Original Article

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Microbiology Section

Guiding Antimicrobial Therapy using Gram Stain in Patients with Ventilator Associated Pneumonia- An Effective Preliminary Diagnostic Tool

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ABSTRACT

Introduction: Empirical antimicrobial therapy is the mainstay of antimicrobial stewardship. The gram stain can be used to guide initial empiric antimicrobial therapy in cases where culture report is not available. This rapid test can therefore be helpful in preventing the initiation of inappropriate therapy and its adverse outcomes.

Aim: To determine the effectiveness of gram stain of tracheal aspirate samples in order to predict the causative microorganism and starting appropriate initial antimicrobial therapy.

Materials and Methods: This cross-sectional study was done for 208 tracheal aspirate samples that were sent to Department of Microbiology, Lady Hardinge Medical College, New Delhi, India, with a request for bacterial culture and Antibiotic Susceptibility Testing (AST) from January 2019 to June 2019. Each sample was inoculated on 5% Sheep blood agar, Chocolate agar and MacConkey agar followed by gram stain preparation and smears. The culture plates were checked after 24 hours for any bacterial growth and further identification was done by gram stain, motility and biochemical tests. The AST was performed as per Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines. All data entry was done on MS excel software and appropriate statistical tests were applied.

Results: Out of total 208 samples significant gram stain findings were seen in 90 cases (43.2%). Out of 208 samples 132 (63.5%) cases grew significant pathogens on culture. Out of these 90 cases, 68 (75.5%) of gram stain finding matched with culture results. The correlation between gram stain and culture was found to be 75.5%.

Conclusion: Gram stain is not only a quick and cost-effective method but also easily available in most laboratories and is highly reproducible. Gram stain, a rapid diagnostic tool, can thus be very useful in antimicrobial stewardship especially for the critically ill patients.

Keywords: Antimicrobial resistance, Endotracheal aspirate, Point of care

INTRODUCTION

One of the factors driving high rates of antimicrobial resistance is high consumption of antibiotics. World Health Organisation (2016-18) study was done to evaluate antibiotic consumption, reported overuse of third-generation cephalosporins in all states across India [1].

Antimicrobial therapy for critically ill patients cannot be withheld for the availability of culture and sensitivity results. Patients in ICU are started with antibiotics empirically while waiting for culture and sensitivity results [2]. Ventilator Associated Pneumonia (VAP) is a serious healthcare infection, associated with increased duration of hospital stay, increased cost of hospitalisation and mortality [3]. Gram stain of Endotracheal Aspirates (EA) can provide valuable guidance while prescribing antibiotic therapy in critical cases where VAP is suspected.

According to a study done by Centre for Disease Dynamics, Economics and Policy (CDDEP) in 2018 (Gandra S et al), one of the highest antibiotic resistance rates among the common community and healthcare associated bacterial infections, were reported from India [4]. CDDEP and Antimicrobial Resistance (AMR) surveillance network of Indian Council of Medical Research (ICMR) evaluated the levels of resistance among various bacteria isolated from bloodstream infections and their data showed high levels of resistance to first line and broad spectrum antibiotics [5,6]. As reported by these studies, resistance to the broad spectrum antibiotics fluoroquinolones and third generation cephalosporin was more than 70% in *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumoniae*, and more than 50% in *Pseudomonas aeruginosa*. There is need to fill the gaps in antimicrobial stewardship programme by introducing diagnostic strategies that will have a direct impact on antibiotic use and hence curb the emergence of antibiotic resistance.

Gram stain of endotracheal aspirates is an easy and cost effective Point Of Care (POC) test that can be used to guide initial empiric antimicrobial therapy in case of non availability of culture report [7-9]. This can thus help prevent the initiation of inappropriate antimicrobial therapy and its adverse outcomes [10]. The aim of this study was to assess the correlation of gram stain examination of endotracheal aspirates to the culture positivity rate in patients suspected of having VAP.

MATERIALS AND METHODS

A cross-sectional study was conducted in a tertiary care hospital in Northern India. Tracheal aspirate samples from patients suspected of VAP admitted in various ICUs (surgical and medical ICUs) in the hospital were sent for microbiological investigation. The study was conducted between January 2019 to June 2019. A total of 208 tracheal aspirate samples were sent during this period.

Inclusion criteria: All critically ill adult patients above the age of 18 years, patients who were on mechanical ventilation for more than 48 hours, the samples from patients clinically suspected to having first episode of VAP and collected within 24 hours of suspicion were included.

Exclusion criteria: Tracheal aspirate samples collected within 48 hours of intubation, have been given antibiotics for more than 24 hours when they meet the inclusion were excluded from the study.

Microbiology Procedure

Gram staining was done for each of the samples received in the microbiology laboratory. The samples were cultured on 5% Sheep

blood agar, Chocolate agar and MacConkey agar as per standard methodology [11]. A semi-quantitative technique was employed using a standard bacteriological loop of size 0.01 mL. The plates were incubated at 35°C in 5-7% CO_2 for 24 hours. After 24 hours culture plates were checked for any bacterial growth and any growth more than 10^3 CFU/mL was reported. Cultures with more than three types of colonies were discarded as contaminants. Bacterial pathogens were identified by gram stain, motility and biochemical characteristics as per standard microbiological techniques [12].

The antibiotic susceptibility pattern was determined for all organisms isolated on culture, irrespective of the gram stain results, using the Kirby Bauer disk diffusion method as per CLSI guidelines [13]. Gram negative bacilli were tested against aminoglycosides (amikacin, gentamicin), β-lactams (cefepime, ceftazidime, ceftriaxone, cefotaxime, piperacillin-tazobactam, ampicillin, amoxicillin-clavulanic acid), carbapenems (imipenem, ertapenem, meropenem), fluoroquinolones (ciprofloxacin) and tetracyclines (minocycline).

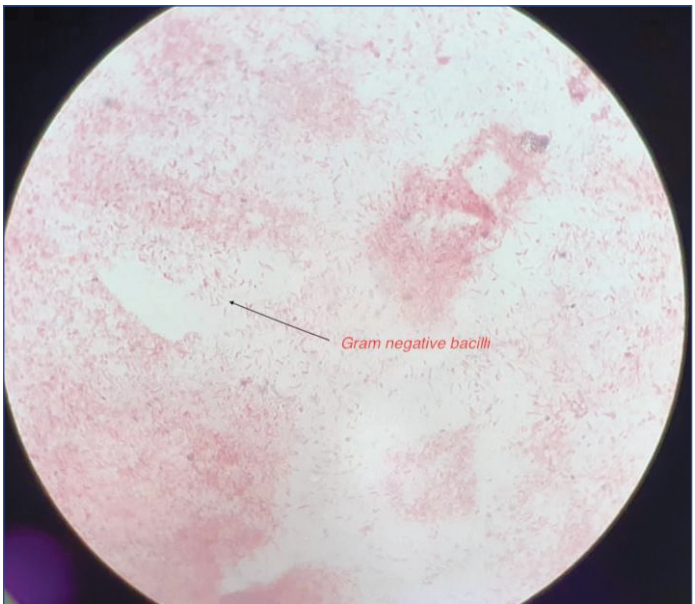
STATISTICAL ANALYSIS

All data entry was done on MS excel software and appropriate statistical tests were applied. Agreement between gram stain and culture results was calculated using Cohen's Kappa (with 95% confidence interval). Pearson's correlation coefficient was calculated between gram stain and culture. To further analyse the validity of results, some additional indices were evaluated using contingency table, namely: sensitivity, specificity, and positive and negative predictive values.

RESULTS

Out of 208 samples, culture positivity was seen in 132 samples (63.5%). Of these 208 samples,123 (59.1%) were males and 85 (40.9%) were females, all adults were above the age of 18 years. Gram staining of the samples revealed gram negative bacilli in 43.2% (90 out of 208) and culture positivity was observed in 68 out of these 90 cases. The correlation (pearsons correlation coefficient, r=0.99) between gram stain and culture was seen in 75.5% of the cases and was found to be statistically significant (p value <0.05).

In study, there was slight agreement between gram stain (shown in [Table/Fig-1]) and bacterial culture finding results (κ=0.202; 95% CI, 0.081-0.323).



[Table/Fig-1]: Gram stain of tracheal aspirate showing gram negative bacilli (10X magnification).

The clinical usefulness of gram stain for tracheal aspirates, sensitivity and specificity, as well as positive and negative predictive values, were calculated [Table/Fig-2].

Statistical tests	(%)
Sensitivity	51.5
Specificity	71
Positive predictive value	75.5
Negative predictive value	45.7

[Table/Fig-2]: Comparison of sensitivity, specificity, positive predictive value and negative predictive values for gram stain and culture results.

Out of 118 samples with negative gram stain finding, 64 (54.2%) grew pathogenic organisms on culture. Twenty three samples had no growth on culture whereas in 53 samples more than three types of organisms were isolated on culture so they were reported as contaminants. Out of the 132 culture positive isolates, most frequently reported organism was *Acinetobacter baumanii* (43.2%), followed by *Klebsiella* spp. (40.9%), *Pseudomonas aeruginosa* (9.1%). The results have been shown in [Table/Fig-3]. Antibiotic susceptibility test results have been summarised in [Table/Fig-4], which showed that most of the isolates were sensitive to ceftazidime and ertapenem.

Organism	Total culture positive isolates (n=132)	Positive finding on gram stain (n=68)
Klebsiella species	54 (40.9%)	25 (36.8%)
Acinetobacter baumanii	57 (43.2%)	31 (45.6%)
Escherichia coli	7 (5.3%)	4 (5.8%)
Pseudomonas aeruginosa	12 (9.1%)	6 (8.8%)
Others (*E. meningoseptica* and *S. marsecans*)	2 (1.5%)	2 (3%)

[Table/Fig-3]: Organism-wise distribution of culture positivity and gram stain positivity.

Antibiotic	Resistance n (%)	Intermediate n (%)	Sensitive n (%)
Amikacin	95 (72)	12 (9.1)	25 (18.9)
Gentamicin	97 (73.5)	2 (1.5)	33 (25)
Ceftazidime	75 (56.8)	4 (3.03)	53 (40.1)
Cefepime	115 (87.1)	11 (8.3)	6 (4.5)
Cefotaxime	119 (90.1)	10 (7.5)	3 (2.2)
Piperacillin-tazobactam	110 (83.3)	9 (6.8)	13 (9.8)
Ampicillin	124 (93.9)	3 (2.2)	5 (3.7)
Amoxy-clavulanic acid	129 (97.7)	3 (2.2)	0
Imipenem	105 (79.5)	14 (10.6)	13 (9.8)
Ertapenem	76 (57.5)	3 (2.2)	53 (40.1)
Meropenem	111 (84.1)	2 (1.5)	19 (14.4)
Ciprofloxacin	119 (90.1)	3 (2.2)	10 (7.5)
Colistin	0	0	132 (100)
Cotrimoxazole	98 (74.2)	8 (6.06)	26 (19.6)
Polymyxin B	0	0	132 (100)
Minocycline	95 (72)	0	37 (28)

[Table/Fig-4]: Antibiotic susceptibility results of all 132 gram negative organisms.

DISCUSSION

Appropriate early therapy for VAP plays a critical role in improving clinical outcomes. Although culture of endotracheal aspirate is considered the gold standard for confirming the causative organisms of VAP, its inherent limitation is that culture results are rarely available before 72 hours. Gram stain of these specimens, however, can provide immediate information about the causative pathogenic bacteria and so is a promising method that will help in assessing likelihood of VAP.

The present study found that out of 208 endotracheal aspirates, 63.4% were culture positive and all were gram negative bacteria (lactose fermenters as well as non fermenters). This could be because of high colonisation rates of gram negative bacteria in our

hospital setting. Badr MA et al., revealed that in majority cases of VAP the causative organism was a gram negative bacteria (68.6%), with *Klebsiella* predominating the positive culture (34.3%) [14]. Another study from in 2019 had reported *Klebsiella* species to be predominant pathogen in ICU similar to present study [15].

According to studies aerobic gram negative bacilli account for more than 60% of VAP cases [7,16-18]. The prevalence of gram negative bacteria in developing VAP in NICUs has been estimated to range from 60-97% with *Pseudomonas, Klebsiella* and *Acinetobacter* organisms being the predominant organisms, which was in agreement with present study [14,19].

Gram positive organisms were not seen in present study as low level colonization by gram positive cocci like Methicillin Resistance *Staphylococcus aureus* (MRSA) has been reported from the hospital, indicating low prevalence rate of these organisms in present hospital. Over the past decade a falling trend in the prevalence of gram positive cocci in the hospital has been observed. This can be supported by a study done by Kapoor L et al., reporting only 20% prevalence rate of gram positive cocci in the hospital. [20]. Another study in 2019 reported gram positive cocci in only 35% of cases admitted in ICU [15].

In present study a correlation rate of 75.5% was observed between gram stain and culture positivity. This was in agreement with a study done by Yoshimura J et al., in 2017 who have reported a similar rate of 74.5% [9]. The author states this study to be the first to provide evidence that Gram stain of endotracheal aspirates may potentially be effective in lowering the broad-spectrum antimicrobials use without affecting appropriate coverage rates compared to a guidelines-based method. A study done by Namais N et al., in Atlanta, USA showed that gram stain correlated with the culture in 53.9% of the cases [21]. Another study done in USA by Kopelman TR showed that Gram Stain results correlated with culture results in 60% of cases [22]. Although other studies have reported that overall agreement between gram stain and pathogenic bacteria culture results to be poor [3,22,23]. According to some authors the lack of correlation could be because of technical issues with gram staining procedure- over decolourisation, excessive heating, inadequate smear preparation or excessive washing between steps being a few reasons. Another reason could be the administration of antibiotic therapy before endotracheal aspirate sampling was done leading to subsequent bacterial cell wall damage [23].

The present study established a good correlation rate which could be because of the fact that as a protocol endotracheal aspirate samples were collected prior to antibiotic therapy and careful gram staining procedure was adopted in the laboratory. Thus gram stain can be used as a reliable surrogate for guiding antibiotic therapy in patients and so should be available as a POC test in the ICU settings.

In the present study, out of 118 samples with gram stains showing no organisms, 64 (54.2%) grew pathogenic organisms on culture. This was in contrast to other studies where lower rates were reported (Albert M et al., 14%) and (TR, 28.2%) [3,22]. The authors concluded that while statistically it may sound helpful but in practice to stop antibiotic therapy based on a negative finding on grams can in turn be detrimental and lead to increased morbidity and mortality. Similarly based on present study findings, a negative gram stain finding does not warrant de-escalation therapy in suspected VAP patients and therefore is not a reliable diagnostic tool in such cases.

A few samples were reported as contaminants because more than three types of organisms were recovered from culture. The likely explanation of this could be the presence of oropharyngeal organisms in the endotracheal aspirates indicating improper collection method.

Most studies have been done to demonstrate the effectiveness of the gram stain of endotracheal aspirate for diagnosing VAP [23,24]. The study has highlighted the usefulness of gram stain in predicting VAP among critically ill patients without impairing the patient outcome, in a cost effective way.

Limitation(s)

Follow-up of whether the cases with culture positive findings in the study actually went on to develop VAP could not be done due to resource constraints.

CONCLUSION(S)

Increasing resistance to antimicrobials over the past decade due to incorrect empirical therapies used in patient management makes this the question of the hour- do we need creative diagnostic tools to reduce this critical waiting period between the sample reaching the laboratory to generation of culture reports? A simple bedside gram stain, a test which is easy to perform and interpret, if incorporated into the antimicrobial stewardship decision making process can help overcome AMR. Future research needs to be done and studies that specifically evaluate correlation of gram stain and culture of respiratory specimen are necessary.

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