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Can sputum gram stain be used to predict lower respiratory tract infection and guide empiric antimicrobial treatment: Experience from a tertiary care hospital

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ABSTRACT

Background: The mortality associated with lower respiratory tract infection is high. Indiscriminate use of antimicrobials leads to alteration of respiratory tract flora and development of multi-drug resistance. Rapid diagnostic tests to confirm infection can guide the clinicians about antimicrobial treatment. So the present study was planned to evaluate the role of direct gram stain examination as a rapid and simple test to help clinicians for appropriate patient management.

Methods: The present study was conducted on 1000 respiratory specimens which were processed using conventional microbiological techniques. Gram stain smear and culture results were compared statistically to assess the sensitivity, specificity, positive and negative predictive value. The agreement between gram stain smear examination and culture was calculated using kappa statistics.

Results: Potential pathogens were obtained from 28 of 209 deeply coughed out sputum samples (13.3%) and from 19 of 315 saliva mixed sputum samples (6%). Out of 473 tracheal aspirates, 115 (24.3%) had potential pathogens. The sensitivity for predicting infection was higher for good quality sputum samples (54%) as compared to poor quality sputum samples (37%). The gram stain and culture of tracheal samples had a good agreement for predicting infection whereas there was only moderate agreement for sputum sample.

Conclusion: Gram stain smear examination from respiratory samples can be used to guide empiric antibiotic therapy pending final culture sensitivity results if the attending physicians ensure appropriate sample collection and transport. In absence of these supportive measures smear examination should not be relied upon for empiric treatment.

1. Introduction

Lower respiratory tract infections (LRTIs) whether community acquired or hospital acquired are one of the common causes of morbidity and mortality worldwide in all age groups (Reimer and Carroll, 1998). The mortality is even higher in cases of hospital acquired pneumonia to the range of 25 to 60% depending on the timing of initiation of empiric treatment with antimicrobials (Rello et al., 2002; Kollef, 1993; Werarak et al., 2010).

Clinicians initiate empiric antibiotic therapy in suspected cases of LRTI to reduce the duration of hospitalization and mortality of patients. Mostly the guide to initiate therapy is based on clinical scoring such as Simplified Acute Physiology Score II and also the rapid point of care test such as presence of biomarkers to confirm infection (Ramirez et al., 2008; İşgüder et al., 2017).

It has been observed that initiation of antimicrobial therapy alters the respiratory tract flora (Thakkar et al., 2012). Moreover the problem of antibiotic resistance is increasing with indiscriminate use of antibiotics. In most of the studies done in this decade the antibiotic resistance in gram negative organisms to third generation cephalosporins and even carbapenem is seen to range from 40 to 90% and in gram positive the resistance to beta lactams ranges from 30 to 60%.(Ahmed et al., 2013; Olugbue and Onuoha, 2011; Kaul et al., 2007)In view of high mortality and rise of multi-drug resistant organisms, the dependence on early point of care test in management of pneumonia is increasing (İşgüder et al., 2017; Thakkar et al., 2012; Ahmed et al., 2013; Olugbue and Onuoha, 2011; Kaul et al., 2007).

Gram stain smear examination directly from the specimen done properly and interpreted accurately by trained microbiologists is a useful tool for providing early diagnosis, supporting antimicrobial

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stewardship, assessing specimen quality to help in culture correlation and guiding clinicians regarding patient management (Thomson, 2016; Samuel et al., 2016). But the role of smear examination in respiratory sample is not fully elucidated as the sample variability is quite significant (Nagendra et al., 2001).

Many studies have reported that there is no correlation between gram stain smear and culture sensitivity results and they conclude that irrespective of gram stain findings empiric antibiotic should be started to reduce mortality (O'Horo et al., 2012; Raghavendran et al., 2007). On the other hand, some studies have found that gram stain smear can be used as good indicator to guide initial antibiotic therapy, decrease mortality and shorten the duration of hospitalization in cases of pneumonia (Rosón et al., 2000; Yoshimura et al., 2017). Most of these studies have been done in hospital settings in suspected cases of ventilator associated pneumonia. A meta-analysis evaluating the utility of sputum gram stain smear in diagnosing community-acquired pneumonia by Rio-Pertuz et al. (Del Rio-Pertuz et al., 2019) included 20 studies and concluded that sputum gram stain is a sensitive and highly specific test for identifying pathogens in adult patients with CAP. Most of these studies had evaluated a small number of patients and only *Streptococcus pneumoniae* cases were included. Only Anevlavis et al. (Anevlavis et al., 2009) reported the use of gram stain in over a thousand patients and included all pathogens but used a different criteria from our study to define 'good' and 'poor' quality specimen. Very few studies have evaluated the role of gram stain findings of tracheal aspirate compared to culture results in case of hospital acquired pneumonia and most have focussed on Staphylococcal pneumonia (Seligman et al., 2015; Gottesman et al., 2014). There are limited studies that have evaluated the role of gram stain smear examination to guide treatment from cases of community and hospital acquired pneumonia.

We planned to carry out this study to evaluate the utility of direct gram stain smear examination and look for the correlation of gram stain with culture from community and hospital acquired pneumonia to help clinicians guide the empiric management of patients. Bartlett's grading system was used to assess the quality of sputum as it takes into account the ratio of WBCs to squamous epithelial cells as well as the presence of mucus in the specimen (Table 1) (Bartlett et al., 1978). It has also been found to miss fewer potential pathogens than other criteria (Wong et al., 1982).

2. Materials and methods

The present study was done in our 1531-bedded tertiary care super specialty hospital from December 2016 to March 2017. Our hospital receives referred patients from in and around Delhi, India.

A total of a thousand (1000) respiratory samples (sputum, tracheal aspirate, broncho-alveolar lavage (BAL), bronchial wash) received from December 2016 to March 2017 in microbiology laboratory were included in this study. The sputum samples received from outpatients and inpatients of the hospital within first two days of hospitalization were considered as suspected community acquired pneumonia. Tracheal aspirates and other such respiratory samples were collected after 48 h of admission from patients who were intubated in our hospital and were considered as from cases of suspected hospital acquired pneumonia. As such patients who have been intubated outside the hospital are not admitted in our hospital. The demographic details of the patients were maintained in the records.

Table 1
Bartlett's criteria to assess quality of sputum sample.

Number of neutrophils/low power field (10×)	Grade
< 10	0
10 to 25	+1
> 25	+2
Presence of mucus	+1
Number of Squamous Epithelial Cells/Low power field(10×)	
< 10	+ 2
10 to 25	-1
> 25	−2
Total score	

The samples were processed in the microbiology laboratory using standard conventional microbiological techniques. Briefly the sputum, tracheal aspirate and other respiratory samples were examined grossly and then mixed properly and homogenized. A gram stain smear was made and sample was inoculated on 5% sheep blood agar and MacConkey agar. Irrespective of the quality of the samples, all the samples were cultured. The plates were incubated aerobically at 37 °C overnight. Identification of significant isolates was done from the culture plate following standard microbiological techniques which involved morphological study of the colonies, gram stain reactions, and a battery of biochemical tests as required.

The direct gram stain smear from samples was examined by a trained microbiologist and if required it was cross checked by a second observer for the presence of polymorphs, epithelial cells and bacterial forms. Sputum samples were evaluated based on Bartlett's grading system (Bartlett et al., 1978) and a score was given (Table 1).

All the smears were retained until the cultures were reported, and all those samples which had discrepant results in cultures their respective smears were reviewed for similar bacterial forms if it was missed during the first time.

Average number of epithelial cells and neutrophils in 20–30 microscopic low power fields was calculated and the total score was given. A final score of 0 or less indicated lack of active inflammation or salivary contamination (poor quality sample), and a score of 1 and above was considered as good quality sample (deeply coughed out good quality sputum sample).

This study was approved by the institutional ethics committee.

3. Data analysis and statistical methods

All data entry was done on MS Excel software and appropriate statistical tests were applied. Various statistical tests used including frequency tables, sensitivity, specificity, positive and negative predictive value were used to compare two test. The agreement between culture and gram stain was also evaluated by kappa (κ) score. Kappa value of one showed perfect agreement and value of zero showed no agreement. A κ score between 0.21 and 0.40 was considered as fair agreement, that between 0.41 and 0.60 was moderate and between 0.61 and 0.80 was taken as good and that showing between 0.81 and 1 showed very good agreement (Cohen, 1960).

4. Results

A total of 1000 consecutive respiratory samples were included for final analysis. The number of sputum samples and tracheal samples were almost equal and most of the samples were received from adults as shown in Table 2.

Based on Bartlett's screening criteria, out of 524 sputum samples processed, 209 (39.8%) were good quality samples and 315 (60.11%) were poor quality samples (contaminated with saliva). Potential pathogens were obtained from 28 of 209 good quality samples (13.3%) and from 19 of 315 from poor quality samples (6.03%). Out of 473 tracheal samples, 115 (24.3%) grew potential pathogens. Analyzing all the 1000 respiratory samples 16.4% (164/1000) were positive for culture. Most of the isolates were seen in the admitted patients and the distribution of pathogens is shown in Table 3.

On analyzing direct smear and culture results of the sputum samples and tracheal samples it was observed that the correlation between the predominant morphotype on gram stain and culture positivity was higher for tracheal aspirates and for gram negative organisms as shown in Table 4.

Table 2
Demographic details of respiratory samples (N = 1000).

Category	Number (%)
Sex	
Males	634 (63.4%)
Females	366 (36.6%)
Age	
Adults (13 to 59 years)	921 (92.1%)
Pediatrics (0 to 12 years)	46 (4.6%)
Elderly (> 60 years)	26 (2.6%)
Samples	
Sputum	524 (52.4%)
Tracheal aspirate	473 (47.3%)
BAL	3 (0.3%)
Location	
Intensive care unit, ICU	471 (47.1%)
Inpatients	403 (40.3%)
Outpatients	86 (8.6%)

Table 3
Distribution of bacterial pathogens from the respiratory samples (N = 164).

Organism	Number of patients (%)			Total no (%)
	ICU	In-patients	Out patient	
Acinetobacter baumannii	43	4	1	48 (29.3%)
Pseudomonas aeruginosa	37	10	1	48 (29.3%)
Klebsiella spp	20	9	1	30 (18.3%)
Escherichia coli	2	8	0	10 (6%)
Staphylococcus aureus	4	5	1	10 (6.1%)
Streptococcus pneumoniae	3	2	0	5 (3%)
Non fermenters	3	0	0	3 (1.8%)
Proteus spp	3	0	0	3 (1.8%)
Enterococcus spp	0	2	0	2 (1.2%)
Enterobacter spp	0	2	0	2 (1.2%)
Total	116	44	4	164 (100%)

The sensitivity of gram stain for respiratory samples was less whereas the specificity and negative predictive value was high as shown in Table 5.

The gram stain smear and culture of tracheal samples had a good agreement for predicting lower respiratory tract infection whereas there was only moderate agreement between gram stain and culture performed on sputum samples, either good quality or bad quality (Table 6).

5. Discussion

Studies have shown that initial choice of antibiotic regimen is of utmost importance in predicting the mortality and clinical outcome of patients with pneumonia especially ventilator associated pneumonia. Initiation of broad spectrum antimicrobial treatment is associated with alteration of lower respiratory tract flora and increased probability of developing drug resistance (Werarak et al., 2010; Thakkar et al., 2012).

It is seen that the frequency of side effects is significantly lower in patients who receive pathogen targeted antimicrobial therapy as compared to those receiving broad spectrum antimicrobial therapy (Werarak et al., 2010; Fukuyama et al., 2014). Only a few point of care tests such as sepsis biomarkers are available to predict infection but they are costly and the majority of these do not have good sensitivity and specificity to guide initial antimicrobial therapy in such cases. With these said objectives we planned to evaluate the role of gram stain as a rapid and economical test to guide initial antimicrobial regimen from suspected cases of community acquired and health care associated pneumonia.

In the present study the frequency of good quality sputum sample was a mere 40% and higher percentage of samples were determined to be poor quality sputum sample(contaminated with saliva) as assessed by Bartlett's scoring criteria. The frequency of good quality sputum sample varies in different studies. The yield of good quality sputum sample was seen to be much higher in another study at about 71% (Fukuyama et al., 2014). The reason for the higher percentage of good quality sputum samples in that study was because of the protocols followed in the sample collection techniques in their setting. The sputum sample collection was done before initiation of antibiotic therapy and nasotracheal suctioning was used when the expectorated sample could not be collected thus reducing the chances of salivary contamination. Moreover the sample was immediately processed to reduce the chances of contamination.

The poor yield observed in our study was also seen in other studies (Rosón et al., 2000; Miyashita et al., 2008). The lower yield of good quality sputum samples in our study could be because proper techniques for sample collection were not always followed. Most of the sputum samples were collected by patients themselves probably without receiving proper instructions. A study comparing the quality and culture positivity in self collected versus supervised sample collection may help to find the deficiencies in sample collection techniques in our setting and may help in standardizing sample collection protocols.

The culture positivity in the present study was approximately 19% in sputum samples or from cases of community acquired pneumonia and 24% from tracheal aspirates or suspected cases of hospital acquired pneumonia as defined earlier in the study. The culture positivity has been reported to be as high as 70% in a good quality sputum sample in one study (Miyashita et al., 2008). Studies have also reported the culture positivity in cases of LRTI samples to range from 40 to 80% (Olugbue and Onuoha, 2011; Miyashita et al., 2008; Uzoamaka et al., 2017). This lower rate of culture positivity in the present study as compared to other studies points towards indiscriminate use of sputum and other respiratory samples for culture in patients suspected of having LRTI. Many cases in our settings could have lacked clinical and/or radiological evidence of pneumonia and still culture and sensitivity testing was requested for these patients. Other reasons for such low culture positivity could be viral causes or exposure to previous antibiotics in our population as has been seen by other observers also (Rosón et al., 2000). The prevalence of gram negative organisms such as *Acinetobacter* spp., *Pseudomonas aeruginosa and Klebsiella* spp. was seen in more than three-fourths of our patients. The prevalence of gram negative organisms is reported to be higher in cases of pneumonia by many other authors as well (Werarak et al., 2010; Raghavendran et al., 2007; Uzoamaka et al., 2017). Some authors have observed a higher prevalence of gram positive organisms in suspected LRTIs (Rosón et al., 2000; Fukuyama et al., 2014). The prevalence of gram negative organisms is seen to be higher in low middle income countries (LMICs)

Table 4
Correlation between direct gram stain smear and culture of various respiratory samples.

Type of sample (n = total samples)	Smear + Culture +	Smear +Culture -	Smear -Culture +	Smear -Culture -
Good quality sputum samples (n = 209)	15	4	13	177
Poor quality sputum samples (n = 315)	7	5	12	291
Tracheal aspirates (n = 473)	55	43	60	315
Gram negative organisms (n = 1000)	61	36	84	819
Gram positive organisms (n = 1000)	9	27	8	956

Table 5
Comparison of sensitivity, specificity, positive predictive value, negative predictive value for gram stain and culture.

Type of sample (n = total samples)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Good quality sputum samples (209)	53.57	97.7	78.94	93.15
Poor quality sputum samples (315)	36.8	98.3	58.3	96
Tracheal aspirates (473)	47.8	88	56.12	84
Gram negative organisms (1000)	42.06	95.7	62.9	90.7
Gram positive organisms (1000)	52.9	97.25	25	99.17

Table 6
Comparison of Kappa value for agreement between gram stain smear and culture from various samples.

Type of sample	Kappa value for agreement
Good quality sputum sample	0.464
Poor quality sputum sample	0.437
Tracheal aspirates	0.761
Gram negative organism	0.439
Gram positive organism	0.339

(Raghavendran et al., 2007; Uzoamaka et al., 2017). Because of the diversity of pathogens responsible for LRTIs it is imperative to know the causative agent for effective patient management.

In this study it was seen that the sensitivity of gram staining was higher in cases of good quality sputum sample (54%) as compared to poor quality sputum sample (37%). This difference in sensitivity of good quality sputum sample versus poor quality sputum sample reiterates the need for collecting appropriate samples taking all precautions to avoid salivary contamination. The other reason for the lower sensitivity of gram stain besides bad quality sample could also be because of previous antimicrobial exposure in our study population. Receiving antibiotics before sputum and other respiratory sample collection adversely affects the performance of gram stain as has been seen by other observers also (Fukuyama et al., 2014; Miyashita et al., 2008).

The sensitivity of sputum gram stain was less whereas the specificity was higher. The sensitivity of gram stain from tracheal aspirates and other respiratory samples also was around 48% whereas the specificity was 88%. The low sensitivity and high specificity has been reported by other authors as well (O'Horo et al., 2012; Raghavendran et al., 2007; Rosón et al., 2000; Miyashita et al., 2008).

There was only moderate agreement between organisms observed on direct sputum gram stain and those recovered from culture as seen by kappa statistics. The agreement for tracheal aspirate samples was however good. The reasons for the lower agreement seen could also be because of salivary contamination in sputum sample which may show organisms on gram stain but they may not be present in significant count on the culture plate. As has been earlier reported, all organisms seen on gram stain are not associated with lower respiratory tract infection and they might not have been reported on culture (O'Horo et al., 2012). The reason for better agreement for tracheal aspirate samples could probably be lower oropharyngeal contamination during sample collection.

The negative predictive value of gram staining from sputum and other respiratory samples was higher (around 90%) as compared to positive predictive value (60%). This trend has been seen by other observers also (Raghavendran et al., 2007). The sensitivity of gram stained smears for detecting gram negative morphotype was only 42% and that for gram positive morphotype was higher around 53%. The accuracy of gram stain for predicting predominant morphotype is seen to be different in different studies (O'Horo et al., 2012; Raghavendran et al., 2007; Rosón et al., 2000; Yoshimura et al., 2017; Fukuyama et al., 2014; Miyashita et al., 2008). Some studies have seen the accuracy of gram positive to be better whereas others have seen it to be better for gram negative organisms (O'Horo et al., 2012; Raghavendran et al., 2007; Rosón et al., 2000; Yoshimura et al., 2017; Fukuyama et al., 2014; Miyashita et al., 2008). In our setting and other LMICs where gram negative organisms are the major causative organisms for causing lower respiratory tract infection a positive gram stain can help to guide the initial choice of antimicrobial but a negative gram stain report cannot be used to rule out infection. Looking into the higher mortality associated with gram negative organisms, gram stain examination should not be relied upon as a rapid point of care test to guide treatment.

6. Conclusion

We therefore conclude that gram stain examination of sputum and other respiratory samples can be used to guide initial choice of antibiotic therapy only if the patients or the attending physicians take proper care during sample collection such as sample is collected before antimicrobial exposure and clinical and/or radiological evidence of pneumonia is present in the patient before sending the sample for culture. Besides these measures rapid transport and processing of samples and screening of gram stained smear by trained microbiologists is equally important to increase the value of gram stain as point of care test to guide initial antimicrobial therapy. In the absence of these supportive measures, gram stain should not be relied upon to initiate antimicrobial therapy as mortality associated with lower respiratory tract infection can be higher if not treated promptly. The limitation of the present study is that no interventions could be done in terms of guiding the patients about proper sample collection and see how many of the previous poor quality samples could be converted into good quality samples. A comparison of the results before and after the training would have been desirable to assess the impact of gram stain in predicting the predominant morphotype based on sputum sample quality.

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Declaration of Competing Interest

None.

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