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Evaluation of Gram Stain Error Rates of Clinical Specimens

Rabia Sajjad, Gohar Zaman*, Irfan Ali Mirza, Wajid Hussain**, Umar Khurshid**, Asim Shehzad*****

Combined Military Hospital, Attock/National University of Medical Sciences (NUMS) Pakistan, *KRL Hospital, Islamabad Pakistan,
**Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan,
***Fauji Foundation Hospital, Rawalpindi Pakistan

ABSTRACT

Objective: To determine important errors in initial Gram staining of clinical specimens and evaluate the types of Gram-stain errors.

Study Design: Cross-sectional study.

Place and Duration of Study: Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Aug 2018 to Feb 2019.

Methodology: Gram staining and culture inoculation of all specimens were performed per recommended guidelines. The Gram stain results on day-0 were compared with the results of the culture on day-2. There was no discrepancy if similar organisms were obtained on culture as on Gram stain. Nevertheless, if the Gram stain and culture results were not similar, it was termed a discrepancy. The consultant microbiologist reviewed all discrepant slides, and if not resolved, possible causes of error were sought, and the results documented.

Results: Of the total 300 clinical specimens, errors were observed in the initial gram staining of 29 specimens (9.7%), whereas 271(90.3%) specimens were error-free. Upon evaluating these 29 errors, 11(38.0%) were observer errors which were resolved when reviewed by a consultant microbiologist. 14(48.0%) were technical errors, and 4(14.0%) results were discrepant due to the presence of anaerobic organisms, missed on initial aerobic cultures.

Conclusion: The frequency of Gram stain errors in our study (9.7%) is not very high; nevertheless, it can have severe consequences in critical samples from seriously ill patients if wrong empirical antimicrobial treatment is begun based on a wrong initial Gram stain result.

Keywords: Clinical specimens, Errors, Gram stain

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INTRODUCTION

Bacterial infections are a major cause of morbidity and mortality worldwide. Respiratory tract infections, urinary tract infections, diarrhoea, wound infections, meningitis, and bloodstream infections, are all important bacterial infections that consume a lot of healthcare resources.[1,2] According to the United States National Ambulatory Medical Care Survey 2015, the number of physician visits for infectious and parasitic diseases was 16.8 million in USA.[3] In Pakistan, a developing country, the infectious disease burden is even more severe. According to World Health Organization, lower respiratory infections are the second leading cause of death in Pakistan, after ischemic heart disease.[4] Community-acquired pneu-monia is more common in extremes of age. However, limited data is available regarding their prevalence in Pakistan. The prevalence of ventilator-associated pneumonia has been studied in Pakistan, with around 33.5% of all patients being ventilated.[5] Much work has been done in microbiology to diagnose these quickly and efficiently, yet the gold standard for most bacterial infections remains culture and sensitivity, which is time-consuming.[6]

In the microbiology laboratory, despite a magnitude of advancements, Gram stain remains the cornerstone of diagnostics.[7] Developed first by a Danish bacteriologist Hans Christian Gram in 1884, the Gram staining procedure classifies bacteria into two major groups; Gram positive and Gram negative, depending upon structural differences in bacterial cell wall.[8] The fast track machines and automated systems are a big help in diagnosis, yet the fundamental of all microbiology lies in Gram staining.[8] It is a simple, economical and very quick means of providing the treating physician/surgeon with a clue to start the empiric antibiotic therapy for the patients until the culture and sensitivity report is available.[7,9]

Despite the important nature of the test, it has yet to be given due attention regarding the establishment of its standardization and reliability.[7,10] In this study,

Correspondence: Dr Rabia Sajjad, Pathology Department, Combined Military Hospital, Attock, Pakistan
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we evaluated the performance of Gram staining in our laboratory, comparing the initial Gram stain results with the subsequent culture results.

METHODOLOGY

This study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from August 2018 to February 2019, after approval from the institutional review board (FC-MIC16-3/READ-IRB/18/1326). The sample size was calculated using the WHO sample size calculator with the statistical assumptions of a 95% confidence level, taking the prevalence of Gram stain error rates to be 5%.[7] The sample size was calculated to be at least 300 clinical samples for this study. A total of 300 clinical specimens were included in the study using simple random sampling.

Inclusion Criteria: The clinical samples including pus, tissue, fluids, cerebrospinal fluid (CSF), sputum, bronchoalveolar lavage (BAL), non-directed bronchial lavage (NBL), endobronchial (EB) washings and tracheal aspirates submitted to AFIP Rawalpindi for bacterial cultures were included in the study.

Exclusion Criteria: The clinical samples of blood, urine and stool submitted to AFIP Rawalpindi for bacterial culture were not included in the study. All swabs, including pus swabs and high vaginal swabs, were not included in the study.

All the specimens were processed according to recommended guidelines by the American Society of Microbiology (ASM).[11] No discrimination was made based on age and gender. The smear was prepared with the specimen and stained with Gram stain.

The specimen was then inoculated onto appropriate culture plates for bacterial cultures. The Gram-stained slide was observed, as routinely done by microbiology residents. For this study, only microorganisms were considered, and the cellular components in the slide were not commented upon. Any bacteria/yeast noted after observing around 30 to 35 fields in the microscope were noted. This was done on day-0 when the specimen was received in the lab to be compared against the culture results after 48 hours. The media plates inoculated with the clinical specimen on day-0 were interpreted regarding the type of bacterial growth, on day-2, after standard incubation as per protocols.

The results of the Gram stain obtained on day-0 were compared with the results of the culture on day-2. If a similar organism was obtained on culture as seen on Gram stains, like Gram-positive cocci (GPC), Gram-positive rods (GPR), Gram-negative cocci (GNC), Gram-negative rods (GNR) or yeast, there was no discrepancy. However, if the results of the Gram stain and culture did not correspond, like the organisms seen on the Gram stain and culture were different, or no organism was seen on the Gram stain. However, the culture revealed growth, or an organism was seen on the Gram stain, but the culture did not show growth. Therefore, it was termed a discrepancy. In a discrepancy, the slides were again reviewed by a consultant microbiologist. If the discrepancy was solved and the organism was seen on a slide review, it was considered an observer error. If the discrepancy still needed to be solved, a repeating slide was prepared, stained with Gram stain and reviewed. If the discrepancy was solved on repeat staining, it was most likely a technical error. Therefore, possible error causes were sought, and the results were documented.

The data obtained were entered into SPSS (version 24) software for statistical analysis. Descriptive statistics were calculated for both qualitative and quantitative variables. For types of clinical specimens, the frequency and percentage of Gram stain findings and culture results were calculated. The percentage of discrepancy in Gram stain and culture results was calculated. Percent error rates of various types were also calculated.

RESULTS

Of the 300 clinical samples studied, the maximum was pus samples (42.3%), followed by respiratory samples (Table-I).

Table-I: Breakdown of Clinical Specimens (n=300)

Clinical Specimens	n(%)
Pus	127 (42.3%)
Sputum	52 (17.3%)
Endobronchial washing	13 (4.3%)
Bronchoalveolar lavage	14 (4.7%)
Non-directed bronchial lavage	29 (9.7%)
Fluid	25 (8.3%)
Tissue	33 (11.0%)
Tracheal secretion	4 (1.3%)
Cerebrospinal fluid	3 (1.0%)
Total	300 (100%)

Upon direct gram stain, the most often seen microorganisms were Gram-positive cocci (GPC) followed by Gram-negative rods (GNR). Mostly single organisms were seen in slides (81%, n=243), multiple (2-3) organisms were seen in 40 slides (13.3%), whereas no organism was found in 17 slides. The exact

breakdown of isolates was given in Table-II. In 271 cases, Gram stain findings on day-0 correlated with culture results on day-2. The error rate was 9.7%. These 29 discrepancies were further analyzed to find the types of errors.

Table-II: Direct Gram Stain findings at Day-0 (n=300)

Organisms on Gram Stain	n(%)
Gram positive cocci	115(38.3%)
Gram negative rods	97(32.3%)
Gram positive cocci and Gram negative rods	27(9.0%)
Gram positive cocci, Gram negative rods and yeast cells	2(0.7%)
Gram positive cocci and yeast cells	2(0.7%)
Yeast cells	9(3.0%)
Gram negative coccobacilli	22(7.3%)
No organism seen	17(5.7%)
Gram negative rods and yeast cells	9(3.0%)
Total	300 (100%)

A consultant microbiologist reviewed these 29 slides. In 11 cases, the organism was seen in the slide when reviewed by the consultant. Therefore, it was an observer error. In 14 cases, there was a technical error in gram staining due to which the organism was not visualized, and a new slide was made from an original sample that revealed the organism. On further evaluation of the errors, various reasons were found, as mentioned in Table-III.

Table-III: Reasons of Gram Stain Errors (n=300)

Reason of error	n(%)
Missed second organism	3 (10.3%)
Wrong numbering	2 (7.0%)
Gram positive cocci-artefacts	4 (13.8%)
Scanty organisms present	7 (24.1%)
Dilute carbol fuchsin, Gram negative rods not stained	10 (34.5%)
Anaerobic organism	3 (10.3%)
Total	29 (100%)

Most errors were seen in interpreting GNRs as the quality of Carbol fuchsin used as counter stain needed to be revised. It was either too dilute or old. This led to missing GNRs on slides. Using proper quality control in Gram staining rectified this problem. Replacing Carbol fuchsin with Safranin as a counter stain produced much better and more reliable results and a clear-cut outline of Gram-negative organisms.

DISCUSSION

Medical errors are responsible for a significant mortality rate in the US and account for the third highest after heart disease and cancer.[11,12] Lab errors also contribute to medical errors. As a significant proportion of diagnoses are confirmed upon lab tests, lab errors must be reduced to a minimum for total quality assurance.[13]

The statistical analysis of our study reveals that error in gram staining of clinical specimens was recorded in 9.7% (n=29), which is comparable with a multicenter study conducted in the USA by Samuel *et al.* that recorded 5% of all Grain stain results discrepant from the culture results.[7]

Guarner *et al*.[14] conducted a study for improving Gram stain proficiency in hospital and satellite laboratories that do not have microbiology. They found that initially, gram staining was read correctly only 71%-77% of the time. However, after repeated training, the correct reading of Gram stain results was seen 77%-99% of the time. Error rates initially were 23%-29% which were then reduced to 1%-23%. Since this study was not at a microbiology centre but rather a small hospital, the results are not comparable to our study and are far below our results.

The literature reveals that the Gram stain is an example of a microbiology test that requires interpretation by the medical laboratory technologist.[15,16] The process of performing Gram stains may be manual or automated, and the methods involved vary between laboratories, but ultimately the challenge remains in accurately reading and reporting Gram stains. This can be complicated by several variables.[17,18] The quality of the stains, quality of the specimen, method of fixation, organism viability and inherent variations in staining of the organisms present in the specimen as documented by Samuel *et al.* in their multicenter study on the incidence of Gram stain errors.[7] In our study, technical errors (48%) were more than observer errors (38%). This implies that the expertise of the staff performing the test should be enhanced, and new staff must be adequately trained before starting routine gram staining in the lab.

Sautter *et al.* commented upon the growing trend of consolidation/centralization of microbiology laboratories in the US. This would mean that many hospitals in peripheral areas are left without dedicated microbiology staff [16]. However, gram stain would still be performed at these peripheral hospitals as it is easy, does not require specialized equipment, and can guide initial therapy. However, the limited volume of specimens for Gram stains processed by laboratory staff at these sites makes it challenging to develop proficiency in Gram stain performance and interpretation in these peripheral areas, far from the central

microbiology labs. In addition, the same problem exists in Pakistan as there are very few well-equipped centres for microbiology in the country.

LIMITATIONS OF STUDY

The limitation of our study was that the collected specimens were only from one centre. Therefore, the sample size was too small to conclude the proficiency of Gram staining of clinical specimens.

CONCLUSION

The frequency of Gram stain errors in our study (9.7%) was not very high. Nevertheless, it can have severe consequences in critical samples from seriously ill patients if a wrong empirical antimicrobial treatment is begun based on a wrong initial Gram stain result.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

RS & GZ: Data acquisition, study design, drafting the manuscript, critical review, approval of the final version to be published.

IAM & WH: Conception, data analysis, data interpretation, drafting the manuscript, approval of the final version to be published.

UK & AS: Critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Hofer TP, Kerr EA, Hayward RA. What is an error? Eff Clin Pract 2000; 3(6): 261-269.
2. Török E, Moran E, Cooke F. Oxford handbook of infectious diseases and microbiology. Oxford University Press 2016. [Internet] available at: https://www.amazon.com/Handbook-Infectious-Diseases-Microbiology-Handbooks/dp/019967132X
3. National Ambulatory Medical Care Survey: 2015 State and National Summary Tables. CDC/National Center for Health Statistics. [Internet] available at: https://www.cdc.gov/nchs/fastats/infectious-disease.htm. (Accessed on April 23, 2018).
4. Pakistan: WHO statistical profile.Country statistics and global health estimates by WHO and UN partners. [Internet] available at: http://who.int/gho/mortality_burden_disease/en/. (Accessed on April 23, 2018).
5. Rafiq MY, Ikram A, Afzal A, Zaman G, Usman B, Ayyub M et al. Ventilator Associated Pneumonia Among Patients on Mechanical Ventilation at Tertiary Care Centres. Pak Armed Forces Med J 2018; 1(1): 75-79.
6. Boyles TH, Wasserman S. Diagnosis of bacterial infection. S Afr Med J 2015; 105(5): 419-421.
7. Samuel LP, Balada-Llasat JM, Harrington A, Cavagnolo R. Multicenter Assessment of Gram Stain Error Rates. J Clin Microbiol 2016; 54(6): 1442-1447.
8. Murray PR, Rosenthal KS, Pfaller MA. Medical microbiology,9th Edition. Elsevier Health Sciences; 2015. [Internet] available at: https://www.elsevier.com/books/medical-microbiology/murray /978-0-323-67322-8
9. Thomson RB Jr. One Small Step for the Gram Stain, One Giant Leap for Clinical Microbiology. J Clin Microbiol 2016; 54(6): 1416-1417. doi: 10.1128/JCM.00303-16.
10. Yoshimura J, Kinoshita T, Yamakawa K, Matsushima A, Nakamoto N, Hamasaki T, et al. Impact of Gram stain results on initial treatment selection in patients with ventilator-associated pneumonia: a retrospective analysis of two treatment algorithms. Crit Care 2017; 21(1): 156.
11. Leber AL. Clinical Microbiology Procedures Handbook, 4th Edition, ASM Press; 2016. [Internet] available at: https://www.wiley.com/en-us/Clinical+Microbiology+Pedures+Handbook,+3+Volume+Set,+4th+Edition-p-978155581 8807
12. Carver N, Gupta V, Hipskind JE. Medical Error. In: StatPearls Treasure Island (FL): StatPearls Publishing; 2020. [Internet] available at: https:// pubmed.ncbi.nlm.nih.gov/28613514/
13. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med 2006; 44(6): 750-759. doi: 10.1515/CCLM.2006.123.
14. Guarner J, Street C, Matlock M, Cole L, Brierre F. Improving Gram stain proficiency in hospital and satellite laboratories that do not have microbiology. Clin Chem Lab Med 2017; 55(3): 458-461. doi: 10.1515/cclm-2016-0556.
15. O'Horo JC, Thompson D, Safdar N. Is the gram stain useful in the microbiologic diagnosis of VAP? A meta-analysis. Clin Infect Dis 2012; 55(4): 551-561. doi: 10.1093/cid/cis512.
16. Sautter RL, Thomson RB Jr. Consolidated clinical microbiology laboratories. J Clin Microbiol 2015; 53(5): 1467-1472.
17. Bartholomew JW. Variables Influencing Results, and the Precise Definition of Steps in Gram Staining as a Means of Standardizing the Results Obtained. Stain Technol 1962; 37(3): 139-155,doi:10.3109/10520296209117723
18. Adams A. Studies in Gram Staining. Stain Technol 1975; 50(4): 227-231. doi: 10.3109/10520297509117063