Analysis Of Sputum Culture And Gram Staining In Subjects With Lower Respiratory Tract Infection: A Microbiological Assessment

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Abstract

Background: To diagnose the LRTIs (lower respiratory tract infections) in the microbiological laboratory, the most frequently employed method is the microscopic examination of expectorated sputum samples. These sputum samples are generally contaminated with the normal flora that is resident of the oropharynx. To manage and diagnose lower respiratory tract infections, sputum collection, culture, and microscopy are vital.

Aim: The present study was conducted to analyse the sputum culture and gram staining in subjects with lower respiratory tract infections.

Materials & Methods: The study included 140 sputum samples where culture and gram-staining were done. Microscopic examination of the gram-stained sputum smears was done to assess the presence of epithelial cells, pus cells, or organisms. Bartlett's grading system was used to assess the quality of the expectorated sputum samples. Bacterial isolates were identified using standard protocols. On Mueller Hinton agar, the Kirby Bauer disc diffusion method was used for testing antibiotic susceptibility.

Results: In 140 processed sputum samples, it was seen that there were 55% (n=77) acceptable samples and 45% (n=63) non-acceptable samples. Total potential pathogens obtained from the study samples were 62.85% (n=88) samples were from acceptable samples, in 76.13% (n=67) potential pathogens were isolated, whereas, from non-acceptable samples, potential pathogens were isolated from 23.86% (n=21) samples. On assessing the organisms isolated from the study subjects, it was seen that the most common isolated organism was Klebsiella pneumoniae isolated from 28.40% (n=25) samples followed by Pseudomonas aeruginosa in 14.77% (n=13) samples, Staphylococcus aureus in 13.63% (n=12) study samples, Escherichia coli in 12.5% (n=11) samples, Streptococcus pyogenes in 7.95% (n=7) samples, Klebsiella oxytoca in 6.81% (n=6) study samples, Streptococcus pneumoniae in 5.68% (n=5) study samples, Acinetobacter baumannii, Citrobacter koseri, and Enterobacter aerogenes in 3.40% (n=3) study samples

Conclusion: The present study concludes that in subjects with Lower respiratory tract infections, good quality sputum must be obtained, and initial screening of the sputum should be done to obtain.

Keywords: Bartlett's grading system, Gram stain, non-acceptable category, Sputum culture, sputum sensitivity

INTRODUCTION

The most common infectious disease with the highest mortality and morbidity rates globally are the LRTIs (Lower respiratory tract infections). To diagnose the LRTIs (lower respiratory tract infections) in the microbiological laboratory, the most frequently employed method is the microscopic examination of expectorated sputum samples. These sputum samples are generally contaminated with the normal flora that is resident of the oropharynx. Hence, the variety of species in the sputum culture overgrows and prevents true pathogen determination. The majority of the time, sputum isolated is watery saliva that is sent to the laboratory instead of purulent sputum which in turn, leads to false results.[1]

To manage and diagnose lower respiratory tract infections, sputum collection, culture, and microscopy are vital. To routinely diagnose lower respiratory tract infections, sputum sample collection, sputum culture, and microscopy are important. Also, sputum culture and staining are the procedures recommended conventionally to diagnose the LRTIs. However, some clinicians advocate that to diagnose LRTIs definitively, proper microscopic examination and Gram

staining of the sputum are needed based on the correct guidelines, whereas, other clinicians suggest that culture and gram-staining are neither sensitive nor specific to diagnose the lower respiratory tract infections.[2]

To conventionally diagnose the causative microorganisms, isolation and culture are needed in the respiratory specimens after incubation on the suitable media, identification of isolates and antibiotic susceptibility tests are the conventional steps to get suitable results. Hence, along with the treatment success, it also allows using appropriate antibiotic therapy along with decreasing the chances of coinfection. Empirical management using broad-spectrum antibiotics is usually advocated by the current guidelines.[3]

For the assessment of the expectorated sputum, gram staining is usually used as it is readily available and inexpensive that can accurately detect the causative microorganisms if conducted at a qualified laboratory by experienced and expert personnel on good-quality specimens. Gram-staining also helps in the sputum culture results interpretation. To rapidly detect bacterial pathogens, gram staining of the expectorated serum has shown reliable and sensitive results.[4] The present study was conducted to analyse the sputum culture and gram staining in subjects with lower respiratory tract infections.

MATERIALS AND METHODS

The present study was conducted to analyse the sputum culture and gram staining in subjects with lower respiratory tract infections. The study was carried out after obtaining clearance from the concerned Ethical committee. The study population was comprised of the sputum samples sent to the Department of the Microbiology of the Institute for examination. After explaining the detailed study design, informed consent was taken from all the subjects in both written and verbal form.

The study included 140 sputum samples. The exclusion criteria for the study were samples obtained from the paediatric subjects and the samples that were repeated from the same subjects. For all the included 140 subjects, culture and gram-staining were done. The sputum samples following gram staining were microscopically assessed for the presence of epithelial cells, pus cells, and microorganisms. The epithelial cells and pus cells/ neutrophils were assessed under 20-30 low power fields in the microscope and the calculation was done to find the average number of pus cells and epithelial cells.

This was followed by scoring the total of assessed pus cells and epithelial cells. On scoring, lack of active inflammation (inacceptable sputum sample) or salivary contamination was considered for scores of less than/ equal to 0, whereas, the scores of 1 or more were taken as an acceptable sample of the sputum. For all 140 sputum samples, incubation in Mac Conkey agar, Chocolate agar, and Blood agar was done at 37^{o}C overnight.

Inoculation plates were assessed for growth presences after 24 hours of inoculation. To evaluate the growth, bacterial isolates were identified using standard protocols. For testing the antibiotic susceptibility, on Mueller Hinton agar, the Kirby Bauer disc diffusion method was used. Isolating the pathogenic organisms from the specimen was indicative of the culture-positive results. Isolation of insignificant or scanty growth from some specimens was taken as culture-negative results. Om isolating the mixed growth of the significant organisms, the counting was done based on the predominant growth and the results were formulated.

RESULTS

The present study was conducted to analyse the sputum culture and gram staining in subjects with lower respiratory tract infections. The study included 140 sputum samples which following gram staining was microscopically assessed for the presence of epithelial cells, pus cells, and microorganisms. The present study used Bartlett's criteria[5] to assess the presence of epithelial cells, pus cells, and microorganisms which are described in Table 1. It was seen that for Neutrophils number/10XLPF, Grade 0 was considered for <10, for 10-25, the grade was +1, for >25, the grade was +2, and was +1 for mucus presence. For Epithelial cells number/10XLPF, for the presence of 10-25 and >25 the grades were -1 and -2 respectively.

Using Bartlett's screening criteria, among the 140 processed sputum samples, it was seen that there were 55% (n=77) acceptable samples and 45% (n=63) non-acceptable samples. Total potential pathogens obtained from the study samples were 62.85% (n=88) samples where from acceptable samples, in 76.13% (n=67) potential pathogens were isolated, whereas, from non-acceptable samples, potential pathogens were isolated from 23.86% (n=21) samples as shown in Table 2.

On assessing the organisms isolated from the study subjects, it was seen that the most common isolated organism was Klebsiella pneumoniae isolated from 28.40% (n=25) samples followed by Pseudomonas aeruginosa in 14.77% (n=13) samples, Staphylococcus aureus in 13.63% (n=12) study samples, Escherichia coli in 12.5% (n=11) samples, Streptococcus pyogenes in 7.95% (n=7) samples, Klebsiella oxytoca in 6.81% (n=6) study samples, Streptococcus pneumoniae in 5.68% (n=5) study samples, Acinetobacter baumannii, Citrobacter koseri, and Enterobacter aerogenes in 3.40% (n=3) study samples each as depicted in Table 3.

DISCUSSION

The present study was conducted to analyse the sputum culture and gram staining in subjects with lower respiratory tract infections. The study included 140 sputum samples which following gram staining was microscopically assessed for the presence of epithelial cells, pus cells, and microorganisms. The present study used Bartlett's criteria[5] to assess the presence of epithelial cells, pus cells, and microorganisms which are described in Table 1. It was seen that for Neutrophils number/10XLPF, Grade 0 was considered for <10, for 10-25, the grade was +1, for >25, the grade was +2,

and was +1 for mucus presence. For Epithelial cells number/10XLPF, for the presence of 10-25 and >25 the grades were -1 and -2 respectively. Bartlett's criteria were also used in the studies by Oberoi A et al[6] in 2006 and Daniel M Musher[7] in 2004 where authors also validated the grading by Bartlett's screening criteria.

With Bartlett's screening criteria, among the 140 processed sputum samples, it was seen that there were 55% (n=77) acceptable samples and 45% (n=63) non-acceptable samples. Total potential pathogens obtained from the study samples were 62.85% (n=88) samples were from acceptable samples, in 76.13% (n=67) potential pathogens were isolated, whereas, from non-acceptable samples, potential pathogens were isolated from 23.86% (n=21) samples. These results were consistent with the studies of M R Shariatzadeh et al[8] in 2009 and Nawfal Ali Mubarak[9] in 2012 where authors reported comparable pathogen isolates from expectorated sputum samples as in the present study.

For assessment of the organisms isolated from the study subjects, it was seen that the most common isolated organism was Klebsiella pneumoniae isolated from 28.40% (n=25) samples followed by Pseudomonas aeruginosa in 14.77% (n=13) samples, Staphylococcus aureus in 13.63% (n=12) study samples, Escherichia coli in 12.5% (n=11) samples, Streptococcus pyogenes in 7.95% (n=7) samples, Klebsiella oxytoca in 6.81% (n=6) study samples, Streptococcus pneumoniae in 5.68% (n=5) study samples, Acinetobacter baumannii, Citrobacter koseri, and Enterobacter aerogenes in 3.40% (n=3) study samples. These results were in agreement with the findings of Lloveras JJ et al[10] in 2010 and Ziyade N et al[11] in 2010 where authors reported similar microorganisms to be isolated from the expectorated sputum of their study subjects.

CONCLUSION

Within its limitations, the present study concludes that in subjects with Lower respiratory tract infections, good quality sputum must be obtained and initial screening of the sputum should be done to obtain it. The present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample size and longer monitoring period will help reach a definitive conclusion.

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TABLES

Criteria	Subgroup	Grades
Neutrophils number/10XLPF	<10	0
	10-25	+1
	>25	+2
	Mucus presence	+1
Epithelial cells number/10XLPF	10-25	-1
	>25	-2
	Total	

Table 1: Bartlett's criteria used in the study subjects

Characteristics	%	N
Total samples processed	100	140
Acceptable samples	55	77
Non-acceptable samples	45	63
Potential pathogens		
Total	62.85	88
Acceptable samples	76.13	67
Non-acceptable samples	23.86	21

Table 2: Pathogens isolation and Bartlett's criteria in the study subjects

Organisms	%	n
Enterobacter aerogenes	3.40	3
Citrobacter koseri	3.40	3
Acinetobacter baumannii	3.40	3
Streptococcus pneumoniae	5.68	5
Klebsiella oxytoca	6.81	6
Streptococcus pyogenes	7.95	7
Escherichia coli	12.5	11
Staphylococcus aureus	13.63	12
Pseudomonas aeruginosa	14.77	13
Klebsiella pneumoniae	28.40	25
Total	100	88

Table 3: Organisms isolated from the expectorated sputum of the study subjects