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Detection of anaerobic wound infection by analysis of pus swabs for volatile fatty acids by gas-liquid chromatography

P. J. REED AND P. J. SANDERSON

From the Division of Communicable Diseases, Clinical Research Centre, and Department of Microbiology, Northwick Park Hospital, Harrow, Middlesex, UK

SUMMARY Swabs were able to absorb enough extractable volatile fatty acids from broth cultures of anaerobic organisms for detection and analysis by gas-liquid chromatography (GLC). Similarly, volatile fatty acids were often detected in swabs dipped into liquid pus. Fifty-three liquid pus specimens were then investigated fully to determine if GLC analysis of swab samples gave the same result as microbial culture of the specimens and GLC analysis of the liquid pus. Anaerobic bacteria failed to grow from 36 and volatile fatty acids were not extracted from swabs of 31 of these pus samples but *were* extracted from swabs of five. Anaerobic bacteria were isolated from 17 of the specimens, and in 15, volatile fatty acids were also detected in the swab samples; in two, volatile fatty acids were absent from both swab samples and liquid pus. In this study, results by culture and GLC analysis of swabs were similar in 87% of specimens.

The volatile fatty acid products of anaerobic bacterial metabolism can be detected by gas-liquid chromatography (GLC) (Holdeman and Moore, 1977). This technique has been used for the rapid diagnosis of the presence of anaerobic organisms in clinical liquid pus specimens (Phillips *et al.*, 1976).

However, it is sometimes difficult to obtain samples of pus, and this paper describes studies in which volatile fatty acids were sought in swabs moistened with clinical specimens of pus in the laboratory. The diagnosis of anaerobic infection by this method was compared with microbial culture and with examination of liquid pus by GLC.

Material and methods

PREPARATION OF CULTURES

Anaerobic organisms isolated from clinical specimens were grown in cooked meat broths before inoculation into pre-reduced brain heart infusion broths and incubated anaerobically for two to six days at 37°C.

PUS SAMPLES

These were collected from the microbiology laboratory after routine culturing procedures had been completed.

SWABS

These were sterile Hospiswabs (Medical Wire and Equipment Co Ltd, Corsham, Wilts, UK) as routinely used in the hospital. They were tested for extractable volatile fatty acids and found to yield small amounts of acetic and isovaleric acids, which originated in the wooden stick on which the cotton wool was wound. The isovaleric acid extracted from a swab head was not normally more than 20% of the concentration of this acid in an extract of 1 ml of standard mixed acid solution. Allowance for this was made when assessing the volatile fatty acid yielded by pus swabs.

SWAB SAMPLES

A swab was immersed in the culture or pus, drained of excess on the inside of the container, and replaced in its holder. It was stored at room temperature for at least 2 hours to simulate the delay likely between ward collection and processing in the laboratory.

EXTRACTION PROCEDURE

Cultures and pus

An aliquot (1 ml) was extracted with an equal

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volume of ether and a drop (approximately 0·02 ml) of sulphuric acid (50% v/v). The two phases were briefly mixed and separated—the broths by standing, the pus by centrifugation—and the ether phase was collected.

Swabs

The swab head was snapped from its stick into a small bottle. Ether (1 ml) and sulphuric acid (1 drop of 5% v/v) were added. The bottle was capped and vortex mixed for approximately 20 seconds.

CHROMATOGRAPHY

A Varian 3700 chromatograph with dual flame ionisation detectors was used with a Servoscribe 1s recorder. The glass column was 5 ft × 2 mm (id), acid washed, packed with 5% Carbowax 20M TPA on Gas Chrom Q 100-120 mesh. Operating temperatures were: injector 150°C, column 80°C, and detector 200°C. The carrier gas was nitrogen at 40 ml/min. A 5 μl syringe (Scientific Glass Engineering Pty, Ltd, 657 North Circular Road, London NW2 7AY) was used to inject 1 μl samples of the ether extracts.

The minimum concentrations of volatile fatty acids considered significant when analysing cultures and pus were those of the standard mixed acid solution. When analysing swabs any volatile fatty acids present, after allowing for natural content, were considered significant.

Standards

A mixed acid solution was made containing acetic and propionic acids at approximately 1 μmol/ml, and isobutyric, butyric, isovaleric, and valeric acids at approximately 0·1 μmol/ml. This solution was prepared for chromatography in the same way as were the cultures.

Results

DETECTION OF ANAEROBES BY EXTRACTION OF SWABS DIPPED INTO CULTURES

Twenty-four brain heart infusion broth cultures and corresponding swabs were analysed (Table). In 18 pairs, volatile fatty acids were detected in both the broth culture and the corresponding swab. A *Peptococcus* sp was the only organism not detected in the broth culture. Some strains of this organism produce acetic acid *only* (Holdeman and Moore, 1977), which is not diagnostic since this volatile fatty acid is also produced by aerobic organisms (Phillips *et al.*, 1976).

DETECTION OF ANAEROBES BY EXTRACTION OF SWABS DIPPED INTO PUS

The summary of findings for the 53 swabs and corresponding pus specimens, together with microbial culture, is given in the Figure. Twenty swabs positive for volatile fatty acid were found, 15 out of 17 that were positive by culture for anaerobes and five from the 36 that were culture-negative for anaerobes. Another three samples positive for volatile fatty acid were found from the remaining culture-negative samples on analysis of the liquid pus. These eight culture-negative but GLC-positive samples may represent (i) early infection or post-treatment states, (ii) an infection where culture has failed, or (iii) possible background levels of volatile fatty acids in damaged tissues. Anaerobic organisms isolated from the anaerobic culture-positive group of 17 samples (with frequency of isolation in parentheses) were: *Bacteroides fragilis* (8), *Bacteroides melaninogenicus* (4), *Bacteroides corrodens* (1), *Bacteroides* spp (3), *Clostridium perfringens* (4), *Eikenella corrodens* (1), *Lactobacillus* sp (1), *Peptococcus* spp (2), *Peptostreptococcus* spp (3). The two culture-positive specimens which were negative for volatile fatty acids on analysis of swabs

Table *Detection of volatile fatty acids in swabbed broth cultures of known anaerobic organisms*

Cultures		*VFA detected by GLC*	
Organism	*Number*	*Broth culture*	*Swab*
Bacteroides fragilis	3	3	**2***
Bacteroides melaninogenicus	1	1	1
Clostridium chauvoei	4	4	3
Clostridium difficile	1	1	1
Clostridium fallax	1	1	1
Clostridium sordellii	1	1	1
Clostridium tertium	1	1	1
Clostridium spp	4	4	2
Fusobacterium spp	2	2	2
Peptococcus spp	2	1	1
Peptostreptococcus sp	1	1	1
Veillonella spp	3	3	2
Total	24	23	18

VFA = volatile fatty acids.
*The figures in bold type indicate failure to detect consistently the cultured organism.

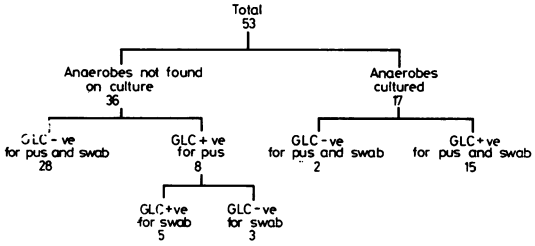

Figure *Summary of analysis of liquid pus specimens and pus swabs for volatile fatty acids by gas-liquid chromatography.*

and liquid pus yielded *Cl. perfringens* and *B. fragilis* in pure cultures. It has previously been shown by Gorbach *et al.* (1976) and Holdeman and Moore (1977) that these organisms can sometimes be present without producing significant volatile fatty acids.

Discussion

Analysis of swabs by GLC has detected volatile fatty acids typical of anaerobic bacteria in 15 out of 17 (88%) samples shown to contain anaerobes by culture. Additionally, five other swabs that were culture-negative for anaerobes had volatile fatty acids suggestive of anaerobic infection. Thus the technique shows a net increase in detection of anaerobic involvement in infections which, in conjunction with the speed with which results are obtained, makes this a worthwhile rapid screening technique where liquid pus is not available.

Preliminary work using swabs taken from wounds into transport medium was carried out. This showed that, although the transport medium is unlikely to interfere with the analysis, the extensive dilution of the volatile fatty acids into it drastically reduces the rate of detection. A trial is now required using swabs transported in a dry state to assess the consistency of detection of volatile fatty acids from swabs taken from wounds.

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Requests for reprints to: Mr P. J. Reed, Division of Communicable Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK.