Clinical Analysis of Sputum Gram Stains and Cultures to Improve the Quality of Sputum Cultures

Dong-Hyun Lee¹ and Sunjoo Kim²

¹Department of Laboratory Medicine, Gyeongsang National University Hospital, Jinju; ²Department of Laboratory Medicine, Gyeongsang National University Changwon Hospital, Changwon, Korea

Corresponding author:

Sunjoo Kim
Department of Laboratory Medicine,
Gyeongsang National University
Changwon Hospital, 11 Samjeongja-ro,
Seongsan-gu, Changwon 51472, Korea
Tel +82-55-214-3072
Fax +82-55-214-3087
E-mail sjkim8239@hanmail.net

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Background: Sputum Gram stains and cultures are standard tests for the diagnosis of lower respiratory tract infections. Analysis reports of the sputum quality are scarce in relation to cultures as well as clinical diagnosis.

Methods: A total of 1,523 sputum specimens, requested for culture, were evaluated for their quality using the modified Murray–Washington (MW) grouping system in association with the culture results. The clinical diagnosis was investigated for the culture positive cases.

Results: There was a significant difference in bacterial growth (odds ratio, 2.01; $P=0.0164$) and diagnosis of pneumonia (odds ratio, 4.18; $P=0.002$) between the acceptable groups (groups 4–5) and the unacceptable groups (groups 1–3). However, one-quarter did not belong to the current MW group. More than half of the sputum cultures were in group 6, with the lowest positive rate (18.0%). Overall, 10% of the sputum cultures were related to a clinical diagnosis of pneumonia.

Conclusions: The MW grouping system should be revised, because one-quarter did not belong to the current group. Further evaluation of group 6 will be needed. Improvement of sputum cultures is required to enhance the diagnosis of pneumonia.

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Key Words Pneumonia, Culture, *Streptococcus pneumoniae*, Quality improvement

INTRODUCTION

Lower respiratory tract infections including community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP) are major public health concerns, causing a variety of complications and high mortality [1-3]. More than 1.5 million adults are estimated to be hospitalized due to CAP, with an estimated 100,000 deaths annually in the United States [4]. Chest X-ray is the primary test

for the diagnosis of lower respiratory tract infections [5,6]. Sputum culture is also a confirmative test to isolate the causative organisms of lower respiratory tract infections, such as CAP, bronchiolitis, and bronchitis, and therefore, is routinely performed in clinical microbiological laboratories [5,7]. Sputum Gram staining is associated with sputum culture results [7-10], and high-quality sputum provides useful information or guides the monotherapy [1,5,11,12]. However, the clinical usefulness

of sputum cultures and Gram stains is questioned due to possible contamination of oropharyngeal normal flora and the complexity of etiological pathogens [6,10]. Adequate collection of sputum must be accomplished to obtain proper test results. Deeply expectorated sputum could serve as a high-quality specimen. Rinsing the mouth with water and coughing deeply early in the morning is recommended [8]. However, it is not easy to obtain a high-quality specimen due to the lack of proper education or poor patient conditions. Some reports demonstrated that up to one-third of the patients with bacterial pneumonia were unable to produce sputum [13,14].

Several criteria have been proposed to evaluate the quality of sputum samples. These criteria have different combinations and cutoffs for the minimum number of squamous epithelial cells (SEC) and/or polymorphonuclear leukocytes (PMN) per low-power field (LPF; 100× objective) [15-17], but none of these guidelines are considered superior. Currently, the modified Murray-Washington (MW) grouping system is primarily used to evaluate the quality of sputum in Korea [17]. This grouping system consists of six groups according to the numbers of SEC and PMN per LPF. There is a consensus that groups 1-3 are unacceptable, whereas groups 4 and 5 are acceptable. However, controversy persists regarding the acceptability of group 6, which shows less than 10 SEC and PMN each, per LPF. In the original MW grouping system, group 6 (<10 SEC) was categorized as acceptable [17]. Bartlett [16] also used SEC and PMN to score sputum quality. Although these grouping systems have been used for several decades, their clinical utility has not been well established. An in-depth analysis of culture results and clinical data is needed in relation to the classification of the sputum groups. Additionally, we found that some samples do not belong to groups 1-6, and no research has been undertaken, to date, for the interpretation of these unclassified groups. Concomitant blood culture is recommended to increase the chance of isolating causative CAP organisms in addition to sputum cultures [5,6]. However, some guidelines recommend only blood cultures instead of sputum cultures for the diagnosis of CAP [6].

We evaluated the clinical relevance of sputum cultures using the MW grouping system and examined the frequency of bacterial growth in each group. We also analyzed the results of requested concomitant blood cultures.

MATERIALS AND METHODS

1. Study population

From December 2016 to February 2017, a total of 1,523 consecutive sputum specimens, requested for cultures and Gram stains, were included from Gyeongsang National University Hospital. Electronic medical records were reviewed to analyze the requested concomitant blood cultures, presence of pneumonia, prior antibiotic use before sputum culture, and antibiotic change after receiving the sputum culture results from the patients with positive cultures. The clinical diagnosis was based on clinical signs and symptoms of lower respiratory tract infections and the presence of a new infiltrate, on chest radiographs, that was at least segmental and not due to any preexisting conditions or other known causes.

This study was approved by the institutional review board of Gyeongsang National University Hospital (IRB approval no., GNUH 2019-10-027-002).

2. Microbiological laboratory tests

Gram stain smears were prepared from the most visually purulent portion of each sputum specimen. The sputum quality was assessed microscopically and each sputum sample was grouped, as outlined by the MW grouping system [17]. The most purulent portion of each specimen was inoculated onto blood, chocolate, and MacConkey's agars. Then samples were streaked out by a medical technician using a standard 4-quadrant streaking method and incubated at 35°C with 5% CO₂ for 48 hours. Agar plates were examined at 16-24 hours and 48 hours, and the predominant organisms were identified and quantified, by an experienced technician, according to the furthest quadrant with visible colonies. Respiratory potential pathogens included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae*,

Streptococcus agalactiae, *Streptococcus pneumoniae*, *Nocardia* spp., *Moraxella catarrhalis*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, and Gram-negative bacilli [1,11]. Normal throat flora, such as α - or γ -*streptococcus*, coagulase-negative staphylococcus, *Micrococcus* spp., *Neisseria* spp., *Corynebacterium* spp., were discarded. Blood cultures were performed using the BacT/Alert 3D system (bioMérieux, Marcy-l'Etoile, France) with FA plus (aerobic) and FN plus (anaerobic) bottles. An amount of 5–10 mL was inoculated into each bottle. Two sets were produced for each episode. Biochemical identification and antibiotic susceptibility tests were conducted using the Vitek-2 system (bioMérieux). To standardize reporting across each technician, uniform standard operating procedures, on-site training, and internal and external quality controls were established.

3. Statistical analysis

Frequencies are expressed as a percentage (%) of the categorical variables. We compared the results for bacterial growth and pneumonia between the groups using the chi-square test and Fisher's exact test. All of the statistical analyses were performed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA). *P*-values <0.05 were considered statistically significant.

RESULTS

1. Patient demographics and sputum characteristics

A total of 1,523 sputum specimens were requested for culture during the study period. The ratio of males to females was 1.6:1 (Table 1). The percentages of expectorated sputum, suction sputum, transtracheal aspirates, and bronchial aspirates were 87.0%, 8.3%, 3.8%, and 0.9%, respectively. Respiratory potential pathogens were identified in 22.6% of the samples. A total of 87.8% of sputum culture positive cases (*n*=344) were concurrently requested with the blood cultures. The blood cultures (37.2%) were requested from the emergency department among the sputum culture positive cases, indicating that the majority of the specimens came from in-patients. The positive rate of blood cultures was 8.9%, and 6.6%

Table 1. Characteristics of the study population, types of sputum, concomitant blood cultures, and antibiotic usage

Characteristic	Value
Median age (yr)	70
Male:female	1.6:1
No. of sputum specimens	1,523
Specimen types	
Expectorated sputum	1,325 (87.0)
Suction sputum	127 (8.3)
Transtracheal aspirates	58 (3.8)
Bronchial aspirates	13 (0.9)
No. of bacterial identifications	344 (22.6)
No. of co-ordered blood cultures	302 (87.8)
Positive blood cultures in positive sputum culture cases	27 (8.9)
The same species as sputum culture	8 (2.3)
Different species from sputum culture	19 (6.6)
Antibiotic use in positive sputum culture patients	
Prior antibiotic usage before sputum culture	97 (28.2)
Antibiotic change after confirmation of sputum culture result	79 (23.0)

Values are presented as number or number (%).

of the blood cultures yielded different species from the sputum cultures. Prior antibiotic usage before sputum culture accounted for 28.2%, and antibiotic changes after receiving the sputum culture results were observed in 23.0% of the sputum culture positive patients.

2. Murray–Washington grouping system

The proportion of sputum samples belonging to MW groups 1 to 6 were 2.9%, 2.0%, 6.2%, 3.5%, 1.0%, and 56.9%, respectively, among the 1,523 samples (Fig. 1).

Respiratory potential pathogens grew in 31.8% (14/44), 23.3% (7/30), 33.7% (32/95), 44.4% (24/54), 60.0% (9/15), and 18.0% (156/867), respectively, for each group (Table 2). The proportion of specimens that did not belong to any of the MW groups was 27.5% (418/1,523), and bacteria grew in 24.4% (102/418) in this unclassified group. The group of SEC 10–25, PMN <10 was the most prevalent among the unclassified groups. The growth of

respiratory potential pathogens was significantly higher in the acceptable groups compared to unacceptable groups (odds ratio [OR], 2.01; 95% confidence interval [CI], 1.13–3.56; $P=0.0164$). In addition, the diagnosis of pneumonia was significantly higher in the acceptable groups (OR, 4.18; 95% CI, 1.64–10.60; $P=0.002$).

Fig. 1. Distribution (%) of MW groups (n=1,523). Unclassified groups which is not included in groups 1–6 accounted for 27.5%. Abbreviation: MW, Murray and Washington.

The unclassified group showed no significant differences in the growth of potential respiratory pathogens ($P=0.0833$) but showed significant differences in the diagnosis of pneumonia compared to the unacceptable groups ($P=0.0168$). There was no significant difference in etiological pathogens, such as gram-positive, gram-

Fig. 2. Comparison of bacterial growth rates and diagnosis of pneumonia among the unacceptable (groups 1–3), acceptable (groups 4–5), and not included in the MW groups. Abbreviation: MW, Murray and Washington. * $P<0.05$, ** $P<0.01$.

Table 2. Growth of respiratory potential pathogens and clinical diagnosis of pneumonia according to MW groups and the among unclassified groups using the MW grouping system

	No. (%)	Bacterial growth (%)	<i>P</i> -value	Pneumonia (%) [*]	<i>P</i> -value [†]
Total	1,523	344		150	
MW grouping system	1,105	242		105	
Group 1	44 (2.9)	14 (31.8)	<0.001	1 (7.1)	0.006
Group 2	30 (2.0)	7 (23.3)		1 (14.3)	
Group 3	95 (6.2)	32 (33.7)		11 (34.4)	
Group 4	54 (3.5)	24 (44.4)		15 (62.5)	
Group 5	15 (1.0)	9 (60.0)		4 (44.4)	
Group 6	867 (56.9)	156 (18.0)		73 (46.8)	
Not included in the MW grouping system	418 (27.5)	102 (24.4)	0.0833	45 (44.1)	0.0168
SEC 10–25, PMN <10	182 (12.0)	34 (18.7)		11 (32.4)	
SEC 10–25, PMN 10–25	78 (5.1)	24 (30.8)		10 (41.7)	
SEC <10, PMN 10–25	158 (10.4)	44 (27.8)		24 (54.5)	

Abbreviations: MW, Murray and Washington; SEC, squamous epithelial cells; PMN, polymorphonuclear leukocytes.

^{*}The percentage of pneumonia cases was calculated by dividing with number of bacterial growth positive patients. [†]The *P*-value was calculated between each MW group and not included groups compared with the acceptable groups (groups 4–5).

negative, and fungi, according to the MW groups (data not presented) (Fig. 2).

DISCUSSION

Clinical decisions for antibiotic treatment of lower respiratory infections are aided by the results of Gram stains and sputum cultures [1,9,11]. However, sputum Gram stains are sometimes unreliable and ambiguous [8,18]. Therefore, the optimal collection of high-quality sputum specimens and the correct isolation and identification of colonies are important [8,10]. However, obtaining an acceptable specimen is sometimes difficult because of the possibility of contamination from normal oropharyngeal flora [7]. In addition, microbiological expertise is required for the appropriate selection of the colonies of potential respiratory pathogens. Sputum cultures are rather subjective and difficult to standardize technically [10,18]. The yield of sputum cultures was 22.6% in this study. Among these potential respiratory pathogens (N=344), 43.6% were associated with pneumonia. Therefore, overall 10% of the sputum specimens were related to the clinical diagnosis of pneumonia.

Expectorated sputum is the most common lower respiratory tract specimen collected by microbiology laboratories. Expectorated sputum is often difficult to obtain as many specimens are mixed with upper airway specimens. In our study, the unacceptable groups (1–3) accounted for 11.1%, whereas the acceptable groups (4–5) accounted for only 4.5%. In contrast, group 6 was predominant (56.9%), and a surprisingly large proportion (27.5%) did not belong to any of the groups in the current MW grouping system (Fig. 1).

In other studies, the percentage of acceptable quality specimen ranged from 40%–70%, as compared to 4.5% in our study. However, these studies differed from ours with respect to the subjects, who were patients with pneumonia [9,12], and the criteria for a good sample, which was “SEC <10/LPF” [19].

The bacterial growth rate was highest in the acceptable groups 4–5 (47.8%) and lowest in group 6 (18.0%)

($P<0.001$). However, pneumonia diagnosis in acceptable groups (46.8%) was significantly higher than that in unacceptable groups (24.5%) ($P=0.004$). Group 6 fared worse than the acceptable group in terms of bacteria growth detection but better in terms of pneumonia diagnosis. Therefore, further research on group 6 is needed, and new interpretation of the MW grouping system is necessary.

Blood culture was established as a major part of the investigation of CAP [20]. However, the necessity of mandatory blood cultures remains controversial for the diagnosis of CAP [21]. More evidence-based studies are needed to elucidate the advantage of routine blood cultures for the diagnosis of CAP or HAP. The positive rate of blood cultures, among the patients who had potential respiratory pathogens, was 8.9%, and a large portion had a different pathogen from the sputum culture in our study.

There are several limitations to this study. This is a retrospective study rather than a prospective study. The medical records were reviewed only for the patients showing positive results in their sputum cultures, and not for all the patients. There was no differentiation between CAP and HAP. Mortality, immunocompromised patients such as those suffering from neutropenia, prior other medical treatments, or clinical severity were not analyzed. One-quarter of the subjects received antibiotics before sputum collection, which might have affected the sputum culture results. Active antibiotic stewardship was not applied at our institute, thus lowering the change of antibiotics even after receiving the antibiotic susceptibility test results.

In conclusion, approximately 10% of the sputum cultures were related to the clinical diagnosis of pneumonia. The lower yield might have been due to the inappropriate requests for sputum cultures or prior antibiotic usage. One-quarter did not belong to the current MW groups. More than half of the sputum samples were in group 6 and showed the lowest positive rate. More large-scale studies will be needed on MW group 6. Improvement of sputum culture quality is required to enhance the diagnosis of pneumonia. The beneficial effects of additional

blood cultures should be carefully reassessed and further infections.
studied, for the diagnosis of lower respiratory tract

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