Original Article

Significance of Gram's stain in the diagnosis and management of Lower respiratory tract infections

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ABSTRACT

Respiratory tract infections are one of the leading causes of ill health worldwide. For the diagnosis of LRTI, expectorated sputum is the most commonly used specimen.

Objective: To establish the importance and relationship of Gram's staining and positivity of sputum culture in lower respiratory tract infections

Study Design: Experimental study.

Place and Duration of Study: This study was carried out in the Dept. of Microbiology Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, from January 2001 to September 2001.

Materials and Methods: Sputum sample of one hundred clinically suspected cases of lower respiratory tract infections attending OPD or admitted in the wards were included in the study. Early morning sputum samples were collected in sterile container. Gram's staining culture and sensitivity were carried out according to the standard methods.

Results: It was very interesting to note that a direct relationship exists between Gram's staining and positivity of culture. Number of pus cells seen per HPF was directly proportional to the isolated bacterial pathogen. <15 pus cells /HPF had 13.2% cases positive for bacterial pathogen. Pus cells15- 20/HPF had 54.5% cases positive for bacterial pathogen and specimens in which there were >20 pus cells/HPF, 90% cases were positive for bacterial pathogen.

Conclusion: Gram's staining is a simple and cost effective method that could provide a basis for culture positivity of the specimen. Higher the number of pus cells in sputum sample greater was the culture positivity. Based on Grams staining results it would be possible to start empiric therapy and alter the therapy after the sensitivity of isolates if needed.

Key Words: Gram stain, Pus cells, Sputum, H.influenzae, S.pneumonae.

INTRODUCTION

Although LRTIs were a major cause of morbidity and mortality, diagnosis of these infections was often complicated by the contamination of specimens with upper respiratory tract secretions during collection. Gram's staining could provide a basis for determining the extent to which identification and susceptibility testing of organism recovered from specimen. [1]

Despite remarkable advances in the identification of new microbial pathogens and antimicrobial agents, a few diseases were so characterized by disputes about diagnostic evaluation and therapeutic decisions. [2]

Identifying the etiologic agents responsible for pneumonia remained a challenge, primarily because of difficulty in obtaining adequate samples for culture and in differentiating infection from colonization and lack of reliable diagnostic methods. [3]

The diagnostic value of Gram's staining and culture of expectorated sputum had been debated for more than two decades. The value of bacteriological assessment of sputum was controversial during lower respiratory tract infections. [4]

In addition, recommendations had been made that sputum culture results be correlated with direct Gram's stain results in order to provide more clinically relevant information.[5, 6, 7]

Finally, the Gram's stain was a useful tool in laboratory quality assurance. Comparison of Gram's stain and culture results could reveal errors in procedure, specimen collection and/or transport issues, or specimen identification and tracking errors. [8]

Treatment of LRTI could be very simple and cost effective if etiological agent was isolated accurately. Unfortunately neither a standardized laboratory method nor a standard timing for the collection of sample exists. Because of easy availability of Gram's staining and culture rather than blood culture, antigen detection, or nucleic acid amplification, Gram's staining could be a better alternative.[9] The sensitivity of Gram's staining was between 50-96% and specificity from 12 to 100% (Rein et al; 1978)[10].

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from January 2001 to September 2001. During this period sputum samples were analyzed. Samples were collected from clinically diagnosed cases of lower respiratory tract infections (LRTI)

Patients above 14 years of age were included in the study. Early morning Sputum was collected in a sterile

container, Patients on antibiotics and samples that contained only saliva were excluded from the study.

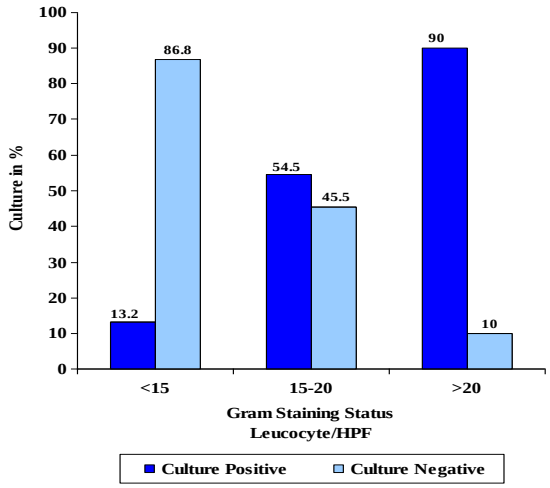
Macroscopic examination: Samples collected were observed by naked eye and were described as purulent, muco-purulent, mucoid and muco-salivary.

Microscopic examination: Expectorated sputa were screened microscopically before inoculation. Gram's staining smear from purulent portion of the specimen was done and observed under low power magnification to determine the number of epithelial cells and or neutrophils. Samples containing more than 10 squamous epithelial cells were discarded. [11, 12, 13] Three categories were made for neutrophil count i.e. neutrophil count < 15 /HPF, 15-20 /HPF and >20 /HPF. Ziehl Neelsen staining was done routinely to see any probable association of tuberculosis along with LRTI.

Culture: To obtain as pure culture as possible and to reduce the number of commensals, washing of purulent part of the sputum was done in 5ml of sterile physiological saline. Sputum was inoculated on Blood, Chocolate and Mac Conkey's agar. S.pneumonae was further confirmed by Optochin sensitivity and H.influenzae by applying factor X, V and XV discs. Sensitivity to antibiotics was observed by standard methods.

RESULTS

Relationship between pus cells/HPF and culture positivity for bacterial pathogen was seen and Gram's staining results were divided in to three categories, specimen having <15 pus cells/HPF, 15-20 pus cells/HPF and >20 pus cells/HPF and it was observed that in sputa which contained <15 pus cells/HPF had 13.2% cases positive for bacterial pathogen, specimen which had pus cells between 15 and 20/HPF had 54.5% cases positive for bacterial pathogen and specimens in which there were >20 pus cells/HPF, 90% cases were positive for bacterial pathogen.



* Statistically significant

Figure No.1: Relationship between number of pus cells and culture positivity

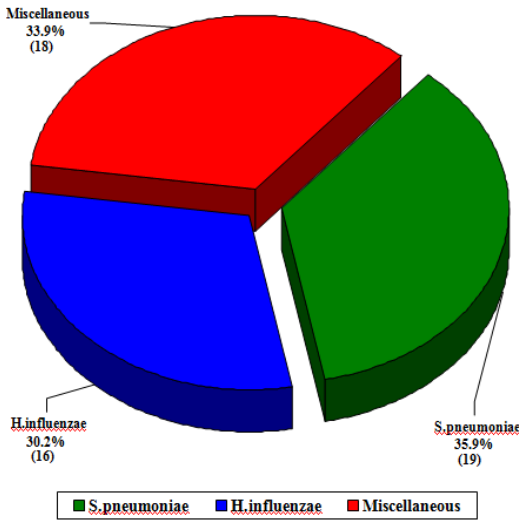


Figure No.2: Distribution of 53 lower respiratory tract bacterial pathogens

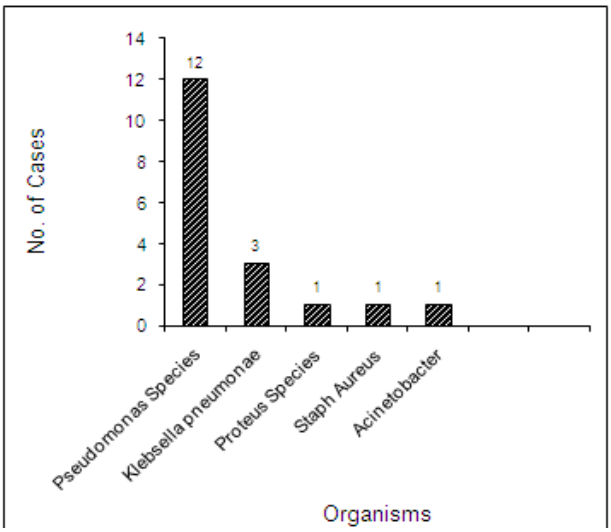


Figure No.3: Distribution of miscellaneous organisms (n=18)

It was observed that higher the number of pus cells/HPF better was the yield for culture positivity (Fig1).

Of 53 positive cultures 19 (35.9%) were S. pneumonae, 16 (30.2%) were H. influenzae and 18(33.9%) were miscellaneous organisms (Fig-2)

Of the miscellaneous pathogens, 12 were Psedomonas species, 3 were Klebsiella pneumonae, and one each was Proteus species, Staphyllococcus aureus and Acinetobacter Fig-3

DISCUSSION

For the diagnosis of LRTI, expectorated sputum remained the most commonly used sample which could be obtained easily and non- invasively. [9]

Clinical laboratory had an important role in the diagnosis of LRTI because of diverse etiological agents, contamination of the oral flora and complex patho-physiology of respiratory system.

Sputum microscopy suggested that presence of pus cells was a good indicator for LRTI. There was direct relationship between pus cells per HPF and isolated pathogen. Only 13% of sputa showed culture positivity in samples who had < 15 pus cells/HPF as opposed to those who had 15-20 pus cells/HPF, and more than 20 pus cells/HPF,54.4% and 90% culture positivity respectively. Higher the number of pus cells greater was the rate of LRTI as it was observed by Niederman et al [14](1993) in their study. Bartlett and Mundy (1995)[2] in their work had mentioned that Gram's stain showing multiple pus cells with large numbers of bacteria that had the morphologic characteristics of likely pulmonary pathogens were an appropriate basis for making initial therapeutic decisions. Roson B et al (2000)[13] in their study mentioned that specimen possessed more than 25/HPF leucocytes were considered as good quality sputum samples, on the other hand Buenviaje MB (1989)[15] did not show any correlation between number of pus cells and quantitative colony counts. Nihan Ziyade and Aysegul Yagci (2010)[9] in their study reported the comparable results to present study, he mentioned that higher the leucocytes count greater was the culture positivity of sputum . Culture positive sputum in leukocyte count less than 10/HPF were 16.7%, while positivity was 88% in specimen which had leukocyte count greater than 25/HPF. Roch N (2007)[16] also suggested that leucocytes count and direct sputum examination should be performed routinely.

Of the positive cultures, 19 (35.9%) had grown *S.pneumonae*, 16 (30.2%) *H.influenzae* and 18 (33.9%) were miscellaneous organisms.

Amongst miscellaneous group the commonest was *Pseudomonas* Sp., 12 cases; next to it was *Klebseilla species*, 3 cases and one each *Proteus* species, *Staphylococcus aureus*, and *Acinetobacter*. Fang et al (1990)[17] studied 359 cases collected from multiple centers and described that the 32.9% were miscellaneous organisms which was comparable to present study. Macfarlane et al (1993)[18] in data of 206 patients, in 113 (54.8%) bacterial pathogens were isolated and 30% were *S.pneumoniae* which was comparable to our data they found *Haemophilus influenzae* in 7.7% cases and viruses were seen in 4.3% cases. Viruses were not seen in our study. Allegra L (2005)[19] in their study mentioned growth of Gram positive organisms in 38% of cases expectorated purulent sputa. Lloveras J J et al (2010)[4] reported 57% culture positivity in their study , this data was comparable to our results however the most commonly found organism in their study was *Staphylococcus aureus* this showed the known variability in the spectrum of micro-organism in different parts of the world. *H. influenzae*, *Pseudomonas aeroginosa* and *Streptococcus pneumonae* were the most common micro organisms reported by Ziyade N and Yagei A (2010)[9] in their study. Same study endorsed the usefulness of Gram's stain and culture in diagnosis of LRTI.

CONCLUSIONS

Higher the number of pus cells in sputum, greater was the culture positivity. Gram's staining was a simple and cost effective method that could provide a basis for culture positivity in patients of LRTI.

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