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Bronchoalveolar Lavage Gram Stains for Early Bacterial Identification in Pneumonia: Should They Stay or Should They Go?

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OBJECTIVES: The primary endpoint was to determine the sensitivity and specificity of the bronchoalveolar lavage Gram stain in predicting culture results. Secondary endpoints included determining the proportion of Gram stains from bronchoalveolar lavages that accurately identify culture isolates and the duration of antibiotic treatment before bronchoalveolar lavage collection.

DESIGN: Retrospective, observational study.

SETTING: Four ICUs at a single academic medical center.

SUBJECTS: Patients at least 18 years old admitted to an ICU with a diagnosis of pneumonia, collection of a bronchoalveolar lavage sample, and receipt of antibiotics.

MEASUREMENTS AND MAIN RESULTS: Two-hundred five isolates were included. Gram stains for Gram-positive and Gram-negative isolates showed high specificity, 97.3% and 100%, respectively, but lower sensitivity at 61.9% and 54.2%, respectively. The positive predictive value and negative predictive value were 77.2% and 95.7% for Gram-positive isolates and 100% and 84.4% for Gram-negative isolates, respectively. Gram stains correctly identified isolates on the bronchoalveolar lavage culture in 61.9% of Gram-positive organisms and in 54.2% of Gram-negative organisms.

CONCLUSIONS: Gram stains accurately identified causative organisms in a limited number of patients making the utility of the Gram stain an uncertain modality for predicting causative respiratory pathogens from bronchoalveolar lavage samples.

KEY WORDS: antibiotics; bronchoalveolar lavage; de-escalation; diagnostics; Gram stain; pneumonia

Pneumonia is one of the leading causes of infection-mediated death and ranks as the eighth leading overall cause of death in the United States when combined with influenza (1). Targeted antibiotic therapy provides for optimal treatment while limiting antimicrobial collateral damage. However, cultures and subsequent sensitivities routinely take days to result, prolonging the duration of empiric broad-spectrum antibiotics until there is sufficient microbiological evidence to narrow therapy.

Gram stains are performed on initial samples with results reported days ahead of culture results, expediting de-escalation of antimicrobial therapy. The Infectious Diseases Society of America's guidelines for nosocomial pneumonia and community-acquired pneumonia state that practitioners may use high-quality Gram stains in conjunction with other clinical markers to guide empiric therapy, although these are considered weak recommendations due to limited evidence (2, 3).

Equipoise exists for the use of Gram stains to narrow antimicrobial therapy. Studies evaluating the utility of Gram stains from endotracheal aspirate and bronchoalveolar lavage (BAL) samples to guide antibiotic therapy for

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ventilator-associated pneumonia (VAP) patients consistently found that Gram stains are of limited use to narrow antimicrobial therapy (4–6). In contrast, other studies demonstrated that the absence of Gram-positive bacteria on a Gram stain obtained from BALs and endotracheal aspirates had a high negative predictive value (NPV), allowing for de-escalation of antimicrobials (5, 7).

Given the inconclusive results found in the literature, the present study aimed to determine if Gram stains from BAL cultures correlate with final positive BAL culture results in critically ill patients.

MATERIALS AND METHODS

Setting

This retrospective cohort study was conducted at the University of Texas Southwestern (UTSW) Medical Center, an academic medical center with a university hospital in Dallas, TX. The study (STU-2019-1349) was determined to be exempt by the UTSW Institutional Review Board.

Study Design and Population

The study cohort included patients at least 18 years old who met the Centers for Disease Control and Prevention/National Healthcare Safety Network's (CDC/NHSN's) clinical definition of pneumonia while in the surgical, medical, cardiovascular, or neurosurgical critical care units, had a BAL culture obtained between June 1, 2012, and June 1, 2019, and received antibiotics following diagnosis of pneumonia (8). Patients were excluded from the study if they had cystic fibrosis or if the BAL sample had more than 10 squamous epithelial cells per low power field (LPF) or fewer than 25 neutrophils/LPF.

The primary endpoint was the sensitivity and specificity of the Gram stain in predicting BAL culture results. Secondary endpoints included the proportion of Gram stains from the BAL that accurately identified the respiratory isolates on the final culture results as well as the effect of hours of antibiotic duration prior to BAL sample collection on the concordance of the Gram stain results with culture results.

Definitions

Pneumonia was defined per the CDC/NHSN's definition (8). To meet the definition, patients had to fulfill imaging test criteria which required two or more serial chest images with at least one of the following: new and persistent or progressive and persistent infiltrate, consolidation, or cavitation. Patients also had to have at least one of the following: fever (> 38°C), leukopenia (≤ 4,000 WBC/mm^3) or leukocytosis (≥ 12,000 WBC/mm^3), or altered mental status with no other recognized cause in adults greater than or equal to 70 years old. Finally, patients had to display at least two of the following: new onset or purulent sputum or changes in respiratory secretion amounts or character; new onset or worsening dyspnea, tachypnea, or cough; rales or bronchial breath sounds; or worsening gas exchange (8).

Immunosuppression was defined as the use of steroids equivalent to prednisone 20 mg per day or higher for greater than 2 weeks; use of biologics or cytotoxic chemotherapy; diagnosis of leukemia, lymphoma, or HIV positive with cluster of differentiation 4 count less than 200 cells/μL; history of solid organ or hematopoietic stem cell transplant with the administration of immunosuppressant medications; neutropenic (absolute neutrophil count or total WBC count < 500 cells/mm^3); or a history of splenectomy. This definition was based on the CDC/NHSN's definition of immunocompromised patients (8). Solid organ transplant was defined as the patient having received the transplantation of a heart, lung, liver, or kidney prior to BAL collection. Appropriate antibiotic coverage was defined as an antibiotic that would cover bacterial isolates grown from the BAL based on culture and antibiotic sensitivity results and was started prior to culture collection.

Data Extraction

During the period reviewed, there were 15,579 BALs performed, inclusive of both inpatients and outpatients. Out of the total BALs collected, 205 isolates met inclusion criteria. The following patient data were extracted from the medical record database or calculated: gender, age, smoking status, Charlson Comorbidity Index, patient comorbidities such as chronic obstructive pulmonary disease and interstitial lung disease (ILD), receipt of any solid or hematopoietic stem cell transplant, presence of acute respiratory distress syndrome (ARDS), ventilation status, dialysis status, and select patient laboratory values. Additionally, microbiological data, antibiotic data (drug choice and duration), and immunosuppressant medications were collected.

All data were extracted from the Epic Hyperspace electronic medical record system at UTSW.

Statistical Analysis

Statistical analyses were performed using SAS Statistical Software Version 9.4 (SAS Institute, Cary, NC). Descriptive statistics including medians and ranges are reported for numerical values. Categorical values are reported as absolute numbers as well as percentages. Comparison of continuous measurements was made using the Wilcoxon rank-sum test. Categorical measurements were compared using a chi-square contingency table analysis. A significant statistical difference was defined as a p value of less than or equal to 0.05, and reported p values are two-sided.

Sensitivity, specificity, NPVs, and positive predictive values (PPVs) were calculated using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA). These were calculated for Gram stain identification of Gram-positive cocci (GPC) with isolation of GPC on culture, Gram stain identification of Gram-negative rods (GNRs) with isolation of GNR on culture, and Gram stain without an organism identified with no isolation of pathogenic organism on culture. Cohen's kappa coefficient was used to determine agreement between the Gram stain and the BAL culture result. A score of 0.61–0.8 was considered good agreement, and a score of 0.81 or greater was considered excellent agreement.

RESULTS

Of the 15,579 BALs conducted in the 7-year study period, 8,849 were conducted while the patient was admitted to the hospital; the remaining were outpatient procedures. Of these, 5,989 (67.7%) were excluded for the patient not being admitted to the ICU. Nineteen percent were excluded for not having a diagnosis of pneumonia. An additional 11% were excluded for having greater than or equal to 10 squamous epithelial cells/LPF or less than 25 neutrophils/LPF, having cystic fibrosis, or having only a fungal isolate on final culture. The final cohort included a total of 205 isolates from 183 BAL cultures, with 20 cultures having more than one isolate. Eighty-seven isolates grew nothing on culture, 38 grew normal respiratory flora (NRF), and 80 had a positive bacterial identification.

Baseline characteristics for patients based on final culture result are listed in **Table 1** and are similar across each group. The majority of patients in each group were male. The median age ranged from 58 years for those with sterile cultures to 63 years for those with GNRs on final culture. The majority of patients in all groups received antipseudomonal and antimethicillin-resistant *Staphylococcus aureus* antibiotics. The biggest differences across groups included those with ILD, immunosuppression, and transplants, with the lowest percentage of patients with these conditions seen in the NRF group. For patients with ARDS, only one isolate (4.8%) grew GPCs compared with roughly 20% of patients with isolates in the other groups.

Table 2 compares the sensitivity, specificity, PPV, NPV, and kappa coefficient of Gram stains showing GPCs, GNRs, and cultures on which no organism was seen. The negative cultures included those with no organism seen on final culture and those that grew NRF. Gram stains for Gram-positive and Gram-negative isolates showed high specificity, 97.3% and 100%, respectively, but lower sensitivity at 61.9% and 54.2%, respectively. The PPV and NPV were 77.2% and 95.7% for Gram-positive isolates and 100% and 84.4% for Gram-negative isolates, respectively. Gram stain identification of cultures which had no organism seen had a higher sensitivity of 92.8% and lower specificity of 68.8%. The PPV and NPV of the Gram stain for these isolates were similar, at 82.3% and 85.9%, respectively. The kappa coefficient for each group showed good agreement.

Of the 80 isolates with positive cultures included in the study, 45 (56.3%) were correctly identified by Gram stain (**Table 3**). From these isolates, 17 bacteria were identified and included Gram-positive isolates *Enterococcus faecalis*, *Enterococcus faecium*, *S. aureus*, *Staphylococcus epidermidis*, and *Streptococcus pneumoniae* and Gram-negative isolates *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Pseudomonas* species, *and Serratia marcescens. S. aureus* was the most common Gram-positive isolate, comprising 13 of the 21 Gram-positive isolates, with 69.2% of these isolates correctly identified on Gram stain. *Pseudomonas* species was the most common Gram-negative isolate, comprising 12 of the 59 Gram-negative isolates, with 58.3% of the isolates correctly identified (data not shown).

Thirty different antibiotics were administered to patients in the study, including penicillins, cephalosporins, fluoroquinolones, aminoglycosides,

TABLE 1.
Baseline Characteristics of Patients With Clinically Diagnosed Pneumonia

Baseline Characteristics	Gram-Positive on BAL Culture (*N* = 21)	Gram-Negative on BAL Culture (*N* = 59)	Sterile BAL Culture (*N* = 87)	Normal Respiratory Flora on BAL Culture (*N* = 38)
Male, *n* (%)	18 (85.7)	47 (79.7)	56 (64.4)	28 (73.7)
Female, *n* (%)	3 (14.3)	12 (20.3)	31 (35.6)	10 (26.3)
Age, yr, median (range)	61 (24–74)	63 (23–82)	58 (18–81)	60 (25–85)
Smoker, *n* (%)	14 (66.7)	36 (61.0)	41 (47.1)	22 (57.9)
Immunosuppressed, *n* (%)	13 (61.9)	26 (44.0)	61 (70.1)	15 (39.5)
Charlson Comorbidity Index, median (range)	9 (2–18)	10 (2–18)	7 (0–18)	8 (3–16)
Chronic obstructive pulmonary disease, *n* (%)	4 (19.0)	12 (20.3)	7 (8.0)	4 (10.5)
Interstitial lung disease, *n* (%)	8 (38.0)	29 (49.2)	22 (25.3)	2 (5.3)
Mechanical ventilation, *n* (%)	11 (53.4)	55 (93.2)	57 (65.5)	23 (60.5)
Acute respiratory distress syndrome, *n* (%)	1 (4.8)	12 (20.3)	17 (19.5)	7 (18.4)
Dialysis, *n* (%)	3 (14.3)	21 (35.6)	15 (17.2)	7 (18.4)
Antipseudomonal coverage, *n* (%)	17 (81.0)	39 (66.1)	78 (89.7)	32 (84.2)
Antimethicillin-resistant *Staphylococcus aureus* coverage, *n* (%)	17 (81.0)	35 (59.3)	66 (75.7)	29 (76.3)
Any transplant, *n* (%)	10 (47.6)	21 (35.6)	42 (48.3)	6 (15.8)
Heart transplant, *n* (%)	5 (23.8)	13 (22.0)	6 (6.9)	1 (2.6)
Lung transplant, *n* (%)	5 (23.8)	8 (13.6)	30 (34.5)	2 (5.3)

BAL = bronchoalveolar lavage.

macrolides, tetracyclines, vancomycin, linezolid, daptomycin, clindamycin, metronidazole, and carbapenems. Thirty-eight isolates (47.5%) had appropriate antibiotic coverage prior to BAL collection. Twenty-three of these isolates (60.5%) were correctly identified on Gram stain and had a median appropriate antibiotic exposure prior to BAL collection of 22.6 hours (range, 1.8–284.7 hr). Fifteen of these isolates (39.5%) were incorrectly identified on Gram stain and had a median appropriate antibiotic exposure time of 71.5 hours (range, 3.2–573 hr) prior to BAL collection. There was no statistically significant difference between the duration of appropriate antibiotic coverage prior to BAL collection for those isolates which were correctly identified by Gram stain and those which were not ($p = 0.052$). There was also no statistically significant difference between appropriate antibiotic coverage duration prior to BAL collection for

TABLE 2.
Predictive Value of the Gram Stain in Correctly Identifying Bronchoalveolar Lavage Culture Results

Gram Stain Result Identification on Culture	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %	Cohen's Kappa Coefficient (κ)
Gram-positive cocci	61.9	97.3	72.2	95.7	0.632
Gram-negative rod	54.2	100	100	84.4	0.628
No organism identified	92.8	68.8	82.3	85.9	0.638

TABLE 3.
Proportion of Gram Stains Which Correctly Identifies Respiratory Isolates From Bronchoalveolar Lavages in ICU Patients With Clinical Pneumonia

Culture Result	Gram Stain Correctly Identifies Isolate on BAL Culture, *n*	Gram Stain Incorrectly Identifies Isolate on BAL Culture, *n*	Proportion of Gram Stains That Correctly Identifies Isolate on BAL Culture
Gram-positive cocci on final BAL culture ($n = 21$)	13	8	0.619
Gram-negative rods on final BAL culture ($n = 59$)	32	27	0.542
Total ($n = 80$)	45	35	0.563

BAL = bronchoalveolar lavage.

correctly or incorrectly identified Gram-positive or Gram-negative isolates when looking at these groups independently (**Table 4**).

Thirty-five of 80 isolates with positive cultures were incorrectly identified on Gram stain (Table 3). The most common reason for incorrect identification was the lack of an organism seen on Gram stain (n = 25; 71.4%). The remaining 10 isolates were incorrectly identified on Gram stain. Of the 35 incorrectly identified isolates, 15 received appropriate antibiotic coverage prior to BAL collection. Twelve of the isolates with appropriate antibiotic coverage did not have an organism seen on Gram stain and had a median appropriate antibiotic duration of 151.8 hours (range, 8.2–573 hr) prior to BAL collection. Three of the isolates had the incorrect Gram type identified. These isolates had a median appropriate antibiotic exposure of 4.7 hours (range, 3.2–10 hr) prior to BAL collection. Of the 125 cultures with no organism identified, nine were incorrectly identified on the Gram stain. These nine cultures had an organism identified on Gram stain when the Gram stain should have been negative. One Gram stain showed Gram-positive rods, four showed GPCs, and four showed a mixed Gram stain.

DISCUSSION

Despite the high specificity seen with Gram stain identification of GPC and GNR in this study, the relatively lower sensitivity and lower negative culture specificity

TABLE 4.
Impact of Appropriate Antibiotic Coverage Prior to Bronchoalveolar Lavage Collection on Gram Stain's Ability to Correctly Identify Isolate on Bronchoalveolar Lavage Culture in ICU Patients With Clinical Pneumonia

	Gram Stain Correctly Identifies Isolate on BAL Culture		Gram Stain Incorrectly Identifies Isolate on BAL Culture	
Culture Result	Time of Appropriate Antibiotic Coverage in Hours Prior to BAL Collection, Median (Range)	Isolates, *n*	Time of Appropriate Antibiotic Coverage in Hours Prior to BAL Collection, Median (Range)	Isolates, *n*
Gram-positive cocci on final BAL culture	4.3 (2.18–112.92)	7	39.17 (8.23–258.47)	6
Gram-negative rods on final BAL culture	48.66 (1.75–284.67)	16	209.63 (3.23–572.98)	9
Total	22.58 (1.75–284.67)	23	71.25 (3.23–572.98)	15

BAL = bronchoalveolar lavage.
Of the 80 isolates included in the study, 38 had appropriate antibiotic prior to BAL collection and were included in the subgroup analysis. Appropriate antibiotic therapy is defined as an antibiotic that would cover the bacteria based on culture and antibiotic sensitivity results.

limits the use of Gram stains in definitively identifying isolates seen on BAL cultures. The Gram stain had a high NPV for GPCs of 95.7%, indicating a negative Gram stain may be more accurate in ruling out GPCs on final BAL culture. In contrast, GNRs had a lower NPV of 84.4% and higher PPV, indicating GNRs on the Gram stain may be more accurate in ruling in GNRs on the final BAL culture.

Of the 80 isolates identified from final BAL cultures in this study, Gram stains correctly identified 56.3% of isolates, which supports recently published literature (5, 9). The most common reason for a Gram stain to identify an isolate incorrectly in this study was absence of organisms on the Gram stain, occurring in approximately 70% of cases. This is similar to a multicenter study of 6,115 isolates which found that 58% of discrepant results were due to no organism identified on the Gram stain (10).

Studies involving BAL samples found conflicting results on the utility of Gram stains to diagnose pneumonia and de-escalate antibiotic therapy prior to the return of culture and sensitivity results. Studies in the 1990s concluded that BAL Gram stains could be used to aid in VAP diagnosis (11, 12). More recent studies have concluded that Gram stains from BALs should not be used to direct initial therapy. Interestingly, these studies found Gram stains of BAL samples had lower specificities, NPV, and PPV for Gram-positive and Gram-negative isolates than the current study (5, 9, 13). In contrast, the sensitivity varied more between studies, with two studies from 2005 and 2006 having higher sensitivity for Gram-positive and Gram-negative isolates and a 2008 study having similar sensitivity for both isolates when compared with this current study (5, 9, 13). Despite these differences, this study supports the conclusion found in the other studies that the Gram stain result is variable and cannot be used to identify the final BAL culture result.

Despite the varying ability of Gram stains to correctly identify respiratory culture results, other culture sources have shown better accuracy. A study published in 2007 which included 5,983 blood cultures found overall sensitivity ranged from 91.3% to 99.7% and specificity ranged from 98.9% to 100% (14). Cerebral spinal fluid has also had variable Gram stain accuracy reported, but a recent 2013 study with 451 specimens found a sensitivity and specificity of 98.2% and 98.7%, respectively, in patients with *S. pneumoniae*, *Neisseria meningitidis*, or *H. influenzae* (15). Therefore, it is possible that Gram stains from other culture sources may be more accurate in correctly identifying isolates on final culture than those from respiratory cultures.

Antibiotics are often started before a BAL is performed. Antibiotics decrease the bacterial load, which may lead to an insufficient bacterial inoculum and misidentification on the Gram stain (16). The ability of the Gram stain to correctly identify a sputum culture has been shown to be adversely affected by increasing antibiotic duration prior to sample collection (17). The study, which included 105 patients with pneumococcal pneumonia, recommended that sputum Gram stains may only be useful within the first 6–12 hours after antimicrobial therapy was started (17). Given the acuity of ICU patients, it is likely they will be started on antibiotics empirically before respiratory Gram stains and cultures can be obtained, making the usefulness of these diagnostic techniques dependent on the duration of antimicrobial administration prior to sample collection.

Although the study described in this article did find a difference in the median number of hours of appropriate antibiotic exposure prior to BAL collection between isolates correctly identified by Gram stain and those incorrectly identified (22.6 vs 71.3 hr), this difference was not statistically significant ($p = 0.052$). The most common reason for incorrect identification by Gram stain was that no organism was identified on the Gram stain. Incorrectly identified isolates had a median appropriate antibiotic coverage time of 151.8 hours for the isolates which did not have an organism seen on Gram stain, compared with a median appropriate antibiotic coverage time of 4.65 hours for isolates which had the wrong identification on Gram stain, although the sample size was small with 12 and three isolates, respectively. This suggests that increased antibiotic exposure prior to culture collection may lead to a progressive decrease in bacterial load, which in turn would affect an organism's ability to be identified on the Gram stain.

Given the imprecision of Gram stains in accurately identifying bacteria from BAL cultures and length of time cultures take to result, a test with a shorter turnaround time is needed. Rapid molecular testing is already available on several platforms; the tests show

results in hours instead of days, and some include testing for resistance genes (18). Case studies have shown rapid molecular testing to be accurate, even in the setting of recent antibiotic exposure (19, 20). Although there are few large-scale trials assessing the use of rapid molecular diagnostics in patients with pneumonia, one study with 846 BAL and 836 sputum samples found that the sensitivity of the test ranged from 75% to 100% for sputum samples and 85.7% to 100% for BAL samples (21). Rapid molecular diagnostics in patients with pneumonia offer a potential option for those who have time-sensitive results. However, the relatively higher cost of these platforms, especially when start-up costs are taken into account, may limit this testing option. A recent study found that using one of these platforms resulted in identification of a pathogen 42.2 hours more quickly than culture (22). The ability to more quickly identify pathogens and allow for more targeted therapy would be useful in high-acuity and high-resource areas and may help offset some of the cost associated with the test. Additionally, no regulatory bodies require Gram stains to be completed on respiratory cultures. If Gram stain testing was no longer done on these samples, the associated cost savings could also help balance the higher price of rapid diagnostic testing.

This study had several limitations. The first is that the study was retrospective, and the total number of cultures included was relatively small compared with the overall number of BALs that were collected, resulting in possible selection bias. Additionally, this study was a single-center study at an academic medical center with a high organ transplant volume where a large number of BALs are collected and therefore may not be applicable to practice at other institutions. Finally, the low sample numbers limited the statistical analyses performed. These low numbers prohibited an analysis of different species of bacteria.

CONCLUSIONS

The results of this analysis call into question the utility of Gram stains collected from BAL samples for clinical decisions. However, there is still a need for rapid identification of pathogens to limit unnecessary antibiotic exposure, and other modalities should be explored to fill this need.

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