Quality Assessment of Sputum Gram Stain in Relation to Sputum Culture for lower Respiratory Tract Infections in a Tertiary Care centre Kanpur

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Abstract:

Background: Lower respiratory tract infections (LRTIs) are among the most common infectious disease and responsible for the cause of morbidity and mortality worldwide. Microscopic examination of sputum is the most commonly followed method in the laboratory for diagnosing lower respiratory tract infections (LRTI).

Aim and Objectives: Quality Assessment of Sputum Gram Stain in Relation to Sputum Culture for lower Respiratory Tract Infections in a Tertiary Care centre Kanpur.

Material & Methods: This study was a Cross- sectional study carried out at the Department of Microbiology, Rama Medical College Hospital & Research College Kanpur, and Uttar Pradesh, India from January 2021 to December 2021. 150 sputum samples were collected from patients in our hospital during the study period and processed in the central laboratory. Repeated sputum samples from the same patient and samples received from pediatric age group were excluded from this study. Samples were evaluated by gross appearance and subjectively categorized into mucus (mucus strands present) and watery (saliva present).

Results: In this study total 150 sputum samples, 105 (70%) samples were accepted according to modified Bartlett's screening system and 45 (30%) samples were in the not acceptable category. Among acceptable category, 68(64.76%) samples were showed culture positivity. Among non acceptable category, 11(24.44%) samples were showed culture positivity. The most common organism isolated was Klebsiella spp 29(42%), followed by Pseudomonas spp 23 (33.82%), Staphylococcus aureus 9(13.23%), Enterobacter spp 1(1.4%), Escherichia coli 1(1.4%), Citrobacter spp 1(1.4%), Acinitobacter spp 1(1.4%) and Streptococcus pyogenes 3(4.4%).

Conclusion: Sputum quality assessment is a useful tool recommended receiving good quality of sputum and do initial sputum screening for diagnosing clinically relevant lower respiratory tract infections.

Keywords: Sputum, Grams stain, Culture

Introduction

Lower respiratory tract infections (LRTIs) are among the most common infectious disease and responsible for the cause of morbidity and mortality worldwide. Microscopic examination of sputum is the most commonly followed method in the laboratory for diagnosing lower respiratory tract infections (LRTI) [1]. One of the most important uses of the Gram stain is to evaluate the quality of expectorated sputum received for routine bacteriological culture. An acceptable sample yields less than 10 squamous epithelial cells per low power field [2]. The simplest and least expensive sample for the diagnosis of lower respiratory infections is expectorated sputum. The utility of this approach is the subject of controversy, as the sample is contaminated by oropharyngeal flora as it passes through the mouth. When the sample is collected carefully, it can provide useful information for initial therapy of community acquired pneumonia [3].

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Sputum Gram's stain and culture are traditionally recommended procedures for routine diagnosis of LRTIs. But some physicians feel that definite diagnosis of LRTIs depends upon the properly performed sputum Gram's stain and microscopically examination according to the correct guidelines [1]. When significant oropharyngael contamination is evidenced in the cellular content of Gram stained sputum smears, the second sample representing lower respiratory tract must be collected [4]. The microbiology laboratory must use objective criteria by Gram stain screening for purulence before inoculation into culture media [5]. Unless microscopic examination is routinely included, half of all microbiological information rendered on sputum samples is meaningless and subject to misinterpretation of culture results. Hence culture must be guided by microscopic findings. When there is no correlation between culture and smear, the culture report may not indicate the etiology of lower respiratory tract infection. The present study was designed microscopically examination of Gram stained sputum smears and the sputum culture in patients with LRTIs.

Material and Methods

This study was a Cross- sectional study carried out at the Department of Microbiology, Rama Medical

College Hospital & Research College Kanpur, and Uttar Pradesh, India from January 2021 to December 2021. All 150 sputum samples were collected from patients in our hospital during the study period and processed in the central laboratory. Repeated sputum samples from the same patient and samples received from pediatric age group were excluded from this study. Samples were evaluated by gross appearance and subjectively categorized into mucus (mucus strands present) and watery (saliva present) [6].

The neutrophils (pus cells) and epithelial cells were observed under Microscope in 20-30 low power fields and average number of epithelial cells and pus cells calculated. Then the total score of epithelial cells and pus cells arrived at. The final score value of less than or equal to 0 is indicated a salivary contamination of sputum sample or lake of active inflammation (non-acceptable sputum sample). The final score of 1 and above was indicated an acceptable sputum sample (Table:-1 According to the Modified Bartlett's Criteria)

Table:-1 Modified Bartlett's Criteria.

	Criteria	Score
Neutrophils (Pus Cell) Count (Score A)	<10 Neutrophils / 10x Field	0
	10-25 Neutrophils / 10x Field	+1
	>25 Neutrophils / 10x Field	+2
Macroscopic (Score B)	Mucoid, Mucopurulent, Purulent or blood Stained	+1
Squamous Epithelial Cell Count (Score C)	<10 Squamous Epithelial Cell / 10x Field	0
	10-25 Squamous Epithelial Cell / 10x Field	-1
	>25 Squamous Epithelial Cell / 10x Field	-2

The total score is Calculated using: - TOTAL SCORE = SCORE A + SCORE B + SCORE C

Each specimen was first mixed with an applicator swab and then inoculated on to blood agar, CLED agar and Mac Conkey agar plates and a smear was prepared for Gram staining. Each stained smear was examined microscopically under low power, oil immersion and the cellular components were evaluated. All the samples were processed regardless of the appearance of the stained smears. Organisms were identified by standard protocols and antibiotic susceptibility of recommended drugs was performed by Kirby-Buer disc diffusion method. Viridians group streptococci, CoNS, Diphtheroids and some Neisseria species were considered as normal respiratory flora [5].

Results

In this study total 150 sputum samples, 105 (70%) samples were accepted according to modified Bartlett's screening system and 45 (30%) samples were in the not acceptable category. Among acceptable category, 68(64.76%) samples were showed culture positivity. Among non acceptable category, 11(24.44%) samples were showed culture positivity. The most common organism isolated was Klebsiella spp 29(42%), followed by Pseudomonas spp 23 (33.82%), Staphylococcus aureus 9(13.23%), Enterobacter spp 1(1.4%), Escherichia coli 1(1.4%), Citrobacter spp 1(1.4%) and Acinitobacter spp 1(1.4%). Streptococcus pyogenes 3(4.4%). Klebsiella spp. was the commonest isolated organism followed by Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes.

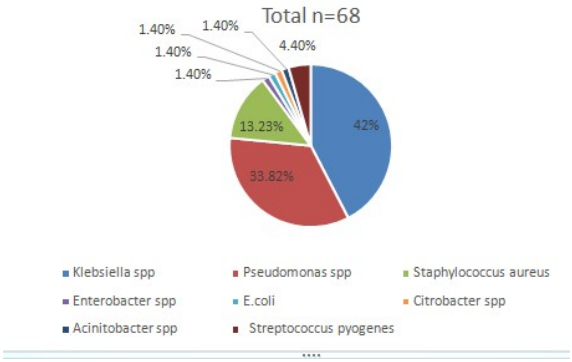

Figure 1: Organism Isolates

Discussion

Author's	Year	Study
Mariraj j et al[7]	2011	Total out of 100 samples, 79(79%) were accepted and 21(21%) were found to be unacceptable by the criteria of Bartlett. Potential pathogens were recovered from 50(63.2%) out of 79 accepted samples and 2(9.5%) out of 21 rejected samples. These data suggest that microscopic examination is mandatory in sputum microbiology
Archana et al [1]	2021	Out of 130 sputum samples, 72 (55.4%) samples were acceptable based on Bartlett's screening system and 58(44.6%) samples were in the not acceptable category. Among acceptable category, 64(78.05%) samples were showed culture positivity. Among non-acceptable category, 18(21.95%) samples were showed culture positivity. Klebsiella pnemoniae-31.71% was the commonest isolated organism followed by Pseudomonas aeruginosa-14.63% and Staphylococcus aureus - 13.41%.In this study authors recommended to receive good quality of sputum and do initial

		sputum Screening for diagnosing clinically relevant LRTIs.
Gorica Popova et al [8]	2019	Among the acceptable category, defined by Bartlett's grading system, 132 (63.2%) samples showed culture positivity of which Streptococcus pneumonia 48 (36.4%) was most commonly isolated, followed by Moraxella catarrhalis 22 (16.7%) and Haemophilus influenza 21 (15.9%). Among the non-acceptable category, 185 (14.5%) samples were culture positive of which most commonly isolated were Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa with 64 (34.6%), 54 (29.2%) and 28 (15.1%), respectively. Sputum quality assessment is a useful tool for distinguishing the true respiratory pathogens from possible colonising flora for which antibiotic treatment should not be highly considered .
In Present Study	2022	In this study total 150 sputum samples, 105 (70%) samples were accepted according to modified Bartlett's screening system and 45 (30%) samples were in the not acceptable category. Among acceptable category, 68(64.76%) samples were showed culture positivity. Among non acceptable category, 11(24.44%) samples were showed culture positivity. The most common organism isolated was Klebsiella spp 29(42%), followed by Pseudomonas spp 23 (33.82%), Staphylococcus aureus 9(13.23%), Enterobacter spp 1(1.4%), Escherichia coli 1(1.4%), Citrobacter spp 1(1.4%) and Acinitobacter spp 1(1.4%). Streptococcus pyogenes 3(4.4%).

Conclusion

Sputum quality assessment is a useful tool for recommendation to receive good quality of sputum and do initial sputum screening for diagnosing clinically relevant lower respiratory tract infections.

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