e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(1); 1299-1303

Original Research Article

Study of Bacterial and Fungal Profile of Sputum Samples in a Tertiary Care Hospital: A Prospective Analysis

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Received: 01-10-2025 / Revised: 15-11-2025 / Accepted: 21-12-2025
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Conflict of interest: Nil

Abstract

Background: Gram-stained sputum samples can be examined under a microscope to help diagnose lower respiratory tract infections in patients. When determining if a sputum sample is suitable for cultivation in the lab, Gram stain is crucial. Determining the sample's representativeness for the targeted collecting site is helpful. The purpose of this study was to evaluate the application of Gram stain in sputum examination in diagnostic microbiology and to connect Gram stain results with culture.

Methods: From February 2025 to July 2025, a total of 133 sputum samples were quality assessed using Bartlett's grading system. The total scoring was done and sample showing score of 1 and above were cultured and identified based on colony characteristics, gram staining morphology and biochemical reactions.

Results: One hundred and thirty-three sputum samples were collected from patients with suspected lower respiratory tract infection. Of the 133 samples, 110(79%) were accepted and 23 (21%) were found to be unacceptable by Bartlett criteria. Potential pathogens were grown in 84 samples in the acceptable category. Normal respiratory flora were grown in 26 samples. Out of 84 samples, 63 samples were positive for bacterial growth and 21 showed fungal growth. Out of 63 bacterial growth, 44 were from in-patients and 19 were from out-patients. Among these bacterial isolates, 23 isolates were Pseudomonas aeruginosa followed by 16 isolates were Klebsiella pneumoniae, 10 isolates were E.coli, 6 isolates were Staphylococcus aureus, 2 isolates were Streptococcus species, 3 isolates were Proteus mirabilis and 3 isolates were Serratia marcesens.

Conclusion: All the sputum samples should be subjected to gram staining before culture to differentiate true pathogens from contaminating flora on culture.

Keywords: Gram staining, Bartlett scoring, Pathogens.

DOI: 10.25258/ijcpr.18.1.219

Introduction

Sputum culture is the most feasible and economical method of diagnosing lower respiratory tract infections. However, the sample's trustworthiness is compromised by the possibility of saliva and oral bacteria contaminating it during the collecting process. Additionally, it avoids the waste of time and resources on processing contaminated samples that are not useful for patient treatment.[1] Increasing the use of microscopy prior to culture may increase the specificity of the sputum culture in diagnosis of respiratory infections. Poor correlation between the culture and gram stain does not provide relevant information required concerning the aetiology of lower respiratory infection, leading to delay and indecisiveness in the management of patients.[2] Identification of a pathogen by isolation from sputum culture cannot be reliably considered the etiologic pathogen in the respiratory infection unless the contamination by oropharyngeal flora is ruled out. The presence of such a contamination can be assessed by microscopy of the specimen and relative quantitation of the number of squamous epithelial cells in the sample. Contaminated samples do not provide reliable diagnostic information for further management of the patients. Interpretation of sputum culture without preliminary screening for possible contamination may lead to

misinterpretation and confusion in the management of patients with respiratory infection.[3] The present study was designed to examine whether the clinical microbiology laboratory should play an active role in interpreting the quality of sputum specimens based on Gram stained smears, prior to inoculation into culture media. Various scoring systems are used for screening sputum sample for acceptance of quality, each employing different cut-offs for the number of epithelial cells and neutrophils per low per field.[4]

Before being inoculated into the culture media, all sputum samples must undergo a standard Gram stain in order to be identified as purulent. When treating patients with lower respiratory tract infections, the misuse of culture is avoided by using microscopic analysis of sputum samples.[5]

Materials and Methods

The microbiology department of Sheikh Bhikhari Medical College and Hospital in Hazaribag, Jharkhand, received 133 sputum samples for this prospective study between February and July of 2025. After being received at the lab, the samples were processed in 30 minutes.

The macroscopic examination of sputum samples were done initially. From the most purulent or mucoid portion of the specimen, a smear was made and plates like 5% sheep blood agar, chocolate agar, MacConkey agar and Saborauds Dextrose Agar were inoculated. The smear was stained with Gram's staining and was examined for the presence of polymorphs, epithelial cells, bacterial forms and fungal elements.

Sputum samples were graded according to Bartletts scoring, there should be more than 25 polymorphonuclear leukocytes and less than 10 squamous epithelial cells on low-power (x 100). SEC is found only in the upper respiratory tract, so this finding suggests oropharyngeal-contamination, whereas the presence of polymorphonuclear leukocytes, suggests material derived from the site of active infection.

The total score was made by calculating the average number of epithelial cells and neutrophils in 20-30 LPFs and sample showing score of 1 and above should be considered as an acceptable sample and a score of 0 or less should be considered as inflammation or contamination (non-acceptable sample). The inoculated culture plates were incubated overnight at 37^0C in a carbondioxide incubator.

The next day, Gram's stain was done from the growth and examined.

The organisms were identified based on colony characteristics, gram staining morphology. Respiratory potential pathogens included Staphylococcus aureus, Streptococcus pyogens, Streptococcus agalactiae, Streptococcus pneumoniae, Hemophilus influenzae, and Haemophilus parainfluenzae and gram negative bacilli. Normal throat flora, such as or γ-Streptococcus, CoNS, Neisseria sps, Corynebacterium sps were discarded.

Conventional biochemical (IMViC) was done to detect upto the level of species. Batch wise testing was made to check the Quality control (QC) for freshly prepared biochemicals as well as agar plates by using CLSI guidelines. For QC, recommended bacterial strains were used like Escherichia coli ATCC 25922, and ATCC 25923 Staphylococcus aureus, ATCC Candida albicans for yeast and ATCC Aspergillus niger for molds.

The results obtained were analyzed using MS Excel, 2024 version, with counts, percentages and pivot tables.

Descriptive measures (frequencies and percentages) were used to summarize study variables.

Results

Among 133 samples, 110(92%) sample showed more than 25 polymorphonuclear cells and less than 10 epithelial cells.

Among 44 sputum samples from in-patients, 7(8%) were microscopy positive, 13(15%) were positive on culture and 28(33%) were both microscopy and culture positive. Among 40 sputum samples from out-patients, 5(6%) were positive on sputum microscopy, 7(8%) were positive on culture and 24(28%) were positive on both microscopy and culture.

Among 133 patients, 43 (32%) were aged from 40 to 60 years followed by 27(20%) patients ranges from 20 to 39 years, 55 (41%) cases were aged more than 60 years and remaining 8 (6%) patients are below 20 years of age. In our study population, male 79(59%) was the predominant when compared to females 54 (41%) in our study population (Table 2) 59 (11.15%) samples were received from the Out-Patient Department (OPD) and 51 (88.85%) from. In-Patient Department (IPD).

Out of 84 samples, 63 samples were positive for bacterial growth and 21 showed fungal growth. Out of 63 bacterial growth, 44 were from in-patients and 19 were from outpatients.

Among these bacterial isolates, 23 isolates were Pseudomonas aeruginosa followed by 16 isolates were Klebsiella pneumoniae, 10 isolates were E.coli, 6 isolates were Staphylococcus aureus, 2 isolates were Streptococcus species, 3 isolates were Proteus mirabilis and 3 isolates were Serratia marcesens.

Among 21 fungal isolates 14 isolates were Candida albicans and 6 isolates were non-albicans Candida (3-Candida tropicalis, 2-Candida parapsilosis, 1-Candida glabrata), 1-Aspergillus niger. Out of the 21 fungal growth, 12 was positive in male patients and 9 from female patients.

Table 1: Sample distribution according to Bartlett's scoring

Bartlett's scoring	No. of sample	Percentage
Acceptable category	110	92%
Non-acceptable category	23	8%

Table 2: Male was the predominant when compared to females in our study population

	In-patient (%)	Out-patients (%)	Total (110)
Pathogens	44 (40%)	40 (36%)	84 (76%)
Normal respiratory flora	7 (6%)	19 (17%)	26 (23%)
Total	51 (46%)	59 (54%)	110 (100%)

Table 3: Pathogens

	In-patient (%)	Out-patients (%)	Total (110)
Microscopy positive	7 (8%)	5 (6%)	12 (14%)
Culture positive	13 (15%)	7 (8%)	20 (23%)
Microscopy and culture postive	28 (33%)	24 (28%)	52 (62%)

Table 4: Age and sex wise distribution of sputum samples

Age group	Male	Female	Total (n=133)
<20	5(4%)	3(2%)	8(6%)
20-39	16(12%)	11(8%)	27(20%)
40-60	30(23%)	13(10%)	43(32%)
>60	28 (21%)	27(20%)	55(41%)
Total	79(59%)	54(41%)	133 (100%)

Table 5: Distribution of bacterial isolates in sputum sample

Name of isolate	In-patient isolate n(%)	Out-patient isolate n(%)	Total n(%)
Pseudomonas aeruginosa	13(15%)	10(12%)	23(27%)
Klebsiella pneumoniae	12(14%)	4(5%)	16(19%)
Escherichia coli	8(10%)	2(2%)	10(12%)
Staphylococcus aureus	4(5%)	2(2%)	6(7%)
Streptococcus sp	2(2%)	0	2(2%)
Proteus mirabilis	2(2%)	1(1%)	3(4%)
Serratia marcescens	3(4%)	0	3(4%)
Total	44	19	63

Table 6: Distribution of fungal isolates in sputum sample

Name of isolate	Male n(%)	Female n(%)	Total n(%)
Candida albicans	8(10%)	6(7%)	14(17%)
Candida tropicalis	2(2%)	1(1%)	3(1.3%)
Candida parapsilosis	1(1%)	1(1%)	2(2%)
Candida glabrata	0	1(1%)	1(1%)
Aspergillus niger	1(1%)	0	1(1%)
Total	12	9	21

Discussion

While 92% of the samples in our study were acceptable and 8% were not (Table 1), 79% of the samples in a study by Mariraj et al. were acceptable and 22% were not, and 77% of the samples in a study by Rana et al.[6] Daniel Musher et al.[7], on the other hand, reported a low approval rate of 31%.[8] Sputum Gram smears can be used to determine the cause of pneumococcal pneumonia, according to research by Parry et al.[9] In contrast, Ewig et al. stated in their study that Gram stain had a low diagnostic yield since only a small percentage of matched samples showed positive growth in culture and that sputum collection cannot be utilized to diagnose community-acquired pneumonia.[10]

Among 44 sputum samples from in-patients, in our study, 7(8%) were microscopy positive, 13(15%) were positive on culture and 28(33%) were both microscopy and culture positive. Among 40 sputum samples from out-patients, in our study, 5(6%) were positive on sputum microscopy, 7(8%) were

positive on culture and 24(28%) were positive on both microscopy and culture (Table 2).

In our study elderly age is more commonly affected but in a study by Bindu Nair et al. mean age of 20.9 and equal number of males and females included in the study were affected.[11] In our study population, Male 79 (59%) was predominantly infected when compared to females 54(41%) which might be explained by the fact that men are more commonly involved in outdoor activities and occupation increasing the risk of infection (Table 3). In this study, 7 bacterial pathogens were isolated from good quality sputum samples which includes S. aureus, E.coli, P.aeruginosa, K.pneumoniae, Streptococcus spp, Proteus spp, and Serratia marcessens. In our study the most commonly isolated pathogen was Pseudomonas aeruginosa but in a study done by Murdoch et al, the most commonly isolated organism was Streptococcus pneumonia.[12] It may be due to the sputum samples that were collected from patients on long term antibiotic therapy or from patients with HAP. This difference can also be due to the differences in study design, type of lesion, geographical location and climatic conditions.

Total culture positivity in the present study was 100%. 57% Culture positivity was reported in other studies by Jean Lloveras et al. and 79% culture positivity in studies done by Daniel Musher et al.[13,14] But only 5% of culture positivity was reported by Ravichandran et al.[15]

The most common pathogen causing lower respiratory tract infection as isolated was 23 isolates were Pseudomonas aeruginosa followed by 16 isolates were Klebsiella pneumoniae, 10 isolates were E.coli, 6 isolates were Staphylococcus aureus, 2 isolates were Streptococcus species, 3 isolates were Proteus mirabilis and 3 isolates were Serratia marcesens. (Table 4).

Candida species are the most common fungal pathogens in humans. Edwards reported the 8%–10% of all nosocomial infections are caused by Candida sps.[16] In our study infection with Candida albicans is 14(17%) and nonalbicans candida 6(7%). In a study done by Nseir et al. it was reported that, antifungal therapy significantly reduced risk of Pseudomonas aeruginosa pneumonia.[17] But in a German study by Lindau et al. it was reported that patients treated with antifungal therapy had higher mortality and pneumonia rates.[18] In a study by Terraneo et al no association between antifungal therapy and clinical outcome was reported.[19] Harriott and Noverr reported synergistic relationship between S. aureus and C. albicans in biofilm formation.[20] Delisle et al. in his study reported that hospital stay duration and mortality was more in patients who harbored Candida in their respiratory tract.[18]

The previous study reported that pulmonary mycosis was more common in women compared to men.[21] Another study reported that more than 60% fungal culture were positive among men,[22] which is similar to our study where more number of Candida were isolated from male patients (Table 5). Malnutrition and long term usage of antibiotics serves as an important risk factor for fungal infection as it reduces the normal flora. C.tropicalis causes infection in patients with preexisting lung disease.[23] Which is similar to our study where among non-albicans Candida, C.tropicalis is reported more in number. In the, immunocompromised host C. tropicalis is more capable of deeper tissue invasion than C.albicans. [24]

In a study done by Bulpa et al. on COPD, 3.6% of cases due to A. niger were reported among patients with invasive pulmonary aspergillosis.[25] In our study 1(1%) case of Aspergillus niger has been reported (Table 5).

Conclusion

Prior to culture, all sputum samples should undergo gram staining in order to distinguish between contaminated flora and actual infections.

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