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Difficulties in interpretation of culture results in sinusitis*†

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SUMMARY

In this article, we try to explain the difficulties met in the bacteriology laboratory when dealing with samples to diagnose sinusitis. The sampling techniques are very important since there are 3 main types of specimen. The transportation also needs to be done in a proper way, because fastidious organisms stop growing if optimal conditions are not achieved, and on the contrary, the commensal flora can overgrow the pathogens. The processing in the laboratory itself follows certain rules in order to reach some standardisation. Nevertheless, the culture results are sometimes difficult to interpret. Thus, the clinician as well as the bacteriologist, should relate laboratory results to clinical history.

Key words: Sinusitis, specimen types, specimen transportation

INTRODUCTION

Bacteriology is not an exact science. In terms of effectiveness of the laboratory, nothing is more important than the appropriate selection, collection, transportation and management of a specimen. Errors and mistakes can occur at several moments. When specimen collection and handling are not priorities, the laboratory can contribute little to patient care. The most important points are 1) sampling techniques 2) transportation 3) processing in the lab 4) culture results and 5) susceptibility testing. Consequently, all members of the medical staff' involved in these processes must understand the critical nature of maintaining specimen quality. Specific criteria for collection and transportation should be incorporated into each department's nursing manual.

METHODS

Specimen sampling techniques

In our hospital, we use the following indications for culturing sinus fluid samples:

- when clinical failure of the treatment is obvious (at the risk of having changed the flora during the first therapy);
- in intensive care patients suffering from sepsis;
- when suspecting a mycosis;
- during surgery for chronic sinusitis;
- in case of clinical studies with bacteriological outcome;
- on the occasion of epidemiological studies.

A specimen for bacteriology is best taken before any antibiotic treatment. Once antibiotics have been administered, cultures can become negative rapidly.

There are 3 main types of specimen (Figure 1). Type 1 is coming from a deep infectious process with only one possible way of access: a puncture through the skin or the mucosa where a normal flora resides. These commensals need to be eliminated before puncture or aspiration by adequate disinfection techniques. Skin can be disinfected with iodine alcohol or an alcoholic solution, mucosæ can not (Gwaltney, 1995). Skin disinfection looks more simple than it is. The positivity rate for blood cultures lies around 15%: half are false positives or contaminants. Disinfection of mucosa is only partial. An adequate sample in ENT is therefore difficult to obtain (Brook, 1981).

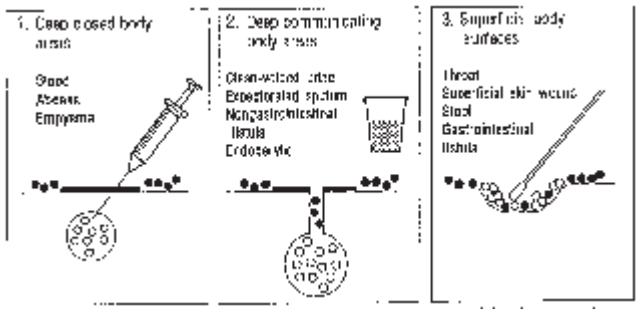

Figure 1. There are 3 categories of bacterial culture specimen.

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Every specimen needs further processing: transport, lab administration, opening of the collecting device, streaking and swabbing for culture and microscopy, drying in air, incubation, reading and further steps in the analysis. At every step contamination can take place, usually with bacteria from the normal skin flora (coagulase negative staphylococci, corynebacterium, propionibacterium) or moulds.
For blood culture the material used is sterile, for sinus aspiration also, although home (= in the hospital) sterilisation – a lesser controlled process than the industrial one- is very common.

When free fluid cannot be obtained from the sinus cavity, a sinus puncture is accompanied by an injection with saline or water to remove the mucus or the pus. The saline or water must be sterile and remain sterile till the last drop. Several manipulations are necessary: at every point a contamination can take place, be it with skin flora from the examinator, mucosal flora from the patient, bacteria suspended in the air. For endoscopy several other pitfalls have to be stressed. First of all there is the equipment used. Before sterilisation it must be thoroughly cleaned and brushed to remove all organic material. The best sterilisation technique is autoclaving. If this is not possible, ethylene oxide or glutaraldehyde is used (Doyle et al., 1991; Golden et al., 1986). Both methods need to be controlled carefully. Particularly, the use of glutaraldehyde is a delicate technique; the concentration is critical, the product is toxic, the necessary submersion time has to be respected and rinsing with sterile water afterwards is necessary. Recontamination can occur at many steps. At the end, under the best available conditions, the product has been sterilised, but is not guaranteed to be sterile. As far as known, infectious incidents with scopes are mainly described in pneumonology and gastro-enterology. There are no arguments to believe that ENT endoscopic material behaves in another way. In fact, the presence of a bacterial infection of the sinus cavity and its specific microbial aetiology can be determined only by culture of an exudate or a rinse obtained directly from the sinus by puncture through the lateral nasal wall of the inferior meatus and aspiration through the puncture needle or by the so called maxillary antroscopy, performed through the fossa canina (Gwaltney, 1995; Hartog et al., 1995). Cultures of nasal pus or of sinus exudates obtained by rinsing through the natural sinus ostium or by endoscopy give unreliable information because of contamination with resident bacterial flora in the nose. Since sample collection by sinus endoscopy has not been shown to avoid specimen contamination, it is considered not acceptable for aetiologic studies in maxillary and frontal sinusitis (Gwaltney, 1995).
Concerning ethmoidal sinusitis, no data are available, but there is no reason that it would be different in this case. No matter how carefully the endoscopy is performed, the possibility of changing a type 1 specimen into a type 2 specimen is present. Indeed, mucosal flora can be pushed into the cavity one likes to explore. For protected brush specimen in pneumonology, one tries to rely on the nature of the microorganisms isolated (the so-called strong pathogens) and on the number ($\geq 10^3$ CFU/ml for the commensals before considering a pathogenic role) (Jimenez et al., 1993; Karma et al., 1979; Pollock et al., 1983; Villers et al., 1985; Violan et al., 1993).
Some authors take 10^4 CFU/ml or 10^5 CFU/ml as cut-off value for the decision whether the bacteria found are pathogens or commensals (Kahn and Jones, 1987). This value thus depends on the use of a dilution step in the sampling or preparation technique. In ENT also, a quantitative culture of the specimen is useful to help detect bacterial contaminants accidentally introduced into the specimen. Most bacteria causing active sinus infection are present in titres of at least 10^5 CFU/ml, while titres of contaminants in a freshly processed specimen are usually considerably less (Gwaltney, 1995). The use of anaesthetics can have antibacterial activity, one possible reason for unexpected negative cultures. For example lidocaine can inhibit the growth of fungi and bacteria, even mycobacteria (Strange et al., 1988; Thorpe et al., 1987).
A type 2 specimen is always more difficult to culture and it is equally difficult to interpret the culture results. For urine samples, the Kass criteria (10^5 CFU/ml urine = significant bacteriuria), although many exceptions exist, are still applied. For sputum, the lab incorporates selective media, a liquefaction technique and quantitative culturing or a washing method in sterile saline. The isolated microorganisms, can belong to the normal flora and be the pathogen. A type 2 sample in ENT is a sinus exudate obtained by rinsing through the natural sinus ostium.
A type 3 specimen is the most difficult one for interpretation. A normal flora and the pathogens are mixed. The clinician can try to eliminate the commensals by swabbing and rinsing and try to reach the best part for sampling (pus, inflammation) (Almadori et al., 1986). The lab will use selective media to facilitate the isolation of the classic pathogens. Type 3 samples in ENT are throat smears and nasal pus. Many times the nasal swab will reflect what is going on in the sinus, but the interpretation of each species isolated can be very difficult.

Specimen transportation

It all starts with the collection device chosen. For bacteriology the material must be sterile. The labelling of the specimen with patient's name and the filling in of the lab order form must be done correctly. One should know when to use a transport medium. In case of doubts or questions the lab must be contacted. Anyhow the transport must be prompt. Especially for anaerobic bacteria, the conditions during transportation are of very great importance to the yield of the cultures. Previous investigators report a variation from 6 to 100% in the number of patients with anaerobic bacteria in their sinuses, depending on the methods used to collect, transport and culture the samples (Hartog et al., 1995; Van Cauwenberghe et al., 1993). Bacteriology on itself remains a slow process. Fastidious organisms stop growing if optimal conditions are not achieved. A delay of 2-3 hours is permissible, afterwards the quality of the sample is deteriorating. The storing temperature is also important. In general, for a type 1 ENT specimen a sterile device is needed with an adequate amount of material to be analysed or a small amount in a transport medium. Storing temperature depends on the sample

and the number of species to be isolated. For blood cultures ± 35° C is advocated, as every bacteria present should be detected. If an incubator is not available, room temperature is best. A sinus aspirate, however, if obtained for culturing aerobes only, is best stored in a refrigerator (4-10° C): most pathogens are fastidious and multiplying pneumococci easily autolyse. In order to preserve both aerobic and anaerobic strains in good condition, fluid samples can be sent to the laboratory in special transport tubes (Hartog et al., 1995). For biopsies, being a small specimen in which aerobes and anaerobes must be detected, prompt transport in an adequate medium seems necessary (Doyle and Woodham, 1991).

A type 2 specimen needs a sterile collection device or a swab. Usually only aerobes will be looked for: the contaminating commensal flora contains also anaerobes. If desiccation can be prevented (e.g. by fast transport) than the transport medium can be omitted, if not a transport medium and storage in a refrigerator are strongly advised. The purpose is to keep the pathogens, who are usually fastidious microorganisms, in the same number as at the time of collection.

Type 3 specimen are usually swabbed. If no prompt transport available, a transport medium and storage in a refrigerator are advocated.

Processing in the laboratory

In routine most labs will look for the expected pathogens: pneumococci, *H. influenza, M. catarrhalis, S. aureus,* streptococci (Almadori et al., 1986; Gwaltney, 1995; Muntz and Lusk, 1991). Therefore a blood agar in ambient air and a chocolate agar incubated in 5% CO_2 are usually chosen (Jimenez et al., 1993; Pollock et al., 1983). Selective media can be used e.g. mannitol-salt-agar for staphylococci . If possible a microscopic examination should always be performed. This is one of the few rapid methods available in bacteriology yielding good information about the aetiologic agent(s).

Extra orders are needed for culturing yeasts and fungi and also for the anaerobes. As already mentioned, anaerobes can only be looked for in type 1 samples with special precautions to be taken (Hartog et al., 1995; Muntz and Lusk, 1991).

Culture results

Cultures need overnight incubation before allowing a presumptive diagnosis. Most identifications and antibiograms for the aerobes take 48 hours, sometimes longer. For anaerobes it is difficult to obtain results before 4 days: they grow slower and further analysis takes more time (Brook, 1989; Doyle and Woodham, 1991).

If microscopy is done, it is very important to compare the result with culture results. This is a parameter for internal quality control. The number of polymorfonuclear cells gives an idea about the degree of purulence and other cell types give a clue to the origin of the specimen. If microscopy is not available the isolation of commensals makes the interpretation difficult.

For type 2 and 3 specimen, whatever species are isolated, stating the pathogenic or the commensal nature of the isolated bacteria remains a good guess. Strong pathogens isolated in high number allow support as to the aetiologic origin of the infection. A strong pathogen in small numbers will receive more importance than a commensal in high numbers. The bacteriologist will perform susceptibility tests on what he considers to be important. The clinician tends to stick to every possible isolate if he is convinced of the bacterial origin of the infection.

Type 1 specimen, such as the sinus aspirate can give the same interpretation problems as type 2 and 3 samples although one is convinced of the better specimen quality. Indeed, some authors consider the large number of staphylococci found in their patients unlikely to be caused by contamination since the sinus fluid specimens were obtained by puncture through the maxillary sinus wall (Hartog et al., 1995) There is also the problem of the species missed: a well organised pus can be sterile. On the other hand “funny” species can be isolated e. g. Pseudomonas spp. without a chronic disease history. These can be contaminants originating from the material and water used for the aspirate. The isolation of mucosal flora (coagulase negative staphylococci, non-haemolytic streptococci, alpha-haemolytic streptococci, ordinary neisseriae) usually points in the direction of a contamination (Kahn and Jones, 1987; Muntz and Lusk, 1991), except in some cases where for example staphylococci are isolated in too high numbers to be caused solely by contamination (Hartog et al., 1995). Further investigations are recommended here. A Gram-stain of the specimen is very important for comparison with the culture results. For material obtained by endoscopy with biopsies the problems usually lie with the small sample volume and the lack of microscopy; with the quality of the transport medium; the quality of the lab processing, particularly for the anaerobes; and with the contamination that can always occur changing the type 1 specimen into a type 2 or 3. Clinicians give even more importance to the results obtained. There is surely not enough experience to be able to draw sound conclusions from these type of specimen. In analogy with protected brush specimen in pneumonology we dare state that this technique is NOT advisable as a culture sampling technique.

It is difficult for the clinician to assess the quality of the lab. The experience of the lab personnel, its dedication to the job are important parameters. The quality of the media and systems used are determinative for the results obtained. There is a lot of home cooking and brewing, of smells and appearances making bacteriology sometimes more an art than a science.

Susceptibility testing

Susceptibility testing is an approximation or an estimation of an arbitrary value expressed in mcg per ml or mg per L determining susceptibility, resistance and an intermediate category which is in fact admitting an ignorance. There are no universal standards for these categories (except for penicillin versus pneumococci). Widely accepted standards are the N.C.C.L.S. criteria (USA); but Sweden, Germany, France, the UK etc. have their

own criteria. There are differences in the techniques used, in the chosen breakpoints, in the disk content, in the zone sizes to be obtained, in the medium, in the incubation conditions. Internal controls are necessary, but not always easily organised. It can be difficult to detect and to control an unknown resistance mechanism. The individual result is important for the individual patient but is not available on the crucial moment of choosing the empirical therapy. It can be more worthwhile to have good epidemiological data, including susceptibility patterns, allowing different first choices for different patient categories.

CONCLUSION

Many pitfalls still exist in the different steps of a specimen for bacteriology. Good cooperation between the clinician and the lab is a necessity, particularly if new techniques are evaluated or in other investigation conditions. Bacteriological culture results never give only a “yes” or a “no” category. There are always results that are doubtful or difficult to interpret. One should remain critical towards these results. Solitary publications have to be confirmed by other investigators before taken for certain. Jumping to conclusions and extrapolation of results from elsewhere to the own situation can be very dangerous.

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