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Assessment of Expectorated Sputum for Bacteriological Analysis Based on Polymorphs and Squamous Epithelial Cells: Six-Month Study

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Samples of sputum were examined microscopically to determine their suitability for routine culture. When the number of squamous epithelial cells per field was less than 10, the number of bacterial species generally fell within the range of one to four. Squamous epithelial cells were not always a true indication because some unmarked transtracheal specimens showing more than 10 squamous epithelial cells also gave a range of isolation falling between one and four. When the presence of 25 or more polymorphs was used as the parameter, the number of bacterial isolates generally fell within the range of one to three, but this resulted in positive overbiasing with consequent rejection of valid specimens. Later it was found that when a differential system using both polymorphonuclear cells and squamous epithelial cells was applied, a significant number of specimens could be salvaged which would otherwise have been discarded.

Sputum specimens are among the most frequently rejected by micobiology laboratories because of a failure to meet minimum standards set by quality control (2). Some authorities even suggest that little is to be gained by processing sputum specimens (1). Others comment on the unreliability of attempting to diagnose pneumococcal pneumonia from gram-stained smears or cultures (3).

The etiological agents of infections of the lower respiratory tract may be isolated by culturing secretions from this area. Except where these specimens can be taken from resected tissue in accordance with Koch's first postulate, this is by no means easy to accomplish.

Potential pathogens of the upper respiratory tract may contaminate secretions of the lower respiratory tract, giving rise to erroneous results, and inconsequential organisms may overgrow pathogens, resulting in false-negative results.

Attempts have been made to eliminate contamination by upper respiratory flora by using methods which include transtracheal (TT) aspiration, tracheal puncture, bronchoscopy, needle aspiration of the lung, and lung biopsy (4). However, these may be impractical for routine practice and may be accompanied by considerable risk, including death (5).

Recent literature described methods for categorizing sputa after examination of a stained smear. Murray and Washington (4) screened sputa according to the numbers of squamous epithelial cells (SEC) observed at a magnification of ×100 and found that the mean number of bacterial species isolated was greater than four in specimens which contained more than 10 SEC per field. In another method the sputum is given a rating number in accordance with the quantitative presence of polymorphs, mucus, and SEC (2).

Van Scoy (7), speaking from the viewpoint of a clinician, proposed a method in which both leukocytes and epithelial cells were used as a means of assessing sputum. Working independently, we have come up with the same recommendation and are presenting laboratory data to substantiate it.

Our investigations covered a period of 6 months. Initially, the screening method applied was that described by Murray and Washington. This was superceded by the differential method described here in which both polymorphonuclear cells (PNC) and SEC were used.

MATERIALS AND METHODS

Specimens of sputum from patients in the Victoria General Hospital were submitted to this institution for examination. Endotracheal specimens were aspirated by a catheter passed through the endotracheal tube, and TT specimens were collected from a TT catheter inserted through the cricothyroid cartilage. In both cases, specimens were submitted to the laboratory with minimum delay.

None of these patients was attached to inhalation equipment.

The numerical range for the bacterial species included both potential pathogens and saprophytes.

The presence of saliva, mucus, blood, and pus was determined macroscopically. Where possible, a purulent portion of the specimen was selected and smeared on a microscope slide. The method of Murray and Washington for screening sputa was originally used, but this was later superceded by the method described below. If three of the five fields contained fewer than 25 PNC, a search was made for SEC. In this case if three of the five fields contained fewer than 10 SEC, the specimen was accepted for analysis. Those falling outside of both of these limits were discarded, and the following report was submitted: "This specimen has been screened and has been shown to contain an excess of saliva and is therefore unfit for bacteriological examination."

Satisfactory specimens were then seeded to sheep blood agar and incubated aerobically overnight in 5 to 10% CO_2. Primary pathogens isolated included *Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Haemophilus influenzae,* and *Klebsillia pneumoniae,* which were identified by routine biochemical and serological techniques (6).

RESULTS

TT specimens. In a study of 72 TT specimens cultured by approved techniques, it was found that 90% of the specimens yielded a numerical range of bacterial species which fell between one and three.

Endotracheal tube specimens. Similarly, in a study of 21 endotracheal tube specimens, in which approved techniques were used, 87% gave a numerical range for the bacterial species isolated which fell between one and three.

From these findings, it was concluded that a specimen which has been subjected to an ideal screening procedure should yield a numerical range of bacterial species approaching that of either the TT or the endotracheal tube specimens, provided that the same approved culturing procedures have been applied.

Sputa screened by PNC and SEC. A total of 100 unselected stained smears from carefully collected specimens of sputum were examined, using the presence of 25 or more PNC as the parameter to determine the suitability for bacteriological analysis. It was found that the number of bacterial species isolated from each fell within the range of one to three for 90% of the specimens.

Likewise, when 1,168 sputa were screened by using the criteria of Murray and Washington, 75% of the specimens gave a value which fell between one and three.

Of the two procedures available, it was decided initially to use the SEC screening parameter. Later a change was made to the PNC parameter, primarily as a result of our findings with TT specimens. In our hands the PNC parameter gave positive overbiasing, leading to an undue rejection of specimens. It was then decided to apply both parameters in a differential way as described above. When both parameters were used in the screening of 2,387 sputa, the following findings were obtained. A total of 529 (22%) were rejected when PNC screening was used, whereas 366 (15%) were rejected when both PNC and SEC were used.

DISCUSSION

From our studies it seemed that either parameter would have been suitable for the screening of sputum specimens. However, when both parameters were applied individually to routine practice, the results obtained did not seem to reflect this. When we applied the SEC parameter as described by Murray and Washington, there were instances when erroneous findings were obtained, this being clearly demonstrated in the case of seven samples of TT specimens which had been taken according to approved technique. In these cases more than 10 SEC per low power field were noted. These specimens would have been routinely discarded, had it not been for the fact that they were TT specimens, which are routinely analyzed regardless of their cytological findings. Cultural studies of these TT specimens yielded a bacterial species range which fell within the limits of tolerance, i.e. one to three. We adopted the PNC parameter as a result of these findings and also as a result of the fact that PNC counts might offer a better index of infection. However, our findings seemed to show that we were overbiasing and discarding specimens which should rightly have been examined. It was only when both parameters were used in a differential way that this fact became apparent. Of a total of 2,387 specimens studied, 529 or 22% would have been discarded had we used the PNC parameter alone. When the differential parameter was applied, the discard rate dropped to 366 or 15%, a saving of some 7%.

It is probable that, due to lack of homogeneity and other factors inherent in sputum, no single parameter in itself will serve as an adequate means of assessment.

In our hands, this differential parameter has been satisfactory. This program has now been in operation for the past year. Our discard rate has fallen from 24.5% when the program commenced to 18% at the present time, i.e., a saving of 7%. To date, however, this screening procedure has been applied only to specimens intended for nontuberculous bacteriology.

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