**Saudi Journal of Medical and Pharmaceutical Sciences** **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

# Isolation, Identification and Antimicrobial Sensitivity Pattern of Bacterial Isolates from Tracheal Aspirate of ICU Patients of Tertiary Care Hospital In Western, Rajasthan, India

**Sony Singh[1*], R. S. Parihar[2], Gaurav Sapra[3], P. K. Khatri[4]**
[1]Post Graduate Resident, Department of Microbiology, Dr. S. N. Medical College, Jodhpur, Rajasthan, India
[2]Professor, Department of Microbiology, Dr. S. N. Medical College, Jodhpur, Rajasthan, India
[3]Senior Resident, Department of Otorhinolaryngology, Dr. S. N. Medical College, Jodhpur, Rajasthan, India
[4]Professor and Head, Department of Microbiology, Dr. S. N. Medical College, Jodhpur, Rajasthan, India

**Original Research Article**

***Corresponding author**
*Sony Singh*

**Article History**
*Received: 14.02.2018*
*Accepted: 24.02.2018*
*Published: 28.02.2018*

**DOI:**
10.21276/sjmps.2018.4.2.16

**Abstract:** To analyze the aerobic bacteria isolated from endotracheal secretions of ventilated patients and to evaluate the antibiotic sensitivity pattern and Multi drug resistance of those isolates, present study was conducted in M.D.M. Hospital, Dr. S. N. Medical College, Jodhpur, and Rajasthan, India. Endotracheal secretions received during the study period from Jan 2016 to April 2016 were processed and all the pathogenic isolates were identified as per the standard guideline. Antibiotic sensitivity was performed for the identified pathogens according to CLSI standards. Clinical condition of the ventilated and tracheotomised patients was recorded. A total of 160 Endotracheal isolates was processed and 114(71.25) of the aspirates was showing growth. The most frequently isolated pathogens were Klebsiella sp.(37.7%), Pseudomonas(18.4%), Esch. coli(15.7) and Acinetobacter sp.(10.5%) followed by Citrobactor sp., Staph. aureus, Proteus sp.. All the organisms more or less showed both sensitive and resistance pattern but Acinetobacter sp was resistant to all the antibiotics except the antibiotic imepenem and ciprofloxacin. This study presents the most common microorganisms colonized and their antibiotic resistance pattern. The variability of flora and the potential need to adjust antibiotic coverage based on culture data suggest that surveillance tracheal aspirates are important during exacerbations.
**Keywords:** Endotracheal aspirate, Ventilated patients, Antibiotic sensitivity, Drug resistance.

## INTRODUCTION

An intensive care unit (ICU) is a special department of a hospital or health care facility that provides intensive care medicine. Intensive Care Units cater to patients with the most severe and life-threatening illnesses and injuries; that require constant, close monitoring and support from specialist equipment and medication in order to maintain normal bodily functions [1].The intensive care unit (ICU) often is called the epicenter of infections due to its extremely vulnerable population of reduced host defenses deregulating the immune responses and increased risk of becoming infected through multiple procedures. The use of invasive devices namely intubation, mechanical ventilation, vascular access etc. distorts the anatomical integrity and protective barriers of patients. Administration of several drugs (sedatives, muscle relaxants) also predispose for infections by reducing the cough and swallow reflexes or by distorting the normal non-pathogenic bacterial flora [2].

The international study of infection in ICU which was conducted in 2007, and involved with 1265 ICUs from 75 countries, demonstrated that patients who had longer ICU stays had higher rates of infection, especially infections due to resistant Staphylococci, Acinetobacter, Pseudomonas species and Candida species [3].

Pathogenic mechanisms of infection in ICU patients have been progressively elucidated in the last decade. They are dominated by two processes: colonization of the oropharynx and its contiguous structures, such as sinuses, dental plaque, trachea and gastric reservoir, and aspiration of the contaminated secretions into the lower airway [4]. Both orotracheal and nasotracheal tubes bypass natural host defenses, permit leakage of bacteria and secretions around the cuff into the trachea, damage the ciliated epithelium of the trachea and reduce bacterial clearance [5].

Endotracheal tube biofilm also plays an important role as reservoir for infecting microorganisms. Furthermore, fragments of biofilm may be dislodged and carried further into the lung by ventilator gas flow, and bacteria encased in this biofilm are relatively resistant to the action of antimicrobials and host defenses [6]. Despite advances in patient care, these changing floras complicate therapy by acquiring drug resistance and altering their sensitivity pattern [7].

Therefore updated knowledge of local epidemiological and susceptibility profile is recommended for guiding the clinicians regarding empirical choice of antibiotics and has become mandatory along with adequate clinical diagnosis and bacterial confirmation [8]. Hence the aim and objective of the study is to retrospectively analyze the spectrum of aerobic bacteria isolated from endotracheal aspirates of tracheostomised patients and Patients on Mechanical ventilation, to evaluate the antibiotic sensitivity pattern and Multi drug resistance of those isolates.

## MATERIALS AND METHODS

This is a cross sectional study, it was carried during a period of 4 months from January to April 2016 at M.D.M Hospital, DR. S. N. Medical college, Department of microbiology, Jodhpur, Rajasthan(India). Patient admitted to ICU and CCU, who was on mechanical ventilation, was included in this study. From the ICU, 160 tracheal aspirates were studied. The following data were collected from the patients enrolled in the study: name, age, gender, underlying illness, date of admission, date of endotracheal insertion and endotracheal aspirate collection, duration of hospitalization and mechanical ventilation, date of reintubation, duration and details of prior antibiotic therapy, clinical diagnosis and X-Ray report. Other relevant data were recorded from microbiological studies in laboratory.

ICU doctor collected ETA (endotracheal aspirate) every time. Hand was washed aseptically and sterile gloves were used in each sample collection. After collection of endotracheal aspirate from patients in a sterile tube, samples were immediately (within 1 hours) transferred to the microbiology laboratory for Gram staining and culture.

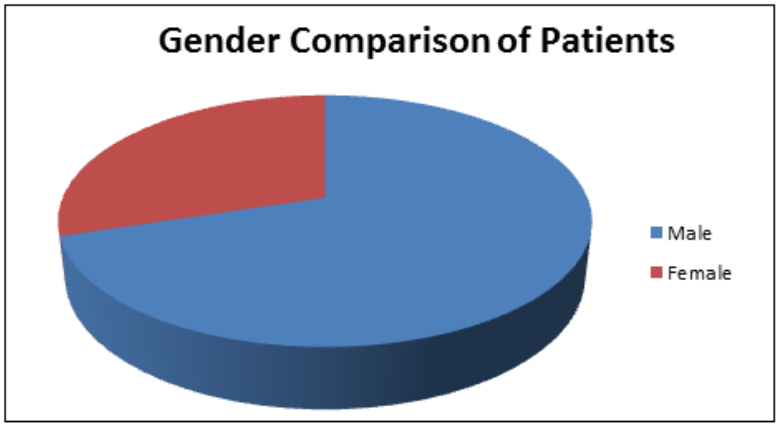
Using an inoculating loop, transfers specimens to glass slides and smears prepared, directly from the most purulent portion of the specimen. The Smears were Gram stained and examined. The smears were examined at low power field and under oil immersion (magnification × 100) for the quantity of squamous epithelial cells, polymorphonuclear neutrophils (PMN) and for the presence of bacteria and immediately plated on the blood agar and MacConkey agar by semi quantitative method and incubated aerobically overnight at 37°C. Organisms were identified as commensal or pathogen as per protocol. Single or mixed growth (two or more than two isolates per specimen) isolated from all the eligible single and consecutive samples were identified by observing the colony characteristic on the blood, MacConkey agar plate, and biochemical reactions using standard microbiological methods. Isolates from repeat culture of previously recruited patients and isolates identified as commensal or contaminants were excluded.

Various biochemical tests were performed in order to measure the viability of biochemical behavior among the strains. According to the manual of methods for general bacteriology by American society of Microbiology, several biochemical tests i.e. Triple Sugar Iron (TSI) Test, Citrate Utilization Test, Motility Indole, Urease (MIU) Test, Oxidase Test, Catalase Test, and Coagulase Test were performed to identify bacteria of interest. After isolation and identification, sensitivity of selected organisms against different antibiotics was studied according to CLSI guidelines. Antibiotic discs used for gram negative organisms were Imipenem, Amikacin, Cefuroxime, Cefexime, Ceftazidime, Ciprofloxacin, Ofloxacin, Gentamycin, Levofloxacin, tobramycin

For gram positive bacteria antibiotic disk of Linezolid, Vancomycin, Ampicillin, Amoxyclav, Imipenem, Amikacin, Cefuroxime, Cefexime, Ciprofloxacin, Ofloxacin, Levofloxacin were used. (Clinical and Laboratory Standards Institute (CLSI) 2015)

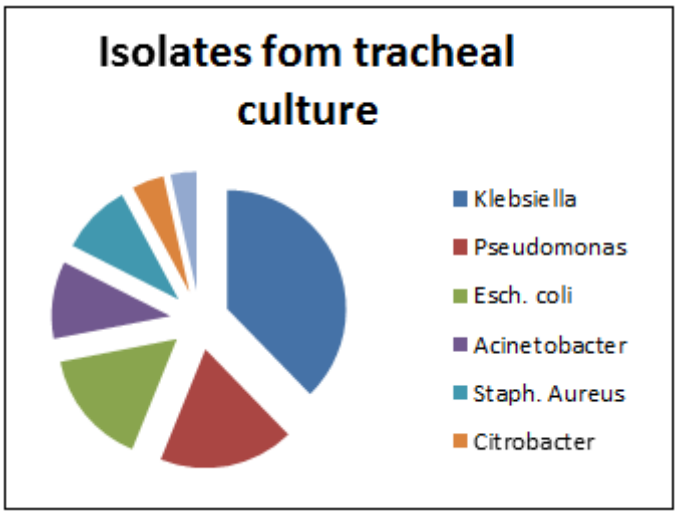
## RESULTS

During the four month of study periods, 160 tracheal aspirates from ICU, CCU admitted patients were Enrolled.114 (71.25%) of 160 the aspirates were showing growth. Among them 80(70.17%) were male and 34(29.82%) were female. Generally most of the patients are admitted with diseases like pulmonary failure, stroke, kidney failure or asthmatic attack. Their ages were between 40 to 70 year.



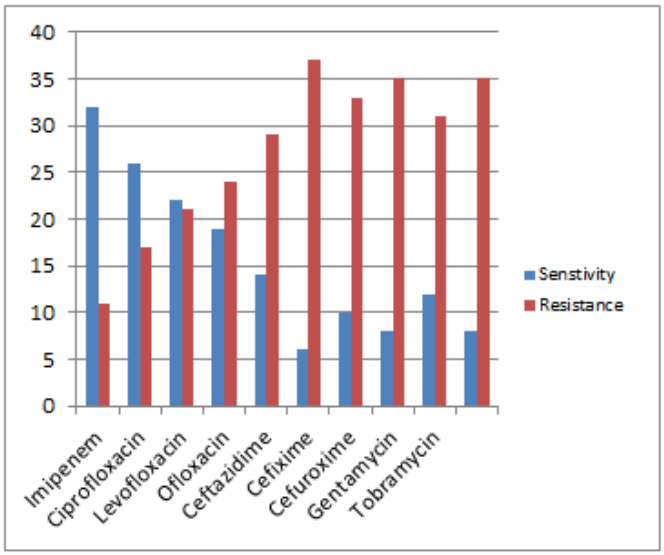

**Graph-1: Gender comparison of patients**

The most common pathogen was Klebsiella sp. 43(37.7%) followed by Pseudomonas aeruginosa 21(18.4%), E. coli 18(15.7%), Acinetobactor sp. 12(10.5%) and 17.6% other organism was isolate these were, Citrobactor sp., Staph. aureus ,Proteus sp.. 14 types of antibiotics disks were uses for sensitivity.

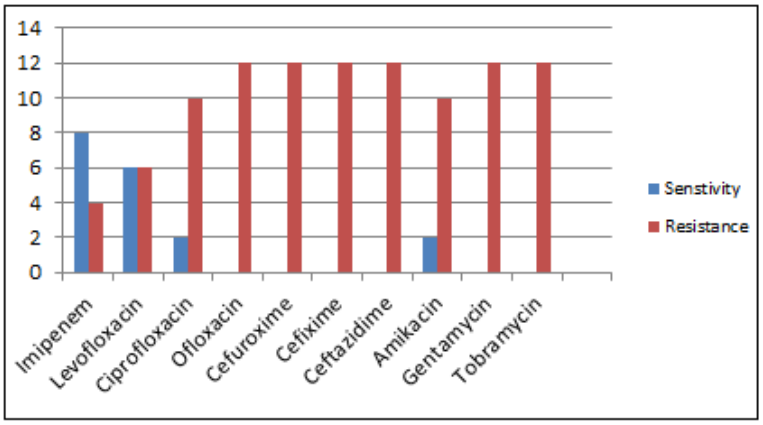



**Graph-2: Isolates from tracheal culture**

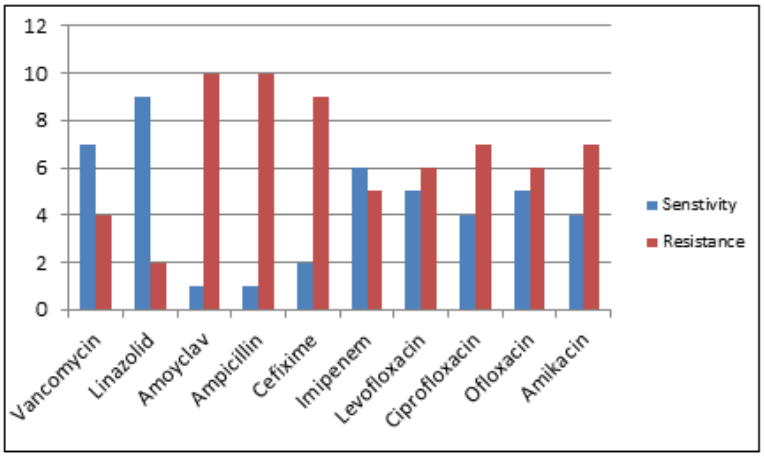
The most sensitive antibiotics was Imipenem shown (74.42%) sensitivity followed by ciprofloxacin and Levofloxacin in gram negetive organisms Among all age, the male patients are found to be higher in number. From the findings it was observed that the antibiotic cephalosporin (Cefexime, Cefuroxime, and Ceftazidime) is most resistant to the organism Klebsiella sp. and Acinetobactor. Among all applied antibiotics except Imipenem and Ciprofloxacin were resistant for Acinetobactor. This is very alarming for the patients infected with Acinetobacter sp.




**Graph-3: Patients Infected by Klebsiella sp and its Antibiotic Sensitivity Pattern**




**Graph-4: Patients Infected by Acinetobacter sp. and its Antibiotic Sensitivity Pattern**




**Graph-5: Patients Infected by Staph. Aureus and its Antibiotic Sensitivity Pattern**

In average, all the antibiotics are sensitive for the infected patients with pseudomonas sp. The antibiotics Imipenem, Flouroquinolones has good sensitivity pattern followed by Aminoglycosides than Cephalosporin.

In case of gram positive organism antibiotics that are most sensitive are Linezolid, Vancomycin, followed by Imipenem, Fluoroquinolones and Amikacin whereas Amoxyclav, Ampicillin, Cephalosporin showed increased resistance.

## DISCUSSION

Infections are among the most important and the leading cause of mortality and morbidity in ICU. Endo-tracheal tubes are susceptible to infection and therefore it is important to be aware of the relevant factors and responsible organisms to take prompt action. The findings of this study would be helpful in selection of appropriate antibiotics. In this study, all the positive colonies obtained from the ET or tracheostomy cases were considered. The study by Simoni *et al*. [9] showed that 100% of samples from airway prosthesis are positive in culture; however, other studies have reported a positive culture rate between 0% and 33% in obtained samples from airway tubes. Cardinosa *et al*. [10] have reported a positive culture result in 89% of their samples and in our study, 82%repoted a positive culture.

In our study, Gram negative bacteria were the most common isolated organisms including Klebsiella and Pseudomonas aeruginosa which is in the same line with the study by N. Shanmuga Vadivoo *et al*. [11]. However, in Cardinosa *et al*. study, during the first 24 hours of admission, Gram positive bacteria were the most common cause of infection and this could be as a result of the time of sampling which in Cardinosa study was within the first 24 hours of admission but in our study the sample was done after the first 24 hours i.e. following intubation.

In contrast, Rello *et al*. [12] in their study have demonstrated that P. aeruginosa is the most common causative organism for infection of ET and this could be the result of large number of patients with chronic obstructive pulmonary disease (COPD) and long time of intubation and even a previous history of antibiotic therapy. It should be noted that although there are minor variations in relative frequency of obtained organisms, different studies have reported varying sources of isolations as in our study we did report colonization of organisms into the ET tube while other studies may have considered ET-related infections in their study analysis.

In our study most gram negative organisms were sensitive to Imipenem and Ciprofloxacin and the maximum resistance was shown with Cefexime, Gentamycin and Amikacin. Acinetobacter was found to be the most resistant gram negative organism. In a study by Nazal –Matunog [13], most of the Gram negative bacteria were sensitive to ciprofloxacin compared with 3% resistant cases; there was Amikacin resistance in 9.7% of the cases with the highest resistance to Cefamandole (57%), Cefotaxime (50%), and Tobramycin (50%). N. Shanmuga Vadivoo, et al. revealed that the most resistant Gram negative isolate is Acinetobacter with highest resistance to Ceftazidime and Gentamycin. The concern which is similarly subject to all the studies is the increasing prevalence of resistant Acinetobacter in critically ill patients which may lead to significant mortality as well as huge widespread to other wards of the hospital.

In a study by Simoni et al. all of the included samples consisted of more than 1 pathogen while in our study each culture has only one pathogen. However, in the same line with our results, all of the organisms in their study were aerobics including S. aureus and P. aeruginosa.

One of the limitations of this study was the fact that although antibiotic resistance pattern is important to intensive care physician, this is also heavily influenced by the antibiotic usage pattern in the study patients prior to obtaining the sample and also overall antibiotic usage pattern in the study institution. In this regard, the utility of the reported results to other centers is limited.

In conclusion, this study presents the most common microorganisms colonized from endotracheal tube of hospitalized patients and their pattern of antibiotic resistance. As our study showed, Klebsiella is the most common microorganism isolated from endotracheal tube. Gentamycin was also the most prevalent antibiotic revealing resistant pattern. Moreover, most of the microorganisms were sensitive to Imipenem and Ciprofloxacin.

## CONCLUSION

Gram negative organisms mostly susceptible to Carbapenem and Fluoroquinolones group of antibiotics which were the predominant isolates in our critical care setup. There is an alarmingly high rate of resistance to cephalosporin's, beta-lactamase inhibitors, and Aminoglycoside. Although Imipenem is still sensitive against most pathogens but resistance is rising. Judicious use of older and newer antimicrobial agents is essential to prevent the emergence of multi drug resistant bacteria in the ICU. A local antibiogram for each hospital, based on bacteriological patterns and susceptibilities is essential to initiate empiric therapy, to prevent poor outcomes and help in framing the appropriate institutional antibiotic policy.

## REFFERENCE

1. Hoque, L., Kamal, S. M., & Ahmed, Z. (2013). Isolation, identification and antimicrobial sensitivity pattern of bacterial isolates from tracheal aspirate of ICU patients of central Dhaka, Bandladesh. *Int j res appl nat soc sci*, *1*(7), 11-6.
2. Dewan, E., Biswas, D., Kakati, B., Verma, S. K., Kotwal, A., & Oberoi, A. (2015). Epidemiological and mycological characteristics of candidemia in patients with hematological malignancies attending a tertiary-care center in India. *Hematology/oncology and stem cell therapy*, *8*(3), 99-105.
3. Shulman, L., & Ost, D. (2005). Managing infection in the critical care unit: how can infection control

make the ICU safe?. *Critical care clinics*, *21*(1), 111-128.

4. Torres, A., El-Ebiary, M., & Raño, A. (1999). Respiratory infectious complications in the intensive care unit. *Clinics in chest medicine*, *20*(2), 287-301.
5. Craven, D. E., Kunches, L. M., Kilinsky, V., Lichtenberg, D. A., Make, B. J., & McCabe, W. R. (1986). Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. *The American review of respiratory disease*, *133*(5), 792-796.
6. Adair, C. G., Gorman, S. P., Feron, B. M., Byers, L. M., Jones, D. S., Goldsmith, C. E., ... & Webb, C. H. (1999). Implications of endotracheal tube biofilm for ventilator-associated pneumonia. *Intensive care medicine*, *25*(10), 1072-1076.
7. Kaul, S., Brahmadathan, K. N., Jagannati, M., Sudarsanam, T. D., Pitchamuthu, K., Abraham, O. C., & John, G. (2007). One year trends in the gram-negative bacterial antibiotic susceptibility patterns in a medical intensive care unit in South India. *Indian journal of medical microbiology*, *25*(3), 230.
8. Silva Júnior, J. M. D., Rezende, E., Guimarães, T., Campos, E. V. D., Magno, L. A., Consorti, L., ... & Mendonça, J. S. D. (2007). Epidemiological and microbiological analysis of ventilator-associated pneumonia patients in a public teaching hospital. *Brazilian journal of infectious diseases*, *11*(5), 482-488.
9. Simoni, P., & Wiatrak, B. J. (2004). Microbiology of stents in laryngotracheal reconstruction. *The Laryngoscope*, *114*(2), 364-367.
10. Cendrero, J. A. C., Solé-Violán, J., Benitez, A. B., Catalán, J. N., Fernández, J. A., Santana, P. S., & de Castro, F. R. (1999). Role of different routes of tracheal colonization in the development of pneumonia in patients receiving mechanical ventilation. *Chest*, *116*(2), 462-470.
11. Vadivoo, N. S., Santharam, P., Sudha, K., Kalaiselvi, G., Padmavathi, B. K., Usha, B., ... & Jaiswal, N. K. (2014). Dynamic bacterial profile of endotracheal aspirates and its sensitivity pattern-a cause of concern. *International Journal of Current Research and Review*, *6*(10), 112.
12. Rello, J., Ausina, V., Ricart, M., Puzo, C., Quintana, E., Net, A., & Prats, G. (1994). Risk factors for infection byPseudomonas aeruginosa in patients with ventilator-associated pneumonia. *Intensive care medicine*, *20*(3), 193-198.
13. Nazal-Matunog, C. L., Rodriguez, L., Mercy, G., Alberto, L., Adrian, C. P., & Remedios, C. (1993). Nosocomial Pneumonia in Mechanically Ventilated Patients. *Phil J Microbiol Infect Dis*, *22*, 11-16.