Thesis Title Re-evaluation of Laboratory methods to Improve Routine Sputum Microbiology

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ABSTRACT

A total of 637 expectorated sputum specimens (ES) collected from hospitalized patients in Siriraj Hospital submitted to Bacteriology Laboratory were intensively studied using macroscopic appearance, the composition of polymorphonuclear leucocytes (PMN) and squamous epithelial cells (SEC) per low power field (LPF), semiquantitative Gram-stained smear and culture to determine grades of pathogenic bacteria and normal flora, isolation and identification of organisms. Previously reported methods and criteria for reading and interpreting laboratory results of ES were re-evaluated to determine suitable, economical and effective procedures for using in routine laboratory practice.

Screening criteria based on numbers of PMN and SEC present in ES appeared to be more reliable than macroscopic appearance in the screening specimen quality. However, ES classified by macroscopy as saliva could be discarded. The more simplified and applicable screening criteria for specimen acceptability; > 25 PMN and ≤ 10 SEC/LPF or PMN:SEC ratio of ≥ 5:1 if sample contains 10-50 SEC/LPF; was proposed with the support of bacteriologic data. Four-category grading criteria of semiquantitative Gram-stained smear and culture were also proposed in this study, to establish practical method of quantity determination of potential pathogens and normal flora. Results of well matching of the corresponding grades indicate their usefulness in ES etiologic diagnosis. In addition, Gram stain was confirmed to be more important than culture for interpreting the results. Acid-fast stain was shown to be essential for ES with numerous PMN, highly suggestive of bacterial infection but yielding negative result by Gram stain.

Of 637 ES, only 223 (35%) yielded positive findings with single (20%) and mixed (15%) putative etiologic agent(s). Most of organisms recovered were Gram-negative bacilli, indicative of specimens collected from patients with nosocomial infection.

ES yielded negative results accounted for 65% of total samples. Among these 414 negative specimens, 270 (42% of total samples) carried potential pathogen(s) without evidence of infection and some of which exhibited characteristic of bacterial colonization. Similarities in many bacteriological aspects between positive ES and

those in colonization group were observed such as the findings of specimens containing single and mixed potential pathogens, distribution of bacterial species, and patterns and grades of growth. The four most common species recovered from four specimen groups; samples showing infection and colonization with single and mixed organisms; were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, other nonfermentative Gram-negative bacilli and *Staphylococcus aureus*. These findings emphasized the significant role of sputum smears in diagnosis of lower respiratory tract infections (LRTI) especially for specimens carrying single potential pathogen.

Gram-stained smear combined with culture on chocolate agar if slender Gram-nrgative bacilli characteristic of *Haemophilus* is observed on smear, is recommended for isolation of *Haemophilus influenzae*. Blood agar with staphylococcal streak technique could be used as alternative method, but only 69% sensitivity. MacConkey agar was shown to yield better result than blood agar in isolation of mixed Gram-negative bacteria.

In conclusion, results obtained in this study indicate that step by step and systematic approach of several combined laboratory methods of sputum bacteriology are needed for the improvement of etiologic diagnosis of LRTI.