ORIGINAL ARTICLES

Evaluation of Housestaff Physicians' Preparation and Interpretation of Sputum Gram Stains for Community-acquired Pneumonia

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Objective: *To evaluate the preparation and interpretation of sputum Gram stains by housestaff physicians in the assessment of patients with community-acquired pneumonia.*
Design: *A prospective, multicenter study.*
Setting: *Two university-affiliated hospitals in Pittsburgh.*
Patients: *Ninety-nine cases of clinically and radiographically established pneumonia occurring in 97 patients.*
Diagnostic test assessment: *Housestaff and microbiology personnel prepared a Gram stain for each case of pneumonia. Housestaff assessed the presence and identity of a predominant microbial organism on the slides they prepared. Two senior staff microbiologists, blinded to patient and preparer, evaluated all slides for preparation, sputum purulence, and identification of the predominant organism. Two reference standards were used to assess the sensitivity, specificity, and predictive values of housestaff's Gram-stain interpretations: 1) senior staff microbiologists' determinations of the microbes present using the slides without benefit of culture results, and 2) the etiologic agent derived from results of sputum culture, blood culture, or serology.*
Measurements and main results: *Housestaff physicians completed a Gram stain in 58% of the pneumonia episodes. Gram stains were not made in 42% of cases, primarily because patients were unable to produce sputum. Fifteen percent of housestaff's smears were judged inadequately prepared, compared with 3% for the laboratory personnel ($p < 0.01$). Housestaff obtained purulent sputum samples significantly more often than did nursing personnel (58% versus 38%; $p < 0.01$). Housestaff's Gram stains were 90% sensitive for detecting pneumococcus, with a 50% false-positive rate. The sensitivity of the Gram stain was less for identification of* Haemophilus influenzae *than for identification of* Streptococcus pneumoniae. *A single antimicrobial agent was chosen as initial therapy for 50% of the patients in whom housestaff identified a predominant organism, compared with 30% in whom a predominant organism was not identified ($p \leq 0.05$).*
Conclusions: *Although housestaff obtained purulent sputum samples more frequently than did nursing personnel, they made systematic errors in the preparation and interpretation of Gram-stained slides. Housestaff physicians should receive formal training in the preparation and interpretation of Gram stains; the specific defects elucidated in this study warrant special attention.*
Key words: *Gram stain; pneumonia; housestaff physicians.* J GEN INTERN MED 1991;6:189–198.

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THE SPUTUM GRAM STAIN is routinely advocated for the evaluation of patients with community-acquired pneumonia.[1-3] In the hands of trained laboratory personnel, it has been shown to be valuable in determining the etiologies of certain common types of bacterial pneumonia.[4-8] In addition, this diagnostic procedure is inexpensive, noninvasive, and rapidly performed. Since the results are available within minutes, a tentative microbiologic diagnosis can quickly be established for guidance in selecting initial narrow-spectrum antibiotic therapy. This holds a distinct advantage over other commonly used diagnostic tests, including sputum culture, blood culture, and serology, the results of which are not available for days or even weeks from the time of presentation of the patient. Therefore, the sputum Gram stain is assumed to be an integral part of all practicing physicians' diagnostic armamentaria.

In hospitals with residency training programs, medical housestaff physicians typically perform sputum Gram stains in their evaluations of patients with community-acquired pneumonia. Housestaff physicians often independently prepare and read their stains, especially after hours, when laboratory services may not be immediately available. Although this practice can play a crucial role in the early management of patients with pneumonia, there is only limited information on housestaff physicians' abilities to perform and interpret the results of this test.[9, 10]

This prospective multicenter study addressed the following questions regarding housestaff utilization of the sputum Gram stain in patients hospitalized with community-acquired pneumonia: 1) How often are sputum Gram stains prepared? 2) Are these stains prepared adequately from a technical perspective? and 3) How do housestaff perform in interpreting these stains for etiologic diagnosis of bacterial pneumonia?

METHODS

We evaluated medicine housestaff physicians' utilization of the sputum Gram stain as part of an ongoing study of the pathogenesis of community-acquired pneumonia and the prognosis of patients hospitalized with it.[11] Patients were prospectively enrolled at two Pittsburgh hospitals between July 1, 1986, and March

1, 1987. The study sites included the Pittsburgh Veterans Affairs Hospital and Presbyterian University Hospital, the two major teaching hospitals affiliated with the University of Pittsburgh.

Selection of Cases

All adults (aged > 16 years) admitted to the medical services of the participating institutions with a diagnosis of pneumonia or an acute infiltrate on the admission chest radiograph were screened by the chief medical resident. Community-acquired pneumonia was defined by the presence of all of the following entry criteria: 1) acute onset of at least one "major" or two "minor" clinical criteria suggestive of pneumonia. The "major" criteria were cough, sputum production, and fever, while the "minor" criteria were dyspnea, pleuritic chest pain, altered mental status, pulmonary consolidation by physical examination, and a total leukocyte count $>12,000/mm^3$; 2) presence of new radiographic evidence of pulmonary infiltration; and 3) admission from the patient's home or a nursing home.

Assessment of the Sputum Gram Stain

All patients meeting the criteria for community-acquired pneumonia were enrolled in this study. Housestaff physicians obtained sputum samples and prepared Gram-stained slides. Nurses also obtained sputum samples and sent them to the diagnostic microbiology laboratory, where laboratory personnel prepared smears.

All Gram stains prepared by the housestaff and laboratory personnel were collected by the hospital's chief medical resident within 24 hours of admission of the patients. Each slide was numerically coded to mask the identity of the patient and the slide preparer. Each house officer who prepared and interpreted a smear completed a standardized questionnaire that asked the following questions:

1. Was there a predominant organism present on the slide? (A predominant organism was defined as representing >50% of the organisms seen in a given microscopic field.[8])
2. What was the presumed identify of the predominant organism, if one was present?

All smears were independently reviewed by two senior staff microbiologists. Each microbiologist, blinded to the identify of the patient and the slide preparer, completed a questionnaire with the following questions pertaining to each slide:

1. Was the Gram stain adequately prepared?
2. Was the sample purulent?
3. Was there a predominant organism present on the slide?
4. What was the presumed identify of the predominant organism, if one was present?

FIGURES 1 - 4 (*facing page*):

FIGURE 1 (*top, left*). Gram stain of sputum showing lancet-shaped gram-positive diplococci. Housestaff physicians were consistently able to make the correct reading as determined by two reference standards—senior staff microbiologists' readings and microbiological evaluation (sputum culture, blood culture, serology).

FIGURE 2 (*top, right*). Gram stain of small gram-negative coccobacillary organisms that yielded *Haemophilus influenzae* on culture. Housestaff physicians were able to make the correct reading in only 58% of cases in which the senior microbiologists reported gram-negative rods as the predominant organism.

FIGURE 3 (*bottom, left*). Sputum Gram stain showing gram-positive cocci, including some diplococci and slender gram-negative bacilli. Sputum cultures showed "mixed" flora, and clinical evaluation revealed aspiration pneumonia. Housestaff physicians tended to interpret these slides as showing predominantly "pneumococcus."

FIGURE 4 (*bottom, right*). Gram stain showing both gram-positive cocci and small faintly-staining gram-negative coccobacillary forms (both *Streptococcus pneumoniae* and *Haemophilus influenzae* were isolated from culture). Housestaff physicians were consistent in identifying the gram-positive cocci but often overlooked the gram-negative organisms.

In the assessment of slide preparation, the microbiologists determined whether sputum smears were too thick or too thin and whether the stains were over-decolorized or under-decolorized. Sputum was defined as purulent if there were <10 epithelial cells and >25 leukocytes per 100× microscopic field.[12] Microbiologists used the same criteria as the housestaff to define a predominant organism.

A final interpretation for each question relating to a given slide required agreement by the two microbiologists. In the event of disagreement on any question, a third microbiologist independently reviewed the slide and completed the questionnaire. In such cases, the final microbiologic reading considered as the reference standard was determined by agreement of two of the three.

Patient Microbiologic Evaluation and Cause of Pneumonia

Patients underwent microbiologic testing consisting of sputum cultures for bacteria, including Legionella, blood cultures, direct fluorescent antibody testing for *Legionella pneumophilia* and *Legionella micdadei*, and acute and convalescent serologic testing for *L. pneumophila*, *L. micdadei*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae* (TWAR). A specific microbial diagnosis was assigned if one of the following predefined criteria was present:

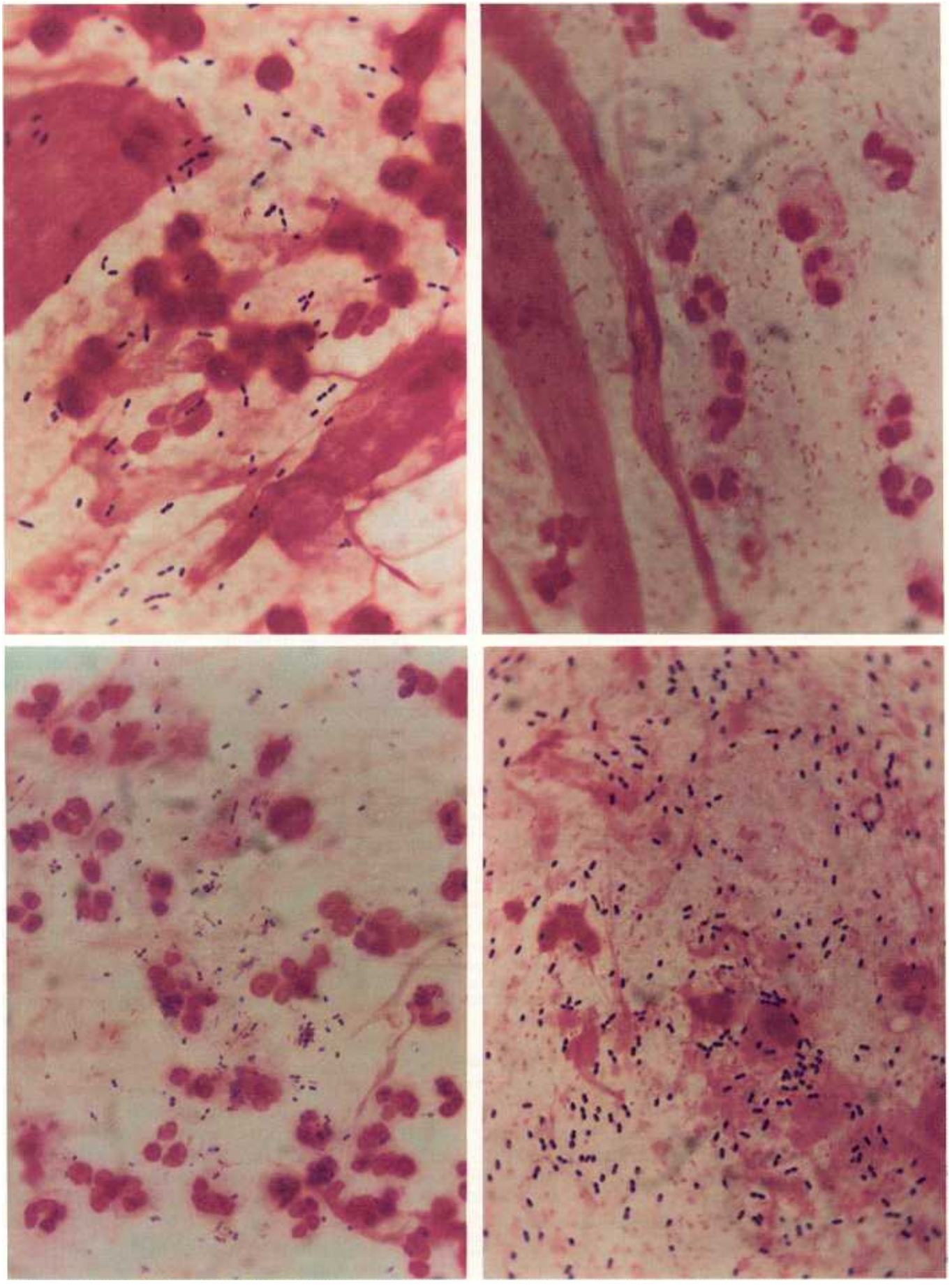


TABLE 1

Housestaff's and Laboratory Personnel's Skills in the Preparation of Gram-stained Smears of Sputum*

	Smear Adequate†	Smear Inadequate
Housestaff	84.8% (84/99)	15.2% (15/99)
Laboratory personnel	97.0% (96/99)	3.0% (3/99)

*$p < 0.01$, chi-square statistic.

†A smear was defined as adequate if it was properly applied to the slide and properly stained. The adequacy of slide preparation was determined by the consensus opinion of at least two senior microbiologists who were blinded to the identities of the patient and the slide preparer.

TABLE 2

Purulence of Sputum Samples Obtained by Housestaff and Nursing Personnel, Assessed by Two Senior Staff Microbiologists*

Sputum Sample† Obtained by	Adequately Prepared Slides	Purulent Sputum‡ Samples
Housestaff	84/99	58.3% (49/84)
Nursing personnel	96/99	37.5% (36/96)

*$p < 0.01$, chi-square statistic.

†Housestaff sputum samples were obtained by the housestaff physicians and microbiology samples were obtained by nursing personnel.

‡Sputum was defined as purulent if it contained <10 epithelial cells and >25 leukocytes per 100× microscopic field.

1. A blood culture positive for a pulmonary pathogen without another apparent source
2. A pleural fluid culture positive for a pulmonary pathogen
3. Heavy or moderate growth of a predominant bacterial pathogen on sputum culture, in the absence of a positive blood or pleural fluid culture
4. Light growth of a bacterial pathogen where the Gram stain performed by laboratory personnel revealed a bacterium consistent with the culture results in the absence of criteria 1–3
5. *Legionella pneumophilia* or *L. micdadei* defined by the presence of a positive direct fluorescent antibody test, a diagnostic sputum culture, a positive blood culture, or a fourfold rise in the IgG-specific antibody titer for one of these organisms following the episode of pneumonia
6. *Chlamydia pneumoniae* (TWAR) defined by a fourfold increase in antibody titer
7. *Mycoplasma pneumoniae* defined by isolation from sputum culture or fourfold seroconversion of antibody titer.

The identities of the causative organisms remained undetermined for patients whose sputum cultures revealed normal oral flora or no growth or light growth of multiple organisms, and for those who did not fulfill any of the above conditions.

Analysis

The diagnostic performance of the Gram stain of sputum was assessed by determining its sensitivity, specificity, and predictive values (positive and negative).[13] Two distinct reference standards were used to calculate these measures of test performance. The first standard was that of two senior staff microbiologists' determinations of the identity of the predominant organisms on the housestaff's slides (without benefit of culture results). The second standard was the final etiologic diagnosis based on the results of the microbiologic evaluation (sputum culture, blood culture, serology). For the latter standard, we evaluated only those patients whose purulent sputum samples were collected by the nursing staff and received in the microbiology laboratory, since sputum culture results reflect more accurately lower respiratory tract secretions in patients who have undergone similar cytologic screening.[7, 12]

Differences in proportions between patient subgroups were analyzed using the chi-square statistic.[14] A p value of ≤ 0.05 was considered significant.

RESULTS

During the study period, 170 cases met the criteria for community-acquired pneumonia. Seventy-one cases were excluded from the current study for the following reasons: 1) Gram stains were not made available because patients were unable to produce sputum (32 cases); 2) housestaff failed to save their Gram-stained slides (26 cases); 3) the microbiology laboratory did not receive a sputum sample (9 cases); and 4) housestaff failed to do a Gram stain (4 cases). Thus, 99 cases were evaluated in the study, each with a slide prepared by a housestaff physician and one prepared by microbiology laboratory personnel.

The 99 cases occurred in 97 patients; two patients had more than one episode of pneumonia in which a Gram-stained specimen was prepared and collected. Eighty-two percent (80/97) of the patients were male and 79% (77/97) were white. The mean age was 62 years, with a range of 17–90 years. The majority of patients had at least one comorbid illness, including cigarette smoking in 54% (52/97), alcohol abuse in 38% (37/97), obstructive pulmonary disease in 36% (35/97), and coronary artery disease in 20% (19/97).

Table 1 compares the Gram stain preparation skills of the housestaff and the microbiology laboratory personnel. Of the smears prepared by the housestaff, 15% were inadequately prepared, as compared with 3% for the laboratory personnel ($p < 0.01$). Of the 15 specimens inadequately prepared by housestaff, seven were improperly applied to the glass slides (five were too

thick and two were too thin) and eight were improperly stained (five were under-decolorized and three were over-decolorized). All three smears inadequately prepared by laboratory personnel were applied too thinly to the slides.

Table 2 compares the sputum purulence of samples obtained by the housestaff and those collected by nurses and submitted to the microbiology laboratory for patients with adequately prepared stains. Housestaff successfully obtained purulent samples in 58% of the cases, compared with 38% submitted to the microbiology laboratory ($p < 0.01$).

Table 3 shows the diagnostic performances of housestaff's Gram stains using the senior microbiologists' smear interpretation as the reference standard. The microbiologists evaluated all housestaff slides that were properly stained and purulent. Of the 99 housestaff slides, 15 were improperly stained and 35 were nonpurulent, leaving 49 slides for evaluation. The microbiologists felt that 19 were diagnostic of *H. influenzae* pneumonia, ten, of *Streptococcus pneumoniae* pneumonia, and two of infections with aerobic gram-negative bacilli, and 18 were non-diagnostic. The sensitivity of the housestaff's Gram stains for detecting *S. pneumoniae* was 90% (Table 3). The positive predictive value for detecting *S. pneumoniae* was 50%, with a corresponding false-positive rate of 50%. Housestaff smears were less sensitive but more specific in the detection of *H. influenzae* as compared with *S. pneumoniae*. Housestaff failed to identify correctly either of the two smears containing gram-negative bacilli.

Table 4 shows the diagnostic performances of housestaff Gram stains using the results of the etiologic diagnosis based on the microbiologic evaluation (sputum culture, blood culture, serology) as the reference standard. To maximize the reliability of the reference standard, only housestaff slides that had corresponding purulent laboratory samples were evaluated. Of the 99 laboratory slides, three were improperly stained (and purulence could not be assessed) and 60 were nonpurulent, leaving 36 slides for evaluation. In this group the two most common final diagnoses were *H. influenzae* pneumonia (ten cases), and *S. pneumoniae* pneumonia (seven cases). Other predominant organisms detected included *Staphylococcus aureus* (two cases), *Pseudomonas aeruginosa* (two cases), *Pasteurella multocida* (one case), *Moraxella (Branhamella) catarrhalis* (one case), *Chlamydia pneumoniae* (TWAR) (one case), *L. pneumophila* (one case), and mixed organisms (two cases). A microbiologic diagnosis was not established for ten patients. Again, housestaff smears had a high sensitivity, but a low positive predictive value, for detecting *S. pneumoniae*. The negative predictive values for detecting *S. pneumoniae* and *H. influenzae* (95% and 92%, respectively) were substantially higher than their positive predictive values (43% and 73%) (Table 4), suggesting that housestaff Gram stains were more useful in excluding than in establishing these etiologies of pneumonia. Housestaff properly identified both cases of *S. aureus* pneumonia but failed to identify either case of pneumonia due to gram-negative bacilli.

Nine patients had a diagnosis established by a posi-

TABLE 3

Housestaff's Gram Stain Performances Using the Senior Staff Microbiologists' Smear Interpretations as the Reference Standard*

Etiologic Agent	Sensitivity	Specificity	Predictive Value	
			Positive	Negative
Streptococcus pneumoniae	90% (9/10)	77% (30/39)	50% (9/18)	
Haemophilus influenzae	58% (11/19)	90% (27/30)	79% (11/14)	

*This reference standard was based on the microbiologists' identifications of the predominant organisms on the 49 housestaff slides that were properly prepared and purulent. Ten slides were considered diagnostic of *S. pneumoniae* pneumonia and 19 of *H. influenzae* pneumonia. Of the remaining 20 slides, two were diagnostic of infections with gram-negative rods (numbers too small to analyze statistically), and 18 did not identify a bacterial pathogen.

TABLE 4

Housestaff's Gram Stain Performances Using the Microbiologic Evaluation as the Reference Standard*

Etiologic Agent	Sensitivity	Specificity	Predictive Value	
			Positive	Negative
Streptococcus pneumoniae				
Haemophilus influenzae				

*This reference standard was based on patients' diagnoses as determined by the results of the microbiologic evaluation (sputum culture, blood culture, serology). The 36 patients with purulent sputum samples received in the microbiology laboratory were evaluated; seven had *S. pneumoniae* and ten had *H. influenzae* pneumonia.

TABLE 5

Influence of the Gram Stain Result on the Subsequent Selection of Initial Antibiotic Therapy

	Number of Initial Antibiotics Selected*	
	One	>One†
Predominant organism identified		
Streptococcus pneumoniae‡	19 (53%)	18 (47%)
Haemophilus influenzae§	8 (57%)	6 (43%)
Staphylococci	0 (0)	3 (100%)
Streptococci	1 (50%)	1 (50%)
TOTAL	28 (50%)	28 (50%)
No predominant organism identified	13 (30%)	30 (70%)

*The number of antibiotics selected by housestaff and used within 24 hours of admission.

†Of the 58 patients who received more than one agent, 32 received two agents and 26 received three agents.

‡$p < 0.05$, chi-square statistic, when compared with cases without a predominant organism identified.

§$p = 0.07$, chi-square statistic, when compared with cases without a predominant organism identified.

tive blood culture. Housestaff obtained purulent sputum from six of these patients. Among these six, housestaff identified two of three *S. pneumoniae*, one of one *S. aureus*, none of one *streptococcus species*, and none of one *H. influenzae* bacteremic pneumonias.

Housestaff identified a predominant organism on their Gram stains and specified its microbial etiology in 57% (56/99) of cases. Thirty-seven predominant organisms were identified as *S. pneumoniae*, 14 as *H. influenzae*, three as staphylococci, and two as streptococci. Overall, a single antibiotic was chosen as the initial treatment in 50% (28/56) of cases in which a predominant organism was identified, compared with 30% (13/43) of those without a predominant organism identified ($p = 0.05$) (Table 5). Patients with *S. pneumoniae* or *H. influenzae* as the predominant organism received single-agent therapy nearly twice as often as did those without a predominant organism identified. Of the 58 patients initially treated with more than one agent, 32 were initially treated with two antimicrobial agents and 26 with three agents. Fifty-four percent (14/26) of the patients receiving triple antibiotic therapy did not have a predominant organism identified.

DISCUSSION

Community-acquired pneumonia affects five million people and is responsible for over 500,000 hospital admissions in this country annually.[15] Authorities have traditionally recommended that a Gram stain of sputum be part of the routine evaluation of all patients seen with pneumonia.[1-3] The Gram stain has special value in that it can be more rapidly performed and interpreted than other diagnostic tests; as a result, it can

FIGURES 5-8 (*facing page*):

FIGURE 5 (*top left*). Gram stain showing slender gram-negative rods (culture yielded *Pseudomonas aeruginosa*). The Gram stains of the two cases of aerobic gram-negative bacilli pneumonia occurring in the study were misinterpreted by housestaff physicians.

FIGURE 6 (*top, right*). Gram stain shows many leukocytes but no organisms. Sputum cultures were non-revealing, but fourfold seroconversion to *Chlamydia pneumoniae* (TWAR) was subsequently demonstrated. The housestaff physician correctly interpreted the slide as consistent with "atypical" pneumonia and included erythromycin for therapy. The patient's condition responded to therapy.

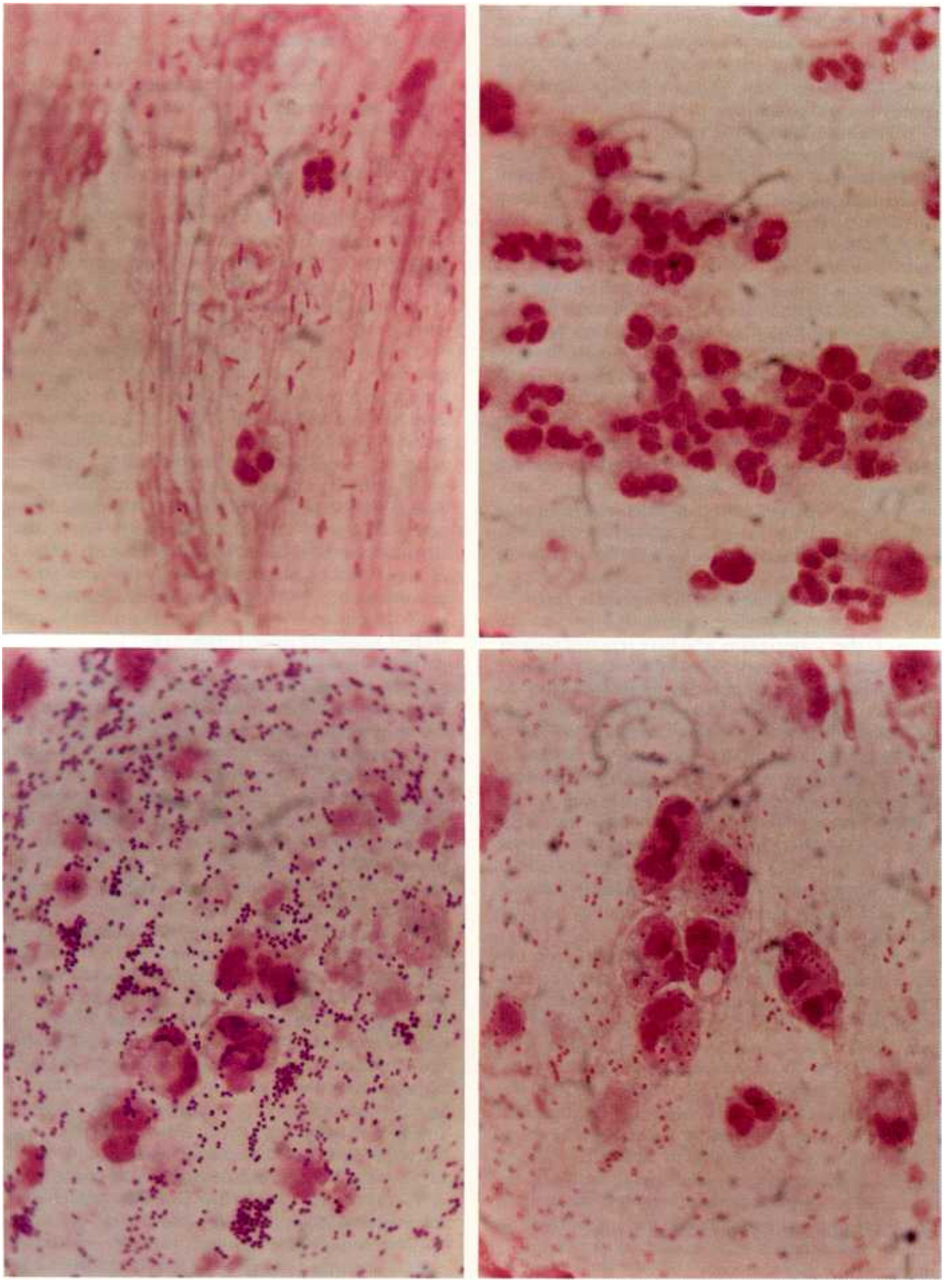
FIGURE 7 (*bottom, left*). Gram stain showing clusters of gram-positive cocci (sputum and blood cultures yielded *Streptococcus aureus*). This slide was correctly interpreted by the housestaff physician.

FIGURE 8 (*bottom, right*). One case of *Moraxella (Branhamella) catarrhalis* pneumonia was encountered in this study. Gram stain showed large numbers of gram-negative cocci (including intracellular organisms). Sputum culture revealed *Moraxella catarrhalis*. Interestingly, although the housestaff physician correctly identified the predominant organisms as gram-negative cocci, he answered "query *H. influenzae*" in response to the presumed identity of the organism.

be used to suggest an initial etiologic diagnosis and to guide initial antibiotic therapy prior to obtaining the results of cultures. This test may also be helpful in assessing prognosis in patients with community-acquired pneumonia inasmuch as it allows separation of patients with pneumococcal pneumonia from those with pneumonia caused by gram-negative organisms.[9]

Given the important role of the Gram stain in the management of patients with pneumonia, housestaff are routinely encouraged to utilize this procedure. Yet there is uncertainty regarding the competence of housestaff physicians in the performance of this diagnostic test. This study was undertaken to evaluate the ability of medical housestaff to prepare and interpret a sputum Gram stain in the management of patients with community-acquired pneumonia.

Our study has several important differences from previous investigations of the sputum Gram stain in patients with community-acquired pneumonia. This study was specifically designed to assess the preparation and interpretation of Gram-stained smears by housestaff physicians rather than by trained laboratory technicians or microbiologists.[5,6] Although prior studies have evaluated housestaff performing various aspects of this diagnostic test,[9,10] we evaluated all aspects, including sputum collection, smear and stain preparation, and interpretation. Finally, our methodology included strict definitions for interpreting the microscopic findings, the establishment of a well-defined



set of two reference standards, and a blinded assessment of the diagnostic test. These methodologic points are considered essential in the formal evaluation of a diagnostic test,[16] yet many reports have not conformed strictly to these guidelines.[4, 5, 9, 10]

We also evaluated the extent to which this test achieved its intended purpose in clinical practice, or its "effectiveness." In our cohort, 19% of patients could not produce sputum, and 25% failed to have a sputum Gram stain prepared by housestaff. Of the 99 Gram stains evaluated, 51% (housestaff's) to 64% (microbiologists') smears were either improperly prepared or nonpurulent. Thus, the sputum Gram stain was prepared in less than four-fifths of our pneumonia population and properly implemented in less than half. We believe that the utilization and effectiveness of the sputum Gram stain may well be even less in community settings and those without the scrutiny of an ongoing investigation. Thus, optimism related to the promising efficacy of the Gram stain must be tempered by the ability and willingness of physicians to employ this diagnostic test in the real world.

Certain aspects of the housestaff's performance were commendable. The physicians obtained purulent sputum samples nearly twice as often as did nursing personnel. Nearly two-thirds of the samples received in the laboratory were considered to represent upper airway secretions or saliva, consistent with the observations of other investigators who have reported nonpurulent samples in up to 60% of laboratory sputum specimens.[8] Obtaining purulent sputum, variably defined as containing $<10-25$ squamous epithelial cells with or without >25 polymorphonuclear leukocytes per $100\times$ field, is important because Gram stains of such specimens are more likely to identify lower respiratory tract pathogens and such samples have yielded culture results comparable to transtracheal aspirates.[7, 12, 17-19] This suggests that sputum collection should be performed by physicians or other personnel specifically trained in collection techniques; this finding has particular importance to community hospitals without housestaff physicians.

Housestaff were also generally capable of identifying *S. pneumoniae* and *H. influenzae*, the two most common bacterial pathogens in community-acquired pneumonia, regardless of the reference standard employed (Tables 3 and 4; Figs 1 and 2). Their sensitivity for detecting *S. pneumoniae* ranged from 86 to 90%, and for *H. influenzae* from 58 to 80%, rates similar to those of trained laboratory personnel in previous studies (Tables 3 and 4).[5, 20] Finally, housestaff were able to identify the proper pathogen in half of the cases of patients with bacteremic pneumonia who produced sputum. This early recognition provided the basis for prescribing specific antimicrobial therapy for those patients who may have been at greater risk of mortality or suppurative complications.

We did uncover several systematic errors in Gram stain preparation and interpretation by the housestaff physicians. The ability of physicians to adequately prepare a Gram stain was inferior to that of the microbiology laboratory personnel. Specimens prepared by the housestaff that were too thickly applied to the slides were often under-decolorized, and those applied too thinly were over-decolorized. This inadequate preparation should not be surprising, given that almost all housestaff had learned their staining techniques in medical school and had not been provided with a refresher course prior to residency. Competence in smear preparation and staining would appear to be a minimal prerequisite for the proper application of this diagnostic test.

Housestaff also appeared to overdiagnose *S. pneumoniae* while underdiagnosing *H. influenzae* and other gram-negative rods. Our review of the slides suggests that this high false-positive rate for detecting *S. pneumoniae* occurs because alpha-hemolytic streptococci and other gram-positive normal oral flora are often mistakenly identified as *S. pneumoniae* by inexperienced observers, an observation also suggested by others[4, 8] (Fig. 3). Alternatively, the high false-positive rate may have been artificially elevated, since *S. pneumoniae* is not isolated from the sputa of up to 50% of patients with proven pneumococcal pneumonia,[21-23] although our review of the individual slides showed that the housestaff tended to overread "pneumococci" when any gram-positive cocci were visible (Fig. 4). Another potential explanation for this misinterpretation is that housestaff often look at areas of the slide that are inappropriate given the presence of epithelial cell contamination.

We suspect that the difficulty housestaff experience in detecting *H. influenzae* and other gram-negative rods stems from the lower contrast of gram-negative organisms due to the similar background color, the pleomorphic shape, and the small size of these microbes. This point is exemplified in Figure 4, in which the gram-negative coccobacillary forms, although plentiful, can be overlooked in the presence of the more distinctive gram-positve diplococci.

Statistical analysis of Gram stain interpretation in specimens from patients with aerobic gram-negative bacillary pneumonia was not performed because of the small number of cases in this study, but it was clear that the housestaff had problems correctly identifying these organisms even though they were easily visible (Fig. 5). In "atypical pneumonias" resulting from Legionella, Chlamydia (including TWAR), and Mycoplasma, Gram stains generally reveal numerous leukocytes with rare to absent microorganisms (Fig. 6). In one case of *S. aureus* bacteremia, the first clue to microbial identity was provided by the Gram stain of the sputum (Fig. 7). Interestingly, the specimen from the one case of culture-confirmed *Moraxella (Branhamella) catarr-*

halis seen in this study had a highly distinctive Gram stain, revealing large numbers of gram-negative cocci, often abundant within the cytoplasm of polymorphonuclear leukocytes (Fig. 8).

It is notable that housestaff chose single-agent antibiotic therapy significantly more frequently in managing patients with a predominant organism identified on the admission sputum Gram stain (Table 5). Nevertheless, 50% of these patients were prescribed multi-agent broad-spectrum therapy when a single agent would have sufficed. The reluctance of housestaff to prescribe narrow-spectrum treatment for such patients apparently resulted from a lack of confidence in their Gram stain interpretation and its applicability.

Three aspects of our methods and results warrant further discussion. First, we chose two distinct reference standards to judge housestaff's Gram stain performances. The first reference standard was based on a standardized microbiologic evaluation (sputum culture, blood culture, and serology). A clear-cut weakness of our study was that in rare circumstances the microbiology department's Gram stain was used to assist in establishing the microbiologic reference standard, leading to the possibility of circular reasoning. However, its use was unlikely to introduce bias into our assessment of housestaff Gram stain performances because we relied on the laboratory's specimens and stains rather than on the housestaff's specimens and stains per se. Furthermore, evidence suggests that incorporating microscopic Gram stain findings improves the reliability of sputum culture results, thereby strengthening the microbiologic reference standard.[8] The second reference standard used the results of the senior microbiologists' Gram stain interpretations, because the diagnostic value of sputum culture in pneumonia is often questioned.[8, 21 24] We point out that the results of housestaff performances were similar regardless of the standard employed (Tables 3 and 4).

Second, our findings emanate from a single training program and may not be generalizable to housestaff training in other settings. However, less than 15% of our housestaff had graduated from the University of Pittsburgh School of Medicine, and the remainder had trained at a multitude of institutions throughout the United States, suggesting that deficits in Gram stain preparation and interpretation may be widespread.

Third, although our finding that housestaff physicians do less well in interpreting Gram stains than do trained microbiologists is intuitively obvious, our results have clinical relevance. Often, especially after hours when laboratory services are not immediately available, the Gram stain prepared by the physician can be pivotal in guiding initial antibiotic therapy. LaForce has even argued that for community-acquired pneumonia, the initial microbiologic evaluation should be limited to the Gram stain of sputum and that culture results can be misleading.[25]

The specific problems we identified can be used to tailor educational interventions aimed at improving Gram-stained slide preparation and diagnostic performance by physicians. Based on our findings, we make the following recommendations: All housestaff physicians should receive formal training in preparing the sputum Gram stain early in their first year, with periodic reinforcement during the training period. For programs in which housestaff are expected to make their own slides, the training should cover the techniques of sputum collection and slide and stain preparation. Special attention should be directed at the assessment of the adequacy (purulence) of a sputum sample. Identification of the morphologic features of common bacteria using the microscope should be reviewed. Microscopic subtleties that allow differentiation of *H. influenzae* from other gram-negative rods and *S. pneumoniae* from other gram-positive organisms should be emphasized. In their early encounters with patients with pneumonia, housestaff should prepare and examine Gram-stained smears under the guidance of microbiologists or other experienced personnel. Findings generated by housestaff should be considered preliminary; smears should be reexamined by experienced personnel for clinical confirmation and teaching purposes. The development of a program designed to achieve proficiency in this test during training will help promote its use throughout physicians' practices.

Finally, once the performance and interpretation of the sputum Gram stain are mastered, the physician should be encouraged to apply those skills to rational management. A more rigorous and confident use of the Gram stain may allow focused narrow-spectrum therapy for selected cases of pneumonia (e.g., penicillin for smears showing pneumococci) and curb the growing trend for unnecessarily broad-spectrum and expensive empiric antibiotic therapy. Such a program should directly improve housestaff physicians' diagnosis and care of patients who have pneumonia.

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