

## Antibiotic Susceptibility Pattern of the Potential Pathogens of Ventilator Associated Pneumonia in the Endotracheal Tubes of Critically Ill Patients

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### Abstract

An endotracheal (ET) tube offers surface for potential pathogens to attach and produce biofilm. These potential pathogens are typically hospital flora with a broad range of antibiotic resistance. The study aimed to characterize the biofilm-producing flora in ET tube of critically ill patients. Following extubation, ET tubes were retrieved aseptically from 100 different patients and promptly transported in a sterile ziplock bag. Each ET tube was cut into three different sections; inner lumen was scraped out and inoculated on Blood agar, MacConkey agar, and Chocolate agar. Colonies produced on media were tested for antibiotic susceptibility testing by applying disc diffusion and Colistin minimum inhibitory concentration (MIC). Out of 100 ET tubes, monomicrobial growth was observed in 62, polymicrobial growth in 14, and no growth in 24 specimens. A total of 93 potential pathogens were isolated including 25 (26.89%) *Acinetobacter species*, 23 (24.73%) *Klebsiella species*, 15 (16.12%) *Pseudomonas species*, 13 (13.98%) *E. coli*, 6 (6.45%), *Staphylococcus aureus*, 4 (4.3%) Coagulase Negative *Staphylococcus species* (CoNS), 2 (2.15%) *Proteus species*, 1 (1.07%) *Enterobacter species* and 4 (4.3%) *Candida species*. Imipenem and Colistin proved to be among the most successful antibiotics against gram negative isolates. Only 1 out of 25 *Acinetobacter species* was resistant to Colistin. Methicillin resistance emerged in two *S. aureus* and three CoNS strains. Microorganisms usually adhere themselves to the surface of ET tubes. They may act as potential pathogens for the onset of Ventilator Associated Pneumonia (VAP) and are resistant to commonly administered antibiotics in hospitals. A technique to reduce or prevent the risk of biofilm development is crucial.

### Keywords

Critical Care Unit, Multi-drug resistant, Minimal Inhibitory Concentration, Mechanical Ventilation, Nosocomial infections.

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## INTRODUCTION

A biofilm consists of microbial cells adhered to each other or to the surface of an organic or inorganic structure, along with self produced matrix consists of extracellular biopolymers that provides protection from environmental stressors (1). Biofilm has gained significant importance, especially in the intensive care units (ICUs) of hospitals, when found on indwelling medical devices and implants such as catheters, mechanical heart valves, pacemakers, prosthetic joints and contact lenses. Most of the common pathogenic bacteria, as well as yeasts, have the ability to form biofilms on indwelling medical devices. Almost 60-80% of infections have been reported to be associated with microbial biofilms while more than 50% of nosocomial infections around the globe are reported to be associated with biofilms developed by bacterial pathogens on the medical devices (2). Among the indwelling medical devices used in critical care units, the Endotracheal Tube (ET) is an important risk factor for the onset of Ventilator-associated pneumonia (VAP). It is commonly acknowledged that VAP is caused by two mechanisms, namely aspiration of oral-gastric contents and the formation of a microbial biofilm on the ET Tube (3).

An ET tube is flexible, disposable device used to facilitate pulmonary air

exchange and relieve pulmonary distress in patients through mechanical ventilation. Intubated patients are more vulnerable to VAP because the hydrophobic surface of the polyvinyl chloride (PVC)-ET tube provides an ideal condition which allows pathogens to cling and thrive, resulting in bacterial colonization of the entire internal surface (4,5). Previous studies have demonstrated such biofilm formation on the interior surface of ET tubes inserted into critically ill patients. Furthermore, the dislodgement of this biofilm, especially during mechanical ventilation, can introduce bacteria directly into the lower respiratory tract, particularly the lungs, and may contribute to the pathogenesis of VAP (6,7).

The ET tubes play a role as a reservoir for potential VAP pathogens by providing a surface to which they can adhere and develop biofilms (8). Biofilm formation by polymicrobial flora, with well-organized antibiotic-resistant structures detectable along the inner lumen of the ET tubes, can develop rapidly within twenty-four hours after intubation (8,9,10). Previous reports indicated that 70% of patients with VAP had identical pathogens present within the biofilm of the ET tubes, as encountered in the clinical specimens collected from the lung (11). This suggests that the biofilm represents a significant and persistent source of pathogenic bacteria. The current prospective experimental study was

designed to isolate, identify and characterize the antibiogram of potential pathogenic flora associated with VAP, specifically within the biofilm formed within the inner lumen of the ET tubes of critically ill patients who were admitted in the ICUs of a tertiary care teaching hospital.

## **MATERIALS AND METHODS**

The current study was conducted in the Bacteriology laboratory of Microbiology department at Dr. S.N. Medical College & Associated hospitals, Jodhpur. Ethical Committee: A written permission was obtained from Ethical Committee of the Institute prior to conduct the study (Letter No. 1/Acad/MC/JU/18/5170).

### **Sample Collection**

A total of 100 ET tubes were collected from critically ill patients admitted to ICUs, and intubated for more than 48 hours. Only one ET tube was collected from each individual patient. ET tube samples were collected after obtaining written informed consent from the relative of patients in the presence of treating physician or nursing staff. ET tubes were collected and promptly transferred in a sterile ziplock plastic bag to Microbiology department without any delay.

### **Sample Preparation**

ET tubes were cut into 3 sections from distinct regions, each measuring 1 cm in length. The inner lumen of each 1 cm cut section of ET tube were scrapped off

using a sterile scrapper to collect the biofilm (if formed) and transferred into test tube consisting 1 mL of sterile phosphate buffered saline (PBS). The PBS containing the specimen was vortexed for 20-40 seconds by adding sterilized glass beads to disrupt any biofilm aggregates. The vortexed samples were further serially diluted into 1:10 and 1:100 in PBS for semi-quantification of microbial colonization of the ET tube.

### **Bacterial Identification**

The undiluted (vortexed sample) and diluted suspensions of each ET tube cut section were streaked on 5% sheep Blood agar, Chocolate agar and MacConkey agar (Himedia Pvt Ltd., Mumbai) using semi-quantitative methods, and the growth was counted as colony forming units per mL after overnight aerobic incubation at 37°C. The isolated bacteria were identified following the standard operating procedures of the laboratory, which included colony morphology, Gram staining, motility testing and routine set of biochemical tests.

### **Antibiotic Susceptibility Testing**

The identified bacteria were further tested for antimicrobial susceptibility testing using Kirby-Bauer disc diffusion method on Muller Hinton agar plates and microbroth dilutions for determining the Colistin MIC (Difco) following the Clinical and

Laboratory Standards Institute (CLSI) guidelines 2018 (12).

### Statistical Analysis

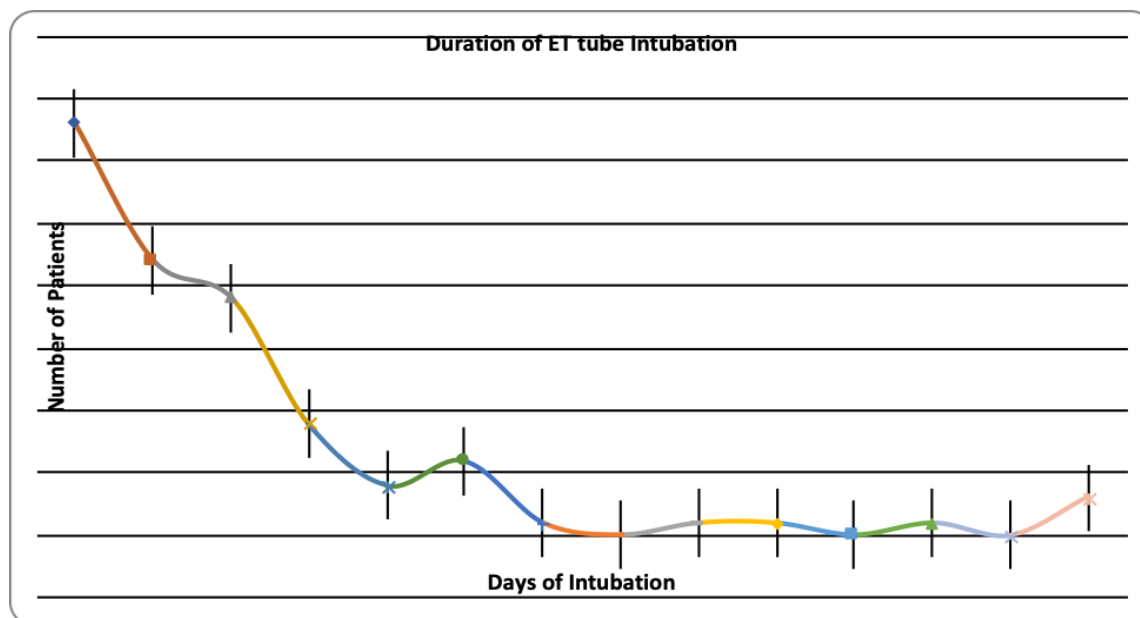
The data was entered into a Microsoft Excel sheet and later analyzed using SPSS version 26. Descriptive analysis of all the outcomes was employed. The Pearson Chi square test was used for the comparative analysis, and a P-value  $<0.05$  was considered statistically significant.

## RESULTS

A total of 120 ET tubes were received during the study period. Twenty samples were rejected as they did not fulfill the inclusion criteria. Among the 100 ET tube

samples included in the study, they were collected from 100 individual patients admitted to the intensive care units with an intubation period of more than 48 hours. Gender differences were observed among the patients included in the study. Males ( $n=67$ ) outnumbered females ( $n=33$ ) with a gender ratio of male to female of 2:1.

The ET tubes were collected from the intubated patients for a period of 48 hours or more, upon removal of the ET tube as indicated by the attending doctor. Most of the patients admitted to the ICUs in the study settings were intubated with ET tube for shorter durations based on medical necessity.



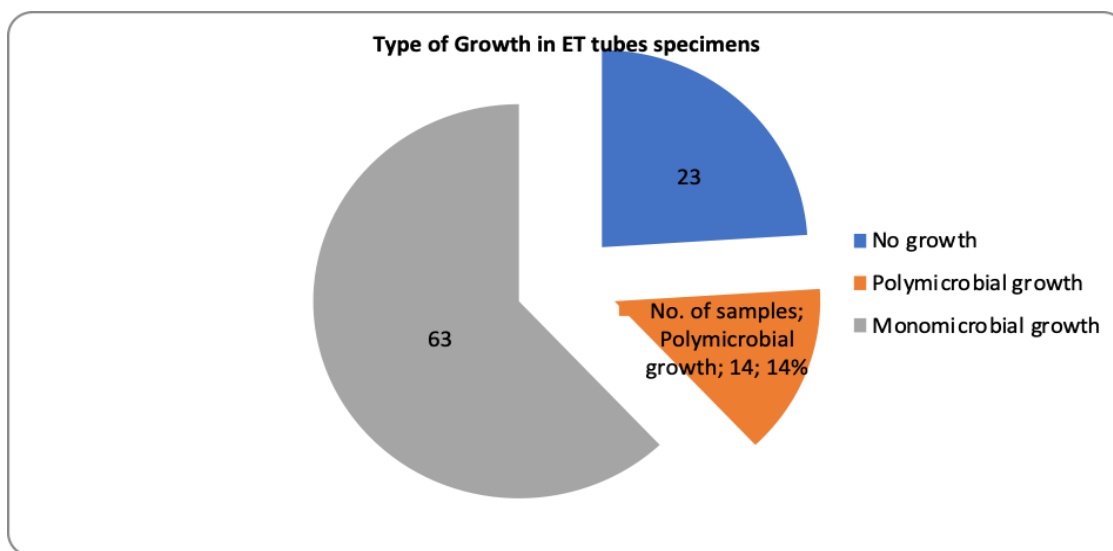
**Figure 1.** Duration of endotracheal tube intubation in patients

Out of 100 ET tubes processed in the study, 23 ET tubes did not yield any microbial growth. Monomicrobial growth (i.e. one type of microorganism) was found

in 63 out of 100 ET tubes, while growth of more than one microorganism (polymicrobial growth) was observed in 14 out 100 ET tubes (Figure 2).

Among the microorganisms which were isolated from ET tube samples, following organisms were identified. *Acinetobacter* species were isolated from the majority of ET tube samples, followed by

growth of *Klebsiella* species. Most of the isolates (n=52) were able to extend their biofilm in the inner or middle part of the same ET tube (Table 1).



**Figure 2.** Various types of growth observed in ET tube biofilm specimens included in the study.

**Table 1.** Number of Potential Pathogens Isolated from Various Cut Sections of the ET Tubes

	No. of Isolates	Inner	Middle	Outer	Inner & Middle	Middle & Outer	All parts
<i>Acinetobacter species</i>	25	7	3	0	12	0	3
<i>Klebsiella species</i>	23	2	1	0	19	0	1
<i>Pseudomonas species</i>	15	1	2	0	6	0	6
<i>E. coli</i>	13	2	1	0	7	1	2
<i>Proteus species</i>	2	0	0	0	2	0	0
<i>Enterobacter species</i>	1	1	0	0	0	0	0
<i>S. aureus</i>	6	1	0	0	3	0	2
Coagulase-negative staphylococci (CoNS)	4	1	1	0	2	0	0
<i>Candida species</i>	4	0	2	0	1	1	0
<b>Total</b>	<b>93</b>	<b>15</b>	<b>10</b>	<b>0</b>	<b>52</b>	<b>2</b>	<b>14</b>

Fifteen potential pathogens were isolated only from the innermost part of ET

tubes, while ten were found in the middle part. Fifty-two isolates were found in both

the inner and middle part of ET tubes. Only two organisms were located in middle and outer part of same ET tubes, while 14 isolates were able to spread throughout the ET tubes and form biofilm. A high rate of antibiotic resistance was observed in Gram-negative bacilli against Ceftriaxone,

Ciprofloxacin, Amikacin and Gentamycin. Imipenem and Colistin has shown high susceptibility rates. (Table 2). Methicillin resistance was tested with using Cefoxitin disc and was found in two isolates of *S. aureus* and three isolates of Coagulase-negative *Staphylococcus* species (Table 3).

**Table 2.** Antibiotic Susceptibility Pattern of the Gram-Negative Bacilli Isolated from ET Tube Specimens

	AK	GEN	CIP	CTZ	CTR	IMP	PIT	CL
<i>Acinetobacter</i> species (25)	13	11	07	08	04	21	15	24
<i>Klebsiella</i> species (23)	14	14	06	09	04	17	13	23
<i>Pseudomonas</i> species (15)	11	10	06	08	00	13	11	15
<i>E. coli</i> (13)	07	07	04	06	05	09	07	13
<i>Proteus</i> species (2)	02	02	01	00	00	02	02	00
<i>Enterobacter</i> species (1)	00	00	00	00	00	01	00	01

Abbreviations: AK = Amikacin; GEN = Gentamycin; CIP = Ciprofloxacin; CTZ = Ceftazidime; CTR = Ceftriaxone; IMP = Imipenem; PIT = Piperacillin-Tazobactam; CL = Colistin

**Table 3.** Antibiotic Susceptibility Pattern of Gram-Positive Cocci Isolated from ET Tube Specimens.

	<i>Staphylococcus aureus</i> (06)	<i>Staphylococcus</i> species (04)
Vancomycin Screen Agar	All Negative	All Negative
Penicillin G	01	01
Erythromycin	02	02
Clindamycin	02	02
Doxycycline	05	02
Cefoxitin	04	01
Ciprofloxacin	02	04
Linezolid	06	04

\* No growth was observed in vancomycin screen agar (i.e. all isolates were susceptible to vancomycin)

## DISCUSSION

The biofilm formed inside the ET tube may serve as persistent source of infection and the organisms associated with the biofilm in indwelling medical devices. The surface of the cuff that folds around a

traditional ET tube when inflated in the trachea creates potential channels through which secretions can drain and also provides access for pathogens to inoculate lower respiratory tract (8,13). The number of days of mechanical ventilation is related to a



higher the risk of acquiring VAP among the patients in ICUs (14).

In the present study, ET tubes were collected from 100 individual patients who fulfilled the inclusion criteria. The number of male patients was almost double that of female patients. The reason for gender difference as a risk factor for VAP may be related to the differences in the hormones, the distribution of infectious pathogens, the effects of gender-related gene polymorphisms on drug immune responses, and the differences in the complications between men and women (15).

In the current study, most of the patients had ET tubes inserted for a shorter duration. The majority of the patients were in the category of 2-4-day intubation period, followed by 5-7 intubation period. Most of these patients were intubated for shorter duration as per their medical need. A total of 93 probable potential pathogen isolates which were isolated from 77 ET tubes samples while no growth was observed in 23 ET tube specimens. In a previous study by Cairns et al. (16) from New Zealand, where they reported the growth in 20 samples out of 25 ET tubes collected from the ICU patients which is similar to the yield rate of current study (16). Among the 77 ET tubes with the growth of microorganisms, 14 had more than one type of potential pathogen. Multiple organisms

have also been reported in 26% of specimens in a previous study of VAP patients (17). Multiple species biofilm is often present in chronic infections, but the exact cell-to-cell communications among them are not well understood.

Among the 93 probable potential pathogens isolated in the present study, Gram Negative bacteria (GNB) outnumbered Gram positive cocci (GPC) and *Candida* species. The most common organism isolated was *Acinetobacter species* (n=25), followed by *Klebsiella species* (n=23), *Pseudomonas species* (n=15) and *E. coli* (n=13). Previous studies conducted in India have also reported similar results where predominance in lower respiratory tract specimens of the patients had GNB as pathogen (18,19,20). The reason for GNBs being the most common isolates may be linked to gut colonization and antimicrobial exposure. Critically ill patients in ICUs are exposed to the hospital isolates, and eventually, the oral flora of patients shifts to a predominance of the hospital flora, which consists mainly of GNB. This flora in the oropharynx of the patient can enter the lower respiratory tract during pulmonary aspiration (21).

The extent of the biofilm in each ET tube was assessed by scrapping and culturing the material collected from three different sites of the ET tube. It was found that the most of the isolates were yielded

from the innermost (closed to the lungs) cut sections as well as the middle part of the same ET tube. The majority of pathogens showed their spread inside the ET tube as the most of the isolates were yielded from more than 1 part of same ET tube. A higher yield of potential pathogens was observed in the inner part (closer to lungs) compared to the outer part (oral portion) of the ET tube. In a previous study, only the middle part of ET tubes was used for bacterial culture where they have reported 80% growth rate in total samples (16). The inner part or middle of the ET tubes inserted in the oral and respiratory tract of the patients provides an environment with suitable temperature and moisture for pathogens to thrive. However, the lack of nutrients and presence of other secretions compel them to form biofilm.

The pathogens associated with VAP are usually highly antibiotic-resistant isolates, thus termed potentially drug-resistant or multi-drug resistant bacteria (22). High rates of antibiotic resistance were observed in the current study especially among the GNB. Most of these GNB isolates were resistant to tested fluoroquinolones and cephalosporins. Colistin was reported as the most effective antibiotic in-vitro against all the GNB isolates except *Proteus* species. Only one isolate of *Acinetobacter* species was tested resistant to the Colistin by the microbroth dilution method. The second most

susceptible drug was found to be carbapenems. A similar pattern of antibiotic susceptibility has been supported by previous studies performed on the lower respiratory tract pathogens (20,22,23). Most of the patients admitted in ICUs are usually exposed to antibiotics previously before visiting the tertiary care center which might be reason for higher drug resistance among these isolates. Another reason for drug resistance in these isolates can be the continuous exposure to access and reserve antibiotics in a hospital environment, especially in critical care units, may have led to adaptation.

*Staphylococcus aureus* has the ability to enhance its antibiotic resistance, leading to higher mortality rates. It can also produce toxins which help in stronger adherence to various surfaces including living cells or artificial surfaces such as medical devices (24). There were total 10 isolates of GPC, which were isolated from the ET tube samples in the present study. Methicillin resistance was observed in three isolates of Coagulase Negative *Staphylococcus* species as well as two isolates of *S. aureus*. Most of these isolates were resistant to Penicillin, followed by Erythromycin, Clindamycin and Ciprofloxacin. Only one isolate of *S. aureus* was seen not responding in-vitro to Doxycycline. None of the isolates were resistant to Linezolid and glycopeptides like Vancomycin and Teicoplanin. A previous



study by Tilouche et al (25) reported a 14% prevalence of MRSA from VAP patients, which is lower than in the current study (25).

**Limitation:** Molecular methods for detecting genes responsible for antibiotic resistance were not used. The correlation of pathogens isolated from biofilm inside the ET tubes with microbiological culture testing from lower respiratory tract specimens, such as ET aspirate or Bronchoalveolar lavage of the individual patients was not performed, which could have put light on the pathogenic role of isolated microorganisms.

## CONCLUSIONS

Potentially pathogenic microorganism frequently forms biofilm within the inner lumen of ET tube of critically ill patients, if intubated for more than 48 hours. Most of these potential pathogens are highly drug-resistant and can seriously complicate the

condition as well as the treatment if biofilm is dislodged and drained directly into the lungs during mechanical ventilation. Newer approaches are required to control or inhibit the biofilm production by these potential pathogens within the ET tubes.

## AUTHOR CONTRIBUTIONS

PKK: Conceptualization, Methodology. VN: Methodology, Data curation, Writing-Original draft preparation, Software. NK: Validation. NM: Acquisition of data, or analysis and interpretation of data. VS: Writing-Reviewing and Editing.

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Not applicable.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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