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Original Research Article

Analysis of Diagnostic Value of Gram Stain in Comparison to Culture of Sputum Samples at A Tertiary Care Hospital

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Abstract

Introduction: Sputum Gram stain and culture are routinely done to determine the causative organism in case of lower respiratory tract infections. Sputum culture takes much more time to give results as compared to Gram stain that's why Gram stain is valuable in guiding empirical treatment for the patients but Gram stain report alone is not always reliable.

Aim: Aim of this study was to analyze the diagnostic performance of Gram staining in comparison to sputum culture results for lower respiratory tract infections.

Materials and methods: Study was performed in a 690-bed, tertiary-care hospital of northern India. A total of 477 expectorated sputum samples which were collected in sterile containerswere included in this study.

Results: A total 311(65%) samples were identified as a good sample, and the count of fair and poor samples were 109(23%) and 57(12%) respectively. Poor sputum samples gave positiveGram stain results and negative cultures more frequently as compared to good & fair samples (p<.05) whereas good quality sputum samples more frequently gave Gram stain results that were compliant with the culture results either positive or negative (p<.05). In good quality sputum samples (311) number of culture positive and Gram stain positive or true positive were 172(55%). The sensitivity, specificity, positive predictive value and negative predictive value for Gram staining in good grade samples were 95.03%, 28.46%, 64.91% and 80.43% respectively (p<0.00001).

Conclusion: Consistent interpretation of Gram stain results can be challenging. Correct interpretation of Gram stain results by the physician can improve the choice of antibiotics and thus can greatly reduce morbidity and mortality in critically ill patients.

Keywords: Gram stain, culture, sputum, lower respiratory tract infection.

Introduction

In 1884 a Danish physician, Hans Christian Joachim Gram, developed Gram stain [1]. In field of clinical microbiology Gram stain is one of the most important, rapid & inexpensive diagnostic method and is used very frequently to determine probable microorganism causing the infectious disease [2]. Based on Gram stain

bacteria can be gram-negative bacilli (GNB), gram-negative cocci (GNC), gram-positive bacilli (GPB), and gram-positive cocci (GPC).

Sputum Gram stain and culture are routinely done to determine the causative organism in case of lower respiratory tract infections [3]. Sputum culture takes much more time to give results as compared to Gram stain, that's why Gram stain is valuable in guiding empirical treatment for the patient [4]. Gram stain is also used as an indicator of sputum specimen quality and acceptability for bacteriological culture. Bartlett devised a grading system for evaluating sputum samples based on the relative number of squamous epithelial cells and segmented neutrophils in Gram stains of sputum samples [5].

Gram stain report alone is not always reliable. Sputum sample can be contaminated by oropharyngeal flora as it passes through the mouth. When significant oropharyngeal contamination is seen in Gram stained sputum smears, a second sample representing lower respiratory tract should be collected [6]. Deeply expectorated sputum is considered as good quality specimen. It is also recommended to rinse the mouth with water and cough deeply early morning to provide sample [7]. Further problems are faced when Gram stain report is misinterpreted. Incorrectly interpreted Gram stains may adversely impact patient care [8-11]. Discordant Gram stain results are seen as following scenarios: positive Gram stain with negative culture (false positive Gram stain) and negative Gram stain with positive culture result (false-negative Gram stain) [9-15].

Aim of this study was to analyze the diagnostic performance of Gram staining in comparison to sputum culture results for lower respiratory tract infections.

Materials and Methods: Study was performed in a 690-bed, tertiary-care hospital of northern India. A total of 477 expectorated sputum samples which were collected in sterile containers were included in this study between the years 2019-2020.

Smear was prepared for Gram staining from the purulent portion of sputum. Stained smear was examined microscopically under low power and oil immersion. Low power magnification was used to detect and quantitate squamous epithelial cells and WBC; however, microorganisms were observed under oil immersion. In Gram stain we noted presence of neutrophils, squamous epithelial cells and bacterial morphotypes (e.g., gram-positive cocci in pairs and clusters) semi- quantitated. Sputum specimens were graded "good" if they had 10 or fewer squamous epithelial cells per low-power field, "fair" with 11 to 19 squamous epithelial cells, and "poor" with more than 19 squamous epithelial cells and less than 10 WBC per low-power field, based on criteria described in table 1[16,17]. Sputum specimens with >19 squamous epithelial cells and <10 WBC per low- power field were considered as "inadequate" and were not included in this study.

Table 1: Criteria for grading the quality of sputum samples

Specimen Grade	Squamous cell /LPF	Neutrophils/LPF
Good	0-10	NA(usually>25)
Fair	11-19	NA(usually>25)
Poor	>19	>10
Inadequate	>19	<10

Each specimen was inoculated on to blood agar, chocolate agar and MacConkey agar plates and incubated at 35-37°C for 48 hours. After culture organisms were identified by standard protocols and antibiotic susceptibility of recommended drugs according to CLSI guidelines was performed by Kirby Bauer disc diffusion method. Enterococcus, Viridians group streptococci, CoNS and Moraxella were considered as normal respiratory flora [18].

Samples which showed mixed growth on culture were considered as negative and only the ones with pure growth were considered as positive. Cultures with less than 5 colonies per plate of potential respiratory pathogens were counted as having only normal oral flora and were considered negative [19].

Results were considered discrepant if : (i) culture demonstrated moderate/many colonies of a particular organism but Gram stain was negative for an organism with same morphology/stain characteristics, or if (ii) Gram stain showed moderate/many bacteria but culture was negative for growth of that organism.

Results:

During study period of one year from 2019-2020 a total 477 expectorated sputum samples were enrolled and were classified according to number of epithelial cells and neutrophils visualized onlow power field. (**Table-1**) A total 311(65%) samples were identified as a good samples, the count of fair and poor samples were 109(23%) and 57(12%) respectively. (**Figure-1**)

The findings of positive gram stain with negative culture were more frequently observed in poor sputum samples as compared to good & fair sputum samples (p <0.05) whereas result of Gram stain in good quality sputum samples were frequently concordant with the culture results (p<0.05). A total 172(55%) good quality sputum samples were observed with culture positive and gram stain positive (true positive), whereas fraction of false negative or culture positive and Gram stain negative samples were minimum in good quality samples. The sensitivity, specificity, positive predictive value and negative predictive value for gram staining in good grade of samples were 95.03%, 28.46%, 64.91% and 80.43% respectively (p <0.00001).

Percentage of culture negative and Gram stain positive or false positive were maximum in fair and poor grade samples. False negative samples were not identified in fair and poor grade samples. Due to this finding the sensitivity and negative predictive value in both grades of samples were 100%. The specificity and positive predictive value for Gram staining in fair grade samples was 3.08% and 41.12%and in poor grade samples was 4.65% and 25.45%.

The overall sensitivity, specificity, positive predictive value and negative predictive value of Gram staining was calculated in all three grades of 477 samples and was found to be 96.23%, 17.23%, 58.86% and 82.00% respectively (p value<0.00001). (table-2, table-3)

In this study potential respiratory pathogens that were isolated were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii, Proteus* species and *Serratia marcessens* among the Gram negative bacilli and *Staphylococcus aureus*, Methicilline Resistant Staphylococcus aureus and *Streptococcus pneumoniae* among the Gram positive cocci.

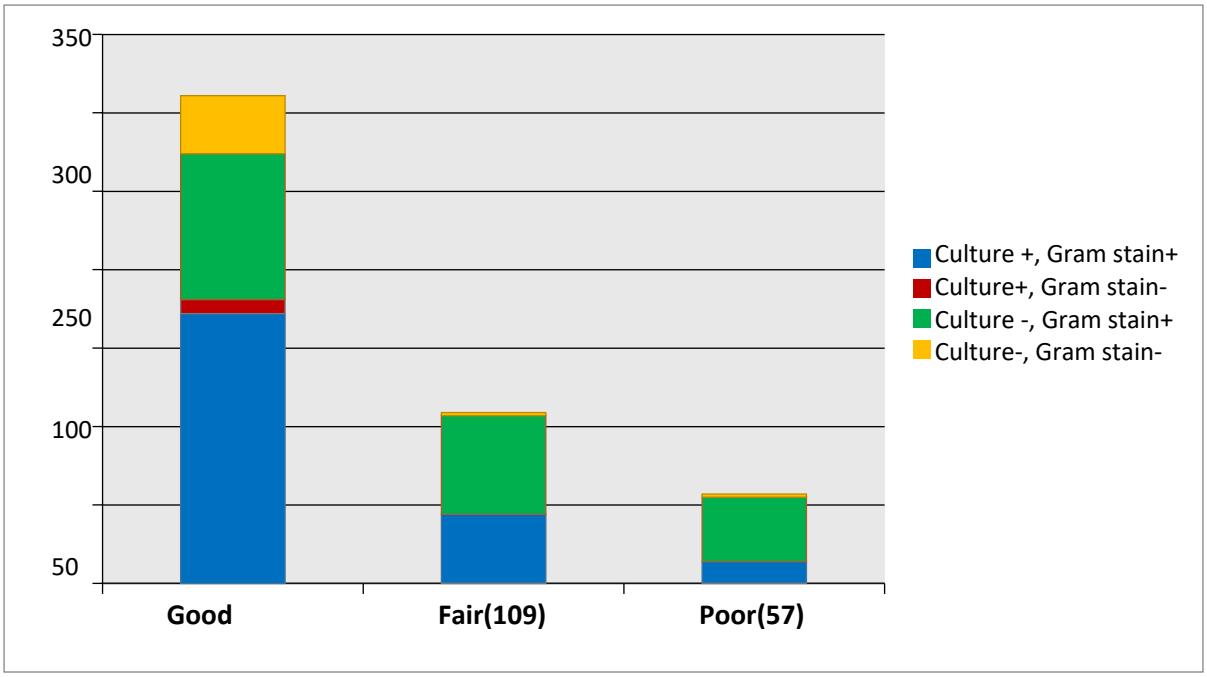

Figure 1: Graphical representation of comparison of sputum quality with Gram stain and cultureresults

Table 2: Comparison of sputum quality with Gram stain and culture results

Grade	Culture +, Gram stain+	Culture - , Gram stain+	Culture +, Gram stain-	Culture -, Gram stain-	Total Number (%)
Good	172 (55%)	93 (30%)	9 (3%)	37 (12%)	311(65%)
Fair	44 (40%)	63 (58%)	0 (0%)	2 (2%)	109(23%)
Poor	14 (24.5%)	41 (72%)	0 (0%)	2 (3.5%)	57(12%)
Total	230 (48%)	197 (41%)	9 (2%)	41 (9%)	477

Table 3: Statistical comparison of Gram stain and culture results

Grade	Sensitivity	Specificity	Positive predictive value	Negative predictive value	p Value
Good	95.03%	28.46%	64.91%	80.43%	p<0.00001
Fair	100%	3.08%	41.12%	100%	-
Poor	100%	4.65%	25.45%	100%	-
Total	96.23%	17.23%	53.86%	82.00%	p<0.00001

Discussion

A proper expectorated sputum sample is a prerequisite for obtaining relevant Gram stain and culture results [20-24]. For this patient needs to be instructed as to how to give a proper sputum sample. If the sputum sample is not of good quality then patient can be asked to give another sample. In case obtaining a second sample is not possible then clinician should use the results of poor sputum sample with caution for initiation of empirical therapy. Roson et al. concluded that a good quality sample could be highly specific for the diagnosis of S. pneumoniae pneumonia, and therefore useful in guiding pathogen-directed antimicrobial therapy [25].

The majorities of discrepant results (41%) in this study were Gram stain positive and culture negative (false-positive). This may happen when organisms are fastidious, nonviable or an anaerobe. If the organism is overgrown by normal oral flora then also it might be missed while reading the culture plates. Another reason could be due to failure to reject specimens that were contaminated with oral flora. These smears were reported to have predominant Gram-negative bacilli, while culture growth demonstrated mixed flora with no predominant organism.

Gram stain negative and culture positive (false-negative) results were obtained in 2% samples. The probable cause for such results could be due to examination of inadequate number of fields, or due to not visually demarcating the area of smear on the slide which is to be observed or because of not being able to distinguish actual organisms from stain debris or background.

Errors in Gram stain report may also arise due to certain technical reasons [7, 10, 26-28]. For example a thick smear might not be very specific because a gram- negative organism might appear as a gram-positive because of the under-decolorized smear whereas a thin smear might be less sensitive because a Gram-positive organism might appear asa Gram-negative one because of the over-decolorization of smear. Such finding was also reported by Yunusa et al. [29]. Another reason for discrepancy in results of sputum Gram stain and culture is variations in morphologic features of bacteria in different environment. For example Staphylococci may be found as diplococci when it has been treated with antibiotics. Pneumococci does not always look lancet shaped and may even be arranged in clusters [30].

In this study Gram stain sensitivity and specificity for good quality sputum samples was 95% and 28% respectively while overall sensitivity and specificity was 96% and 17% for all types of samples. In a study done by Musher et al. sensitivity of Gram stain was found to be 80% for sputum samples that were adequate [31]. Reed and colleagues did a meta-analysis for evaluating the sputum Gram stain sensitivity and specificity in 1996. The results they obtained found the sensitivity range from 15 to 100% and specificity from 11 to 100% [32].

We can improve the Gram stain results by implementing some measures to address potential areas of concern. Laboratory staff should be educated and trained thoroughly on key aspects of smear preparation. For example they should be advised to demarcate the area of smear on the slide. Smears should just be thick enough so that newsprint is visible through the slide. Smears that appear to have inadequate material can be repeated. Also while reporting one should make sure that an adequate number of fields are examined. Another exercise that is suggested so as to reduce the error rates is double review of smears. Though there is no data available on the influence of double review of smears in clinical microbiology, but this approach in other fields has favorable

outcome to a certain extent for example in surgical pathology and cytology [33,34]. It was observed by Meier et al. that by doing double review of slides from breast and prostate cases incidence of misinterpretations was markedly reduced [33]. Further steps like anaerobic testing whenever warranted will also reduce the proportion ofdiscrepant results.

Limitations of this study that need to be pointed out are, firstly the prior antibiotic usage was not taken into account so this might be one of the many reasons contributing to discrepant result due to nonviability of the pathogenic organism. Secondly we have not taken into account the samplesshowing mixed type of growth in culture or Gram stain. Thirdly only a single specimen type is taken into account in this study. For assessing the accurate Gram stain error rates different types of specimen need to be considered. Lastly we have not done anaerobic culture for Gram stain positive and culture negative samples to rule out discrepancy due to anaerobes. To address these issues further studies have to be carried out.

Conclusion:

There is no doubt about the value of a rapid and non-invasive test that can reliably diagnose the etiologic cause of a disease. But consistent interpretation of Gram stain results can be challenging and if we do not know where we stand we cannot begin to improve. So this is just an initial steptoward establishing a benchmark for the incidence of errors during the performance of Gram stains and additional data may be required to establish acceptable ranges for Gram stain performance. Still this study can help clinicians understand the advantages and drawbacks of sputum Gram stain in initiating the empirical antibiotic therapy and of culture in further continuation or alteration of the therapy. Correct interpretation of Gram stain results by the physician can improve the choice of antibiotics and thus can greatly reduce morbidity and mortality in critically ill patients [35]. Also broad spectrum antibiotics will be prescribed less frequently, antibiotic resistance will decrease, since patients will be treated with fewer antibiotics they will have fewer undesirable side effects, and moreover it will be cost effective.

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