(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805
SJIF (Impact factor) 2017= 6.261
Index Copernicus value = 80.90

ORIGINAL ARTICLE

To analysis of sputum gram staining and culture in individuals suffering from lower respiratory tract infections

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ABSTRACT:
Aim: To analysis of sputum gram staining and culture in individuals suffering from lower respiratory tract infections. **Materials and methods:** This study was conducted in Department of Microbiology. A total of 150 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 150 sputum samples. Gram stained sputum smears were observed under microscope for presence of organisms, pus cells and epithelial cells. **Results:** Based on Bartlett's screening criteria, out of 150 sputum samples processed, 90 (60%) were acceptable and 60(40%) were non-acceptable. Potential pathogens were obtained from 100 of 150 samples, of which 75 are from acceptable samples (83.33%), and 25 are from non- acceptable samples(41.67%). Most common isolates obtained were Klebsiella pneumoniae-32%, Pseudomonas aeruginosa- 15% and Staphylococcus aureus - 14%. In this study authors recommended to receive good quality of sputum and do initial sputum screening for diagnosing clinically relevant LRTIs. **Conclusion:** The most prevalent isolates found were Klebsiella pneumoniae, accounting for 32% of the total, followed by Pseudomonas aeruginosa at 15% and Staphylococcus aureus at 14%. This research suggests obtaining high-quality sputum samples and doing initial sputum screening as a means of detecting clinically significant lower respiratory tract infections (LRTIs).
Keywords: Gram stain, Sputum culture, Non-acceptable category, Sputum acceptable category

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This article may be cited as: Ahmed J. To analysis of sputum gram staining and culture in individuals suffering from lower respiratory tract infections. J Adv Med Dent Scie Res 2017;5(12):187-189.

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 Gramstained sputum smears and the sputum culture in patients with LRTIs.

MATERIALS AND METHODS

This study was conducted in Department of Microbiology. Ethical clearance was obtained from the institutional ethical and research committee. A total of 150 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 150 sputum samples. Gram-stained sputum smears were observed under microscope for presence of organisms, pus cells and epithelial cells.

Table 1: Bartlett's Criteria used[3]

Number of Neutrophils/10X LPF	GRADE
<10	0
10-25	+1
>25	+2
Presence of mucus	+1
Number of Epithelial Cells/10X 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 150 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 150 sputum samples processed, 90 (60%) were acceptable and 60 (40%) were non-acceptable. Potential pathogens were obtained from 100 of 150 samples, of which 75 are from acceptable samples (83.33%), and 25 are from non- acceptable samples(41.67%). Most common isolates obtained were Klebsiella pneumoniae-32%, Pseudomonas aeruginosa- 15% and Staphylococcus aureus - 14%. In this study authors recommended to receive good quality of sputum and do initial sputum screening for diagnosing clinically relevant LRTIs.

Table 2: Organisms Isolated

Organism	N0	(%)
Klebsiella pneumoniae	32	32
Pseudomonas aeruginosa	15	15
Staphylococcus aureus	14	14
Escherichia coli	12	12
Streptococcus pyogenes	9	9
Klebsiella oxytoca	7	7
Streptococcus pneumoniae	5	5
Acinetobacter baumannii	2	2
Citrobacter koseri	2	2
Enterobacter aerogenes	2	2
Total	100	100

The organisms obtained from the non-acceptable category (25of 60) included, Pseudomonas aeruginosa-7, Staphyl- ococcus aureus-7, Klebsiella pneumoniae-6, Escherichia coli-4 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, out of 150 sputum samples processed, 90 (60%) were acceptable and 60 (40%) 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 66.67% (100/150). 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 90 acceptable specimens in the present study, potential pathogens were grown in 75 samples (83.33%). Mariraj et al

reported similarly in his study that the potential pathogen was 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 60 samples in the non- acceptable category in the present study, pathogens were grown in 25(41.67%). 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- 32% followed by Pseudomonas aeruginosa-15% and Staphylococcus aureus- 14%, 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.

CONCLUSION

Most common isolates obtained were Klebsiella pneumoniae-32%, Pseudomonas aeruginosa- 15% and Staphylococcus aureus - 14%. In this study we recommended to receive good quality of sputum and do initial sputum screening for diagnosing clinically relevant LRTIs.

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