A Study on Analysis of the Sputum Gram Staining and Culture in Patients with Lower Respiratory Tract Infections Attending a Tertiary Care Hospital

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Abstract

Background: Microscopical examination of expectorated sputum samples is the most commonly followed method in the Microbiological laboratory for diagnosis of lower respiratory tract infections (LRTIs). Sputum sample is usually contaminated with normal resident floral organisms of the oropharynx. For the diagnosis and management of LRTIs, collection of sputum sample, sputum microscopy and culture is very important. The present study was conducted to analyse the importance of the microscopical examination of Gram stained sputum smears and the sputum culture in patients with LRTIs.

Materials & Methods: The place of the study was in the department of Microbiology in a tertiary care hospital. The study period was for one year from Jan 2015 to Dec 2015. Gram staining and culture were done for all the 130 sputum samples. Gram stained sputum smears were observed under microscope for presence of organisms, pus cells and epithelial cells. Quality of expectorated sputum samples were assessed by using Bartlett's grading system. By using standard protocols bacterial isolates were identified. Kirby Bauer disc diffusion method on Mueller Hinton agar was performed for antibiotic susceptibility testing.

Results and conclusion: 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.

Keywords: Bartlett's grading system, Gram stain, Non-acceptable category, Sputum acceptable category, Sputum culture

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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). The sputum samples usually contaminated with normal resident bacteria of the oropharynx. So, a large number of different species overgrow in sputum culture and preventing the determination of the true pathogen[1]. Most of the times sputum is watery saliva which is sent instead of the purulent sputum to the laboratory, leading to erroneous results. For the diagnosis and management of LRTIs, collection of sputum sample, sputum microscopy and culture is very important. 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 microscopical examination according to the correct guidelines. Some others suggest that sputum Gram's stain and culture are neither sensitive nor specific for diagnosis of LRTIs (LRTIs)[2]. The present study was conducted to analyse the importance of the microscopical examination of Gram stained sputum smears and the sputum culture in patients with LRTIs.

Materials and Methods

The present study was conducted in the department of Microbiology, during one year period from January2015 to December 2015. A total of 130 sputum samples were processed during the study period. Repeated sputum samples from the same patient and samples received from paediatric age group were excluded from this study. Gram staining and culture were done for all the 130 sputum samples. Gram stained sputum smears were observed under microscope for presence of organisms, pus cells and epithelial cells. . Quality of expectorated sputum samples were assessed by using Bartlett's grading system and a score was given below.

Table 1: Bartlett's Criteria[3] used:

Number of Neutrophils /10 X LPF	GRADE
<10	0
10-25	+1
>25	+2
Presence of mucus	+1
Number of Epithelial Cells /10 X LPF	
10-25	-1
>25	-2
TOTAL SCORE	

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.

All the 130 sputum samples were inoculated onto Blood agar, Chocolate agar and Mac Conkey agar and were incubated overnight at 37^0C. After 24 hrs inoculated plates were observed for the presence of growth. By using standard protocols bacterial isolates were identified from the growth. Kirby Bauer disc diffusion method on Mueller Hinton agar was performed for antibiotic susceptibility testing. The isolation of significant pathogenic organisms from a specimen indicates culture positive and isolation of scanty or insignificant growth from a specimen considered as culture negative. When mixed growths of significant organisms were isolated, they were counted according to the predominant growth.

Results

Based on Bartlett's screening criteria, out of 130 sputum samples processed, 72 (55.4%) were acceptable and 58 (44.6%) were non-acceptable. Potential pathogens were obtained from 82 of 130 samples, of which 64 are from acceptable samples (78.05%), and 18 are from non-acceptable samples (21.95%).

Table 2: Organisms Isolated:

Organism	N0	(%)
Klebsiella pneumoniae	26	31.71
Pseudomonas aeruginosa	12	14.63
Staphylococcus aureus	11	13.41
Escherichia coli	10	12.19
Streptococcus pyogenes	7	8.54
Klebsiella oxytoca	6	7.32
Streptococcus pneumoniae	4	4.88
Acinetobacter baumannii	2	2.44
Citrobacter koseri	2	2.44
Enterobacter aerogenes	2	2.44
Total	82	100%

The organisms obtained from the non-acceptable category (18 of 58) included, *Pseudomonas aeruginosa*-5, *Staphylococcus aureus*-5, *Klebsiella pneumoniae*-4, *Escherichia coli*-3 and *Klebsiella oxytoca* -1.

Discussion

Microscopical examination of expectorated sputum samples is the most commonly followed method in the Microbiological laboratory for diagnosis of lower respiratory tract infections (LRTIs). Sputum sample is usually contaminated with normal resident floral organisms of the oropharynx. Hence, sputum is considered as least clinically relevant specimens received for culture. Good sputum samples depend on thorough healthcare worker education and patient understanding [4]. The sputum grading system was initially given by Bartlett. This gives an indication whether the specimen represents the site of infection [5].

In the present study, 130 sputum samples were processed. Among the 130 sputum samples 72 samples (55.4%) were acceptable and 8 samples (44.6%) were non-acceptable based on Bartlett's screening criteria. Anevlavis et al and Mariraj et al. had reported similarly in their study that the acceptability percentages were 63% and 79%. In contrast, Daniel Musher et al had reported a low percentage of 31% acceptability. Also Ravichandran et al had reported a low percentage of acceptability that all 74 (100%) of their sputum samples were in the non-acceptable category. Bartlett's sputum grading system is not applicable for lower respiratory tract infections caused by viruses, fungi, Mycobacterium tuberculosis and Legionella species. The importance of micro-organisms recovered from respiratory samples must always be evaluated in light of clinical history[3].

Total culture positivity in the present study was 63.08% (82/130). Culture positivity reported in other studies include- Jean J Lloveras- 57%, Daniel Musher et al- 79%, Somporn et al- 40.95%, Nawfal Ali Mubarak- 41.7% and Aroma Oberoi et al- 32%. On the contrary Ravichandran et al had reported only in 5% of culture positivity.

Among the 72 acceptable specimens in the present study, potential pathogens were grown in 64 samples (78.05%). Mariraj et al reported similarly in his study that the potential pathogen were grown in 63.2% of acceptable samples. In contrast, M R Shariatzadeh et al reported that the potential pathogen were grown only in 33.7% of their acceptable samples.

Among the 58 samples in the non-acceptable category in the present study, pathogens were grown in 18(21.95%). Mariraj et al had reported that out of their 21 non acceptable samples 2(9.5%) were showed positive culture.

Comparison of Gram's stain and culture is used as quality assurance tool for sputum culture. If organisms seen in smear do not grow in culture, or if organisms

that grow in moderate to heavy quantities are not seen in the smear, the smear should be re-evaluated. Gram's stain is a relatively in-sensitive method. Hence small numbers of bacteria in culture may not be visualized in the smear [3].

The most common isolated organism in the present study was *Klebsiella pneumoniae*- 31.71% followed by *Pseudomonas aeruginosa*-14.63% and *Staphylococcus aureus*- 13.41%, which correlates well with other studies [1,6,8,9].

In a study by Mariraj et al, the authors had concluded that Microbiology laboratories may reject for culture, those sputum samples which fail to meet the criteria of Bartlett for purulence, and sputum cultures must be ordered judiciously for documented episodes of LRTIs to provide a meaningful output.

Conclusions

A total of 130 sputum samples were processed during a one year period (Jan. to Dec. 2015). Based on Bartlett's screening procedure, 72 (55.4%) were in the acceptable category and 58(44.6%) were in the non- acceptable category. Potential pathogen was grown in 64 (78.05%) samples in the acceptable category and 18 (21.95%) samples in the non-acceptable category. Most common isolates obtained were *Klebsiella pneumoniae*-31.71%, *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.

Conflicts of Interested: None

Source of Support: Nil

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