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UTILITY OF GRAM STAINING IN COMPARISON TO QUANTITATIVE CULTURE OF TRACHEAL ASPIRATES IN DIAGNOSING VENTILATOR ASSOCIATED PNEUMONIA

Clinical Microbiology

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ABSTRACT

Introduction: Ventilator-associated pneumonia (VAP) is the fatal nosocomial infection in Intensive Care Units (ICU) [1, 2]. VAP prolongs the ICU stay, increasing the economic burden and mortality[1]. Timely and accurate diagnosis of VAP continues to be the challenge in the clinical setting. **AIM:** To evaluate the utility of Gram staining of endo-tracheal aspirates (ETA) & quantitative cultures for predicting VAP & to study the microbiology and susceptibility patterns of MDR isolates. **Methodology:** Data was retrieved retrospectively through the records from Jan 1st 2016- July 31st December 2019). The diagnostic thresholds for ETA quantitative cultures were taken as 10^5cfu/ml. Growth below the threshold was assumed to be due to colonization. Diagnostic threshold for Gram stain results was assumed as $>10^3$cfu. **Results:** Out of total 865 samples 464 were sterile, 255 were culture positive and 146 were colonizers and 90 were polymicrobial. *Acinetobacter baumannii* was found to be the most common pathogen isolated followed by *Klebsiella pneumoniae* .84.09% of isolates were multi drug resistant. The sensitivity of gram staining in our study was 75%, specificity 53.42% with a positive predictive value of 70.9% and negative predictive value of 58.90%. **Conclusion:** Gram staining is useful aid to help to guide empirical antimicrobial therapy in patients with VAP as it can provide immediate information about causative organisms.

KEYWORDS

INTRODUCTION:

Patients in Intensive care units (ICU) are at higher risk of acquiring nosocomial infections, hence complicating the primary disease process resulting in treatment failure, increased hospital stay, high morbidity, mortality. Pneumonia is the second most common nosocomial infection in critical patients, affecting 27% of all critically ill patients. Majority of nosocomial pneumonias are due to presence of mechanical ventilation and that subset of pneumonia is called as ventilator-associated pneumonia (VAP) [3]. VAP is defined as pneumonia that occurs 48 hours or more after endotracheal intubation or tracheostomy, caused by microorganism that was not present or incubating at the time mechanical vantilator was intubated. It can be of two types: (i) early-onset VAP which is defined as VAP that occurs within the first 4 days of ventilation, and (ii) late-onset VAP which is defined as VAP that occurs after 4 days of ventilation.

Depending on the surveillance methods used for the diagnosis of VAP, the risk of this complication ranges from 1.2 to 8.5 cases per 1,000 ventilator-days (0.6%-4%)[4] . The mortality attributable to VAP has been reported to range between 0 and 50% [3,5]. VAP is preventable complication , timely and appropriate diagnosis is important in guiding antimicrobial therapy and decreasing morbidity, mortality and health care cost associated with it. Various severity scoring criteria like clinical pulmonary scoring system(CPIS), modified CPIS, lung ultrasound and pentraxin -3 pulmonary score, etc., have been formulated for accurately diagnosing VAP in clinical settings, they include clinical picture, imaging techniques, microbiological analysis for samples obtained, and biomarkers of host response. Microbiological diagnosis of VAP includes Gram staining and subsequent culture methods of respiratory tract secretions obtained using either endotracheal aspiration or fiberoptic bronchoscopy [6]. culture methods of respiratory tract secretions obtained by various defined methods are considered as gold standard for confirming the causative microorganism ,however the turnaround time (TAT) for identification and antimicrobial susceptibility takes at least 48–72 hours. Gram stain of respiratory specimens can provide quick preliminary report of suspected bacterial pathogen. The effectiveness of the Gram stain of endotracheal aspirate for diagnosing VAP was found useful by several studies. [7,8]. In this study, we assessed effectiveness and correlation of Gram staining and quantitative culture in diagnosing VAP.

AIMS AND OBJECTIVES:

- To evaluate the utility of Gram staining of endo-tracheal aspirates & quantitative cultures for predicting VAP
- To study the microbiology and susceptibility patterns of Multi Drug Resistant isolates.

MATERIAL AND METHODS:

A retrospective study was undertaken from 1st January 2016 to 31st December 2019 at Nizams Institute of Medical Sciences, Hyderabad. All the required clinical data was retrieved retrospectively through the records. A total of 865 endo -tracheal aspirates received from different ICUs were included in the study. The clinical samples included in the study was endo tracheal aspirates conventionally processed by standard laboratory protocol(as per CMPH guideline) at microbiology laboratory.

Microbiology Workup:

Endo-tracheal aspirate specimens were quantitatively (neat dilution, 1:10, 1:100 and 1:1000 dilution) cultured on chromogenic agar and 5% sheep blood agar (biomeriux, France, Marcy'l Etoile) and incubated in aerobic conditions at 37°C for18-24hours. The Gram smear was prepared from the neat dilution .Endotracheal aspirate Cultures showing growth of $\geq 10^5$CFU/ml in microbiology are considered as significant growth (laboratory proven VAP) while cultures with growth $\leq 10^5$CFU/ml are considered as colonizers . Identification and susceptibility testing of isolates from significant growth was done by using Vitek 2 compact system. IDGN ,N280 and N281 panels were used for identification of Gram-negative pathogens and IDGP and P628 panels were used for identification of Gram positive pathogens. The microscopic threshold for diagnosis of VAP with Endo tracheal aspirates > 10 polymorphonuclear neutrophils (PMN) / high-power field (HPF), ≥ 1 bacteria / oil immersion field (OIF).

OBSERVATION AND RESULT:

A total of 865 ETA samples were received from different ICUs across the hospital by the department of Microbiology from Jan 1st 2016- July to 31st 2019. Out of 865 samples 464 ETA samples were sterile after overnight incubation. On further evaluation of remaining 401(865-464) ETA samples, 90 samples were showing more than 3 different organisms indicating possible contamination during sample collection ,while remaining 311 samples included 165 samples had significant growth of $\geq 10^5$CFU/ml and 146 samples had insignificant growth($< 10^5$CFU/ml),hence were considered as colonizers. (Fig.1)

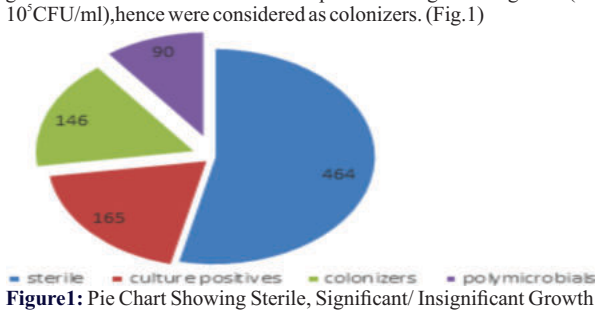

Figure1: Pie Chart Showing Sterile, Significant/ Insignificant Growth Of ETA Cultures

On analyzing the data it was observed that major chunk of ETA samples were received from neurology ICUs 375 out of 865[that included 237 samples from neurosurgery ICU (NSICU) and 138 from neuro-medical (NLICU)], followed by respiratory intermediate care unit (RICU) n=242, Emergency department (EMD) n=95,Acute medical care (AMC) n= 51 and rest of the samples were from other departments of our institute .(Fig.2)

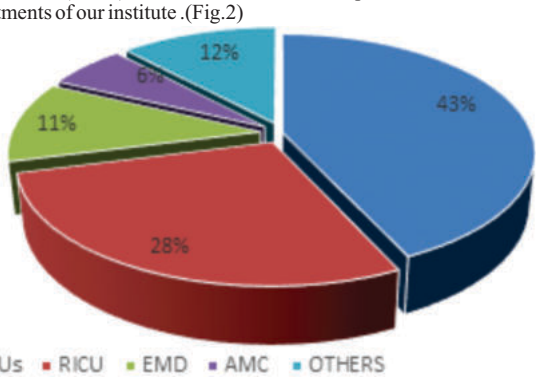

Figure 2: Pie Chart Showing Percentage Of ETA Samples From Various Departments

Of 165 ETA samples with significant growth of $\geq 10^5$CFU/ml, 10 samples were having significant growth of 2 micro-organisms while remaining were having monomicrobial growth.

Gram smear of 311 samples were analyzed that included 165 ETA samples with significant growth and 146 ETA samples with insignificant growth. For a total of 192 ETA samples gram smear were showing plenty of polymorphs with and without micro-organisms taking gram staining thresholds for diagnosis of VAP with ETA: > 10 polymorphonuclear neutrophils (PMN) / high-power field (HPF), ≥ 1 bacteria / oil immersion field (OIF). Positive Gram smear findings and culture positivity (significant growth) observed in 124 ETA samples whereas gram smear were negative for 41 samples with significant growth. However, for 68 of the ETA samples with insignificant growth, gram smear findings were positive(table. 1)

Table 1: Showing Gram Smear And Culture Finding/ Correlation

True positive	**Grams+ve**	**Significant growth**	**124**
True negative	Grams -ve	insignificant growth	78
False positive	Grams +ve	insignificant growth	68
False negative	Grams –ve	Significant growth	41

The sensitivity of gram smear was observed in our study was 75% whereas specificity as low as 53.42%.

CPIS scoring of 311 ETA samples (significant growth samples and insignificant growth samples) were analyzed for clinical presence and absence of VAP by retrospectively reviewing the patient case files .Of 165 samples with significant growth 160 corresponding patients were diagnosed with clinical VAP whereas for remaining 5 samples with significant growth did not have clinical VAP. 130 samples with insignificant growth were in line with clinical absence of VAP ,however 16 ETA samples with insignificant growth were having clinical VAP.(Table.2)

Table 2:showing Correlation Between Culture Findings And Clinical VAP

True positive	Culture≥1 lakh cfu/ml	VAP present	160
True negative	Culture < 1lakh cfu/ml	VAP absent	130
False positive	Culture≥1lakh cfu/ml	VAP absent	5
False negative	Culture < 1lakh cfu/ml	VAP present	16

The sensitivity and specificity of quantitative culture of ETA was observed as 90.91% and 97.7% respectively for Lab. diagnosis of VAP .The positive predictive value of quantitative culture of ETA was found to be 96.97% and negative predictive value was observed as 89.04%.

Microbial profile of pathogenic isolates from samples with significant growth were analyzed, *Acinetobacter baumannii* was found to be the most common isolate 44.31% (n=77), followed by *Klebsiella pneumonia* 23.86%(n=42), *Pseudomonas aeruginosa, 11%(n=20), Escherichia coli 10.22%(n=19)i* and others like, *Enterococcus spp 4%* (n=7), *Staphylococcus aureus 3.8%(n=6)an Serratia marcescens* 2.2%(n=4).(Fig.3)

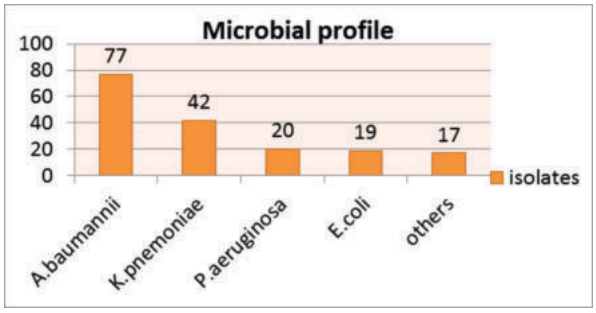

Figure 3 : Column Chart Showing Profile Of Pathogens Isolated

30.85%(n=54) pathogenic isolates were from RICU followed by EMD 17.14%(n=30),NSICU accounted for 11.42%(n=20),AMC 10.28%(n=18),NLICU 9.14%(n=16) and remaining 37.21%(n=37) from other departments.(Fig.4)

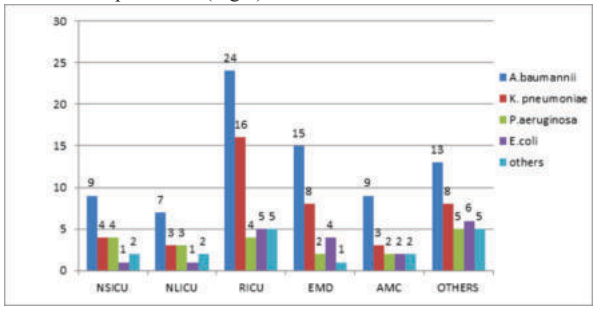

Figure 4: Column Chart Showing Distribution Of Isolates In Different Units

Antimicrobial sensitivity of the pathogenic isolates were analysed for both groups of isolates(gram positive and gram negative)

Highest drug resistance was observed in *Acinetobacter baumannii,* followed by. *K.pneumonia*. All *A.baumannii* isolates were found resistant to cephalosporins where as only 1.5% of them were resistant to Tigecyclin and Colistin where as 4.7% *of k.pneumonia* isolates were also resistant to tigecyclin and Colistin. The resistance pattern of remaining isolates in shown in table 3 and table 4.

Table 3: showing resistance pattern of gram negative isolates

Antibiotics	**Acinetobacter baumannii (n=77)**	**Klebsiella pneumonia(n=42)**	**Pseudomonas aeruginosa (20)**	**Escherichia coli (n=19)**	**Serratia spp (n=4)**
Cefepime	100%	85.7%	28.50%	68.4%	75%
Ceftazidime	100%	85.7%	28.50%	73.6%	75%
Amikacin	74.04%	80.9%	90%	68.4%	75%
Gentamicin	93.5%	80.9%	57.14%	68.4%	75%
Meropenem	90.9%	78%	65%	52.6%	75%
Doripenem	90.9%	78%	65%	52.6%	75%
Levofloxacin	94.80 %	85.7%	90%	84.2%	75%
Cotrimoxazole	94.80%	70%	Intrinsic resistance	68.4%	75%
Colistin	1.2%	4.7%	0	0	Intrinsic resistance
Tigecycline	1.2%	4.7%	Intrinsic resistance	0%	75%

Table 4 :Resistance pattern of Gram positive patterns

Antibiotic	**Enterococcus Spp n=7**	**Staphylococcus aureus n=6**
Benzlypenicillin	18.18%	83.3%%
Oxacillin	not tested	66.6%
Gentamicin	not tested	66.60%
Ciprofloxacin	9.00%	33.33%
Levofloxacin	18.80%	33.33%
Clindamycin	not tested	16.66%
Eythromycin	18.80%	33.33%
Linezolid	100%	100%
Daptomycin	not tested	100%
Teicoplanin	100%	100%

Vancomycin	100%	100%
Tetracycline	100%	66.66%
Tigecycline	100%	100%
TMP/SMX	not tested	66.66%

DISCUSSION:

VAP is a preventable fatal nosocomial infection that occur in patients, on mechanical ventilation. Diagnostic challenges in clinical settings are constant matter of concern. In this study we analyzed the Gram staining and quantitative cultures of ETA in predicting VAP along with other components of CPIS scoring system. Gram staining, a potentially useful aid to help to guide empirical antimicrobial therapy in patients with VAP as it can provide immediate information about causative organisms. However, in our study the sensitivity of Gram staining was found 75% whereas specificity was as low as 53.43%. A study from Zurich has reported sensitivity as high as 96% but specificity was found12%.[9]. In another study from France sensitivity and specificity was found 95and 61% respectively[10]. In our study the sensitivity of quantitative ETA culture was found 90.91% and specificity 97.7%. in a study by Luis Fernando et al reported sensitivity and specificity of 68% and 48% respectively[11]. In a study from india by Noyal M Joseph et al[12], the sensitivity and specificity was found 88.1 %and 84.2% respectively. Due to their high specificity rate, quantitative cultures continue to be the gold standard. In this study we found that gram negative organisms are more prevalent accounting for 92.5% cases of VAP while gram positive pathogens caused only 7.5% of cases. Our findings are similar to the study done by Zorana M. Djordjevic et al, where gram negative organisms accounted for above 90% cases .[13] In a study by Su Young Chi el al from South Korea , gram positive pathogens (*S.aureus*) was found more common than gram negative in VAP, [14]. *Acinetobacter* species are common cause of VAP. Since this organism survives in moist and dry conditions for a prolonged period, it often leads to nosocomial outbreaks.[3]. In our study *Acinetobacter baumannii* accounted for 44.31% (n=77), followed by *Klebsiella pneumonia* 23.86%(n=42), *Pseudomonas aeruginosa, 11%(n=20), Escherichia coli 10.22%(n=19)i* and others like, *Enterococcus spp 4%* (n=7), *Staphylococcus aureus 3.8%(n=6)an Serratia marcescens* 2.2%(n=4). In a study from India by Yogesh Harde et al, the pathogenic profile was almost like our study, with *Acinatobacter baumannii* being most common[15]. Pathogenic spectrum of a study by Zorana M. Djordjevic et al , has minor variation in comparison to our study, in both studies *Acinatobacter baumannii* was found most common pathogen but in later study Pseudomonas sp. was second common pathogen while it was Klebseialla in our study [13]. Baraibar et a,l has reported only 8.1% of VAP due to *Acinatobacter baumannii* in her study which are far less in number than our study [16]. likewise in a study by Mandakini Pawar et al, the *Acinatobacter baumannii* was reported in 16% of cases of VAP.[17] in later study the gram positive pathogens accounted for 16% and no VAP was reported due to fungi ,these findings are quite in line with our findings. In our study 6(3.4%) isolates were *S.aureus* and all of them were sensitive to methicillin whereas in a study by Alok Gupta el al, 8 (23.52%) isolates were *S.aureus* however in later study all *S.aureus* isolates were resistant to methicillin while none of our isolate was resistant to methicillin [18].

The menace of rising antimicrobial resistance in hospital set up across the globe is making treatment failure more prevalent and is also acting as an additional risk factor for increased mortality among critically ill patients. VAP due to a multi drug resistant (MDR) organism is one of the serious complications. Studies have suggested that risk of death is more in patients who have VAP due to a multidrug-resistant pathogen.[2]. In our study highest drug resistance was observed in *Acinetobacter baumannii,* , that agrees with study by Zorana M. Djordjevi et al,[13].

CONCLUSION:

We conclude that gram staining and qualitative cultures have fair correlation , in predicting VAP. Quantitative cultures can aid in differentiating a true pathogen from colonizers. Timely and accurate diagnosis will guide the treating doctor in providing definitive therapy, thereby preventing the irrational use of antibiotics.

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