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Endotracheal Tube Associated Microbiome in the Intensive Care Unit

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Abstract: Background: Exploration of the microbiome within the respiratory tract predominantly centers on the diversity of microorganisms in the airways and lungs. However, there remains a lack of clarity regarding the potential impact of intubation and prolonged stays in intensive care units (ICU) on these microbial communities. **Aim of the study:** The present investigation sought to examine and characterize the microbiome of endotracheal tubes (ETT), along with assessing their susceptibility and resistance to antimicrobial agents. **Patients and methods:** This 12-month cross-sectional study, conducted in the ICU department of Baghdad Teaching Hospital, focused on forty adult patients (aged 18 and above) undergoing mechanical ventilation for diverse reasons. Notably, exclusion criteria involved individuals intubated for less than 3 days or more than 14 days. The research involved collecting data on demographics and the duration of endotracheal tube placement. Additionally, a thorough bacterial culture and sensitivity analysis was conducted on samples obtained from the endotracheal tube. **Results**: In a cohort with a mean age of 48.6 years (SD: 20.0), comprising 67.5% males and 32.5% females, the average duration of endotracheal intubation was 7.3 days (SD: 2.7). Klebsiella pneumoniae prevailed in bacterial cultures (37.5%), followed by Acinetobacter baumannii (15.0%). Fungal cultures were mostly negative (97.5%), with Saprochaete capitata detected in 2.5% of cases. Overall sensitivity was 85%, with amikacin at 37.5%, gentamicin at 25.0%, and cefepime, colistin, minocycline, and piperacillin-tazobactam at 17%. Multi-drug resistance was identified in one pseudomonas and three klebsiella pneumonia cases. **Conclusion**: The study revealed Klebsiella pneumoniae as the predominant pathogen in endotracheal tube cultures, followed by Acinetobacter. High drug sensitivity, especially to Amikacin, was observed, with limited instances of multi-drug resistance (MDR) in Klebsiella pneumoniae cultures.

Keywords: Endotracheal tubes; Intensive care units (ICUs); Microbial diversity; Respiratory tract microbiome.

INTRODUCTION

Nosocomial infections represent a major healthcare problem associated with high mortality and increased costs. Medical device-related infections represent almost one-fourth of nosocomial infections. (Ssekitoleko, R. T. *et al*., 2020) Researchers devote extensive work in order to discover efficient prevention and treatment strategies. The most common type is nosocomial pneumonia with a reported incidence of 6.8-27%.(Magill, S. S. *et al*., 2014)

Nosocomial pneumonia is a hospital-acquired life-threatening infection. Critically ill patients requiring mechanical ventilation in the intensive care unit (ICU) are the group at the highest risk of developing the most severe form, ventilator-associated pneumonia (VAP). (Pneumatikos, I. A. *et al*., 2009)

VAP is defined as nosocomial pneumonia occurring in a patient after 48 hours of mechanical ventilation via an endotracheal tube (ET) or tracheostomy tube.[3] and it is the most common infectious complication in critically ill patients.[4] VAP prevalence varies between 9-65%, mortality rates are high (15-76%), ICU- and hospital-length of stay is increased (by 5-7 ICU days, respectively by 2-3 folds), with significantly increased costs per patient. (Heo, S. *et al*., 2008)

Historically, the lungs were considered sterile. The first culture-independent report of a lung microbiome was published in 2010. (Hilty, M. *et al*., 2010) Early culture-independent studies debated whether bacterial DNA detected in samples from the lung represented a true active resident lung microbiome rather than recent micro-aspiration or contamination due to sampling technique. (Dickson, R. P. *et al*., 2014)

The factors that influence the composition of the lung microbial community are immigration, elimination, and the relative reproduction rate, which is affected by the local environment and growth conditions. Surveys of the lung microbiota indicate that the lower airways contain approximately 100 different taxa, including Streptococcus, Veillonella, and Prevotella. (Pettigrew, M. M. *et al*., 2021)

Endotracheal tubes (ET) serve as reservoirs for infecting microorganisms, forming mixed biofilms with pathogens shortly after intubation, notably within the tube's distal third. (Pneumatikos, I. A. *et al*., 2009) In 1967, Redman and Lockey demonstrated ET bacterial colonization through culturing the distal end, while in 1986, Sottile *et al*. used scanning microscopy to reveal biofilm presence on polyvinylchloride ET inner surfaces. (Diaconu, O. *et al*., 2018) Microorganisms exist

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either as individual planktonic cells suspended in liquid or as sessile communities forming biofilms—a three-dimensional structure of microbial cells enclosed in a self-produced polymer matrix for protection against harsh conditions. (Høiby, N. *et al*., 2015)

ET biofilms contribute to ventilator-associated pneumonia (VAP) through multiple mechanisms: biofilm fragments can disperse and reach the lungs, cells can be aerosolized and aspirated during ventilation, and individual cells can be dislodged by liquids and transported deep into the lungs. (Pan, Y. *et al*., 2017) VAP diagnosis faces challenges due to its complex etiology, including inter- and intra-patient variability, often with unknown causative agents at suspicion. (Cairns, S. *et al*., 2011)

The distinction between colonization and infection is crucial yet difficult. ESKAPE pathogens (multidrug-resistant microbes) and normal oral flora are implicated in VAP, with the biofilm acting as a reservoir of pathogenic bacteria. (Vandecandelaere, I. *et al*., 2015) Despite early biofilm presence and diversity, VAP develops later, influenced by intubation duration. The advanced biofilm stage correlates with pneumonia, suggesting VAP might be more related to ET presence than mechanical ventilation itself. The term "endotracheal tube-associated pneumonia" has been proposed to better reflect its pathogenesis. (Diaconu, O. *et al*., 2018)

PATIENTS AND METHODS

The observational cross-sectional study took place in the Intensive Care Unit (ICU) of Baghdad Teaching Hospital over a 12-month interval, commencing on August 1, 2022, and concluding on August 1, 2023. Forty eligible participants, as per the defined criteria, were enrolled in the study.

Approval for the research was obtained from the Scientific Committee of Anesthesia and Intensive Care under the Iraqi Board for Medical Specialization. Prior to data collection, whenever possible, consent was taken from the patients' relative after receiving a detailed explanation of the study's objectives and assurances regarding data confidentiality.

Target population are those who got admitted to the ICU department and required ETT ventilation at the above-mentioned center during the study period.

A convenient sampling technique was employed during sample selection procedure.

This study encompassed adult individuals, 18 years and older, who were admitted to the Intensive Care Unit and underwent mechanical ventilation for any reason.

Exclusion criteria
1. Patients who got intubated for less than 3 days or more than 14 days were excluded from this research.
2. Patients with chest infection.

Baseline data encompassed patient characteristics such as age and sex. The length of time the endotracheal tube (ETT) remained in place was also documented. Furthermore, the reason for hospital admission for each individual was recorded. Additionally, the administration of antibiotics throughout the ICU stay was documented.

Endotracheal tube (ETT) specimens were collected and sent to the laboratory under three conditions: upon patient demise, during tube replacement, or during tracheostomy procedures. A compact 2x2 cm segment was provided to the hospital laboratory for bacterial and fungal culture as well as sensitivity testing. Strict aseptic measures were followed during the sampling process, focusing on obtaining samples from the internal surface of the ETT.

Bacterial cultivation entailed the separation and characterization of bacterial types, whereas fungal cultivation discerned specific fungal varieties. Sensitivity assays were conducted to assess the responsiveness of the isolated microbes to different antimicrobial treatments.

Continuous variables were expressed as means and standard deviations and categorical variables were expressed as frequency and percentages. R software packages (dplyr, gt_summery and ggplot) were used for data processing, visualization, and statistical analysis ("R version 4.3.0, R Foundation for Statistical Computing, Vienna, Austria").

RESULTS

In this study of 40 patients admitted to the ICU, it was observed that the mean age of 48.6 years with a standard deviation of 20.0 years. Among the patients, 67.5% were male, while 32.5% were female, highlighting a gender distribution in the cohort. The average duration of endotracheal intubation in these patients was 7.3 days, with a standard deviation of 2.7 days.

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The most prevalent cause of admission was Cerebrovascular Accident (CVA), accounting for 22.5% of cases. Other significant admission causes included Myasthenia Gravis (12.5%), Road Traffic Accidents (RTA) at 12.5%, Guillain-Barré Syndrome (GBS) at 10.0%, and Diabetic ketoacidosis (DKA) at 7.5%, and Additional reasons for admission included Cardiac Arrest (5.0%), Chronic Obstructive Pulmonary Disease (COPD) (5.0%), Epilepsy (5.0%), and various other medical conditions, each contributing to 2.5% or less of admissions.

Among the patients, 57.5% received single antibiotics, while 42.5% received a combination of two antibiotics. Regarding the types of antibiotics administered, Ceftriaxone was the most commonly used antibiotic, prescribed to 60.0% of patients. Meropenem followed at 32.5%, Vancomycin at 20.0%, Levofloxacin at 7.5%, and other antibiotics such as Colistin, Azithromycin, Amikacin, Metronidazole, Augmentin, Linezolid, and Tigacyclin were used in smaller proportions, each accounting for 2.5% or less of antibiotic usage. In addition to antibiotics, we observed that Variconazole, an antifungal agent, was used in 2.5% of cases.

Table 1: types of antibiotics and anti-fungal used in treatment of patients in the ICU

Characteristic	**N = 40[1]**
Antibiotics used	
Single	23 (57.5%)
Double	17 (42.5%)
Type of antibiotics	
Ceftriaxone	24 (60.0%)
Meropenem	13 (32.5%)
Vancomycin	8 (20.0%)
Levofloxacin	3 (7.5%)
Colistin	2 (5.0%)
Azithromycin	1 (2.5%)
Amikacin	1 (2.5%)
Metronidazole	1 (2.5%)
Augmentin	1 (2.5%)
Linezolid	1 (2.5%)
Tigacyclin	1 (2.5%)
Antifungal used	
Variconazole	1 (2.5%)
[1]n (%)	

Regarding bacterial culture results, *Klebsiella pneumoniae* was the most prevalent bacterium, accounting for 37.5% of cases, followed by Acinetobacter baumannii at 15.0%. No bacterial growth was reported in 15.0% of samples. Other bacteria identified included *Escherichia coli* (12.5%), *Proteus mirabilis* (5.0%), *Pseudomonas aeruginosa* (5.0%), *Acinetobacter calcoaceticus* (2.5%), *Enterobacter cloacae* (2.5%), non-aureus staphylococci (2.5%), and *Staphylococcus aureus* (2.5%).

In contrast, the fungal culture results showed predominantly negative findings, with 97.5% of samples showing no fungal growth. Saprochaete capitata was detected in only 2.5% of cases.

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Table 2: description of the results from bacterial and fungal cultures

Characteristic	N = 40[1]
Bacterial Culture	
Klebsiella pneumonia	15 (37.5%)
Acinetobacter baumannii	6 (15.0%)
No growth	6 (15.0%)
Escherichia coli	5 (12.5%)
Proteus mirabillis	2 (5.0%)
Pseudomonas aeruginosa	2 (5.0%)
Acinetobacter calcoaceticus	1 (2.5%)
Enterobacter cloacae	1 (2.5%)
Non-aureus staphylococci	1 (2.5%)
Staphylococcus aureus	1 (2.5%)
Fungal culture	
No growth	39 (97.5%)
Saprochaete capitata	1 (2.5%)
[1]n (%)	

The overall sensitivity was found to be 85%, indicating a substantial level of responsiveness to the antibiotics tested. Furthermore, we analyzed the sensitivity of bacterial isolates to specific types of antibiotics. Among these, Amikacin demonstrated a sensitivity rate of 37.5%. Gentamicin followed with a sensitivity rate of 25.0%, and Cefepime, Colistin, Minocycline, and Piperacillin Tazobactam all showed a sensitivity rate of 17.5%. Other antibiotics, such as Imipenem, Doxycycline, Polymyxin B, Trimethoprim, Ceftazidime, Levofloxacin, Meropenem, Tobramycin, and Ciprofloxacin, exhibited varying degrees of sensitivity, ranging from 2.5% to 15.0%. Of particular importance, one case with pseudomonas infection and three case with klebsiella pneumonia infection had been found to multi-drug resistance (MDR).

Table 3: Sensitivity to a specific type of antibiotic

Characteristic	N = 40[1]
Overall sensitivity	34 (85%)
Sensitivity to a specific type of antibiotic	
Amikacin	15 (37.5%)
Gentamicin	10 (25.0%)
Cefipime	7 (17.5%)
Colistin	7 (17.5%)
Minocycline	7 (17.5%)
Piperacillin Tazombactam	7 (17.5%)
Imipenem	6 (15.0%)
Doxycycline	5 (12.5%)
Polymyxin.B	5 (12.5%)
Trimethoprime	5 (12.5%)
Ceftazidime	4 (10.0%)
Levofloxacin	4 (10.0%)
Meropenem	4 (10.0%)
Tobramicyn	3 (7.5%)
Ciprofloxacin	2 (5.0%)
Ampicillin	1 (2.5%)
AncomycinV	1 (2.5%)
Aztreonam	1 (2.5%)
Chloramphenicol	1 (2.5%)
Clindamycin	1 (2.5%)
Daptomycin	1 (2.5%)
Linezolid	1 (2.5%)
Moxifloxacin	1 (2.5%)
Rifampin	1 (2.5%)
Teicoplanin	1 (2.5%)
Tetracycline	1 (2.5%)
Tigecycline	1 (2.5%)
[1]n (%)	

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DISCUSSION

The development of biofilms on endotracheal tubes in mechanically ventilated patients is a crucial concern in intensive care medicine. Biofilms, intricate communities of microorganisms, have the potential to form on the inner surfaces of these tubes, presenting substantial challenges. They not only elevate the risk of ventilator-associated pneumonia (VAP) but also contribute to antibiotic resistance. Gaining insight into the characteristics and composition of these biofilms is imperative for devising effective strategies to prevent and address VAP, minimize the reliance on broad-spectrum antibiotics, and enhance the overall outcomes of mechanically ventilated patients in critical care settings.[14]

In this study of ICU patients with endotracheal tubes, *Klebsiella pneumoniae* was the most prevalent bacterium, accounting for 37.5% of cases, followed by *Acinetobacter baumannii* at 15.0%. Notably, 15.0% of samples showed no bacterial growth. Other bacteria identified included *Escherichia coli* (12.5%), *Proteus mirabilis* (5.0%), *Pseudomonas aeruginosa* (5.0%), *Acinetobacter calcoaceticus* (2.5%), *Enterobacter cloacae* (2.5%), non-aureus staphylococci (2.5%), and *Staphylococcus aureus* (2.5%). Fungal culture results, on the other hand, were mostly negative, with 97.5% of samples showing no fungal growth, and only 2.5% of cases detecting Saprochaete capitata.

The present study data are consistent with several previous research. Hotterbeekx, *et al*., 2015, observed a frequent isolation of *Pseudomonas aeruginosa* from endotracheal tubes, mirroring our findings of *Pseudomonas aeruginosa* in 5.0% of instances. Cifuentes, *et al*., 2022, Indicated a significant occurrence of *Klebsiella pneumoniae* in individuals with ventilator-associated pneumonia (VAP), consistent with our findings of a high prevalence of *Klebsiella pneumoniae* in patients undergoing endotracheal intubation (EET).

Natham, *et al*., 2019, documented the presence of *Pseudomonas aeruginosa* and non-fermenting Gram-negative bacteria (NFGNB), such as *Acinetobacter* and *Klebsiella pneumoniae*, aligning with our observations. Shrestha *et al*.[18] Identified Acinetobacter as the predominant isolate, in accordance with our research results, while Swati *et al*.[19] similarly documented Acinetobacter as the primary isolate, followed by *Klebsiella* spp. Both of these studies offer corroboration for our findings.

Additionally, Lu, *et al*., 2014; noted variations in the composition of flora between patients with and without ventilator-associated pneumonia, providing support for our research emphasis on the microbiome in endotracheal intubation (EET) patients.

This study also revealed the sensitivity of bacterial isolates to specific types of antibiotics, shedding light on the potential treatment options for infections in EET patients in the ICU. Notably, the sensitivity of Amikacin was 37.5%, aligning with the results reported in Kaur, *et al*., 2021's study[21], where Acinetobacter baumannii isolates were sensitive to colistin, which, notably, belongs to the same class as Amikacin. The concurrence in antibiotic sensitivity profiles implies a promising approach for effective treatment, particularly in situations where either Amikacin or colistin could be viable options.

Furthermore, this study identified multi-drug resistance (MDR) in some cases of Pseudomonas and Klebsiella pneumoniae infections. This is a critical finding, as it underscores the growing concern of antibiotic resistance in healthcare settings, which was also noted in Shrestha, *et al*., 2021; and Swati *et al*., 2020 studies. These studies highlighted elevated levels of drug resistance, particularly in Acinetobacter and Klebsiella species, underscoring the imperative for rigorous infection control measures and the exploration of alternative treatment strategies in intensive care unit (ICU) patients.

Malik, *et al*., 2018; documented susceptibility trends among Gram-negative bacteria to combination drugs, a theme pertinent to our study's emphasis on bacterial sensitivity to particular antibiotics. This study findings also resonate with Qi, *et al*.,(2018) study, which identified notable variations in the microbiota composition among patients with Pseudomonas aeruginosa infections. This emphasizes that understanding the microbiome's intricacies can have implications beyond just identifying the most prevalent bacteria; it can guide treatment strategies, as certain bacterial compositions may respond differently to antibiotic therapy.

LIMITATION:

1. The study included a total of only forty cases. The relatively small sample size may limit the generalizability of the findings to a larger population of ICU patients. A larger sample would provide more robust and generalizable results.

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2. Excluding patients intubated for less than 3 days or more than 14 days and those with chest infections may introduce bias and limit the applicability of the findings to a broader range of ICU patients. These criteria may exclude cases with different characteristics and outcomes.

3. The study collected endotracheal tube samples at specific times (upon patient decease, during tube replacement, or tracheostomy). This timing may miss potential variations in colonization or infection that occur between these events. Continuous monitoring or more frequent sampling might provide a more comprehensive view of microbial colonization.

CONCLUSION

This study concludes that:

1. Klebsiella pneumoniae was the predominant pathogen identified through endotracheal tube culture, followed by Acinetobacter.
2. Drug sensitivity, particularly towards Amikacin, displayed high levels in the study.
3. Multi-drug resistance (MDR) was observed in a limited number of Klebsiella pneumoniae cultures.
4. Our study's consistent findings with the literature, particularly regarding prevalent bacteria, antibiotic sensitivity, and the emergence of MDR strains, underscore the importance of ongoing research in this field. As the understanding of the microbiome in EET patients in the ICU continues to grow, it can provide valuable insights into better infection prevention and treatment strategies to enhance patient care and safety.
5. These findings are particularly significant given the current global challenges associated with antibiotic resistance and the need for effective antimicrobial stewardship.

RECOMMENDATIONS

1. Implement and enforce strict infection control protocols in ICU settings to minimize the risk of endotracheal tube-associated infections, particularly those caused by Klebsiella pneumoniae and Acinetobacter baumannii.
2. Emphasize prudent antibiotic use, guided by sensitivity testing results, to prevent the development of multi-drug resistance and maintain the effectiveness of critical antibiotics like Amikacin.
3. Implement ongoing monitoring of microbial colonization in endotracheal tubes to detect trends, emerging pathogens, and changes in drug sensitivity over time. This can inform timely interventions.
4. Consider isolating patients with multi-drug resistant infections to prevent their spread and protect vulnerable patients in the ICU.
5. Conduct additional studies to investigate the factors contributing to the prevalence of Klebsiella pneumoniae and Acinetobacter baumannii in endotracheal tube patients and explore more effective preventive strategies and treatments.
6. Provide education and training to healthcare professionals in the ICU on best practices for endotracheal tube care and infection control measures to reduce the risk of colonization and infection.

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