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Research Article

MICROBIOLOGICAL PROFILE AND ANTIMICROBIAL SENSITIVITY PATTERN OF BACTERIAL ISOLATES FROM ENDOTRACHEAL TUBE ASPIRATES OF PATIENTS IN INTENSIVE CARE UNIT OF A TERTIARY CARE HOSPITAL

Aravind. R and Jeya. M

Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar – 608002

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ABSTRACT

Background: Many reports have appeared in the literature of an alarming increase in hospital infections, many of which are antibiotic resistant and presumably acquired after a patient's admission to the hospital. This is a serious indictment of present-day indiscriminate antibiotic therapy of our hospitals.Endotracheal intubation is a life-saving procedure, but it is associated with a high risk of acquiring respiratory infections.[1] Several factors like new mutations, selection of resistant strains and suboptimal infection control along with the use high level antibiotics influences the rapid spread of extensively drug resistant bugs in these intubated patients.. These infections are associated with significant rise in morbidity, mortality and health care cost.[2,3] It is very essential for the clinicians to be aware of local bacteriological flora and their susceptibility pattern to encourage rational use of antibiotics.

Aim: 1.To identify the microbiological profile of endotracheal tube aspirates received from ICU patients. 2. To study the antimicrobial sensitivity pattern of pathogenic isolates

Materials and Methods: This was a descriptive cross sectional analytic study of endotracheal tube aspirates of patients on mechanical ventilation done from January 2019 - May 2020 and sample size was calculated based on the N - master sample size software system. All the cases were randomly selected for the study. Endotracheal tube secretions are obtained from patients in ICU by using a suction tube. The collected samples were subjected to Gram s stain and culture by standard protocols. The pathogenic isolates were identified by standard biochemical reactions and subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method as per standard CLSI guidelines. Data entry was done in MS Excel.

Results: Out of 150 samples 145(96.7%) were culture positive.5 samples showed no growth. *Klebsiella spp* was the most common organism(32.0%) . *Pseudomonas aeruginosa* was the next most common organism (28.0%), In *Enterobacteriaceae* family *E. coli* (13.3%) *Enterobacter* (2.7) *Citrobacter spp.*(2.0%) *Protues spp.* (2.0), were the most commonly detected isolates. Amongst gram positive bacteria *Staphylococcus aureus* (12.7%) was commonly detected. Most isolates of *Pseudomonas* were multi drug resistant and showed sensitivity to Gentamicin and Ciprofloxacin. The Gram Negative bacilli were mostly sensitive to Amikacin and Piperacillin Tazobactam. The Gram Positive cocci were mostly sensitive to Linezolid and Gentamicin.

Conclusion: We conclude that Ventilator Associated Pneumonia in intubated patients is on the rise and has been continually associated within discriminate and irrational use of antibiotics which contribute to emergence of drug resistant strains. Knowledge of their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotic for therapeutic use and a better outcome.

INTRODUCTION

Many reports have appeared in the literature of an alarming increase in hospital infections, many of which are antibiotic resistant and presumably acquired after a patient's admission to the hospital. This is a serious indictment of present-day indiscriminate antibiotic therapy of our hospitals. Endotracheal intubation and mechanical ventilation are life-saving procedures needed in clinical conditions like sepsis, acute respiratoy distress syndrome and neurological dysfunctions. Patients on mechanical ventilation are at higher

Corresponding author:* **Aravind R
Department of General Surgery, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram-608002

risk of acquiring hospital aquired infection due to interplay of compromised host defence, virulent organism and presence of invasive device. These invasive therapeutic and diagnostic methods may lead to nosocomial infections particularly in Intensive Care Units and Critical Care Units.

Ventilator-associated pneumonia (VAP) is the second-most common hospital-acquired infection (HAI), accounting for 15% of HAIs and has the highest morbidity and mortality. According to a recent review by Morehead *et al.*[5] the incidence of ventilator associated pneumonia was 16.7% for patients incubated longer than 48hrs.Bypassing of the upper respiratory tract and imperfect functioning of mucociliary escalator (due to insertion of tube in trachea) impair the immune system. Besides, leakage of secretion around the tube and opening of the binding site for gram negative bacteria may have cause high rate of colonization. The Etiologic agents widely differ according to the population of patients in an intensive care unit, duration of hospital stay and prior antimicrobial therapy. The tracheostomized patients are colonized timely surveillance for local microbiological data is extremely important in predicting the type of resistance that may be present in the etiologic agent causing a clinical infection. There is a dire need of epidemiological studies for ventilated patients, to know the local microbial flora and their antibiotic profiles for rational use of antibiotics.[4,25] Hence, this study was undertaken to determine the prevalence of pathogenic bacteria in respiratory secretions of ventilated patients and their antibiotic susceptibility patterns in our tertiary care hospital. We also aimed to review available therapeutic options for the treatment of resistant organisms causing VAP, based on evidence from the literature. mostly by gram negative bacteria which may cause either tracheo bronchitis or broncho pneumonia the predominant Gram negative bacteria are *Pseudomonas aeruginosa, Acinetobacter spp. E.coli* and *Klebsiella pneumoniae*[3,6,7]

AIMS AND OBJECTIVES

1. To identify the microbiological profile of endotracheal tube aspirates received from ICU patients.
2. To study the antimicrobial sensitivity pattern of pathogenic isolates

MATERIAL AND METHODS

The study was conducted in the department Of Microbiology at Rajah Muthiah Medical college Hospital at Chidambaram. This was a Descriptive cross sectional study analysis of Endotracheal Aspirates of 150 intubated patients done from January 2019 - May 2020

Inclusion criteria

Endotracheal tube aspirates from Patients who are mechanically ventilated for various reasons in ICU for >48hrs

Exclusion criteria

Endotracheal tube aspirates from Patients who are mechanically ventilated for various reasons in ICU for <48hrs and Patient attendees who are not willing to participate in the study.

Endotracheal tube secretions were obtained from patients in ICU by using a suction tube. The suction tip and the secretions are collected in a sterile container. The collected samples were subjected to Gram's stain and were cultured in Sheep's Blood Agar, Mac Conkey, and Nutrient Agar for routine bacterial isolation following the standard operating procedures. Isolates were identified using conventional methods based on their reaction in biochemical tests.[8] Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method strictly adhering to the standards stipulated in CLSI 2018 guidelines.[9] The following antibiotics (Hi- Media Disc in µg) were tested for Antibiotic Susceptiblity testing - Amoxclav (AC 20/10mcg), Amikacin (AK 30mcg), Ampicilin (AMP 10mcg), Ceftazidime (CAZ 30mcg), Ciprofloxacin (CIP 5mcg), Chloramphenicol (C 30mcg) Clindamycin (CD 2mcg) Co-trimoxazole (COT 20/10mcg), Ceftriaxone (CTR 30mcg), Erythromycin (E 15mcg) Gentamicin (G 10mcg), Imipenem (I 10mcg), Levofloxacin (LE 5mcg), Linezolid (LZ 30mcg), Meropenem (MRP 10mcg), Oxacillin (OX 30mcg), Piperacillin plus Tazobactum (PTZ 100/10mcg), and Tetracycline (TE 15mcg)

RESULTS

A total of 150 samples were processed, out of these 107 (71.7%) were male and 43(28.3%) were female [Table1]. Maximum patients were in 45-60 yrs age group (Table2) Out of the 150 samples 145 (96.7%) were culture positive and 5 samples showed no growth. Among these 145 clinical isolates *Klebsiella spp* was the most common organism (32.0%) and *Pseudomonas aeruginosa* was the next most common organism (28.0%), In *Enterobacteriaceae* family *E.coli* (13.3%) *Enterobacter* (2.7) *Citrobacter spp.* (2.0%) *Protues spp.* (2.0), were the other detected isolates.Amongst gram positive bacteria only *Staphylococcus aureus* (12.7%) were commonly detected. *Acinetobacter baumannii* was about 4.0% among the isolated organisms.[Figure -1]

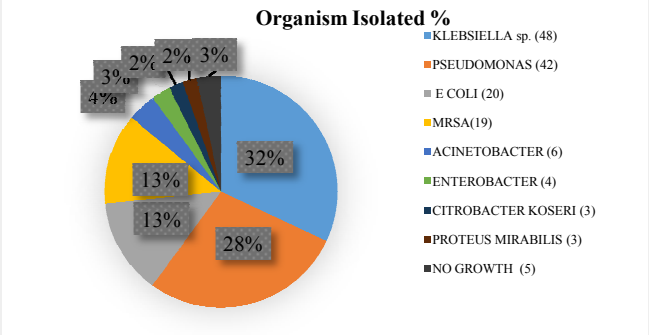

Figure-1 Percentage of Organisms Isolated (N=150)

Table1 Gender Distribution of Number of organism isolated

Gender	Organisms isolated (n=145)		No growth (n=5)	
Males	103	71%	4	80%
Females	42	29%	1	20%

Table 2 Age group distribution and Organism Isolated

Age groups	Organisms Isolated N=145		No growth N=5	
Children (< 10 years), n=10	9	6%	1	20%
Adolescents (10-18 years), n=5	5	4%	0	0%
Adults (18-60 years), n=111	108	74%	3	60%
Elderly (> 60 years), n=24	23	16%	1	20%

Table 3 Antibiotic sensitivity Pattern of the bacteria isolated (N=145, 5 = No Growth)

Drugs/ Organisms	***Klebsiella spp* N=48**		***Pseudomonas* N=42**		***E Coli***	***N=20***	***MRSA***	***N=19***	***Acinetobacter* N=6**		***Enterobacter* N=4**		***Citrobacter***	***N=3***	***Proteus***	***N=3***
Antibiotics	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
AC	25 (52%)	23 (48%)	-	-	11 (55%)	9 (45%)	-	-	-	-	2 (50%)	2 (50%)	1 (33%)	2 (67%)	2 (67%)	1 (33%)
AK	36 (75%)	12 (25%)	30 (71%)	12 (29%)	8 (40%)	12 (60%)			4 (67%)	2 (33%)	3 (75%)	1 (25%)	2 (67%)	1 (33%)	2 (67%)	1 (33%)
AMP	23 (48%)	25 (52%)			13 (65%)	7 (35%)	-	-	-	-	2 (50%)	2 (50%)	0 (0%)	3 (100%)	2 (67%)	1 (33%)
C	-	-	-	-	-	-	1 (5%)	18 (95%)	-	-	-	-	-	-	-	-
CD	-	-	-	-	-	-	11 (58%)	8 (42%)	-	-	-	-	-	-	-	-
CAZ	-	-	11 (26%)	31 (74%)	-	-	-	-	2 (33%)	4 (67%)		-	-	-	-	
CIP	29 (60%)	19 (40%)	36 (86%)	6 (14%)	14 (70%)	6 (30%)	8 (42%)	11 (58%)	3 (50%)	3 (50%)	3 (75%)	1 (25%)	2 (67%)	1 (33%)	0 (0%)	3 (100%)
COT	27 (47%)	31 (53%)	-	-	13 (65%)	7 (35%)	9 (47%)	10 (53%)	2 (33%)	4 (67%)	2 (50%)	2 (50%)	1 (33%)	2 (67%)	3 (100%)	0 (0%)
CTR	16 (33%)	32 (67%)	-	-	8 (40%)	12 (60%)	-	-	2 (33%)	4 (67%)	1 (25%)	3 (75%)	1 (33%)	2 (67%)	1 (33%)	2 (67%)
E	10 (53%)	9 (47%)	-	-	-	-		-	-	-	-	-	-	-	-	-
G	27 (56%)	21 (44%)	30 (71%)	12 (29%)	12 (60%)	8 (40%)	13 (68%)	6 (32%)	3 (50%)	3 (50%)	3 (75%)	1 (25%)	0 (0%)	3 (100%)	2 (67%)	1 (33%)
I	27 (56%)	21 (44%)	23 (55%)	19 (45%)	13 (65%)	7 (35%)	0 (0%)	0 (0%)	3 (50%)	3 (50%)	2 (50%)	2 (50%)	2 (67%)	1 (33%)	1 (33%)	2 (67%)
LE	-	-	23 (55%)	19 (45%)	-	-	-	-	-	-	-	-	-	-	-	-
LZ	-	-	-	-	-	-	16 (84%)	3 (16%)	-	-	-	-	-	-	-	-
MRP	-	-	23 (55%)	19 (45%)		-	-	-	-	-	-	-	-	-	-	-
OX	-	-	-	-	-	-	0 (0%)	19 (100%	-	-	-	-	-	-	-	-
PTZ	30 (63%)	18 (38%)	34 (81%)	8 (19%)	15 (75%)	5 (25%)	-	-	6 (100%)	0 (0%)	4 (100%	0 (0%)	3 (100%)	0 (0%)	2 (67%)	1 (33%)
TE	27 (56%)	21 (44%)	-	-	10 (50%)	10 (50%)	13 (68%)	6 (32%)	3 (50%)	3 50%)	1 (25%)	3 (75%)	1 (33%)	2 (67%)	1 (33%)	2 (67%)

(Sensitive = S ; Resistant = R)

DISCUSSION

Ventilator associated pneumonia (VAP) is one of the most frequently encountered hospital acquired infection in the ICU. The microbial profile of pathogens causing VAP may differ between hospitals and ICUs.[1,3] Our study showed 97.7% growth from endotracheal aspirates which is concurrent with the Gupta *et al*.[10] Out of 145 isolates 87% were Gram negative Bacilli and only 13% were Gram positive cocci.

The common pathogens were *Klebsiella pneumoniae, Pseudomonas aeruginosa, E.coli*, and Gram positive cocci *Staphylococcus aureus. Klebsiella* spp.(34.28%) was the most common isolate followed by *Pseudomonas spp*.(20%) in Rathod *et al* study[11]. There have been many studies done in the Indian subcontinent that have identified the aetiological agents of VAP as well as the susceptibility pattern which has been showing increasing resistance. In our study Gram negative enteric aerobic bacteria were isolated from most of the patients, most common being *Klebsiella* species (32.35%), which was similar to Chandra *et al* study. [12]

In our study most of the organsims showed more than 50% of resistance to Cotrimoxazole and Ceftriaxone antibiotics. *Pseudomonas* showed alarming (45% & 75%) resistance to Ceftazidime and Carbapenams. *Klebsiella spp* showed more than 50% resistance to Ampicillin, Cotrimoxazole and Ceftriaxone. *E.coli* showed more than 50% resistance to Amikacin, Ceftriaxone and Tetracycline.

An increase in resistance was shown by *Pseudomonas* aeruginosa for Ceftazidime. Among the *Enterobacteriaceae* family 67% of *Citrobacter spp* and 54% of *Klebsiella spp*. were found to be ESBL producers which was detected by Double Disc Synergy Test. The emergence of Extended Spectrum Beta-Lactamase (ESBLs) necessitated the increased use of Carbapenems, but nowadays Carbapenems also showing the emergence of multi drug resistant. The ESBL producing isolates were only 60-70% sensitive to Imipenem in this study. VAP due to Gram positive bacteria is an another global problem, this study showed all the gram positive batceria were *Staphylococcus aureus* 19 (100%) isolates, of which all were Methicillin resistant *S.aureus* (MRSA). Most of the gram positive organisms were sensitive to Linezolid as in Rathod *et al*[11].

Resistances to Beta-Lactam class of antibiotics are a common occurrence and pan-drug-resistant strains are beginning to emerge. In our study, *Klebsiella spp* was the most common organism causing VAP, and Amikacin was the most sensitive drug which was concurrent with of Koirala *et al*. [16]

In our study, 19 (45%) of *Pseudomonas spps* were carbapenamase producing strains. Similar observations were made by Dey *et al* (50%) [18] and Goel *et al* (47.06%) [19]. More than 70% of *Pseudomonas* were sensitive to Amikacin, Gentamicin, Ciprofloxacin and Piperacillin-Tazobactam. *Klebsiella* and *E.coli* were sensitive to Ciprofloxacin and Piperacillin - Tazobactam. *Acinetobacter* and C*itrobacter* were 100% sensitive to Piperacillin-Tazobactam. Enterobacter was (75-100%) sensitive to Amikacin, Ciprofloxacin, Gentamicin and Piperacillin-Tazobactam.
Klebsiella and *Acinetobacter* were mostly sensitive (65-70%) to Amikacin and Gentamicin. Ampicillin mostly sensitive to Ecoli (65%). *Klebsiella, Pseudomonas*, and *Enterobacter* mostly sensitive to Quinolones (60-80%). Chloramphenoicol is 95% resistant to *Staphylococcus aureus.* Oxacillin is 100% resistant to *Staphylococcus aureus* and all strains were MRSA. Piperacillin Tazobactum was sensitive to *Pseudomonas, E.coli, Acinetobacter* and *Enterobacter. Acinetobacter* was sensitive to Piperacillin-Tazobactam in 75% of instances.

Quinolones was resistant for *Proteus*. Linezolid was mostly sensitive to *Staphylococcus aureus* as in Golia S *et al*. [15] in which they found that all gram-positive cocci were sensitive to Linezolid. Cephalospsorins was mostly resistant to *Pseudomonas* and *Acinetobacter*. ESBL was produced by these organisms. Carbapenems was only 50 - 60 % sensitive to Gram Negative Organisms. The broad-spectrum antibiotics showed sensitivity to an extent, but there was an alarming rise in the resistance to drugs such as Carbapenems. This was in consistency with the pattern shown in the research by Gupta *et al*[10]

In our study there was increased emergence of multi drug resistant (MDR) organisms like *Acinetobacter spp, Klebsiella spp and Pseudomonas spp* as potential pathogens from endotracheal aspirates especially from ICUs. Several studies have also reported the same bacterial flora. [19-24]

CONCLUSION

We conclude that VAP in mechanically ventilated patients is on the rise and has been continually associated with indiscriminate and irrational use of antibiotics which contribute to emergence of drug resistant strains. Knowledge of their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotic for therapeutic use and a better outcome. A multi disciplinary approach, coordinated participation of microbiologist, clinician, nursing personal and hospital infection control team is necessary for the management of this nosocomial infection. Combined approaches of rational antibiotic therapy might be beneficial to combat high antibiotic resistance in our setup.

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