Sinusitis: an improved regime of investigation for the clinical laboratory

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SUMMARY Clinical material from 200 patients suffering from acute sinusitis was examined cytologically and bacteriologically. Seventy per cent of specimens were collected by aspiration of the middle meatus, 20% by antral wash, and 10% by direct swabbing of the antra. A comparison of the results from these differing techniques is made. The bacteriological methods used and the isolates and their significance are discussed, and suggestions are offered for improving the routine demonstration of presumptive pathogens in this disease.

The collection of satisfactory clinical material from infected nasal sinuses is best undertaken by the ear, nose and throat specialist. However, the far greater number of patients under the care of general practitioners generally rely on the clinical laboratory for specimen collection, and here an adequate routine technique is not always available. Swabs of any type are often contaminated by commensal flora, giving results that are at best suspect and at worst frankly misleading (Axelsson and Brorson, 1973). The aspirate method of collection described in this paper appears to produce adequate material during the acute phase of sinusitis, to demonstrate a significant pathogen in most cases, and to be no more than slightly uncomfortable for the patient. It is also simple to operate with a minimum of experience. The bacterial isolation methods employed have produced fewer sterile culture results than are recorded in previously published surveys (Sparrevohn and Buch, 1946; Palva et al., 1962; Lystad et al., 1964; Montgomery Smith and Smith, 1971; Frederick and Braude, 1974), and evidence that a significant number of pathogens may be missed by standard primary isolation methods is offered.

Patients and methods

Patients
Patients were selected on the following criteria:
(1) A current attack of acute sinusitis with discharging sinus(es). Ideally, where evidence of sinus opacity, by either x-ray or transillumination, was available, this should be noted.
(2) A past history of sinusitis involving at least one previous acute episode.
(3) No intercurrent infection present, e.g. a common cold.
(4) No current antibiotic therapy.

Findings from patients not conforming to these criteria were excluded from the results.

All patients undergoing aspiration at the laboratory were seen by request, and the aspirations were undertaken by the author. Materials supplied by ENT specialists were taken by them either by antral wash or by swabs of frank pus, the latter either from sinus puncture or postoperatively.

Aspiration apparatus
This comprised a sterile Surflow (Terumo) disposable, winged infusion set from which the needle was removed to leave a flexible, thin, plastic tube with an effective length of about 30 cm. The plastic bevel on the far end of this tube was pushed firmly and rotated on to the hub of a sterile, plastic, disposable 20 ml syringe. The whole assembly is shown in Figure 1.

Technique
The collection of antral pus from the middle meatus in the nasal cavity is undertaken in the following way. The patient is asked to lie supine on a medical couch without the aid of any pillow or other support. The head is rotated sideways to rest on the couch at about 45° from the horizontal, this inclination being towards the side of the face on which the...
patient judges the sinus involvement to be most severe. In the absence of a firm opinion, an arbitrary side is chosen. A wait of up to 5 minutes is made to ensure gravitational drainage from the affected sinus(es) or less if the patient reports passage of exudate down the throat. During the waiting period a simple and reassuring explanation of the technique is given, and the point is emphasised that although the procedure may be slightly uncomfortable and that the eyes may water, no actual pain will be felt.

The open end of the plastic tube is passed gently but firmly up the anterior nares to the posterior nares. By steady pressure and manipulation, the tube, being sufficiently rigid, will take the line of least resistance into the middle meatal area. The distance required to reach this area is about 12 cm from the outer edge of the external nares in the adult and proportionately less in the child. The syringe, with the plunger extended and held in the other hand, is manipulated to expel air slowly during this passage to exclude the entry of mucus from the nares into the tube. Once the middle meatus is reached, a steady suction is applied. An initial dry tap usually needs only small back and forth movements of the tube to reach exudate. If the syringe fills with air, the plastic bevel is simply rotated off the syringe hub, the syringe plunger is depressed, and the bevel is reattached. Exudate is clearly visible as it descends in a fluid column below the outer edge of the anterior nares. Accidental passage right through to the edge of the soft palate is quickly noted by the patient who generally gags and coughs. The passage of a fresh tube is found to be preferable in these cases to exclude pharyngeal contamination. Given actively draining sinus(es), the whole procedure takes about 1-2 minutes.

Where a totally dry tap was found, the other side often produced an adequate sample and, failing this, a later aspiration, say the next day, was commonly successful in the acute patient.

The exudate was expelled completely from the tube by repeated sharp depressions of the syringe plunger, on to the primary blood agar culture plate and was sufficient to inoculate all media and make two smears on glass slides for staining.

**BACTERIOLOGICAL PROCEDURES**
Identification of isolates to species level (Fig. 2) was carried out according to the methods outlined by Cowan and Steel (1974).

**ANTIBIOTIC SENSITIVITY TESTS**
Readings of zones of inhibition around discs were measured against the quality control disc template. The antibiotics of most common choice in this condition were used for testing, i.e., penicillin, ampicillin, erythromycin, tetracycline, and cotrimoxazole.

In view of the problems of penetration into the sinuses of some common antibiotics (Lundberg et al., 1968; Editorial, Lancet, 1974; Agbim, 1975; Eneroth et al., 1975) isolates were reported as either 'sensitive' or 'resistant' to specific antibiotics.

Since only two species of anaerobes, of predictable antibiotic response, were isolated, no sensitivity tests were carried out on these strains. Routine sensitivity of *Haemophilus influenzae* strains to antibiotics was not undertaken but, in retrospect, a procedure to demonstrate β-lactamase producers could have been incorporated into the regime to possible clinical advantage (Nelson, 1974).

**Results**
The results are shown in Tables 1-3.

**STAINED FILMS OF EXUDATES**
In the Gram-stained film, 96 showed no conventional organisms on search but pleomorphic, Gram-negative bodies were present in approximately
ANTRAL PUS
(Middle meatal aspirate, washings or pus)

- Direct film
  - Gram stain
  - Bacterial flora—type and quantity. Pus cells—quantity
- Direct film
  - Giemsa stain
  - Presence of significant eosinophilia
- Colombia blood agar 37°C for 24 h in CO₂ incubator
  - Aerobic bacterial isolates
  - Subculture isolates on to Sensitest agar. Add antibiotic discs and incubate 37°C in CO₂ incubator
- Colombia blood agar (prereduced) in Gaspak jar 37°C for 48 h in incubator
  - Anaerobic bacterial isolates
  - Subculture Leventhal agar 24 h at 37°C in CO₂ incubator
- *BHI-sucrose broth 18-24 h at 37°C in CO₂ incubator
  - Reversion of atypical forms to parent type
  - Subculture Leventhal agar 18-24 h at 37°C in CO₂ incubator
  - Subculture Sensitest agar with antibiotic discs. Incubate 24 h at 37°C in CO₂ incubator
  - Subculture Sensitest agar
- Leventhal agar 37°C for 24 h in CO₂ incubator

Fig. 2 Primary methods of bacterial isolation from exudates.

Table 1 Numbers and percentages of organisms isolated from sinus exudates, listed according to methods of collection and species

<table>
<thead>
<tr>
<th>Species</th>
<th>Middle meatal aspirate</th>
<th>Antral wash</th>
<th>Antral pus</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep. pneumoniae</td>
<td>43</td>
<td>6</td>
<td>4</td>
<td>53</td>
<td>27.6</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>29</td>
<td>10</td>
<td>4</td>
<td>43</td>
<td>22.4</td>
</tr>
<tr>
<td>H. influenza</td>
<td>15</td>
<td>6</td>
<td>1</td>
<td>22</td>
<td>11.5</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>13</td>
<td>5</td>
<td>2</td>
<td>20</td>
<td>10.4</td>
</tr>
<tr>
<td>β-haemolytic strep A</td>
<td>9</td>
<td>4</td>
<td>—</td>
<td>13</td>
<td>6.8</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>5.2</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>9</td>
<td>4.7</td>
</tr>
<tr>
<td>Non-haemolytic strep</td>
<td>3</td>
<td>2</td>
<td>—</td>
<td>5</td>
<td>2.6</td>
</tr>
<tr>
<td>Commensal flora</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>2</td>
<td>1</td>
<td>—</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>2</td>
<td>—</td>
<td>1</td>
<td>3</td>
<td>1.6</td>
</tr>
<tr>
<td>H. parainfluenza</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Proteus morganii</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Micrococcus spp.</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Totals</td>
<td>136</td>
<td>39</td>
<td>17</td>
<td>192</td>
<td>100.0</td>
</tr>
<tr>
<td>No growths</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>19</td>
<td>9.5</td>
</tr>
</tbody>
</table>

half of these (see Discussion). The 19 sterile cultures were included in this 'no organism' group with one exception—that in a patient found to have been on non-admitted, self-administered antibiotic therapy.

The Giemsa-stained films of exudate produced no significant eosinophilias, suggesting an absence of any allergic element in the patients examined. No fungal structures were demonstrated in any stained film.

QUALITY OF SPECIMENS

All specimens were graded for the amount of pus present. The ratios were: scanty pus, 62 specimens (31%); moderate pus, 88 specimens (44%); heavy
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Table 2  Atypical bacterial forms isolated from hypertonic broth cultures: species, numbers, percentages, and sources of exudates

<table>
<thead>
<tr>
<th>Species</th>
<th>Middle meatal aspirate</th>
<th>Antral wash</th>
<th>Antral pus</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td>22</td>
<td>3</td>
<td>3</td>
<td>28</td>
<td>43.9</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>14</td>
<td>21.9</td>
</tr>
<tr>
<td>Strep. pneumoniae</td>
<td>4</td>
<td>1</td>
<td>—</td>
<td>5</td>
<td>7.8</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>7.8</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>3</td>
<td>1</td>
<td>—</td>
<td>4</td>
<td>6.2</td>
</tr>
<tr>
<td>β-haemolytic, strep A</td>
<td>2</td>
<td>2</td>
<td>—</td>
<td>4</td>
<td>6.2</td>
</tr>
<tr>
<td>H. parainfluenzae</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>Totals</td>
<td>47</td>
<td>11</td>
<td>6</td>
<td>64</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3  Sex and age classification of patients

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>11-20</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

pus, 50 specimens (25%). It was significant that 13 sterile cultures, or 80% of all sterile cultures, derived from exudates containing only scanty pus.

Seasonal changes

Since sampling was spread evenly throughout the year, any seasonal change in the bacterial flora should have been apparent; no such changes were noted.

Discussion

The predominance of Streptococcus pneumoniae in this series is in agreement with the findings of several previously published surveys (Lystad et al., 1964; Sparrevoorn and Buch, 1946; Urdal and Berdal, 1949; Björkwall, 1950; Montgomery Smith and Smith, 1971). The importance of the second highest isolate is not so clear. The literature reviewed shows a sharp division of opinion regarding Staphylococcus aureus as a cause of sinusitis. Some authors doubt its pathogenic status (Lystad et al., 1964; Kinnman et al., 1967; Dawes, 1971; Chapnik and Bach, 1976) while others acknowledge its place in the disease (Sparrevoorn and Buch, 1946; Reynolds et al., 1964; Rulon, 1970; Montgomery Smith and Smith, 1971; Frederick and Braude, 1974).

This paper presumes pathogenicity for the following reasons:

1. *Staph. aureus* was the sole isolate in 43 cases of acute sinusitis and its incidence was proportionately equal by all three collection methods.

2. Where the organism was grown on standard media, characteristic Gram-positive cocci were almost invariably present, as the sole organism, in the exudates.

3. Where hypertonic broth was required for reversion of atypical strains to parent form (66% of all *Staph. aureus* isolates), approximately half the stained films of exudate showed scanty Gram-variable cocci, many with distortion effects. A further 30% showed pleomorphic, Gram-negative bodies of variable size, and the remainder showed no obvious structures. The absence of presumptive cell wall-deficient organisms, however, does not appear to exclude their presence (Cate, 1974) within the debris of stained films.

It must also be conceded that a commensal role for this organism does not exclude the possibility of a pathogenic role for the same organism in the sinuses, particularly in chronic sinusitis under successive antibiotic treatments. The antibiotic sensitivity patterns of this species sustains this contention in that only five of the 43 isolates were fully sensitive to the five drugs of common choice; 14 strains were resistant to two antibiotics; 12 strains were resistant to three antibiotics; 10 strains were resistant to four antibiotics; and two strains were resistant to all five antibiotics. Only one strain was methicillin-resistant.

Species in general did not appear to be age-related although, in subjects under the age of 10 years, *Strep. pneumoniae* and *H. influenzae* accounted for 68% of isolates, which is in general agreement with
published surveys (Rulon, 1970; Montgomery Smith and Smith, 1971; Chapnik and Bach, 1976).

Some investigators have demonstrated large numbers of anaerobes in sinus exudates (Fredette et al., 1961; Frederick and Braude, 1974; Kidder et al., 1975). With only 13 isolates, this series has produced a disappointingly low percentage (6.8%). This may be due to the fact that cultures were incubated for a maximum of 48 hours, a time limitation imposed for clinical reasons. Further work is proceeding in this area and is hoped to be a subject for later publication.

Strains of organisms damaged by prior antibiotic treatment present problems of laboratory isolation in most pyogenic infections, including sinusitis (Barile et al., 1963; Godzeski et al., 1965; Guze, 1968; Gnarpe and Lundberg, 1971; Bhattacharyya et al., 1972; Sprinkle, 1972). The organisms in this series isolated only from hypertonic broth pose a problem of nomenclature. Although their demonstration required incubation in hypertonic broth, they did not meet the commonly accepted criteria for L-forms, spheroplasts, or protoplasts (Guze, 1968; Feingold, 1969) either in extended serial subculturing requirements or in colonial appearances once reversion to the parent form had been achieved. It is suggested that these isolates are 'atypical bacterial forms', as defined by Charache (1968), and because of their swift reversion to parent form are a presumptive pathogen in this site. This contention is supported by a significant number of clinical remissions in affected patients, obtained by antibiotic treatment (other than those of the penicillin group) directed solely against these organisms. Atypical bacterial forms may reasonably be construed as organisms with defective cell walls within this context. In this series, 64 out of 192 isolates (33%) were demonstrated only by hypertonic broth culture, and material from all three collection methods was included. The broad range of species suggests that such media are essential better to define the bacteriology of sinus exudates.

The 19 sterile exudates were all associated with very recent antibiotic therapy. No more than two organisms were found in any mixed infection and no particular pairing was noted although four out of 11 had an anaerobe as a member, two each of Bacteroides and Peptostreptococcus species.

Commensal flora is generally considered to comprise Neisseria catarrhalis, Streptococcus viridans, diphtheroids, and Staphylococcus epidermidis in the upper respiratory tract. One of the more encouraging features of the described regime is the very low number of commensal-contaminated specimens (2%).

The following conclusions, therefore, seem to be valid:

1. Since there is very little difference between the findings from the three types of specimen collection, middle meatal aspiration offers a safe and useful technique applicable to all age groups.

2. The suspicion that middle meatal specimens routinely risk contamination by commensal flora is not supported by this series.

3. The satisfactory isolation of presumptive pathogens from sinus exudates is dependent on a combination of standard media and hypertonic broth culture.

4. This regime, as a whole, is simple to operate, relatively inexpensive, time-conserving, and fits easily into the routine of the clinical laboratory.

I am grateful to Miss Anne Grigor for technical assistance and to those practitioners who allowed me access to their patients for the purposes of this survey.

References


Requests for reprints to: R C Bridger, Pathology Laboratory, Colston House, 137 Kilmore Street, Christchurch 1, New Zealand.
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