 11 patients examined (45 %; Table 2). Positive PCR amplifications were obtained with 1–3 of the bacterial species-specific primer pairs used. Three sinus samples (patients 3, 4, and 6) were positive by both PCR and culture methods (Table 2). One sample (patient 7) was positive only by PCR, and one (patient 11) was positive only by culture (Table 2). Table 3 shows the genera identified by PCR detection and sequencing of bacterial 16S rDNA, as well as non-cultivable flora of the maxillary sinus.

**Sequencing and phylogenetic analysis.** Plaidsms for sequencing were prepared by using the Wizard Plus SV Miniprep DNA purification system (Promega). DNA sequencing was performed using primer T7, complementary to the vector pGEM-T, by the Biopolymer Facility at the Roswell Park Cancer Institute, Buffalo, NY, USA. Sequences of inserts were compared to the 16S rDNA gene sequences in GenBank using BLAST software. DNA sequences sharing ≥ 98 % identity with known sequences were assigned to that phylotype.

**Table 2.** Detection of bacteria and/or bacterial rDNA in maxillary sinuses of 11 patients with chronic maxillary sinuses by PCR using three primer pairs and culture.

<table>
<thead>
<tr>
<th>Patient</th>
<th>PCR primer pair</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>27F, I492r</td>
<td>63F, I387r</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>+</td>
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<tr>
<td>3</td>
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<td>−</td>
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<tr>
<td>4</td>
<td>−</td>
<td>+</td>
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<td>6</td>
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<td>9</td>
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<tr>
<td>10</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>11</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>
### Table 3. Species identified in bacteria-positive maxillary sinuses by molecular and culture methods

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cloned 16S rDNA PCR</th>
<th>Culture result</th>
<th>Closest published sequence (accession no., similarity)</th>
<th>Primer pair GenBank accession no.</th>
<th>Closest published sequence (accession no., similarity)</th>
<th>Culture result</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Wash, +</td>
<td>Anaerobic, Gram-positive bacilli</td>
<td>Streptococcus mitis (AJ295853 and AF00392, 99 %)</td>
<td>Bact-338, Bact-1525 AY307987</td>
<td>Streptococcus oralis (AJ295853 and AF003932, 99 %)</td>
<td>Anaerobic Gram-positive bacilli</td>
</tr>
<tr>
<td>4</td>
<td>Tissue, +; Wash, +</td>
<td>Enterococcus avium (AF061008, 96 %)</td>
<td>Pseudomonas aeruginosa (AF331663, 99 %)</td>
<td>63f, 1387r AY307995</td>
<td>Pseudomonas aeruginosa (AF331663, 99 %)</td>
<td>Enterococcus avium</td>
</tr>
<tr>
<td>5</td>
<td>Tissue, +; Wash, +</td>
<td>Prevotella gen. (AY005061, 94 %)</td>
<td>Staphylococcus aureus (AB008509, 99 %)</td>
<td>63f, 1387r AY307996</td>
<td>Staphylococcus aureus (AB008509, 99 %)</td>
<td>Prevotella gen.</td>
</tr>
<tr>
<td>6</td>
<td>Tissue, +; Wash, +</td>
<td><em>– –</em></td>
<td><em>– –</em></td>
<td>63f, 1387r AY307997</td>
<td><em>– –</em></td>
<td><em>– –</em></td>
</tr>
<tr>
<td>7</td>
<td>Wash, +</td>
<td>Enterococcus avium (AF061008, 96 %)</td>
<td>Staphylococcus aureus (AB008509, 99 %)</td>
<td>Bact-338, Bact-1525 AY307998</td>
<td>Stenotrophomonas maltophilia (AB008509, 99 %)</td>
<td>Enterococcus avium</td>
</tr>
</tbody>
</table>

- **PCR product too weak for cloning and sequencing.**

### Possible novel species

The majority of the bacterial sequences identified in this study demonstrated > 98 % identity with previously known bacterial species. No previously unrecognized species were recovered, but one clone from patient 4 demonstrated 94 % sequence identity with a *Prevotella* sp. oral clone, and one clone from patient 3 demonstrated 96 % sequence identity with an *Enterococcus avium* strain when > 1000 bp were compared. As < 97 % similarity is considered to differentiate species within genera (Stackebrandt & Goebel, 1994) and < 90 % similarity to represent a species from an unknown genus, these clones may represent novel species related to *Prevotella* or *Enterococcus*.

### Relationship between bacteria detected in the maxillary sinus and the oral cavity by PCR

In patient 3, nine different bacterial clones were identified from the maxillary sinus; the majority of these clones were typically oral species such as *Streptococcus* sp., nutritionally variant streptococci, *Eubacterium* sp. or *Neisseria* sp. In the same patient, an attempt was made to detect the same species in the oral cavity. Indeed, a positive result was obtained with *Streptococcus mitis/Streptococcus oralis* primers, but a negative result was obtained with *Streptococcus gordonii* primers. In patient 4, a *Prevotella* clone with a possible oral origin was detected in the maxillary sinus. Also, in patient 4, attempts to find the same species by molecular methods from the oral cavity gave positive results with *Pseudomonas aeruginosa* as by bacterial culture from maxillary sinuses. In three cases (patients 6, 7 and 11) only one bacterial species was detected, in one case (patient 4) three species were detected, and in one case (patient 3) nine different RFLP patterns were identified among the clones by PCR amplification and cloning, with the majority of the clones identified as *Streptococcus* sp. In patient 3, culture results indicated the presence of anaerobic Gram-positive bacilli, which correlated with PCR results that indicated the presence of *Eubacterium* sp. (Table 3). In patient 4, *Pseudomonas aeruginosa* was detected using both methods, and two additional species were detected by PCR (Table 3). The only species that was not detected by universal PCR was *Staphylococcus aureus*; all three cases that were culture-positive for *Staphylococcus aureus* were PCR-negative for this species. One of the bacteria-positive sinus samples (patient 6) gave so weak an amplification product after both the first and second PCRs using the universal primer pair (27f and 1492r) that it could not be processed further for cloning. As that particular maxillary sinus bacterial isolate was *Staphylococcus aureus*, the *Staphylococcus* 16S rDNA primers (Jaffe *et al.*, 2000) were used in PCR amplification of the maxillary sample. It was found to be positive for *Staphylococcus* rDNA. Sinus samples of two other patients (4 and 11) that were culture-positive but PCR-negative for *Staphylococcus aureus* were also amplified with *Staphylococcus* 16S rDNA primers, but were found to be negative. Fungi were not isolated from the maxillary sinus samples, and were not tested for by molecular methods.
primers and negative results with Staphylococcus aureus and Serratia marcescens primers. In patients 6 and 11, Staphylo-
coccus aureus, which was present in the maxillary sample, was
detected in the oral sample by molecular methods. For
the other species identified in the maxillary sinuses, specific
primers were not available or the primers designed as
described in previous studies did not work in our laboratory
with the matched species DNA.

**DISCUSSION**

In the present study, maxillary sinus tissue samples and/or
washes obtained from adult patients with a history of chronic
maxillary sinusitis were examined for the presence of
bacteria. In addition to bacterial culture, PCR amplification
of bacterial 16S rDNA followed by cloning and sequencing
was employed to detect and identify non-cultivable bacterial
species within the sinus. To determine whether the oral cavity
may serve as a source of bacterial colonization of the
maxillary sinus, an attempt was also made to correlate the
species identified in the maxillary sinus with the dental
plaque from the same patients.

We detected bacteria and/or bacterial 16S rDNA in five of 11
patients (45%). Our results corroborate those of some
previous studies, where bacteria have been detected in
mucosal specimens from approximately 50% of chronic
maxillary sinusitis cases (Jiang et al., 1998; Hwang et al.,
1999). However, other studies have reported that up to 92% of
chronic sinusitis samples contain bacteria (Brook et al.,
1997). The type of specimen and the methods of detection
may contribute to the different results observed. Our results
are based upon analysis of mucosal tissues or maxillary sinus
washes, because no exudates were obtained from these
patients. The results must also be interpreted with caution,
as the present study was limited to 11 subjects. Studies of
larger populations using culture-independent methods are
necessary.

Of the three primer pairs used to amplify bacterial DNA in
our study, primers 27f and 1492r are broadly conserved and
have previously been widely used to detect bacteria in human
biopsies and body fluids (Dymock et al., 1996; Rantakokko-
Jalava et al., 2000). To enhance detection of some specific oral
species such as Prevotella, two additional bacterial-specific
primer pairs that were designed to detect oral species were
used (Kroes et al., 1999; Marchesi & Weightman, 2000). It is
acknowledged that the absence of bacterial DNA in chronic
maxillary sinusitis samples may simply be due to the fact that
the number of bacteria in the sample was below the detection
limit of the PCR method used.

Three maxillary sinus samples were positive by both PCR
and culture methods. One sample was positive only by PCR, and
one was positive only by culture. PCR has previously been
found to be more sensitive than standard culture methods
when mucosal specimens from chronic sinusitis were ana-
lysed for the detection of aerobic bacteria (Keech et al., 2000).
However, in three cases where Staphylococcus aureus was
detected by culture, it was PCR-negative or faintly positive,
and therefore was not suitable for further analysis using
universal PCR primers. Whilst every effort was made to
collect sterile specimens, some of these culture-positive
results could be the result of contamination of the sample
during collection or processing. This may be particularly true
in the case of patient 11, who received moxifloxacin, a
fluoroquinolone that is effective against Staphylococcus
aureus, 2 weeks before sample collection. This sample was
PCR-negative but culture-positive for Staphylococcus aureus.
Alternatively, the Staphylococcus-specific primers used here
may not bind to the strains found in this study.

The species identified in the present study are not commonly
associated with acute bacterial rhinosinusitis by culture
methods (Gwaltney, 1996). The bacteria detected in max-
illary sinuses in this study included several typically oral
species, suggesting that the oral flora may be a source for
bacterial colonization of the chronically inflamed maxillary
sinus. Of these species, viridans streptococci have previously
been identified in chronic maxillary sinusitis by culture
methods (Biel et al., 1998), and elevated antibody levels to
Prevotella intermedia have been observed in patients with
chronic maxillary sinusitis (Brook & Yocum, 1999). How-
ever, the presence of specific antibodies against an organism
does not necessarily define it as a pathogen. Interestingly,
A. defectiva and Granulicatella elegans, previously
classified as nutritionally variant streptococci and members
of the normal flora of the oral cavity, throat, urogenital and
intestinal tracts, were identified by molecular methods from
the same maxillary sinus. These species are rarely cultured
from clinical samples because of their fastidious nature,
which may have contributed to the fact that they were
detected and identified here only by molecular methods.
Recent results from a multicentre study corroborate our
findings by showing that Granulicatella species are associated
with chronic maxillary sinusitis and may contribute to
antibiotic treatment failure (Finegold et al., 2002).

Possible contiguous spread of bacterial flora from the oral
cavity to the maxillary sinus was investigated by attempting
to find the species detected in the sinus also in the oral cavity,
using published species-specific primers. There were no
primers available for some species, or the primers that were
available did not work in our laboratory as described in the
original publications, so our efforts were limited to about half
of the species detected in the sinus. Two of five bacterial
species found in the maxillary sinus were also found in the
oral cavity by PCR using species-specific primers. The same
Streptococcus mitis and/or Streptococcus oralis clone(s) were
found in the oral cavity and the maxillary sinus in one
patient. In another patient, Pseudomonas aeruginosa, identi-
fied in the maxillary sinus, was also found in the oral cavity.
Previous studies suggest that oral colonization by potential
respiratory pathogens may occur, and that dental plaque may
serve as a reservoir for these organisms (Scannapieco et al.,
1992). Unexpectedly, Streptococcus gordoni, a common
colonizer of the oral cavity, was not found in the mouth of
the patient who carried it in the maxillary sinus. Also,
Staphylococcus aureus and Serratia marcescens were not detected in the oral samples. However, this does not rule out the possibility that these species may have been present in very low numbers in our samples and thus below the detection limit for the method.

As PCR does not distinguish viable from dead bacteria, we cannot say that the present cases of chronic maxillary sinusitis are definitely caused by the bacterial species isolated. It is possible that there were only dead bacteria or released DNA left in the sinuses from a past infection by these organisms. Bacterial DNA has been demonstrated to persist in human synovial fluid and tissue samples as long as 10–22 days after the initiation of antimicrobial treatment, whereas all samples became culture-negative by 2–3 days following the start of therapy (van der Heijden et al., 1999). In contrast, studies on chinchillas have shown that bacterial DNA can be found only for 1 day in the presence of middle ear effusion (Aul et al., 1998). The elimination of bacterial DNA may therefore depend on the body site and fluid. The role of bacteria in chronic sinusitis and related disorders has been questioned, and fungal pathogens have been proposed to be responsible for the inflammatory reaction and mucosal response in the sinus. However, in the present study, no fungi were detected in chronic maxillary sinusitis by culture and we did not attempt to detect fungi by molecular methods.

In conclusion, the present results suggest that both aerobic and anaerobic bacteria can be detected in nearly half of chronic maxillary sinusitis cases. The species identified in the present study are rarely associated with acute bacterial rhinosinusitis and include several oral species, suggesting that these are present in the inflamed maxillary sinus. The simultaneous detection of some bacterial species in the maxillary sinus and in the oral cavity shows that the direct connection between these two sites may allow oral bacteria to contribute to non-oral inflammatory conditions.

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REFERENCES


