Microbiology Section

Letter to Editor

Sputum Gram Stain Assessment in Relation to Sputum Culture for Respiratory Tract Infections in a Tertiary Care Hospital

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ABSTRACT

The microscopic examination of Gram stained sputum specimens is very helpful in the evaluation of patients with lower respiratory tract infection. This study was undertaken to assess the utility of Gram stain in sputum examination. One hundred sputum samples were collected from patients with suspected lower respiratory tract infection. Quality of sputum was determined on Gram stained smears by using a modification criteria of Bartlett. Of the 100 samples, 79(79%) were accepted and 21(21%) were found to be unacceptable by the criteria of Bartlett. Potential pathogens were recovered from 50(63.2%) out of 79 accepted samples and 2(9.5%) out of 21 rejected samples. These data suggest that microscopic examination is mandatory in sputum microbiology.

Key Words: Infection, Lung, Gramstain

INTRODUCTION

One of the most important uses of the Gram stain is to evaluate the quality of expectorated sputum received for routine bacteriological culture. An acceptable sample yields less than 10 squamous epithelial cells per low power field [1]. The simplest and least expensive sample for the diagnosis of lower respiratory infections is expectorated sputum. The utility of this approach is the subject of controversy, as the sample is contaminated by oropharyngeal flora as it passes through the mouth. When the sample is collected carefully, it can provide useful information for initial therapy of community acquired pneumonia [2].

Direct Grams stain of clinical material may be used to determine whether a sample is representative of the site of infection. This technique has been applied for evaluation of sputum samples. From the relative numbers of squamous epithelial cells and segmented neutrophils in direct Gram's stains of sputum samples, Bartlett has devised a grading system for evaluating sputum samples [2].

Gram staining is considered key in the work up of sputum from patient with community acquired pneumonia and other lower respiratory tract infections. The sputum samples are often contaminated with saliva and contains resident oropharyngeal microbial flora. Culture of such a sputum sample might yield the organisms present in the saliva [3].

When significant oropharyngael contamination is evidenced in the cellular content of Gram stained sputum smears, the second sample representing lower respiratory tract must be collected [4].The microbiology laboratory must use objective criteria by Gram stain screening for purulence before inoculation into culture media [5]. Unless microscopic examination is routinely included, half of all microbiological information rendered on sputum samples is meaningless and subject to misinterpretation of culture results. Hence culture must be guided by microscopic findings. When there is no correlation between culture and smear, the culture report may not indicate the aetiology of lower respiratory tract infection. The present study was designed to examine whether the clinical microbiology laboratory should play an active role in interpreting the quality of sputum specimens based on Gram stained smears.

MATERIALS AND METHODS

The 100 unselected expectorated sputum samples were examined in the central microbiology laboratory. Samples were evaluated by gross appearance and subjectively categorized into mucus (mucus strands present) and watery (saliva present) [3].

Each specimen was first mixed with an applicator swab and then inoculated on to blood agar, chocolate agar and Mac Conkey agar plates and a smear was prepared for Gram staining. Each stained smear was examined microscopically under low power, oil immersion and the cellular components were evaluated. All the samples were processed regardless of the appearance of the stained smears. Organisms were identified by standard protocols and antibiotic susceptibility of recommended drugs was performed by Kirby Buer disc diffusion method. Viridians group streptococci, CoNS and some Neisseria species were considered as normal respiratory flora [5].

RESULTS

According to the Bartlett's screening criteria, 79 (79%) samples were accepted and 21(21%) samples were rejected from 100 samples. Potential pathogens were recovered from 50(63.2%) samples out of 79 accepted samples. Two (9.5%) samples yielded pathogens from 21 rejected samples. The most common organism isolated was *Klebsiella spp,* followed by *Pseudomonas spp, Streptococcus pyogenes, Staphylococcus aureus, Enterobacter spp, Escherichia coli, Citrobacter spp* and *Acinitobacter spp.*

DISCUSSION

Clinicians are interested in rapid, simple, inexpensive and readily available tests that will assist them in prescribing proper medications for lower respiratory tract infections. Sputum Gram stain served this function in the management of lower respiratory tract infection [6].

When potential pathogen is isolated from the sputum sample, it is often difficult to decide whether the potential pathogen is an etiological agent or represents oropharyngaeal contamination. The amount of oropharyngeal contamination can be judged by evaluating the relative number of squamous epithelial cells in the samples. The samples that are contaminated are less likely to yield interpretable results [7]. Without microscopy, culture results are of unknown relevance and results may be misleading. Hence diagnosing respiratory infection by sputum culture without microscopic examination invites confusion and misinformation [8]. To minimise the effect of oropharyngael contamination on lower respiratory tract secretions, Bartlett, Murray and Washington devised screening criteria based on quantitation of leucocytes and squamous epithelial cells [5]. The use of gram stained smears to assess the quality of sputum samples has received considerable attention as a means for improving the reliability of sputum culture. In 1974, Bartlett proposed that purity of sputum samples be rated according to the relative concentration of polymorphonuclear neutrophiles, squamous epithelial cells and mucus in gram stained smears [4]. Even though sputum culture has been criticised for lack of sensitivity and specificity, comparability of a positive yield on sputum culture with transtracheal aspiration has been demonstrated when the sputum samples have been microscopically adequate [9]. Murray and Washington initially reported that 45% of their specimens were rejected [7]. Joseph and David reported rejection rate of 23 to 25% [5]. In our study, 21% sputum samples were unacceptable. Isolation rate in present study is 63% which is higher compared to 57% isolation rate reported by Jean and Mohammed [10].

CONCLUSION

Our data support the notion that clinical microbiology laboratories may reject for culture, those sputum specimens which fail to meet the criteria of Bartlett for purulence. Sputum cultures must be ordered judiciously for documented episodes of lower respiratory tract infection to provide meaningful report. The microbiology laboratory must use objective criteria by Gram stain screening for purulence before inoculation in to culture media. Hence the routine sputum Gram stain is essential to provide meaningful culture report.

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