Original Research Article

Microbiological profile and the antimicrobial susceptibility pattern in endotracheal tube tip culture/ endotracheal aspirates of mechanically ventilated patients at a tertiary care hospital in Kashmir valley: A cross sectional study.

A B S T R A C T

Introduction:

The leading causes of morbidity and mortality among mechanically ventilated patients admitted to Intensive care units (ICU'S) are Infections particularly respiratory and bloodstream. Mechanical ventilation , while being life-saving comes with the danger of a hospital acquired respiratory infection. The kind and severity of such infections can be affected by a variety of factors including the patients age, the antimicrobial medication given and drug resistance pattern, type of ICU'S and the Extent of Initial illness with co morbidities etc. Antibiotics play a pivotal role in the therapy of such infections, therefore knowing the microbial profile and antimicrobial drug susceptibility pattern(AST) is essential for preventing the spread of such infections. As a result, the current study was undertaken.

Objective: The aim of study was to identify the bacterial isolates in the endotracheal tube tips/tracheal aspirates of mechanically ventilated patients and study their antimicrobial resistance pattern

Materials and Methods:

A descriptive cross-sectional study was conducted in the Department of Microbiology, SKIMS MCH Bemina for a period of 12 months from Sept 2019-April 2020 and July 2021- Dec 2021.Endotracheal tube aspirates/tip cultures of patients were processed by standard methods. Standard microbiological protocols and Clinical and Laboratory Standards Institute (CLSI) 2016 were followed for the isolation, identification and antimicrobial susceptibility testing (AST) of microorganisms.

Results:

A total of 63 samples of ET secretions were collected and proceeded for culture. Out of 63 samples, 53 (84.1%) were positive for bacterial growth. Among 53 positive cultures, 42(79.2%) were Gram negative bacteria , 3 (5.6%)

isolates were Gram Positive bacteria, and 2(3.78%) were yeast.The isolates in Gram negatives included *Acinetobacter baumannii 21* (50%),*Klebsiella pneumoniae 15 (35.72%) Pseudomonas aeruginosa 3(7.15%)*,E.coli 3 (7.15%) .In the 3(5.6%) Gram Positives all(100%) isolates were Staphyllococus aureus(MRSA) and 2(3.78%) were yeasts. Acinetobacter was seen resistant to all antibiotics except colistin, tigicycline and polymixin-b. Most of the Klebsiella isolates were found to be Multi Drug Resistant Strains and were sensitive to colistin, tigicycline, polymixin b and occasionally to Meropenam. Pseudomonas and E coli were sensitive to nearly all the recommended antibiotics and MRSA showed high resistance except to Vancomycin , Linezolid and gentamycin.

Conclusion:

The most common isolated bacteriaS from ETT tips and tracheal aspiration were the *Acinetobacter and Klebsiella sps* with high Resistance pattern to the widely used antibiotics.

Keywords: *Endotracheal aspirates, mechanically ventilated patients, antibiogram of bacterial isolate, AST, VAP*

Introduction:

Mechanical ventilation is a rescue procedure for patients with critical illness and respiratory failure. Research estimates that more than 300,000 patients receive mechanical ventilation in the US annually **[1].** These patients are at high risk for complications and poor outcomes, including Ventilator-associated pneumonia (VAP), Acute Respiratory Distress Syndrome (ARDS) ,and sepsis . Such complications can lead to longer duration of mechanical ventilation, longer stays in the ICU'S and hospitals, increased healthcare expenses, and increased risk of impairment and even death**. [1].**

Patients with risk characteristics, such as extremes of age, underlying disease, prolonged intubation and immunological impairment have greater infection rates. Prolong hospitalization associated with nosocomial infections results in higher rate of morbidity and mortality.**[2]** A Patient in the ICU has a 5 to 7 fold increased risk of acquiring nosocomial infection.**[2]**.Bacterial colonisation of the pharynx and upper airways is initial portal of entry into generally sterile lower respiratory tract**.** Therapeutic techniques like ET incubation aid colonisation which is further enhanced by the formation of microbial biofilm's around ET tubes and their dislodgement following suctions and repeated incubations leading ultimately to Ventilator Associated Pneumonia (VAP) **[3].**

Antibiotic resistance among these ICU infections is also a serious concern, owning to widespread use of broad spectrum antibiotics**.[4]**Due to spread in antimicrobial resistance, very few number of antibiotics are available for treating critically ill patients. The indiscriminate use of antibiotics further hastens the emergence of the multidrug resistant (MDR) superbugs, and creating a new challenge and a major hurdle in treating critically ill patients of ICUs**[5]**
Henceforth a good knowledge of frequent pathogens associated with mechanical ventilation and their antibiotic susceptibility profile is recommended. This will guide and assist the clinicians in making a empirical choice of Antibiotics and thus save precious time in the management of such critically ill patients.As a result , the goal of this study was to determine the Microbiological profile and antimicrobial susceptibility pattern of endotracheal tube tip culture/ endotracheal aspirates in mechanically ventilated patients at intensive care unit.

Materials and Methods:

This descriptive cross-sectional study was conducted in the Department of Microbiology, SKIMS MCH Bemina for a period of 12 months from Sept 2019-April 2020 and July 2021- Dec 2021. . The laboratory records of ETT tips and tracheal aspirate specimens were retrospectively reviewed as well.
63 patients who were mechanically ventilated for more than 48 hours were included in the study. All the patients were screened previously prior to Intubation for SARS COV-2 Infection through RT-PCR and included only confirmed Negative cases. Patients of all age groups regardless of gender, were included in this study. Patients who were incubated following SARS COV 2 infection were excluded from this study. Those patients who were intubated elsewhere and shifted to ICU were also excluded from the study. HIV seropositive patients, pregnant women, patients with clinical suspicion of pre-existing respiratory infections were also excluded from this study. The study was approved by the Institutional Ethical Committee.

Endotracheal secretions and Endotrcheal tube tips delivered to the microbiology lab were Gram stained before being and cultured by on MacConkey agar and blood agar and incubated overnight at 37◦C.
On Blood agar medium and MacConkey agar medium the organism's proliferation was detected. The organisms isolated were identified based using standard microbiological techniques such as colony characteristics, Gram's

stain, Biochemical reactions.Isolates identified as commensals or contaminants were excluded from further process. Antimicrobial susceptibility testing was performed on Mueller Hinton Agar (MHA) (Oxoid UK) by reading the zone of inhibition by Kirby Bauer disc diffusion method as per CLSI guidelines 2016**[6].** Antibiotics disks of HIMEDIA were used according to type of bacterial isolate.

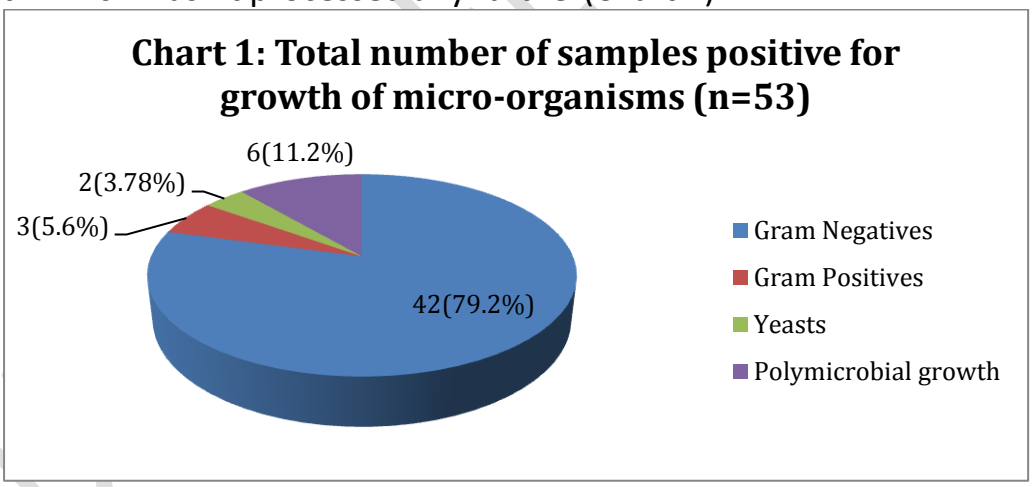
Results:

A total of 63 specimens fulfilling the inclusion criteria were proceeded for culture. Tracheal Aspirate 33 (52.38%) and ETT Tip 30 (47.6%).(Table 1)

Table1: Frequency of ETT tips and Tracheal Aspirates recieved

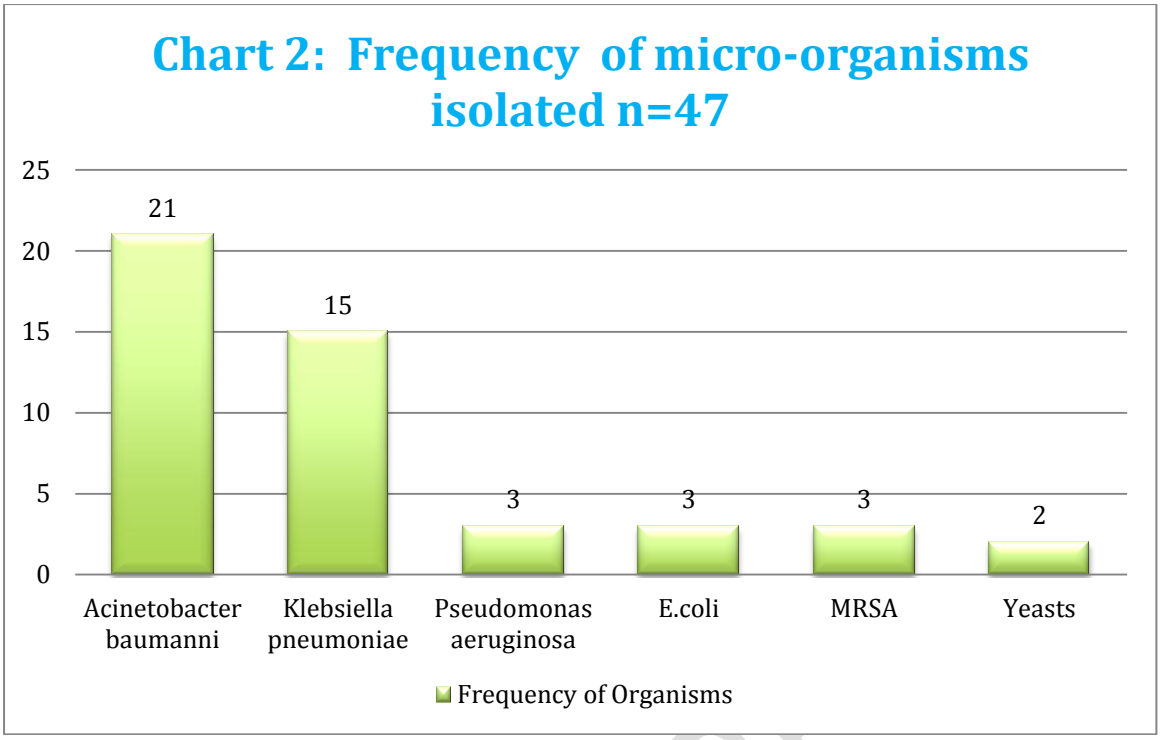
Total no of samples	Type of specimen	
63	Tracheal Aspirate's	ETT's
	33(52.38%)	30(47.6%)

Out of the total 63 samples received, 40 (63.50%) were males and 23(31.75%) were females.55(87.35%) patients among them were over 60 years of age. Among the 63 samples received, 53(84.1%) samples showed positive microbial growth , 10(15.8%) samples showed no growth of microorganisms .42(79.2%) were Gram negative bacteria , 3 (5.6%) isolates were Gram Positive bacteria, and 2 (3.78%) were yeast. 6(11.32%) samples showed polymicrobial growth which wasn't processed any further(Chart 1)



The isolates in Gram negatives were identified as *Acinetobacter baumannii 21* (50%),*Klebsiella pneumoniae 15 (35.72%) Pseudomonas aeruginosa 3(7.15%)*,E.coli 3 (7.15%) .In Gram Positives 3 (5.6% in all positive growth) all isolates were Staphyllococus aureus(MRSA) and 2(3.78% in all positive growth) were yeasts.

(Chart 2)



In this study we found that most of the Gram negative isolates were sensitive to Colistin, Tigicycline and Polymixin B and Meropenam but resistant to Aminoglycosides , Ampicillin, Amoxyclav, and most of the Cephalosporins .7 isolates (4 Kleb*siella pneumonae* and 3 *Acinetobacter baumannii)* were found to be completly resistant to Meropenam.In these 7 isolates 2 isolates of *Acinetobacter baumannii* were found to be resistant to Colistin ,Tigicycline as well in addition to Meropenam, out of which 1 isolate was resistant to polymixin B as well.

The 3 isolates of E.coli were found to be sensitive to nearly all recommended antibiotics except for Ceftriaxone.Pseudomonas 3 isolates were found to be resistant to Ceftriaxone, cefepime, ciprofloxacin, cotrimoxazole (Table 1)

The 3 Gram positive samples isolated were all *Methicillin Resistant Staphyllococcus aureus* and were sensitive only to Vancomycin, Clindamycin, Linezolid and Gentamycin but resistant to majority of other antibiotics including beta lactams. (Table 2)

(Table 2): Antibiotic susceptibility pattern of Gram-negative isolates(N=42)

s.no	Antibiotics	***Acinetobacter Sps n=21***	***Klebsiella Sps n=15***	***E.coli n=3***	***Pseudomonas sps n=3***
1	Ampicillin	**3**(14.2%)	**2**(13.3%)	**1**(33.3%)	**1**(33.3%)
2	Amoxyclav	2(9.5%)	3(20%)	2(66.6%)	1(33.3%)

3	Amikacin	3(14.2%)	6(40%)	3(100%)	2(66.6%)
4	Ceftriaxone	1(4.7%)	2(13.3%)	0(0%)	0(0%)
5	Ciprofloxacin	0(0%)	1(6.6%)	2(66.6%)	0(0%)
6	Cefixime	0(0%)	0(0%)	0(0%)	0(0%)
7	Cotrimoxazole	0(0%)	0(0%)	2(66.6%)	0(0%)
8	Imipenem	17(80.9%)	10(66.6%)	3(100%)	2(66.6%)
9	Meropenem	18(85.7%)	11(73.3%)	3(100%)	3(100%)
10	Tigecycline	19(90.4%)	15(100%)	3(100%)	3(100%)
11	Piperacillin/ Tazobacam	4(19.0%)	3(20%)	2(66.6%)	1(33.3%)
12	Colistin	19(90.4%)	15(100%)	3(100%)	3(100%)
13	Polymixin B	20(95.2%)	15(100%)	3(100%)	3(100%)

(Table 3): Antibiotic susceptibility pattern of Gram-positive isolates (n=3)

s.no	Antibiotics	*MRSA n=3*
1	Ciprofloxaxin	0(0%)
2	Gentamycin	3(100%)
3	Cefixime	1(33.3%)
4	Clindamycin	3(100%)
5	Erythromycin	2(66.6%)
6	Levofloxacin	0(0%)
7	Cefoxitin	0(0%)
8	Vancomycin	3(100%)
9	Linezolid	3(100%)
10	Ceftriaxone	0(0%)
10	Amoxcillin +Calvulanic Acid	1(33.3%)
11	Penicillin	0(0%)

Discussion and Conclusion:

Although life saving, mechanical ventilation carries an equal risk of acquiring Respiratory Tract infections including Ventilator Associated Pneumonia(VAP).These infections can increase the mortality and morbidity of

the patients admitted in ICU'S and may interfere with the normal recovery of the patients and raise healthcare costs.
Because of the worrisome rise in the antibiotic resistance which further jeopardizes the lives of these patients , it's critical to determine the exact cause and the drug susceptibility pattern among them.
In this study among the 63 samples 40(63.5%) were males and 23(31.75%) were females (Table 1) patients which is comparable to the findings of Sannathimmappa et al **[5]**. In our study majority of the patients 55(87.35%) belonged to age group greater than 60 years .Positive growth in the samples was 53(84.1%) which was nearly similar to Malik et al's(83%)**[8],** Deepti Chandra et al's**[9]** (72%) and Khayyam et al's**[10]** (87%) studies. In this study, the most frequent organisms isolated Gram- negative bacilli (79.2%) which was significantly higher as compared to Gram- positive bacteria as mentioned in each study conducted globally and comparable to studies conducted by Jani et al **[11]**(83%) but lesser as compared to work of Kaur et al **[7]** (93.7%) .In our study we found that most of the Gram negative isolates were sensitive to Colistin , Polymyxin B , Tigicycline followed by carbapenems (Imipenem),[Table 1]. Few multidrug resistant Acinetobacter sps and Klebsiella strains were also found [Table 1]. Resistance was frequently seen to Aminoglycosides , Ampicillin, Amoxyclav, and most of the Cephalosporins .[Table 1].
All the isolated *Staphylococcus aureus* were sensitive to Vancomycin ,Linezolid, Clindamycin and Gentamycin and and were MRSA isolates as all of them were resistant to cefoxitin .The Staphylococcus isolates were mostly resistant to majority of other recommended antibiotics. Similar results have been seen in most of the studies conducted worldwide such as Panda et al **[12]** who found high sensitivity of Acinetobacter for polymyxin B, Colistin and Intermediate sensitivity towards Meropenam and Imepenam. Our results also coincide with the studies conducted by Jani et al **[11]** & Jamil et al**[13]** who gave similar resitance patterns for Acinetobater sps. In a study research by Gupta et al**[14]**,Acinetobacter showed resistance to cephalosporins and aminoglycosides which is in accordance to our study. For Klebsiella sps similar sensitivity patterns were observed in many investigations conducted across globe such as Jani et al **[11] and** Chandra D et al **[15].** In our study Pseudomonas sps were found to be 100% sensitive to polymyxin B, Colistin, Tigicycline and meropenam followed by Imepenam and Amikacin (75%) each. Polymyxin B,Tigecycline, carbepenams and Colistin were 100% sensitive for *Eschericha Coli followed by* Piperacillin/ Tazobacam,cotrimoxazole and ciprofloxacin (75%) each. In our study, all isolates of *Staphylococcus aureus* were MRSA(100%) and susceptible to vancomycin and linezolid as found in studies conducted by Samal et al **[16].**

This study informs us about the various pathogens , that are frequent in various ICU'S as well as their common Antibiotic Sensitivity, so that appropriate empirical antibacterial therapy can be initiated for such patients who are already very sick . This study also raises concerns regarding the rise of antibiotic resistance among these patients. Because mechanically ventilated patients are at a greater risk of developing pneumonias each hospital must follow a specific protocol and adhere to stringent antibiotic strategy when treating these patients. Such infections can be avoided and better outcomes can be expected with a strong antibiotic policy and through knowledge of the culprit pathogens. More research on these themes should be conducted at each institution and a hospital based database should be created so that both patients and clinicians benefit, and there is no delay in the management of such patients who are at severe risk of acquiring infections.

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