Biomedical and Biopharmaceutical Research
Abbreviation: Biomed. Biopharm. Res.
Volume: 22: Issue: 02 |Year: 2025
Page Number: 708-712

Interpretation of Direct Gram stain versus culture isolates of Pyogenic infections

Dr. Sowjanya MP[1], Dr. M.Sailaja[2], Dr. S.Uma Devi[3]
[1]Post Graduate, Department of Microbiology, Government Medical College, Anantapur, Andhra Pradesh, India.
[2]Associate Professor, Department of Microbiology, Government Medical College, Anantapur, Andhra Pradesh, India.
[3]Associate Professor, Department of Microbiology, Government Medical College, Anantapur, Andhra Pradesh, India.

Corresponding Author

Dr. Sowjanya MP
Post Graduate, Department of Microbiology, Government Medical College, Anantapur, Andhra Pradesh, India.

Article Received:28-06-2025

Article Accepted:30-07-2025

Abstract

INTRODUCTION: Pyogenic infections are characterized by the inflammation with the collection of pus at the site due to bacterial infections. To know the type of organism and its antimicrobial susceptibility pattern it is important to utilize the microbiology services like staining, culture and sensitivity. In the present study we are trying to enlighten the importance of gram stain and culture testing in pyogenic infections.

MATERIALS AND METHODS: A 3 months (March 2024 to May 2024) prospective observational study conducted with a total of 150 pus samples were collected from outpatient and inpatient clinical departments under aseptic precautions using a sterile syringe aspiration or sterile swabs by following standard protocols. The entire quantitative variables were expressed as number, frequency and percentage. The tests accuracy was measured by sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV).

RESULTS: Out of 150 pus samples studied, direct Gram stain examination was matched with culture isolates in nearly 70% (105 out of 150) of the samples. 30 out of 150 (20%) were Gram stain negative and culture showed pathogens and 5 out of 150 (3.33%) were gram positive and culture negative. Gram staining was evaluated with pus culture by calculating sensitivity, specificity, positive predictive value and negative predictive values with percentages of 77.7%, 66.6%, 95.4%, and 25% respectively.

CONCLUSION: Though the culture is a gold standard and confirmatory test for the diagnosis of infection, the gram stain with a good positive predictive value will help the physicians in the appropriate antibiotic selection, however the physician should focus on hospital antibiogram and microbiotia of the community.

KEY WORDS: Culture, Gram stain, Pyogenic infections.

INTRODUCTION

Pyogenic infections are characterized by the inflammation with the collection of pus at the site due to bacterial infections. Pus is a fluid containing dead and dying white blood cells, bacteria and tissue debris [1]. Pyogenic infections can affect various organs in the body, which can present as skin and subcutaneous infections like boils, furuncles, carbuncles, impetigo, abscesses and also deep seated serious infections like spinal infections, empyema or osteomyelitis. Pyogenic infections caused by bacteria, virus, fungi and protozoa, but widely infected by bacteria in most number of cases, the wide range of bacteria involving in pyogenic infections are *Staphylococcus, Streptococcus, Escherichia coli, Klebsiella, Pseudomonas, Proteus* and many others [2]. Among these bacteria Gram positive bacteria are most probably present in abscesses, impetigo, accidental wound infections, cellulitis, and necrotizing fasciitis, whereas gram negative bacteria are most commonly responsible for blood stream infections, cardiac diseases, intestinal diseases, meningitis, diabetic wounds and surgical site infections [3]. The overall incidence of wound sepsis in India is from 10% to 33% [4]. Pyogenic infections treatment modalities are antimicrobial therapy, incision and drainage, and surgery.

Diagnostic modalities of pyogenic infections which help in the identification of pathogen are Gram stain, AFB stain, Fungal stain, microbiological culture and sensitivity, rapid diagnostics tests, serological tests like ELISA and molecular methods like polymerase chain reaction; electrophoresis.

Antibiotic resistance is a worldwide major public health concern, even ESKAPE pathogens are emerging in pyogenic infections especially in hospital settings. During the last few decades, multidrug-resistant Gram-negative bacterial strains

such as *Acinetobacter baumannii, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) were increasingly associated with pus infections under hospital settings due to extensive overuse and inadequate dose regimen of antibiotics [5].
In such case the selection of antimicrobial agent is a hurdle to clinician to target at accurate treatment. The selection of antimicrobial agent depends on the type of organism, its antimicrobial resistance pattern, acquisition of resistant genes frequency, pharmacodynamics and pharmokinetics of the drug and pathogenesis of infectious disease. Clinically based on the patient condition the physician can choose the appropriate drug after correlating with laboratory and radiological assistance. To know the type of organism and its antimicrobial susceptibility pattern it is important to utilize the microbiology services like staining, culture and sensitivity. In the present study we are trying to enlighten the importance of gram stain and culture testing in pyogenic infections.

Aim & Objectives:
1.To assess the usefulness of Direct Gram's staining for the diagnosis of pyogenic infections.
2.To correlate the observations of direct Gram's stain from pus samples received to their respective culture results.

MATERIALS AND METHODS:
A 3 months (March 2024 to May 2024) prospective observational study conducted in the department of Microbiology, Government Medical College at Anantapur, Andhra Pradesh. A total of 150 pus samples were collected from outpatient and inpatient clinical departments under aseptic precautions using a sterile syringe aspiration or sterile swabs by following standard protocols. If it is swab then two pus specimens were collected from each patient, one for gram stain and another one for bacterial culture and sensitivity, these pus samples were transported immediately to the laboratory under aseptic precautions at appropriate temperature.
All the samples were processed as per the standard guidelines by making the smear of pus sample for gram stain and inoculating pus specimens on culture or agar media plates for further incubation and processing. Identification of bacteria and testing its antimicrobial susceptibility pattern was done as per the protocols and CLSI guidelines.

Gram stain:
0.05ml of well mixed urine was placed on a clean glass slide, left for air drying, heat fixed and then Gram staining done as follows: Application of the Primary Stain (crystal violet) to a Heat-Fixed Smear of Bacterial Culture for 1min. After washing primary stain, Add Gram's Iodine and place for 1min. Third step involves Decolorization with 95% Ethyl Alcohol for 1min followed by final step of Counterstain with Safranin for 1min [6]. For interpretation of Gram stain, at least 20 fields of the smear were examined under oil immersion objectives (100X).
The morphology of bacteria observed and quantified as follows:

S.No.	Numerical/Descriptive	
1	1+/Rare	Less than one bacteria per oil immersion field
2	2+/Few	One bacteria seen per oil immersion field
3	3+/Moderate	2-10 bacteria seen per oil immersion field
4	4+/Many	>10 bacteria seen per oil immersion field

Culture & Sensitivity testing:
Pus samples were inoculated on to nutrient agar, blood agar and MacConkey agar. Plates were incubated at 37◦C for 24 hours. Organisms were identified by series of biochemical reactions standard following standard procedures. Antimicrobial susceptibility testing was performed using Muller-Hinton agar plates by disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines [7].

Data Collection and Statistical analysis:
The data has been collected into spread excel sheet and the results were tabulated. The entire quantitative variables were expressed as number, frequency and percentage. The tests accuracy was measured by sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV).

RESULTS
A total of 150 pus samples were assessed in this study. Out of 150, 135 (90%) were culture positive and remaining 110 (73.3%) were direct Gram stain positive.

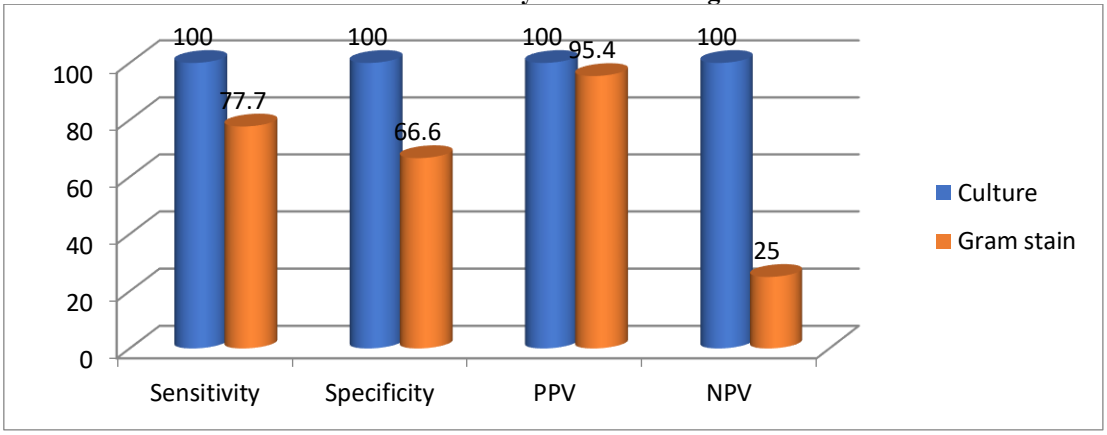
Out of 150 pus samples studied, direct Gram stain examination was matched with culture isolates in nearly 70% (105 out of 150) of the samples. 30 out of 150 (20%) were Gram stain negative and culture showed pathogens and 5 out of 150 (3.33%) were gram positive and culture negative (Table 1).

Table 1. Culture and Gram stain tests correlation

Gram Stain	Culture Positive	Culture Negative	Total
Positive	105	5	110
Negative	30	10	40
Total	135	15	150

Gram staining was evaluated with pus culture by calculating sensitivity, specificity, positive predictive value and negative predictive values with percentages of 77.7%, 66.6%, 95.4%, and 25% respectively (Table 2).

Table 2. Test accuracy of culture and gram stain



DISCUSSION

One of the most common presentations in the outpatient department is pyogenic or wound infections which had a wide clinical spectrum including skin, surgical site infections, soft tissue infections, diabetic wound, and abscesses. The crude mortality rate due to infectious diseases in India is approximately 417 per one lakh persons [8]. It is estimated that antimicrobial resistance kills at least 1.27 million people every year and it could increase up-to 10 million people per year by 2050 [9]. Diagnosing pyogenic infections plays a vital role in management of the patient especially in surgical departments, as the chronic infections might harbor mycobacteria and also the immunocompromised patients or comorbid patients may held up with increased morbidity, mortality, and healthcare expenses. It even aid in the appropriate selection of antibiotic, dosage optimization, and effective treatment strategies. Empirical therapy is a good option to manage a case if there is an availability of hospital antibiogram, if not microbiology culture and sensitivity testing of the specimen with appropriate antimicrobials is needed to improve the patient outcome.

A total of 150 pus samples were assessed in this study. Out of 150, 135 (90%) were culture positive and remaining 110 (73.3%) were direct Gram stain positive. In line with this study Abbas ZG et al [10] did a similar study on diabetic wounds who reported that 92% growth isolated. Bajare B et al [11] reported 68% of culture positivity in pyogenic infections. MW et al [12] noted 63.5% of pyogenic infections yielded pathogenic organisms. Saini S et al [13] out of the total fifty samples/swabs processed, 74% were positive by Gram's staining whereas only 64% were positive by culture.

Abbas ZG et al [10] also did a study on comparing the Gram stain appearance of biopsy smears by matching each with their respective culture results and they used the McNemar test, noted there were just two discordant stain/culture pairs, with 96·4% congruency between Gram stain appearance and culture results; there was no statistically significant difference ($P = 0{\cdot}25$) between the predictive value of Gram stains and cultures in the ascertainment of the identity of the microorganism responsible for the infection.

Out of 150 pus samples studied, direct Gram stain examination was matched with culture isolates in nearly 70% (105 out of 150) of the samples. A study done at Jaipur on pyogenic infections clearly stated about the percentage of specimens yielded same result in gram stain and culture in similar to our study [14]. Abbas ZG et al [10] found 75% were gram positive and culture positive in pyogenic infections.

30 out of 150 (20%) were Gram stain negative and culture showed pathogens which could be contaminants or due to low sensitivity of gram stain when compared to the culture test, gram stain require 100000 of bacteria in one ml of the sample whereas with culture we can diagnose with a bacteria of 100 in one ml of the sample and 5 out of 150 (3.33%) were gram positive and culture negative this could be due to contaminants in the sample or fastidious organisms or anaerobes or due to technical error during sample inoculation [15]. Abbas ZG et al [10] observed out of 128 individual cultures performed, 126 (98·4%) yielded growth of microorganisms in similar to our study. Among 126 isolates, 58 (46·8%) of these positive cultures yielded a single microorganism; 68 (54·0%) yielded mixed growth involving a total of 138 Gram-positive and Gram-negative bacteria and yeasts. Forty-four (75·9%) of the 58 single isolates were Gram-negative bacteria; 13 (22·4%) were Gram-positive bacteria and 2 (3·4%) were yeasts. Of the 68 cultures that yielded mixed growth, 60 (88·2%) included Gram-negative pathogens, 58 (85·2%) included Gram-positive organisms and 17 (27%) included yeasts.
Gram staining was evaluated with pus culture by calculating sensitivity, specificity, positive predictive value and negative predictive values with percentages of 77.7%, 66.6%, 95.4%, and 25% respectively. In this study the observation was high positive predictive value of gram stain, which denotes that this test has potential to provide clear cut information to clinician on the type of organisms whether it is a gram positive or gram negative, cocci or bacilli, based on this clinician can choose the empirical antibiotic within the critical time. Other advantageous of Gram stain are: determination of whether chronic or acute infection, severity of infection based on number of neutrophils in the back ground of staining, detects fastidious organisms those are difficult to grow on culture media, organisms that cannot grow on culture media as the patient is on antimicrobial therapy. It is a rapid and economical test can be performed in low resource health care settings. It requires a semi skilled experience personnel to analyze the gram stain picture.
Strand CL et al [16] assessed the usefulness of reporting direct blood Gram stain results compared with the results of positive blood cultures in 482 episodes and monitored impact on selection of antimicrobial treatment. They found that gram stain results definitive to identify the *"Staphylococcus spp," "Pseudomonas spp and related organisms,"* and *"yeasts"* and were matched perfectly with later culture identification or changed antimicrobials more frequently than when "other *Streptococci*" and "family *Enterobacteriaceae*". Incorrect recognition of *Acinetobacter spp* as *Enterobacteriaceae* family is still the most challenging problem in this context. Ramalatharani S et al [17] did a retrospective study on urinary tract infection, they found gram stain as a more reliable screening test for UTI that is showed by statistical analysis of Sensitivity 93.25%; Specificity 91.6%; Positive predictive value 95.8%; Negative predictive value 86.9%.

CONCLUSION

Gram stain is a rapid, economical, feasible and a sensitive test to screen the pyogenic infections. Microbiologists should focus on the direct microscopic examination along with the culture and sensitivity testing for the early identification of organism. Microbiologists should make sure to run quality controls during the gram stain procedure to avoid misinterpretation of results. Though the culture is a gold standard and confirmatory test for the diagnosis of infection, the gram stain with a good positive predictive value will help the physicians in the appropriate antibiotic selection, however the physician should focus on hospital antibiogram and microbiotia of the community.

REFERENCES

1. Harsh Vinubhai Singel et al. Bacteriological Profile of Pus Samples and their Antibiotic Susceptibility Pattern. Afr.J.Bio.Sc.6.12(2024).
2. The antibiogram of pus cultures in federal tertiary care hospital, Islamabad and its utility in antimicrobial stewardship. Farhan Kursheed1 et al; IRAN. J. MICROBIOL. Volume 16 Number 1 (February 2024) 56-61.
3. Yadav LK, Ansari S, Yadav SR et al. Microbes and Infectious Diseases 2022; 3(2): 332-338.
4. Mantravadi H, Chinthaparthi M, Shravani V. Aerobic isolates in pus and their antibiotic sensitivity pattern: a study conducted in a teaching hospital in Andhra Pradesh. Int J Med Sci Public Health. 2015;4(8):1076–9.
5. 5.Courvalin P. Antimicrobial Drug Resistance: Prediction Is Very Difficult, especially about the Future. Emerg Infect Dis. 2005;11:1503-06.
6. Sobel Kaye JD, Bennett JE, Dolin R, Blaser MJ. Mackie & Mccartney Practical Medical Microbiology. 14 Edn. Elsevier Publications.
7. Performance Standards for antimicrobial susceptibility testing, XXI International Supplement (M100-S27). In: Clinical Laboratory Standards Institutes (CLSI); 2024.
8. Salem-Bakhit MM, Moussa IMI, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug resistant enterococci isolated from clinical specimens. Indian Journal of Medical Microbiology 2012; 30: 44-51.

9. Efstrtiou A. Outbreaks of human infection caused by pyogenic streptococci of Lancefield groups C and G. Journal of Medical Microbiology 1989; 29: 207-219.
10. Abbas ZG, Lutale JK, Ilondo MM, Archibald LK. The utility of Gram stains and culture in the management of limb ulcers in persons with diabetes. International Wound Journal. 2012 Feb 1;9(6):677–82.
11. Bajare B, Dhangar A, Tankhiwale S, Shrikhande S. Bacteriological profile of pyogenic infections and their antimicrobial susceptibility in a tertiary care hospital in central India. mljgoums 2024; 18 (2) :16-18.
12. M W, Naaz S, Hashmiya SS. Evaluation of bacteriological profile of pyogenic infections and their susceptibility profiles at a tertiary care hospital. Int J Med Rev Case Rep. 2022; 6(3): 15-19.
13. Saini S, Katiyar R, Deorukhkar S.Gram Stain versus Culture for Diagnosis of Pyogenic Infections. Pravara Med Rev 2011.
14. Apoorva sharma, Mahima lall, Aditi Sondhi, Mohammed Ashraf Ali Namaji. Interpretation of direct Gram stain versus culture isolates of pus samples received in a tertiary care hospital. Int J Med Microbiol. 39:S30.
15. Lipsky BA, Berendt AR. Principles and practice of antibiotic therapy of diabetic foot infections. Diabetes Metab Res Rev 2000;16 Suppl 1:S42–6.
16. Strand CL. Positive blood cultures, Can we always trust the gram stain?Am J Clin Pathol 2006;126:671-672.
17. Ramalatharani S. A retrospective study of predictive value of Gram staining in the diagnosis of urinary tract infection. International Journal of Medical Microbiology and Tropical Diseases 2022;8(1):24–28.