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ANALYSING THE USEFULNESS OF GRAM'S STAIN OF SPUTUM AND ITS CULTURE IN PATIENTS SUSPECTED OF LOWER RESPIRATORY TRACT INFECTIONS -- STUDY FROM TERTIARY CARE CENTRE.

Microbiology

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ABSTRACT

Introduction: The use of Gram stained smears to assess the quality of sputum has received considerable attention as a means of improving the reliability of sputum culture in diagnosing lower respiratory tract infections(LRTI) **Materials and methods :** The study was conducted in the Department of Microbiology, RIMS, Ranchi for a period of 9months from November 2016 to August 2017. Gram staining and culture was performed on expectorated sputum from clinically suspected cases of LRTI. Barlett's criteria was used for assessing the acceptability of sputum samples . Gram stained smears of sputum were observed under microscope for the presence of organisms, polymorphs and squamous epithelial cells. Culture of purulent part of sputum was performed . Standard microbiological methods were used to identify bacterial isolates . Kirby Bauer disc diffusion method on Muller Hinton agar was performed for antibiotic sensitivity testing. **Results:** A total of 341 sputum samples were collected out of which 232 samples were acceptable. Bartlett's criteria was used to screen the sputum samples based on which 68% were acceptable and 32% were unacceptable. Among acceptable category 180(77.5%) samples showed culture positivity. *Klebsiella pneumoniae*(29.4%) was the most common isolate followed by *Pseudomonas aeruginosa*(21.1%) , *Staphylococcus aureus*(13.9%) and Streptococcus pneumoniae(11.7%) . **Conclusion:** It is important to receive good quality expectorated sputum and not saliva for Gram stain and culture for proper diagnosis of etiological agents of LRTI, so that a high diagnostic yield for clinically suspected LRTI becomes inevitable.

KEYWORDS

Sputum, Gram stain, Bartlett's criteria, Lower respiratory tract infection

INTRODUCTION

Lower respiratory tract infections (LRTI) are one of the common infections that lead to increased morbidity and mortality in hospitalized patients.(1) Sputum is the simplest, rapid and inexpensive sample for the diagnosis of lower respiratory tract infections. Direct microscopic examination of expectorated sputum with culture has been an important determinant in detecting causes of bacterial pneumonia. Gram staining is an important step in the work up of sputum in patients with LRTI.(2) However lower respiratory tract secretions are often contaminated with upper respiratory tract flora present in the saliva.(3) But when samples are collected carefully they may yield useful information in the treatment of LRTI caused by potential bacterial pathogens when isolated from sputum samples. It is often difficult to interpret whether the pathogen is the etiological agent or it represents oropharyngeal contamination when sputum samples are contaminated with saliva . Contaminated samples are less likely to yield interpretable results.(4) Good sputum samples depend on thorough healthcare worker education and patient understanding.(5) To minimise the effect of oropharyngeal contamination on lower respiratory tract secretions, Bartlett, Murray and Washington have set up a screening criteria based on the quantitation of the presence of squamous epithelial cells and polymorphs.(6) The use of Gram stained smears to assess the quality of sputum has received considerable attention as a means of improving the reliability of sputum culture. An acceptable sample yields less than 10 squamous epithelial cells per low power field. (7) Barlett's proposed that purity of sputum samples should be rated according to the relative concentration of polymorphic neutrophils, squamous epithelial cells and mucus in gram stained smears. Sputum samples have been criticised for the lack of sensitivity and specificity. Gram stain can be used to screen the sputum samples before inoculation into the culture media (6). The culture must be guided by microscopic finding .(2) Lack of correlation between culture and smear may not help in finding out the etiological agent of LRTI. Therefore, the study was taken up to analyse the usefulness of microscopic examination of Gram stained smears of sputum and its culture in patients with LRTI.

MATERIALS & METHODS

The present study was conducted in the Department of Microbiology, RIMS Ranchi, for a period of 9months from November 2016 to August 2017. Gram staining and culture was performed on expectorated sputum from clinically suspected cases of LRTI. Barlett's criteria (Table-1) was used for assessing the acceptability of sputum samples .(8) Gram stained smears of sputum were observed under microscope for the presence of organisms, polymorphs and squamous epithelial cells. Polymorphs and squamous epithelial cells were observed under microscope in 20- 30 LPF, average number of the cells were counted and total score was interpreted. The final score of 1 was considered acceptable for samples and those were used for further processing. A value less than 1 indicated salivary contamination of samples and were put into unacceptable category and repeat sampling was advised. Culture of purulent part of sputum was performed on blood agar, Mac Conkey agar plates and incubated at 37°C for 18-24hrs. Identification was based on standard microbiological methods by the characteristic appearance of colonies on the culture media, Gram's staining, hanging drop preparation for motility, and biochemical tests such as catalase, coagulase, oxidase , indole, citrate, urease, triple sugar iron, PPA, methyl red and Voges Proskauer test. Antimicrobial susceptibility testing was performed by Kirby- Bauer disc diffusion method and results interpreted as zone of inhibition as per CLSI guidelines.

RESULTS

A total of 341 sputum samples were collected out of which 232 samples were acceptable. Bartlett's criteria was used to screen the sputum samples based on which 232(68%) were acceptable and 109(32%) were unacceptable. Among acceptable category 180(77.5%) samples showed culture positivity. The various pathogens isolated were *Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa , Escherichia coli, Acinetobacter baumanii, Citrobacter spp. and Enterococcus spp. Klebsiella pneumoniae*(29.4%) was the most common isolate detected from acceptable sputum samples followed by *Pseudomonas aeroginosa(21.1%) , Staphylococcus aureus(13.9%) and Streptococcus pneumoniae(11.7%).*

Comparison of Gram stain and culture can be used as a quality assurance tool for sputum culture. Gram stain does not detect bacteria if their number is small. Small number of bacteria in culture may not be visualised in the smear (9). If organisms are seen in smear but culture does not show growth, or if there is heavy growth in culture but are not seen in the smear, reevaluation is required. Gram's stain is relatively insensitive methods as 10^5 CFU/ml of bacteria must be present to be visualised, so small number of bacteria in culture may not well be visualized .(9) The most common organism isolated in the present study was *Klebsiella pneumoniae* 53(29.4%), *pseudomonas aeruginosa* 38(21.1%), *Staphylococcus aureus 25(13.9%), Streptococcus pneumoniae 21(11,7%), Escherichia coli 18(10%), Klebsiella oxytoca 5(2.8%), Enterococcus spp. 7(3.9%), Acinetobacter baumanii 9(5%) and Citrobacter spp 4(2.2%).*

Table-1. Bartlett's criteria

Number of Neutrophils/10XLPF	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	

Table-2. Organisms isolated from acceptable sputum samples

Organisms	No. of bacteria isolated	% of bacteria isolated
Klebsiella pneumoniae	53	29.4%
Pseudomonas aeruginosa	38	21.1%
Staphylococus aureus	25	13.9%
Streptococcus pneumoniae	21	11.7%
Escherichia coli	18	10%
Klebsiella oxytoca	05	2.8%
Enterococcus spp	07	3.9%
Acinetobater baumanii	09	5.0%
Citrobacter spp	04	2.2%
Total	180	100%

DISCUSSION

This study showed an acceptable percentage of sputum samples to be 68% which is in concordance with study conducted by Anevlavis *et al*., (2011)(10) with acceptability percentage of sputum samples of 63%. Also, Mariraj et al. (2) reported acceptability percentage of 79% respectively. The present study is in contrast to study done by Daniel Musher et al (2004)(11) who showed 31% acceptability. Ravichandran et al. (12) reported an acceptability percentage of zero with all the samples i.e.74 in unacceptable category.

Bartlett's criteria is only useful for bacterial pathogens. Clinical history is the most important guide in determining the presence of microorganisms. The presence of important pathogens from respiratory samples must always be evaluated in the light of clinical history.(9) The culture positivity in the present study was 77.5% which is very near to study done by Akansha Rana et al.(2017) (13) which showed 78.54% culture positivity. Other authors have shown a variable range of culture positivity. Study done by Jean J Lloveras(14) showed a culture positivity of 57%, Daniel Musher et al 79%, Somporn et al 40.95% (15), Nawfal Ali Mubarak 41.7% (16) and Aroma Oberoi et al. 32%(17). Ravichandran reported only 5% culture positivity. Other studies done by Chinnusamy et al. showed 78.05% (18), by Mariraj et al reported a growth in 63.2% and M R Shariatzadeh et al (19) reported growth in 33.7% of acceptable samples. In the present study *Klebsiella pneumoniae* (29.4%) was the most common isolate detected from acceptable sputum samples followed by *Pseudomonas aeruginosa (21.1%), Staphylococcus aureus(13.9%) and Streptococcus pneumoniae* (11.7%). Chinnusamy et al. have reported *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* as potential pathogens derived from acceptable sputum samples with detection rate of 31.71%, 14.63%, 13.41% and 12.19% respectively. Other study done by Akansha Rana et al. have reported *Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae and Pseudomonas aeruginosa* to be the most common organisms detected from acceptable sputum samples with detection rate of 20.22% for *Streptococcus pneumoniae, 14.76% for Streptococcus pyogenes, 16.39% for Klebsiella pneumoniae and 9.84% for Pseudomonas aeruginosa.* In a study done by Anuradha Mokkapati et al.(2013)(20) *Klebsiella pneumoniae* (22.85%) was most common bacteria followed by *Streptococcus pneumoniae (11.49%)* and *Staphylococcus aureus* (10%). In another study conducted by S. Krishna et al(21). The most common organism isolated were Streptococcus pyogenes , followed by *Klebsiella spp, Pseudomonas spp, Staphylococcus aureus, Esherichia coli, Citrobacter koseri,* and *Enterobacter spp.*

CONCLUSION

Gram staining may show the normal resident microbial flora of the lower respiratory tract but for the good quality of sputum. Bacteria isolated from non acceptable samples causing LRTI s is not a good guide of true pathogen and its utility is relatively uncertain. Initial screening of sputum is a must for clinically relevant results. There is also a need to formulate rejection criteria of sputum for good results. Therefore good quality sputum is a must as its further processing by Gram stain and culture can provide a high diagnostic yield in clinically suspected LRTIs. The identification of true pathogens may be identified accurately only if the microbiological laboratories are provided with good quality samples, in this case sputum for the results to be accurate. This will lead to rapid and accurate management of LRTIs and decrease in morbidity and mortality of hospitalized patients, decreased hospital stay thereby decreasing burden on hospitals .

REFERENCES

1. Carroll K.C. Laboratory diagnosis of lower respiratory tract infections: controversy and conundrums. J. Clin. Microbiol.2002; 40:3115-3120.
2. Mariraj J, Surekha Y, Asangi, Krishna S, Suresh B Sonth, Ramesh, Shanmugam. Sputum Gram's stain assessment in relation to sputum culture for Respiratory Tract Infections in a tertiary care hospital. Journal of Clinical and Diagnostic Research, December 2011; 5(8): 1699- 1700.
3. Bindu Nair, Jenny Stapp, Lynn Stapp, Linda Bugni, Jili Van Dalfsen, and Jane L. Burns. Utility of Gram staining for evaluation of the quality of cystic fibrosis sputum samples. J. Clin. Microbiol.2002; Vol 40. No 8
4. Lester K, Wong, Arthur L. Barry and Susan M. Hogan. Comparison of Six Diferent Criteria for Judging the Acceptability of Sputum Specimens . J. Clin. Microbiol.1982; Vol 16. No.4
5. Fuselier PA, Garcin LS, Procop GW. Infections of the Lower Respiratory Tract. In: Betty AF, Daniel FS, Alice SW, editors. Bailey and Scott's Diagnostic Microbiology. Mosb. 2002; 884-898.
6. Joseph R, Lentino and David A. lucks. Nonvalue of sputum culture in the management of lower respiratory tract infections. J. Clin. Microbiol.1987;Vol 25,No 5
7. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology; 12th edn. Missouri: Mosby;2007.
8. Barlett,J.G., Breiman, R.F., Mandell,L.A., File, T.M. Community-acquired pneumonia in adults: guidelines for management. Clin. Infect. Dis.1998;26: 811-38
9. Washington Winn Jr, Stephen Allen, William Janda, Elmer Koneman. Guidelines for collection, transport, processing, analysis and reporting of cultures from specific specimen sources. In: Koneman's colour atlas and textbook of Microbiology, 6th edition. Lippincott, Williams and Wilkins publications, 2006: 68-111
10. Stavros Anevlavis, Niki Petroglon, Athanasios Tzavaras Efstratios Maltzos, Ioannis Pneumatikos, Marios Froudarakis, Eleftherios Anevlavis, Demosthenes Bouros. A prospective study of the diagnostic utility of sputum Gram stain in pneumonia. Journal of Infection, 2009; 59: 83-89.
11. Daniel M Musher, Roberto Montoya, Anna Wanahita. Diagnostic value of microscopic examination of Gram stained sputum and sputum cultures in patients with Pneumococcal pneumonia. Clinical Infectious Diseases, 2004; 39: 165-169.
12. Ravichandran Theerthakarai, Walid El Halees, Medhat Ismail, Roberto A. Solis, M Anees Khan. Non value of Initial Microbiological Studies in the management of non severe Community Acquired Pneumonia.Chest, January 2001; 119(1): 181-184.
13. Akansha Rana, Archana Sharma and Gaganpriya Pandey. Diagnostic value of sputum gram stain and sputum culture in lower respiratory tract infections in a tertiary care hospital. Int. J.Curr. Microbiol. App. Sci. 2017;6(7): 4310-4314
14. Jean Jacques Lloveras, Mohamed–Issam, Shukr, Claude Pinos, Anissa Lindoulsi, Philippe Grima. Usefulness of sputum Gram's stain and culture for diagnosis of pneumonia in Geriatric institution. Journal of IMAB, 2010; 16(3): 20-22.
15. Somporn Srifuengfung, Malinee Sangsawang, Podjanee Komolpis, Chertsak Dhiraputra and Busabawart Chompanee. Bacterial pathogens (non-Mycobacterial) from sputum culture and their anti-microbial susceptibility. March 1998; 29(1): 96-99.
16. Nawfal Ali Mubarak. The findings of sputum culture of intubated mechanically ventilated patients versus non intubated patients in the Intensive Care Unit. Basrah Journal of Surgery, September 2012; 18: 1-5.
17. Aroma Oberoi, Aruna Aggarwal Bacteriological profile, Serology and Antibiotic Sensitivity Pattern of Micro-organisms from Community Acquired Pneumonia. J K Science, April June 2006; 8(2): 79-82.
18. Chinnusamy N., Arumugam V., Vedachalam D. A study on analysis of the sputum Gram staining and culture in patients with Lower respiratory tract Infections attending a tertiary care hospital. Indian J. Microbiol. Res. 2016; 3(1):24-26
19. M R Shariatzadeh & T J Marrie. Does sputum culture affect the outcome of community acquired pneumonia? Eastern Mediterranean Health Journal, 2009; 15(4): 792-799.
20. Anuradha Mokkapati, Madhavi Yalamanchili. Correlation of sputum Gram stain and culture in lower respiratory tract infections. IOSR-JDMS. 2013;Vol 8,Issue 1: 06-09