MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT

–A CROSS SECTIONAL STUDY

By

DR. NALLAGONDA SATYA SAI SRIRAM

DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE, KOLAR

In partial fulfilment of the requirements for the degree of

MASTER OF SURGERY
IN
OTORHINOLARYNGOLOGY

Under the guidance of

DR.S M AZEEM MOHIYUDDIN
MBBS,MS E.N.T, FICS,FACS, MNAMS, SEKHSARIA FELLOWSHIP IN HEAD AND NECK SURGERY,
PROFESSOR AND HEAD
DEPARTMENT OF OTORHINOLARYNGOLOGY
AND
DR.ARVIND NATARAJAN-MBBS,MD,MNAMS
PROFESSOR
DEPARTMENT OF MICROBIOLOGY
DEAN OF ALLIED HEALTH SCIENCES

DEPARTMENT OF OTORHINOLARYNGOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE
TAMAKA KOLAR
MAY 2025

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT– A CROSS SECTIONAL STUDY"** is a bonafide and genuine research work carried out by me under the guidance of DR.S M AZEEM MOHIYUDDIN MBBS,MS E.N.T, SEKHSARIA FELLOWSHIP IN HEAD AND NECK SURGERY, FICS,FACS, MNAMS,PROFESSOR AND HEAD, Department of Otorhinolaryngology and Head and Neck Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar in partial fulfilment of University regulation for the award "**MASTER OF SURGERY IN OTORHINOLARYNGOLOGY**". This has not been submitted by me previously for the award of any degree or diploma from the university or any other university.

Date: Signature of the Candidate

Place:

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT– A CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. NALLAGONDA SATYA SAI SRIRAM** in partial fulfillment of the requirement for the degree of MASTER OF SURGERY IN OTORHINOLARYNGOLOGY as per regulations of SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR. I have great pleasure in forwarding this to the university.

DATE :

PLACE :

DR.S M AZEEM MOHIYUDDIN
MBBS,MS E.N.T, FICS,FACS, MNAMS,SEKHSARIA
FELLOWSHIP IN HEAD AND NECK SURGERY,
PROFESSOR AND HEAD
Department of OTORHINOLARYNGOLOGY AND HEAD AND NECK SURGERY
Sri Devaraj Urs Medical College
Tamaka, Kolar – 563 101

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT– A CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. NALLAGONDA SATYA SAI SRIRAM** in partial fulfillment of the requirement for the degree of MASTER OF SURGERY IN OTORHINOLARYNGOLOGY as per regulations of SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR. I have great pleasure in forwarding this to the university.

DATE :

PLACE :

DR.ARVIND NATARAJAN-
MBBS,MD,MNAMS

PROFESSOR

DEPARTMENT OF MICROBIOLOGY

DEAN OF ALLIED HEALTH SCIENCES

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA, KOLAR – 563101

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled **"MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT– A CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. NALLAGONDA SATYA SAI SRIRAM** under the guidance of DR.S M AZEEM MOHIYUDDIN MBBS,MS E.N.T, SEKHSARIA FELLOWSHIP IN HEAD AND NECK SURGERY, FICS,FACS, MNAMS,PROFESSOR AND HEAD, Department of Otorhinolaryngology and Head and Neck Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar.

DATE :
PLACE: **Signature of the HOD**

Dr. S.M AZEEM MOHIYUDDIN,
MBBS, MS, FACS, FICS, MNAMS,
SEKHSARIA FELLOWSHIP
IN HEAD AND NECK SURGERY
Professor AND HOD
Department of Otorhinolaryngology,
Sri Devaraj Urs Medical College,
Tamaka, Kolar.

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH TAMAKA, KOLAR, KARNATAKA

ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT– A CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr.NALLAGONDA SATYA SAI SRIRAM** under the guidance of DR.S M AZEEM MOHIYUDDIN MBBS,MS E.N.T, SEKHSARIA FELLOWSHIP IN HEAD AND NECK SURGERY, FICS,FACS, MNAMS,PROFESSOR AND HEAD, Department of Otorhinolaryngology and Head and Neck Surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar..

SIGNATURE OF HOD

Dr. S.M.AZEEM MOHIYUDDIN,
M.B.B.S.,M.S, FICS.,FACS.,MNAMS,
SEKHSARIA FELLOWSHIP
IN HEAD AND NECK SURGERY
Professor and HOD
Department of Otorhinolaryngology
Sri Devaraj Urs MedicalCollege,
Tamaka, Kolar.

Date:
Place:

SIGNATURE OF PRINCIPAL

DR. PRABHAKAR .K
M.B.B.S, M.D,
PRINCIPAL
Sri Devaraj Urs Medical College,
Tamaka, Kolar.

Date:
Place:

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SRI DEVARAJ URS MEDICAL COLLEGE

Tamaka, Kolar

INSTITUTIONAL ETHICS COMMITTEE

Members

1. **Dr. D.E.Gangadhar Rao, (Chairman)** Prof. & HOD of Zoology, Govt. Women's College, Kolar
2. **Dr. Sujatha.M.P, (Member Secretary),** Prof. Department of Anesthesia, SDUMC
3. Mr. Gopinath Paper Reporter, Samyukth Karnataka
4. Mr. G. K. Varada Reddy Advocate, Kolar
5. Dr. Hariprasad S, Prof. Dept, of Orthopedics, SDUMC
6. Dr. Abhinandana R Asst. Prof. Dept. of Forensic Medicine, SDUMC
7. Dr. Ruth Sneha Chandrakumar Assoc. Prof. Dept. of Psychiatry, SDUMC
8. Dr. Usha G Shenoy, Asst. Prof., Dept. of Allied Health & Basic Sciences SDUAHER
9. Dr. Munilakshmi U Asst. Prof. Dept. of Biochemistry, SDUMC
10. Dr.D.Srinivasan, Assoc. Prof. Dept. of Surgery, SDUMC
11. Dr.Shilpa M D Assoc. Prof. Dept. of Pathology, SDUMC

No. DMC/KLR/IEC/99/ 2023-24 **Date: 10/04/2023**

PRIOR PERMISSION TO START OF STUDY

The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the synopsis entitled **"Microbial Biofilms On Tracheostomy Tube And Tracheostoma Before And After Treatment - A Cross Sectional Study"** being investigated by **Dr.Nallagonda Satya Sai Sriram, Dr. S.M.Azeem Mohiyuddin & Dr.Arvind Natarajan[1]** in the Departments of E.N.T. & Microbilogy[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

Sujatha. M.P

Member Secretary

Member Secretary
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar.

Chairman

CHAIRMAN
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research, Kolar shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date: Signature of the Candidate

Place: **Dr.NALLAGONDA SATYA SAI SRIRAM**

Sri Devaraj Urs Academy of Higher Education & Research, Kolar

PLAGIARISM CHECK CERTIFICATE

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR, KARNATAKA, INDIA 563103

CERTIFICATE OF PLAGIARISM CHECK

Title of the Thesis/Dissertation	**MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT – A CROSS SECTIONAL STUDY**
Name of the Student	**Dr. NALLAGONDA SATYA SAI SRIRAM**
Registration Number	22EN1097
Name of the Supervisor / Guide	**DR S M AZEEM MOHIYUDDIN**
Department	OTORHINOLARYNGOLOGY
Acceptable Maximum Limit (%) of Similarity (PG Dissertation /Ph.D. Thesis)	10 %
Similarity	09%
Software used	TURNITIN
Paper ID	2682090836
ORCID ID	0009-0003-6337-2733
Submission Date	22/05/2025

N. Sriram
Signature of Student

S. M. Azeem Mohiyuddin 23/5/25
Signature of Guide/Supervisor

S. M. Azeem Mohiyuddin 23/5/25
HOD Signature

PG Coordinator

University Librarian
University Library
Learning Resource Centre
SDUAHER, Tamaka
KOLAR-563103

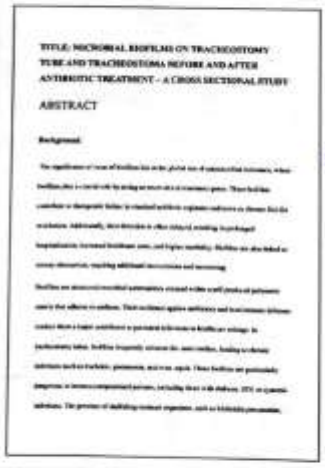
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	DR. NALLAGONDA SATYA SAI SRIRAM
Assignment title:	PG Dissertation - 2025
Submission title:	MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHE...
File name:	D_AFTER_ANTIBIOTIC_TREATMENT_A_CROSS_SECTIONAL_STU...
File size:	2.47M
Page count:	95
Word count:	14,473
Character count:	88,243
Submission date:	22-May-2025 04:08PM (UTC+0530)
Submission ID:	2682090836



TITLE: MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT – A CROSS SECTIONAL STUDY

ABSTRACT

Background

S.M. Azeem Mohiyuddin
23/5/25

Document Viewer

Turnitin Originality Report

Processed on: 22-May-2025 16:09 IST
ID: 2682090836
Word Count: 14473
Submitted: 2

MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND T... By DR. NALLAGONDA SATYA SAI SRIRAM

Similarity Index	Similarity by Source	
9%	Internet Sources:	8%
	Publications:	8%
	Student Papers:	2%

include quoted | include bibliography | excluding matches < 14 words | mode:
quickview (classic) report | print | refresh | download

1% match (Internet from 06-Sep-2018)
http://www.esp.org

1% match ()
Ailing Zhao, Jiazheng Sun, Yipin Liu, "Understanding bacterial biofilms: From definition to treatment strategies", Frontiers in Cellular and Infection Microbiology

1% match (Internet from 11-Apr-2025)
https://pmc.ncbi.nlm.nih.gov/articles/PMC10305407/

1% match (Internet from 21-Feb-2023)
https://link.springer.com/article/10.1007/s12070-021-02598-6?code=4701d62b-ebba-4915-bb18-51e44729c935&error=cookies_not_supported

<1% match (Internet from 27-Apr-2023)
https://WWW.MDPI.COM/2076-0817/9/8/630

<1% match (Internet from 04-Sep-2022)
https://www.mdpi.com/2076-0817/10/10/1345/html

<1% match (Internet from 26-Jun-2024)
https://www.mdpi.com/2079-6382/13/6/509

<1% match (Internet from 28-Jan-2023)
https://www.mdpi.com/2076-0817/10/7/877/htm

<1% match (student papers from 06-Mar-2025)
Submitted to Higher Education Commission Pakistan on 2025-03-06

<1% match (J. BORMAN, J.T. DAVIDSON. "A HISTORY OF TRACHEOSTOMY:", British Journal of Anaesthesia, 1963)

S.M. Azeem Mohiyuddin 23/5/25

I. BORMAN, J.T. DAVIDSON, "A HISTORY OF TRACHEOSTOMY:", British Journal of Anaesthesia, 1963

<1% match (Internet from 15-Apr-2025)
https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-025-03800-3

<1% match (Internet from 06-Dec-2022)
https://bmcmicrobiol.biomedcentral.com/counter/pdf/10.1186/s12866-018-1224-6.pdf

<1% match ("ESICM LIVES 2019", Intensive Care Medicine Experimental, 2019)
"ESICM LIVES 2019", Intensive Care Medicine Experimental, 2019

<1% match ("Posters", Clinical Microbiology and Infection, 2008)
"Posters", Clinical Microbiology and Infection, 2008

<1% match (Internet from 02-Feb-2024)
https://idoc.pub/documents/brotes-2018-1-3no00jwvd3nd

<1% match (Internet from 18-Nov-2022)
https://microbiologyjournal.org/bacterial-colonization-of-intensive-care-unit-environment-and-healthcare-workers-in-a-tertiary-care-hospital-in-kolar-region-india/

<1% match (Internet from 21-Jul-2024)
https://ichgcp.net/de/clinical-trials-registry/NCT04383652

<1% match (Internet from 26-Feb-2023)
https://www.jemds.com/data_pdf/1_Vasant%20bha--.pdf

<1% match (Internet from 09-Nov-2021)
http://v3r.esp.org

S.M. Azeem Mohiyuddin
23/5/25

<1% match (Internet from 26-Jan-2025)
https://assets.cureus.com/uploads/original_article/pdf/324802/20250114-1764483-6qk2tf.pdf

Dr. S.N. Azeem Mohiyuddin, MBBS, MS,(ENT), SEKHSARIA FELLOWSHIP (Head & Neck) F.I.C.S., F.A.C.S., M.N.A.M.S., KMC No: 31667 Prof. of E.N.T. & Head Neck Onco Surgeon Sri Devaraj Urs Medical College Tamaka, KOLAR

<1% match (student papers from 23-Dec-2023)
Submitted to University of Birmingham on 2023-12-23

<1% match (Asima Banu. "Bacteriological Profile Of Burn Wound Infections with Reference to Biofilm Formation", Journal of Bacteriology & Mycology: Open Access, 2017)
Asima Banu. "Bacteriological Profile Of Burn Wound Infections with Reference to Biofilm Formation", Journal of Bacteriology & Mycology: Open Access, 2017

<1% match (Internet from 28-Nov-2023)
https://journalcra.com/archive/201703

University Library
Learning Resource Centre
SDUAHER, Tamaka
KOLAR-563103

<1% match (Internet from 03-Jul-2022)
https://innovpub.org/assets/auArtzFile/60e69b9314657remodified%2002Journals-15-12-20.edited%20-%20Copy.docx

<1% match (Internet from 18-Mar-2022)
https://www.ijpsonline.com/articles/abstract/inhibition-of-methicillin-resistant-emstaphylococcus-aureusem-biofilm-by-ethanol-extracts-of-emsauropus-androgynusem-and-4358.html

<1% match (Internet from 25-Jan-2024)
https://mdpi-res.com/d_attachment/microorganisms/microorganisms-11-01614/article_deploy/microorganisms-11-01614.pdf?version=1687172572

<1% match (Internet from 12-Feb-2024)
https://www.sechenov.ru/science_and_innovation/repo/1526562/PAGEN_1=99

S.M. Azeem Mohiyuddin
23/5/25

TITLE: MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT – A CROSS SECTIONAL STUDY ABSTRACT Background: The significance of issue of biofilms lies in the global rise of antimicrobial resistance, where biofilms play a crucial role by acting as reservoirs of resistance genes. These biofilms contribute to therapeutic failure in standard antibiotic regimens and serve as chronic foci for reinfection. Additionally, their detection is often delayed, resulting in prolonged hospitalization, increased healthcare costs, and higher morbidity. Biofilms are also linked to airway obstruction, requiring additional interventions and monitoring. Biofilms are structured microbial communities encased within a self-produced polymeric matrix that adheres to surfaces. Their resilience against antibiotics and host immune defenses renders them a major contributor to persistent infections in healthcare settings. In tracheostomy tubes, biofilms frequently colonize the inner surface, leading to chronic infections such as tracheitis, pneumonia, and even sepsis. These biofilms are particularly dangerous in immunocompromised patients, including those with diabetes, HIV, or systemic infections. The presence of multidrug-resistant organisms, such as Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, exacerbates the clinical burden. Timely identification, monitoring, and antibiotic sensitivity testing of biofilm-forming bacteria are essential for improving patient outcomes and managing antibiotic resistance. Objectives : 1. To determine the microbial profile in biofilms on tracheostomy tube and tracheostoma. 2. To document the antibiogram of isolates in biofilms. 3. To evaluate the response of biofilm to antibiotic treatment. Methodology :The study also recorded clinical parameters such as duration of ICU stay, underlying comorbid conditions, frequency of tracheostomy tube changes, and type of care (hospital vs home care). Each isolate was subjected to gram staining, culture on selective media, and biochemical identification. The microtiter assay utilized optical density readings at 570 nm to quantify biofilm strength. Patients were categorized based on risk profiles to allow for subgroup analysis. Regular follow-ups and documentation of clinical progress were maintained for each patient to correlate microbial data with clinical outcomes. This prospective cross-sectional study was conducted at R.L. Jalappa Hospital & Research Centre between April 2023 and July 2024. A total of 95 tracheostomized patients were enrolled. Swabs were collected from the tracheostomy tube and tracheostoma on Day 0, Day 7, Day 14, and Day 30 post-procedure. Microbial cultures were performed, and biofilm detection was carried out using the microtiter plate method with crystal violet staining. Antibiotic susceptibility was tested by the Kirby-Bauer disc diffusion method as per CLSI standards. Data analysis was done using SPSS version 24. Statistical significance was considered at $p < 0.05$. Results Patients with multiple comorbidities exhibited more aggressive biofilm progression, especially in the second and fourth weeks. A comparative analysis revealed that patients receiving regular tracheostomy care and timely suctioning showed lower rates of strong biofilm formation. Statistical evaluation further confirmed that strong biofilms were significantly associated with prolonged ICU stays and higher chances of ventilator-associated pneumonia. Interestingly, in patients with oral malignancy, microbial profiles were more polymicrobial, suggesting increased vulnerability due to mucosal

ACKNOWLEDGEMENT

*First and foremost, I thank the **"Lord Almighty"** for showering his blessings and giving me the strength during my post-graduation and providing me everything that I required in completing my dissertation.*

I would like to thank my beloved guide, **Dr. S M AZEEM MOHIYUDDIN, M.B.B.S., M.S, FICS., FACS., MNAMS, SEKHSARIA FELLOWSHIP IN HEAD AND NECK SURGERY, PROFESSOR AND HEAD**, *Department of Otorhinolaryngology and Head and Neck surgery, Sri Devaraj Urs Medical College, Tamaka, Kolar for being the epitome of a teacher, with whom I completed this dissertation with utmost enthusiasm. Her unique teaching style and thought provoking ideas on the topic left a strong impression on me. Her unwavering support and inspiration have been invaluable throughout this journey.*

I am also thankful to **DR.ARVIND NATARAJAN-MBBS,MD,MNAMS, PROFESSOR**, **DEPARTMENT OF MICROBIOLOGY**, **DEAN OF ALLIED HEALTH SCIENCES** , *Sri Devaraj Urs Medical College, Tamaka, Kolar, for his valuable inputs and ideas in the topic and being an extending support throughout.*

My deep gratitude to the entire team of Department of Otorhinolaryngology, **DR . K C PRASAD , DR. SAGAYARAJ A , DR G K NARAYAN (PROFESSORS) , DR KOUSER MOHAMMADIN(ASSOCIATE PROFESSOR), DR.INDU VARSHA G , DR.UJVAL M , DR USHA G , DR. MEENAVALLI ROHITHA , DR DEVIKA SINHA (ASSISTANT PROFESSORS) , DR DIANA ANN JOSE , DR INDRANIL PAUL , DR AKSHAYA, DR ZUALI (SENIOR RESIDENTS)** *and my seniors* **DR SREELEKSHMY, DR MONESHA , DR KRITHIGA, DR GAUTHAM S , DR BOSCO SURIYA, DR HITHYSHREE ,DR CHARUVI , DR SREEJITH** *for their guidance and encouragement throughout the course. The impact and influence of their work on my own study is strikingly obvious in this dissertation*

I am extremely grateful to all my PG colleagues **DR CHANDANA , DR GOKUL , DR RACHANA J , DR PAVITHRA , DR SWATHI , DR DEEPTHI, DR ASMITHA ,** *and my juniors* **DR VYSHNAVI , DR MEENU , DR RISHITHA , DR HANEESHA, DR GAYATHRI, DR SYNAN , DR MANOGNYA, DR SHREYA** *for their assistance and comradeship during my post-graduation course. More importantly, to these people, for listening to and, at times, having to tolerate me over the past three years.*

I am extremely thankful to tutor, PhD scholar(Microbiology), **SAI SRAVAN KUMAR.P,** *for his constant help and guidance throughout the dissertation to which I am deeply obliged.*

Above all, I owe my wholehearted gratitude and love to my parents **MR. N SRINIVAS , MRS N SUJATHA** *and my sister* **MRS N SRI VYSHNAVI** *who have always been an infinite source of inspiration, love, support and encouragement throughout . I thank them for giving me everything in life that I could have ever wished for and bringing out the best version of myself. This dissertation is a defining statement to the unconditional love and encouragement from my entire family*

I would like to extend my deepest gratitude to my dearest friends, ***DR SAI PRITAM.K, DR ASHISH.N, DR TARUN.V, DR TARUN KUMAR.R, DR MEGHANA.M.S*** *who have been my pillars of strength, laughter, and unwavering support throughout this journey. In moments of stress and self-doubt, your encouragement lifted me up; in moments of joy, your presence made everything brighter, Thank you for being my chosen family—your love, loyalty, and belief in me have been the silent forces behind every step of this achievement.*

Last but not the least, I wholeheartedly thank all my patients and their families who submitted themselves most gracefully for this study. To these stoic people who showed great strength despite their suffering, let me say, I am greatly indebted…Thank you and God bless.

Dr.NALLAGONDA SATYA SAI SRIRA

LIST OF ABBREVIATIONS

ABBREVIATION	EXPLANATION
CLSI	Clinical and Laboratory Standards Institute
COPD	Chronic Obstructive Pulmonary Disease
CVA	Cerebrovascular accident
CV	Crystal violet
CDLS	Central Diagnostic Laboratory Services
ELISA	Enzyme-Linked Immunosorbent Assay
EPS	Extracellular polymeric substances
HIV	Human Immunodeficiency Virus
ICMR	Indian Council of Medical Research
ICU	Intensive Care Unit
MDR	Multidrug resistance/Multidrug-resistant
NA	Not applicable
OD	Optical density
ODC	Optical Density Cutoff value
Piptaz	Piperacillin-tazobactam (implied by usage)
SD	Standard deviation
SEM	Scanning electron microscopy
SPSS	Statistical Package for the Social Sciences
TB	Tuberculosis
TCP	Tissue culture plate
TT	Tracheostomy tube
VAP	Ventilator associated pneumonia
XLT	Extended-Length Tracheostomy Tubes

TITLE: MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT – A CROSS SECTIONAL STUDY

ABSTRACT

Background:

The significance of issue of biofilms lies in the global rise of antimicrobial resistance, where biofilms play a crucial role by acting as reservoirs of resistance genes. These biofilms contribute to therapeutic failure in standard antibiotic regimens and serve as chronic foci for reinfection. Additionally, their detection is often delayed, resulting in prolonged hospitalization, increased healthcare costs, and higher morbidity. Biofilms are also linked to airway obstruction, requiring additional interventions and monitoring.

Biofilms are structured microbial communities encased within a self-produced polymeric matrix that adheres to surfaces. Their resilience against antibiotics and host immune defenses renders them a major contributor to persistent infections in healthcare settings. In tracheostomy tubes, biofilms frequently colonize the inner surface, leading to chronic infections such as tracheitis, pneumonia, and even sepsis. These biofilms are particularly dangerous in immunocompromised patients, including those with diabetes, HIV, or systemic infections. The presence of multidrug-resistant organisms, such as Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, exacerbates the clinical burden. Timely identification, monitoring, and antibiotic sensitivity testing of biofilm-forming bacteria are essential for improving patient outcomes and managing antibiotic resistance.

Objectives :

1. To determine the microbial profile in biofilms on tracheostomy tube and tracheostoma.
2. To document the antibiogram of isolates in biofilms.
3. To evaluate the response of biofilm to antibiotic treatment.

Methodology :

The study also recorded clinical parameters such as duration of ICU stay, underlying comorbid conditions, frequency of tracheostomy tube changes, and type of care (hospital vs home care). Each isolate was subjected to gram staining, culture on selective media, and biochemical identification. The microtiter assay utilized optical density readings at 570 nm to quantify biofilm strength. Patients were categorized based on risk profiles to allow for subgroup analysis. Regular follow-ups and documentation of clinical progress were maintained for each patient to correlate microbial data with clinical outcomes.

This prospective cross-sectional study was conducted at R.L. Jalappa Hospital & Research Centre between April 2023 and July 2024. A total of 95 tracheostomized patients were enrolled. Swabs were collected from the tracheostomy tube and tracheostoma on Day 0, Day 7, Day 14, and Day 30 post-procedure. Microbial cultures were performed, and biofilm detection was carried out using the microtiter plate method with crystal violet staining. Antibiotic susceptibility was tested by the Kirby-Bauer disc diffusion method as per CLSI standards. Data analysis was done using SPSS version 24. Statistical significance was considered at $p < 0.05$.

Results: Patients with multiple comorbidities exhibited more aggressive biofilm progression, especially in the second and fourth weeks. A comparative analysis revealed that patients receiving regular tracheostomy care and timely suctioning showed lower rates of strong biofilm formation. Statistical evaluation further confirmed that strong biofilms were

significantly associated with prolonged ICU stays and higher chances of ventilator-associated pneumonia. Interestingly, in patients with oral malignancy, microbial profiles were more polymicrobial, suggesting increased vulnerability due to mucosal breakdown. Antibiotic resistance showed a temporal pattern, with some isolates developing resistance by Day 30 that were previously sensitive. This underscores the importance of continuous microbiological surveillance in dynamic hospital environments.

The cohort included 44 males and 51 females, with a mean age of 57.29 years. Carcinoma of the oral cavity and larynx was the leading diagnosis (62%). Biofilm formation increased progressively, peaking on Day 14. Klebsiella pneumoniae was the most frequently isolated biofilm-forming organism (38%), followed by Pseudomonas aeruginosa (17%) and Acinetobacter baumannii (15%). Biofilm strength varied, with a notable increase in moderate to strong biofilm presence by Day 14. Amikacin and Colistin were the most effective antibiotics, while resistance to cephalosporins and beta-lactams was frequently observed. Among comorbidities, diabetes ($p = 0.001$), HIV ($p = 0.04$), and sepsis ($p = 0.03$) showed significant correlation with biofilm formation. Pneumonitis was observed in 9 ICU patients, correlating with progressive biofilm formation over the month.

Conclusion: The research reaffirms the role of biofilms in complicating post-tracheostomy outcomes and highlights the gap in current prevention strategies. Incorporating routine biofilm screening into ICU protocols can help guide antimicrobial therapy more effectively. In addition, the study encourages development of biofilm-inhibiting materials for tracheostomy tubes, regular training for healthcare providers, and implementation of hospital-wide antibiograms to manage evolving resistance patterns. Strengthening preventive measures in both inpatient and outpatient care is essential for improving the quality of life and survival of tracheostomized patients.

This study demonstrates the significant presence of biofilms on tracheostomy tubes, especially in immunocompromised patients. Klebsiella pneumoniae was identified as the

dominant organism and exhibited high levels of multidrug resistance. The findings highlight the need for regular microbial surveillance, targeted antibiotic therapy, and rigorous hygienic protocols for tracheostomy care. High-risk groups, such as patients with diabetes, HIV, or sepsis, should receive enhanced monitoring to prevent complications like pneumonia. Future research should focus on anti-biofilm strategies and establishing standardized care protocols for long-term tracheostomized patients.

Keywords

Tracheostomy, Biofilm, Klebsiella pneumoniae, Multidrug resistance, Diabetes, HIV, ICU

TABLE OF CONTENTS

LIST OF TABLES:

LIST OF FIGURES:

INTRODUCTION

INTRODUCTION

Bacterial biofilms are the principal contributors to the vast variety of health complications by exhibiting antibiotic resistance. Biofilms are oftenobserved on the exterior of hospice devices and body flesh, in manufacturing and diet dispensation units, and in normal atmospheres.[1] Nearly all microbes can generate biofilms.

As stationary bacteriological groups coated in extracellular polymeric substances (EPS), bacterial biofilms are typically identifiable. Variations in the permanent union of infectious compartments to external surfaces or substrata, or to each other, are characteristics of biofilms. They are fixed in extracellular polymeric substances (EPS) and retain certain phenotypes in the context of genetic factor transcript and progression. A single bacterium or a mix of bacteria, mushrooms, archaea, protozoans, and mildews can form a bacteriological biofilm. Its network assembly handles the problem of disinfectants, nutrients, and vapours.

Planktonic bacteria can collectively system biofilms, which show comparable appearances to medicinal device-associated biofilms.[2]As therapeutic technology improves, more medical devices are used, and patients strive for a good quality of life, biofilms that are attached to medical devices become a clear threat to their health and lives.

Microbes can adhere to almost any remedial material and cause biofilm contaminations linked to therapeutic devices. Device-associated scum frequently arises as a result of action, in which certain bacteria initiate from the host. These bacteria can set up a biotic ampule when they take over a remedial device's surface. Microbes in composite humans that observe and grow on convenient exteriors are linked to the aetiology of infections linked to restorative devices.

Device-associated biofilms are complex microbial communities that form on the surfaces of implanted medical devices, such as catheters, prosthetic joints, and heart valves. These biofilms can consist of a single microbial species or multiple species, depending on factors like the type of device and its duration of use within the host. The formation of biofilms begins when microorganisms adhere to the device surface and secrete extracellular polymeric substances, creating a protective matrix that facilitates further colonization and

resistance to external threats, including antimicrobial treatments and host immune responses.

The most common pathogens associated with medical device-related infections are *Staphylococcus aureus* and *Staphylococcus epidermidis*, both of which are capable of forming robust biofilms. These infections are particularly challenging to treat due to the protective nature of the biofilm, which can shield bacteria from antibiotics and immune system defenses. In some cases, the removal of the infected device is necessary to effectively manage the infection .

Additionally, multidrug-resistant (MDR) gram-negative bacteria, such as *Acinetobacterbaumannii*, *Klebsiellapneumoniae*, and *Pseudomonas aeruginosa*, have been increasingly identified in device-associated infections, especially in complex hospital settings. These pathogens contribute to the persistence and severity of infections, complicating treatment strategies and posing significant public health concerns

In summary, the formation of biofilms on medical devices by various microorganisms, including both gram-positive and gram-negative bacteria, poses significant challenges in the management of device-associated infections. Understanding the mechanisms of biofilm formation and the factors influencing microbial colonization is crucial for developing effective prevention and treatment strategies.[3]

In addition to this, bacteria can stick to many tissue exteriors in figure (e.g., skin, abdominal mucosa, vascular, oral hollow, airway, bone skin, and vagina), which in try can origin non-device -allied biofilm contaminations and lead to various diseases. [4]

Biofilms comprise three-dimensional assemblies consisting of microorganisms surviving on an extracellular atmosphere made of polysaccharidesand proteins. [5] Multiple research findings revealed that 60% of hospitalacquired contaminationstriggeredby biofilm-forming bacteria on medicinaldiplomacies. [6,7]

Tracheotomy is one of the primogenital and maximum frequently done otolaryngological clinicalmeasures. The procedure includes establishing an introductory in anterior wall of windpipe to facilitate the supplement of a tracheostomy tube into the airway lumen.[8–10] Healthcare-associated infections (HCAIs) occurring in affected role with tracheotomyare challenge in contemporary medicine and are notably more prevalent in this demographic compared to others. The chances of infection in these individuals is associated with various predisposing factors, the insensitivity of analytical and beneficialmeasures,

staff precautions, and the increase inconfrontation of viruses to frequently utilized antibiotics. [11]

It is important to note that a wide range of bacterial microflora colonizes tracheostomy tube patients due to the extensive colonization of the tube. In addition to inadequate patient care, this could cause the germs in the lower respiratory tract to move, which would then trigger the inflammatory process.[12]

Respiratory tract impurities that present as per ventilator-associated pneumonia (VAP), which is likely to occur in 4–28% of patients, are the most frequent infections among tracheostomy patients. [13,14]Additionally, the potential for chronic otitis media with expression, in which frequently pragmatic in Ear, Nose, and Throat (ENT) facilities, and wound infections around the stomata are significant complications that may result from this procedure. [15,16]

A clinically momentous category of impurities pertains to those intricately linked to the biomaterials utilized in present daymedication, known as biomaterial-associated infections (BAIs). The capacity of microbes to develop biofilm is crucial in the pathogenesis of corruptions linked to the utilization of materials, which create a suitablesuperficial for microbial annexation.[17]

The prolonged presence of indwelling prostheses in tracheostomized patients results in the inevitable formation of biofilms on tracheostomy pipes. The broadcast of these biofilms to the minorroutezones can lead a serious difficulties such as pneumonia or sepsis.[18] The various ingredients used in tracheostomy tubing demonstrated no variation in their vulnerability to biofilm formation.[19] The extended duration of tracheostomy did not demonstrate a significant correlation with biofilm formation, which was observed as premature as 7 days.[20]

Topical studies have identified Staphylococcus aureusand Acinetobacter species as the prevalent creatures responsible for biofilm formation on tracheal tubes.[21,22] Acinetobacter emerged as the predominant multidrug-resistant organism, exhibiting sensitivity towards Carbapenem and Colistin. In contrast, Staphylococcus aureus demonstrated sensitivity to Linezolid, while Pseudomonas showed responsiveness to Imipenem.[23]

To prevent these issues, it is essential to utilize suitable prophylactic broad-spectrum antibiotics, maintain meticulous tube hygiene during the hospital stay as well as at the patient's residence, and closely monitor immunocompromised patients.

It is essential to identify the organisms that form biofilm in tracheostomy tubes within our institution. This will enable us to tailor and personalize treatment effectively, thereby preventing potential ventilator associated pneumonia (VAP).

OBJECTIVES OF THE STUDY

OBJECTIVES :

1. To determine the microbial profile in biofilms on tracheostomy tube and tracheostoma
2. To document the antibiogram isolates in biofilms
3. To evaluate the response of biofilm to antibiotic treatment

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Background

A biofilm is consortium of microbes like bacteria that can live and reproduce as a group, such as a colony. Biofilm structure plays a dual role in protecting and in the expansion of its colony.

The human figure has a vast microbiome that is huge and multifaceted, comprising bacteria, viruses, and fungi. Most of the microbiota reside in intestinal tract, mucosa, and skin,wherever they assist several biologicalpurposes that vary from metabolism to innate protection. But under confident unfavourable conditions, these symbiotic microorganisms cannot be controlled and can diverge to the formation of biofilms. [24]

Microbeshappen in two stages like planktonic, i.e, free floating, and stalkless, which attach to surfaces. Bacteria display varied traits in amongst these states as infectiousadd-on to surfaces roots drastic variation in the gene expression levels connected with ripening and creation of exopolysaccharides (EPS), which are termed as SLIME. A defensivebarricade is then formed due to this transition that enables bacterial colonization in biotic and abiotic surfaces. This newly formed barrier protects the microbes from the defenceapparatuses of the host and antibiotics. [25–27]

Antonie van Leeuwenhoek was the first to observe bacteria adhering to surfaces, but the term "biofilm" was not introduced until 1978.In that year, J. William Costerton and colleagues published a seminal article in *Scientific American*, titled "How Bacteria Stick," which proposed that bacteria predominantly exist in surface-attached communities encased in a self-produced matrix, a concept that revolutionized our understanding of microbial life

The standing of biofilms was added by American Society of Microbiology in 1993.

Casterton et al. in 1999 then characterised biofilm as an organised people of germs enclosed in a polymeric medium yielded by the bug which is attaching to the surface. [28,29]

The growth of microorganisms in biofilms has been related to a wide variety of human diseases, as well as the colonization of medical equipment. These germs are extremely resilient to the antimicrobic therapies that are available. The process of disease is

initiated by the formation of biofilm through a variety of mechanisms. These mechanisms include the detachment to separate cells of bacteria or cell bunchestotals, manufacture of the endotoxins, amplifieddodging fromshadowing of the host protectedscheme, and the formation of a defensiveblockade that is helpfulto advent of immune-resistant bacteria.

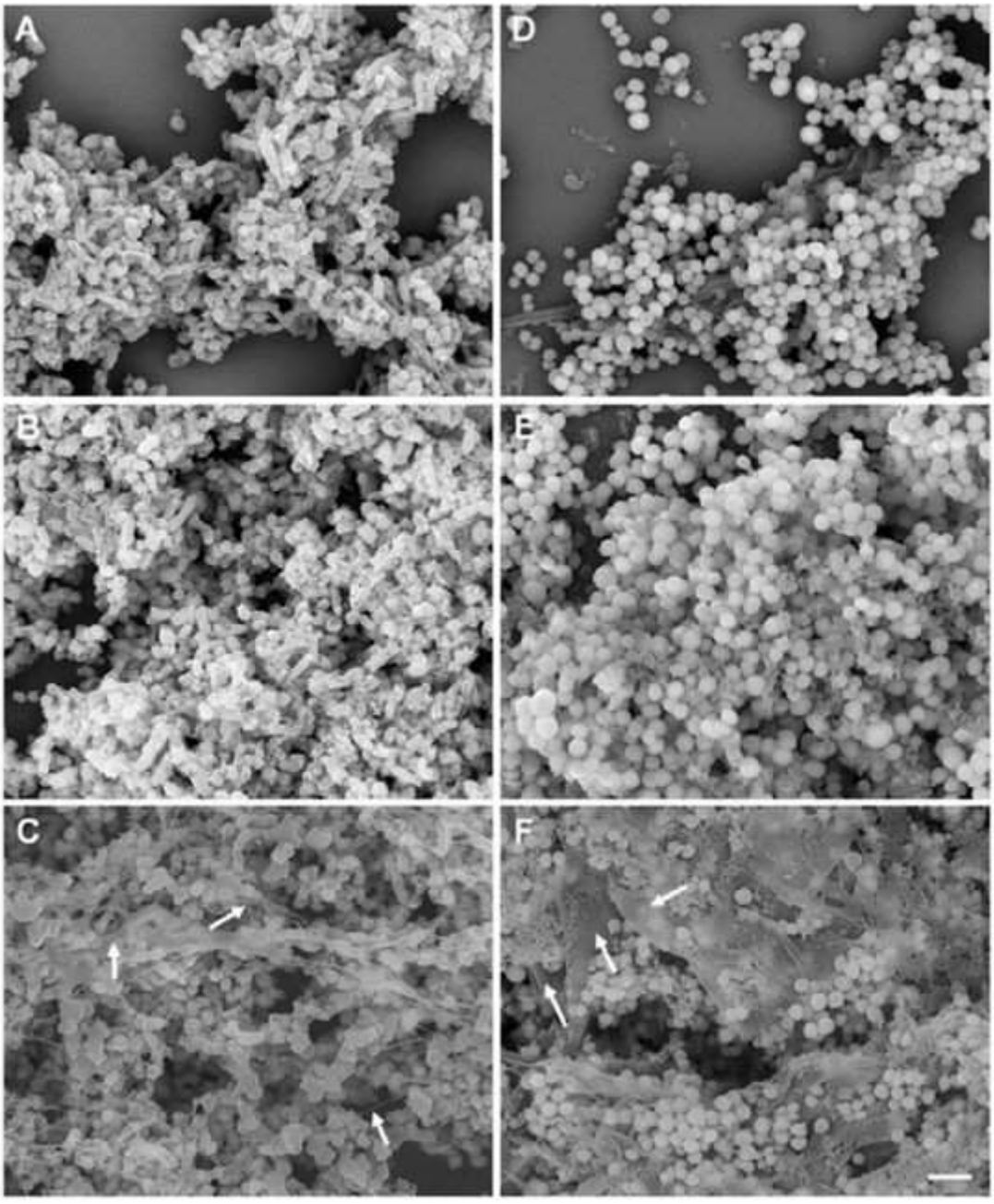


Figure 1: An example of a conventional scanning electron microscopy (SEM) image of biofilm that was developed by M. haemolytic (D153) cultured in colourless RPMI 1640

(A–C) and S. aureus Newbould 305 (NB305) grown in BHI broth (D–F) on circular glass coverslips in 24-well plates at 37 degrees Celsius. Fixative solutions containing 10% formalin (A,D), 2.5% glutaraldehyde (B,E), or Methacarn (C,F) were used to fix biofilms that had been developed on glass coverslips for 48 hours. After that, the samples were further processed for scanning electron microscopy analysis. The white arrows in the diagram represent the EPS layers that are located at top, in the centre, and at bottom of the biofilms (C,F). PLoS One, volume 15, issue 5, page e0233973, will be cited as the source for this figure. This is an open-access article that is available to the public without any copyright restrictions and is reprinted below Creative Commons CC0 public domain dedication.

The recentconsiderate of biofilms is immovablemultifacetedconstruction with solitary or numerousclasses of bacteria, and by-products with lockupsdevoted to the surface thenenclosed by extracellular polymericingredientshaped by microbes. Any shallow givesdampness and nutrients areperfectsetting for biofilm growth. These biofilms may be decent, evil, or neutral.

Biofilms are the agents responsible for 70% of microbial infections. The bacteriaexistingis a part of the biofilm demonstratestypicaltopographies, as collaboration, source seizing, as augmented subsistencein contradiction of action with disinfectants. Amplified endurance and illusionis the host immune system make biofilms accountable aimed attenacious chronic poisons. [30]

Makeup and Nature of Biofilm

Biofilm contains 10% microbes and 90% water. 50%–90% of biofilms' organic component is matrix polysaccharides. Mesh like polysaccharide chains. Communication between polysaccharide hydroxyl groups increases mechanical strength. Biofilms can grow up to 300 micrometers thick with positively charged ions like Ca2+ or Mg2+ forming cross bridges between polymers. However, biofilm polysaccharides can be neutral or polyanionic, like Gram-negative bacteria's EPS. D-glucuronic and D-galacturonic acids, and anionic, are also found in biofilms. [31–38]

These characteristics facilitate the binding of divalent cations to inert polymer strands, enhancing the adhesive strength of mature biofilms..

Gram-positive bacteria have cationic EPS. Most physiological activities in biofilm are controlled by sessile bacteria. The most common biofilm bacteria include Pseudomonas aeruginosa, Staphylococcus epidermidis, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, and S. aureus. [39–49]

Formation of Biofilm

Due to species diversity, bacterial biofilm production stages differ yet are similar. Attachment to the entity, bacterial bond, emission of extracellular polymeric ingredients, colony construction, bacteriological cell escapes and dispersal, biofilm development are all part of the multi-step process. [50]

The complete biofilm formation is depicted in the figure below.

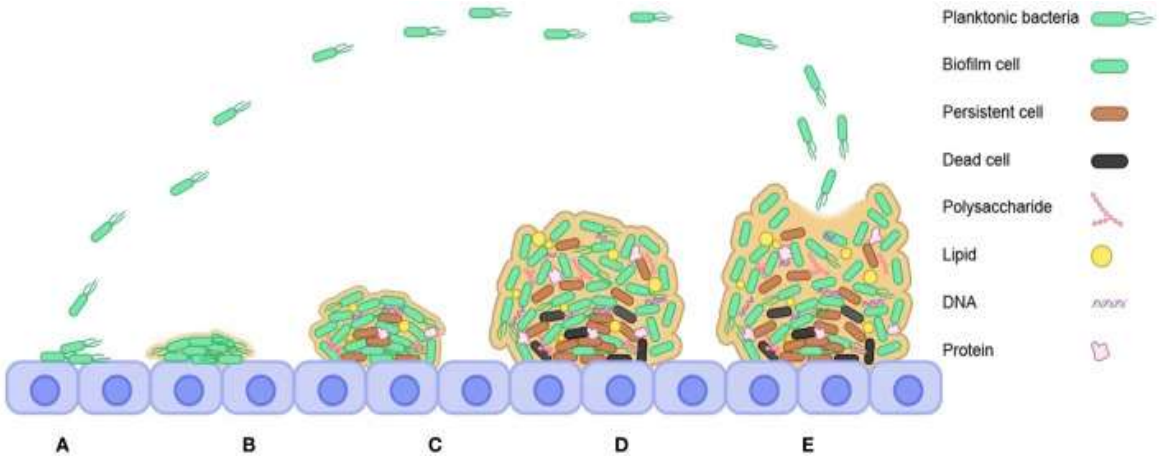


Figure 2: Steps to bacterial biofilm formation.

(A) Reversible attachment.

(B) Irreversible attachment.

(C) Bacterial cells synthesize and secrete EPS.

(D) Maturation. And

(E) Dispersion

Add-on

First, bacteria connect an external surface to form biofilms. Bacteria reachs the surface by Brownian motion, sedimentation or convection,.[51] Chemotaxis is common in bacteria and allows free floating cells of bacteria in fluids move material concentrations to chemically triggered materials (amino acids and sugars) or food bases. It helps microbial cells attach to an object's surface, allowing planktonic bacteria to flourish. The sum of the forces pushing or pulling against surface of cell&as well the object's surface limits how

planktonic bacteria interact with it. [52] Bacteria can't stick if pushing is stronger than pulling. Surface bacteria can stick if the pulling force is stronger than the pulling force.

Non-specific physical forces like Vander Waal forces, electrostatic forces, and hydrophobic communications bind planktonic bacteria to the object's surface, transforming them into stable cells. It is reversible and has weak adherence. Reversible microorganisms are two-dimensional. Biofilm substrates cling to all planktonic bacteria-contact substrates. Substrate roughness, film modulation, and hydrophobicity can inhibit biofilm colonization and production.[53,54]

Hydrophobicity mostly helps bacteria stick to surfaces. Hydrophilic substance reduces bacterial cell surface-substrate repulsion, yet hydrophobic force promotes colonization.

Negative surfaces prevent planktonic bacteria attachment, while positively charged surfaces aid attachment. The surface charge of microbial cells depends on species, age, pH, ionic asset, and culture average.[54]

Irreversible adhesion

In the second phase of biofilm formation, known as irreversible adhesion, bacteria establish firm attachments to surfaces through various interactions.This process is facilitated by short-range forces such as dipole–dipole interactions, hydrogen bonds, ionic and covalent bonds, and hydrophobic interactions.Bacterial cell surface structures, including flagella and fimbriae, play crucial roles in this phase.Flagella enable motility, allowing bacteria to approach and adhere to surfaces, while fimbriae enhance cell surface hydrophobicity and help overcome electrostatic repulsion barriers, promoting stronger attachment to substrates.These interactions collectively contribute to the formation of a stable biofilm, which is essential for bacterial colonization and persistence on surfaces..[52] Flagella can move through liquids and gather on moist solid substrates. Several bacterioplankton species attach cells to surfaces and migrate to favourable environments using these two locomotion strategies. Due of their ability to overcome cell-surface resistance, flagella help planktonic bacteria attach to surfaces. Fimbriae help bacteria connect to surfaces and each other. P. aeruginosa twitches via fimbriae. The QS system promotes bacterial biofilm development in individual cells through intercellular communication [56]. Quorum sensing helps bacteria communicate by

producing and emitting chemical signals. Gram-positive and Gram-negative bacteria utilize distinct signaling molecules to regulate biofilm formation.Gram-positive bacteria primarily employ small peptides known as autoinducing peptides (AIPs) for quorum sensing, while Gram-negative bacteria predominantly use N-acyl homoserine lactones (AHLs).[57]

Bacterial cell synthesizes and secrets

Bacteria produce biofilm in the third stage by synthesizing and secreting extracellular polymeric molecules. A hydrophobic, ion-bridging EPS substrate may increase microbialreduction and biofilm adherence. EPS affects surface bond, bacterial biofilm production, internal assembly, cell gratitude, signal transduction, nutrition acquisition, cell maintenance, and genetic information sharing, according to Rib et al. [58] The second envoi makes bacterial attachment irreversible by creating the extracellular polymeric substance (EPS) milieu and bacterial cell surface constructions. The matrix needs extracellular polysaccharides for biofilm growth. EPS has many enzymes and fimbriae. The extracellular polymeric substance (EPS) matrix includes eDNA and lipids. According to Flemming et al. (2016), the previous link with bacterial cells,later can affect Thiobacillus ferroxidase adhesion.[59]

Maturation

The maturity of microbial biofilms constitutes the fourth step in biofilm growth. Bacterial biofilms occur as microbes multiply besides proliferate inside the extracellular polymeric substance (EPS) milieu, resulting in the creation of tiny microbial colonies and the growth of three-dimensional constructions. The buildup of the EPS milieu and the growth of bacterial colonies lead to modified gene expression, with the resultant products of these genetic factor EPS matrix synthesis. The milieu's construction enables the creation of water channels that operate like the body's circulatory system, supplying nutrients to the cellular community while eliminating unnecessary substances.

Dispersal

The dispersal of bacterial biofilms signifies the conclusion of biofilm growth. Adjustments in the building of mature bacterial biofilms occur subsequent harm. Discharged bacteria can infect other organs and form biofilms. Dispersal courses show disparity due to bacterial specificity. All typically necessitate the dissociation of bacterial cells from small groups, followed by transmission to alternative substrata and following

adhesion to those substrates.[60] Bacterial biofilms may disengage over energetic or inactive mechanisms. Active behaviour involves sowing spreading, wherein biofilm bacteria detach to adapt to environmental changes in response to matrix-degrading enzymes, antimicrobials, and food scarcity. Passive behaviour encompasses external factors such as shear shedding and erosion dispersion. Flaking dispersion refers to the hasty loss of significantpercentage of a microbial biofilm, whereas corrosion dispersal involves the release of a subset of bacterial cells. Kaplan (2010) demonstrated that reduced c-di-GMP expression hinders bacterial biofilm formation and facilitates biofilm detachment. Reserve the c-di-GMP signalling pathway is capable to lead to the dispersion of biofilms.[61]"Temperature, pH, oxygen, and nutrient levels" can influence biofilm dispersal. Low-oxygen environments facilitate biofilm formation in bacteria by rushing the degradation of c-di-GMP. Elevated glucose concentrations suppress c-di-GMP and promote flagellar synthesis, thereby impeding separation[62]

Temperature, blood pH, nutrition availability, quorum sensing mechanisms, Brownian motion, and surface features affect bacterial biofilm development. Multiple strains and signal transduction systems affect biofilm growth. Matrix, regulatory, connective, and bacterial biofilm layers make up the mature biofilm, from inner to outer.

Biofilm formation on medical devices

Microbescan form biofilms on the surfaces of devices, which can result in the growth of communicablebugs within the host creature. Medical grafts have made remarkable progress in the field of medicine, providing innovative opportunities for improving human health and extending longevity. They also promote tissue infections. Biofilm generation in device-related infections begins with non-specific reversible and particular irreversible bacterial adherence. 'Staphylococcus aureus' and 'epidermidis' are the main rinsings.[63]Microorganisms can originate from various sources, including the patient's skin, healthcare workers' skin, or the surrounding environment. *Staphylococcus epidermidis* utilizes specific surface proteins known as adhesins to facilitate attachment to both each other and medical devices. These adhesins enable the bacteria to adhere to host proteins such as fibrinogen, collagen, and fibronectin, which are commonly found on medical devices.

On the other hand, *Staphylococcus aureus* employs a different set of adhesins to bind to host proteins. These adhesins include clumping factors A and B, which bind to fibrinogen, and fibronectin-binding proteins A and B, which bind to fibronectin. Additionally, *S. aureus* expresses a collagen adhesin that facilitates attachment to collagen.

Both *S. epidermidis* and *S. aureus* utilize these adhesins to establish biofilms on medical devices, contributing to persistent infections.

Developing 'device-associated biofilms' requires plasma proteins on medical devices.[64] The amount and type of plasma proteins that attach to device outward depend on their physicochemical qualities. Staphylococcus aureus adheres to medical equipment with adhesins. Adhesins identify device-adsorbed plasma proteins. S. aureus can multiply and create extracellular polymeric substances (EPS) like polysaccharides, proteins, and eDNA. S. aureus population dispersion promotes infection transmission in the final stage. [65] Inhibiting EPS formation, enzyme-mediated EPS breakdown, and surfactants impact bacterial dispersion. [66] Phenol-soluble modulinos (PSM) and extracellular enzymes affect S. aureus biofilm dispersion significantly. PSM is crucial to biofilm diffusion. It upsets the non-covalent requisite forces that maintain the biofilm milieu, creating routes for nutrition delivery to the deeper layers. [67]

Device related infection

Biofilms on medical devices include a variety of microorganisms, which are linked to device infections. Bacteria stick to the medical equipment or tissue at the breaking. Bacteria reproduce, develop, and form EPS-encapsulated biofilms. Bacteria are released from the biofilm simultaneously. Bloodstream transmission can cause infection or localized lesions elsewhere.

'Innovators', 'artificial heart valves', 'cardioverter-defibrillators', and 'cardiovascular implanted electronic strategies' increase infection-related morbidity and mortality. [68]

Non-device related infections

Infections not associated with devices also significantly affect health issues. Examples of non-device-related bacterial biofilm contagions include dental plaque [69], urinary tract contagions[70], cystic fibrosis[70], otitis media [71], infective endocarditis [72],

tonsillitis [73], periodontitis [74], necrotizing fasciitis[75], infective kidney pebbles[76],chronicprovocative diseases [77] and bacterial vaginitis[78].

Methods for detecting biofilms

Nuclear medical imagerytechnology :

Nuclear medicine imagery is still used to detect infectious diseases [79].'Indium-111''Technetium-99m', , and 'iodine-125' label compounds. Limitations include limited target receptor expression on microbes, 'non-specific adsorption', 'complex radiochemical synthesis'[80]. Recent research showed that the maltodextrin transport system, a bacterial metabolic product, can outward label and trace jots to identify biofilm impurity[81]. Nuclear medicine imaging needs dedicated equipment, operator exercise, and patient fallout[82]. Researchers created an MH18F nuclear imagery agent that can contribute in bacterial carbohydrate digestionand ingested by maltodextrin transporters [81], detecting biofilms early.

Ultrasonic technology :

Kujundzic et al. (2007) found that ultrasonic technology can track the growth of microbiological colonies, including bacteria, on thedevice surfaces in real time. [83]It improves bacterial biofilm detection when paired with other methods, according to Vaidya et al. [84]. Ultrasonography echo sauces and microbubble disparity agents made arevolutionized diagnostic ultrasonography medicine [85]. Ultrasonic medical imagery has improved with disparity media, enabling more accurate diagnoses. A tailored ultrasonic contrast medium can identify healthy and sick tissues [86]. In vivo detection and targeting of bacterial biofilm substrates is difficult due to their acoustic impedance, similar to human tissue. Ultrasound and customized ultrasound contrast agents detect bacterial biofilms early to improve therapeutic success. Non-invasive imaging with ligand-targeted ultrasonic contrast agents can detect primary and twilight bacterial biofilms. These compounds can board, image, and notice S. aureus biofilm milieu production in vitro.[87]

Crystal Violet Staining :

Crystal violet (CV) staining is most commonly used staining technique for quantifying biofilm microtiters grown in the polystyrene porous dishes. After CV staining, scanning electron microscopy shows biofilm structure. The prolonged incubation time and reduced bacterial biofilms after numerous washings limit this staining approach. It cannot identify biofilms quickly. Castro et al. (2022) found CV staining extremely successful for

the ‘single-species biofilm’exposure. In bacterial vaginosis, analysing numerous biofilms may be biased.[88]

BACKGROUND OF TRACHEOSTOMY

Tracheostomy was conducted before modern medicine. Albucasis (1013-1116) sutured a tracheal wound and showed that it could restore in a servant girl who attempted self-destruction by slashing her gullet, advancing tracheostomy. During his medical studies, Avenzoar (1126-1198) successfully operated on a goat [89]

The first successful human case was Brasavola (1546). He saved a patient from suffocating from a windpipe abscess. [89] Fabricius (1617) explained the procedure's technicalities after Brasavola's instance. It was called the "Scandal of Surgery," and he declined the operation. He advised vertical skin incision and tube use.

Goodall (1934b) notes that first successful tracheotomy in a child was conducted by Caron in 1766, aimed theseelimination of a foreign body—a bean. Bretonneau illustrated the potential of tracheotomy in the management of Diphtheria was addressed through a surgical intervention that successfully saved the life of a 5-year-old girl, as documented by Bretonneau in 1826.[89] In 1833, Trousseau, a pupil of his, reported that he has conducted 200 operations successfully saved over fifty children suffering from progressive diphtheria (Nelson, 1957)[90] This report successfully addressed the concerns of sceptic’s and critics regarding the process, leading to tracheotomy being broadly embraced by the medical community. Trousseau also developed the dilator to facilitate the expansion of the tracheal opening during the insertion of the cannula. His instrument continues to be utilized in contemporary settings. Additionally, he highlighted the benefits of prompt and intentional surgical intervention, recognizing that the primary goal oftracheostomy is to ensure an unhindered airway. He subsequently advised tracheostomy for stenosing conditions of larynx, including TB and syph.

EMBRYOLOGY OF TRACHEA

embryology and development of the trachea represent a sophisticated process that commences in the initial weeks of gestation. The trachea develops from the endodermal layer of the embryonic germ disc. In the fourth week of gestation, the respiratory diverticulum, commonly referred to as the lung bud, originates since the ventral wall of the

foregut, which serves as the precursor to the gastrointestinal tract. The lung bud extends in a caudal direction, and at its distal end, it bifurcates to give rise to the primary bronchial buds. The bifurcation persists as the buds extend further to create the bronchial tree. The trachea originates from the proximal section of the respiratory diverticulum. The tracheoesophageal septum develops to create a separation between the trachea and the esophagus, thereby establishing distinct pathways for respiration and digestion. [91]

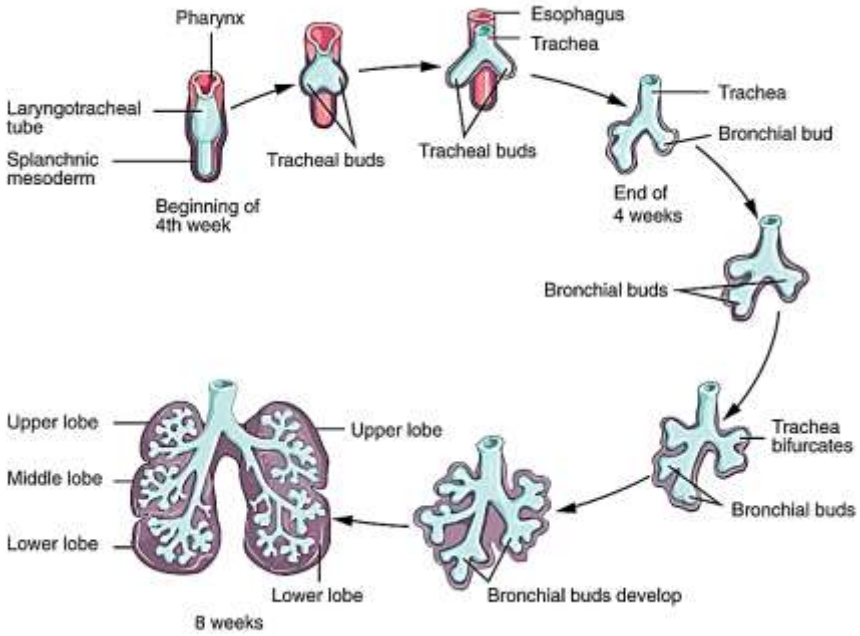


Figure 3: Early stages of Trachea development

During development, the tracheal epithelium, originating from the endoderm, undergoes differentiation into the ciliated pseudostratified columnar epithelium that is typical of the adult trachea. The mesodermal tissue in the surrounding area plays a vital role for the development of tracheal cartilage, conjunctive tissue, and flat muscle. By 10th week of gestation, trachea exhibits clear structural differentiation while it continues to undergogrowth and maturation during fetal development.

Tracheal anatomy

The trachea is an integral component of the conducting airway system, beginning just beneath the larynx linked to the cricothyroid gristle at the C6 level via the cricotrachealmuscle. It is a flexible tube made up of incomplete cartilaginous rings that are interconnected by membranous structures.

The structure comprises 16 to 20 hyaline cartilaginous rings. The posterior border of the trachea is constituted by the trachealis muscle, while the tracheal rings are characterized as U or D-shaped structures composed of hyaline cartilage on the anterior and lateral walls. The sheath is composed of pseudostratified ciliated columnar epithelium along by goblet cells.

The carina, located at the T5 level posteriorly and the sternal angle anteriorly, bifurcates into the right and left primary bronchi. These bronchi are asymmetrical, with the right being wider and shorter compared to the left. [92]

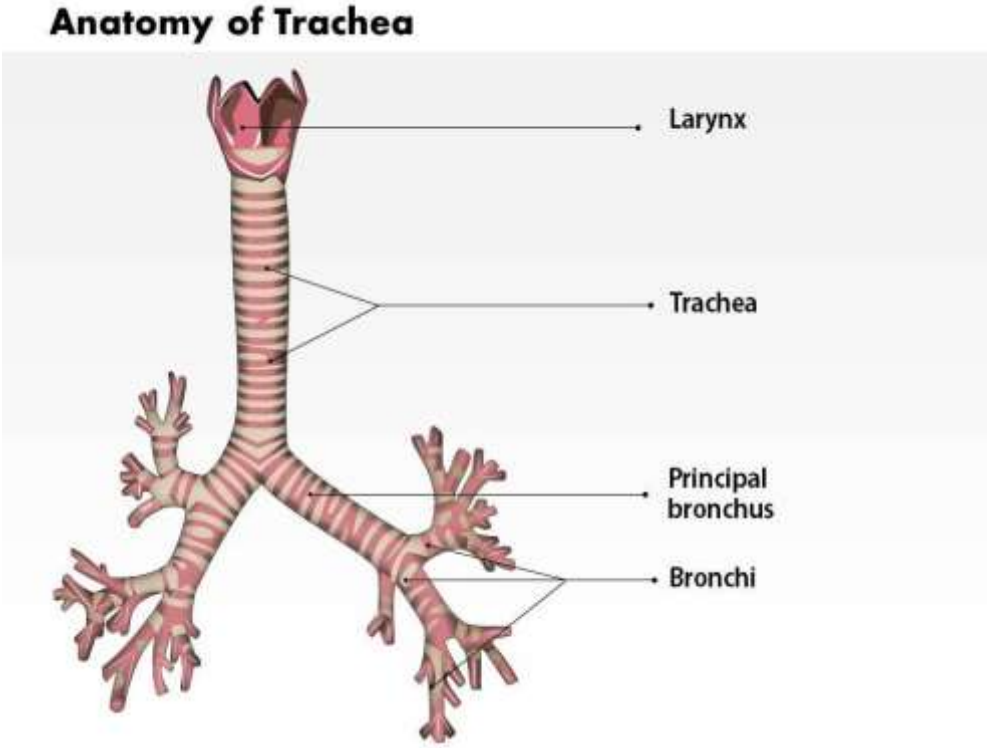


Figure 4: Anatomy of Trachea

The right bronchus is positioned at a steeper angle and is more directly aligned with the trachea, measuring 25 degrees from the vertical, in contrast to the left bronchus, which is at a 45-degree angle from the vertical.

FUNCTIONS OF TRACHEA

The air conduit serves a fundamental purpose by facilitating a clear and efficient pathway for air to flow in and out of the lungs. The trachea serves as a conduit between the larynx, commonly known as the voice box, and the bronchi, which direct airflow to the lungs.

The trachea features a lining composed of a mucous membrane, which includes cilia and cells responsible for mucus production, serving as a protective barrier.

The mucus serves to capture dust, bacteria, and various foreign particles, while the cilia facilitate the upward movement of these trapped particles toward the throat, enabling them to be either coughed out or swallowed, thereby safeguarding the respiratory system from infection and irritation.

The C-shaped cartilage rings provide structural integrity to the trachea, ensuring it remains open consistently while also permitting a degree of flexibility. The ability to adapt is crucial during swallowing, neck movement, and breathing, as the trachea can make slight adjustments to its shape and diameter. During respiration, particularly during forceful inhalation or exhalation, the trachea has the ability to modify its diameter. The trachealis muscle plays a crucial role by contracting or relaxing to regulate airflow, thereby contributing to effective respiration.

The trachea, while not directly responsible for sound production, serves a crucial function by facilitating the airflow to and from the larynx, the primary site of sound generation.

TRACHEOSTOMY

A tracheostomy is a medical procedure that involves creating anmock airway in the cervical trachea. At earliest well-documented instance of a tracheostomy (or tracheotomy) can be traced in the 15th century, though there is a speculation that the process have been conductedfar back 2000 BC. This process is routinely conducted in contemporary medical practice. Though, the term ‘tracheostomy’ developed encompasstogether the procedure and the scientific state of possessing a tracheostomy tube.[93]

The most common sign for a tracheostomy is to enable weaning off ventilation in exhaustive care patients.

Other motives for tracheostomy include:

- upper airway impediment;
- safe and preserve a safe airway in cases where the upper airway is unsafe (e.g. wounds to the face, head and neck);
- bronchial toilet and ooze removal;
- airway fortification (e.g. neuromuscular disorders)

Tracheostomy may be conducted via open dissection through the anterior neck or a percutaneous approach. The percutaneous tracheostomy technique has become increasingly favouredsubsequently its inception in 1980s, recognized as an effective substitute to surgical tracheotomy while eliminating the necessity transfer to the functioning theatre and achieving similar outcomes. The Seldinger technique is the most commonly utilized and documented method for performing percutaneous tracheostomy.[94,95]

TYPES OF TRACHEOSTOMY TUBES

There are various **types of tracheostomy tubes**, each designed for specific clinical situations. Here's a detailed overview:

1. **By Material**

a. Plastic (PVC or Silicone) Tracheostomy Tubes

- Materials: Polyvinyl chloride (PVC), polyurethane, or medical-grade silicone.
- Features:
  - Soft and flexible → reduces risk of tracheal injury.
  - Thermosensitive: becomes softer at body temperature.
  - Disposable (some types).
- Applications:
  - Used in both short- and long-term care.
  - Most common in ICU and home care settings.
- Examples:
  - Shiley™ (Covidien/Medtronic)
  - Portex® (Smiths Medical)
- Advantages:
  - Radiopaque line allows X-ray visualization.
  - Silicone variants are biocompatible and suitable for long-term use.
- Disadvantages:
  - Not as durable as metal.
  - Some may stiffen over time with repeated sterilization (PVC).[94]

b. Metal Tracheostomy Tubes

- Materials: Stainless steel or sterling silver.
- Structure: Often double-lumen (outer + inner cannula), with an obturator for insertion.
- Applications:
    - Used for long-term tracheostomy.
    - Suitable for patients with firm tracheas or when plastic is not tolerated.
- Examples:
    - Jackson tracheostomy tubes.
- Advantages:
    - Reusable, durable.
    - Less bulky.
- Disadvantages:
    - Cannot be used during MRI.
    - Rigid → may cause trauma.
    - Not compatible with most modern ventilators.

2. **By Cuff Design**

a. Cuffed Tracheostomy Tubes

- Features:
    - Inflatable balloon seals the airway against aspiration.
    - Required for positive pressure ventilation.
- Cuff types:
    - High-volume, low-pressure: spreads pressure over a larger area to reduce tracheal damage.
    - Low-volume, high-pressure: used rarely, higher risk of mucosal damage.
    - Foam cuffs: self-inflating, used in tracheomalacia or after radiation therapy.
- Indications:
    - Ventilator-dependent patients.

    - High risk of aspiration (e.g., poor swallowing reflex).
- Care note:
    - Cuff pressure must be monitored (20–30 cm H_2O) to avoid tracheal necrosis.

b. Uncuffed Tracheostomy Tubes

- Features:
    - No balloon cuff.
    - Allows free airflow around the tube.
- Applications:
    - Common in pediatrics, where the airway is naturally tight.
    - Used in stable, non-ventilated adults.
    - Weaning from tracheostomy.
- Risks:
    - Less protection against aspiration.[94]

3. **By Cannula Configuration**

a. Single Cannula Tubes

- Structure: One tube, directly inserted into the trachea.
- Applications:
    - Low secretion patients.
    - Used in home care or pediatric tracheostomies.
- Disadvantages:
    - Cleaning is more difficult.
    - If it gets blocked, entire tube must be replaced.

b. Double Cannula Tubes

- Structure:
    - Outer cannula remains in place.
    - Inner cannula is removable for cleaning.
- Advantages:

    - Facilitates frequent suctioning and cleaning.
    - Improves patient safety — if the inner tube gets blocked, it can be removed without replacing the entire device.
- Common in hospitals and long-term ventilation.[95]

4. **By Fenestration**

a. Fenestrated Tubes

- Structure: Has holes (fenestrations) in the curvature of the tube.
- Function:
    - Allows air to pass through the vocal cords, enabling speech when the cuff is deflated.
    - Allows gradual transition to breathing through mouth/nose.
- Needs:
    - Often used with a speaking valve.
- Risks:
    - Secretions may block fenestrations.
    - Risk of granulation tissue forming at fenestration site.
- Used for:
    - Speech therapy, weaning trials.

b. Non-fenestrated Tubes

- No openings in the tube.
- Ideal for:
    - Mechanical ventilation.
    - Patients at risk for aspiration.
- Safer for critically ill patients.

5. **By Length and Anatomical Variation**

a. Standard Length Tubes

- Most common, fit standard tracheal anatomy.

b. Extended-Length Tracheostomy Tubes (XLT)

- Indicated for:
  - Obese patients.
  - Tracheal deformities, or neck swelling.
- Two types:
  - Distal XLT: extra length at the tracheal end.
  - Proximal XLT: extra length at the neck end.[95]

6. **Special Purpose Tubes**

a. Adjustable Flange Tubes

- The neck flange (neck plate) is adjustable.
- Allows precise placement of the tube for patients with unusual neck anatomy, post-head/neck surgery.

b. Percutaneous Tracheostomy Tubes

- Used during bedside percutaneous tracheostomy (e.g., Ciaglia technique).
- Often have a tapered tip, soft introducer, and dilator set.
- Short-term, ICU use.

c. Speaking Valves (e.g., Passy-Muir Valve)

- One-way valve placed over the tube.
- Let'sinhalation via the tube and exhalation through upper airway (mouth/nose), enabling speech.
- Requires patient to be alert and able to manage secretions.[95]

Table 1: Basic description of Tracheostomy tube types

Type	Key Features	Common Use
Cuffed	Inflatable cuff, seals airway	Ventilated patients
Uncuffed	No cuff	Pediatric, low aspiration risk
Fenestrated	Holes for airflow	Allows speech
Non-fenestrated	No holes	Mechanical ventilation
Single Cannula	One tube	Simple, less secretion
Double Cannula	Inner + outer tube	Easier cleaning
Metal	Rigid, reusable	Rare, long-term
Plastic/Silicone	Flexible	Common in all settings
Extended-Length (XLT)	Longer tubes	Obese or anatomical variance
Adjustable Flange	Movable neck plate	Post-surgical use

TRACHEOSTOMY PROCEDURE

The initial assessment of surgical landmarks, including 'notch of thyroid', 'cricoid cartilage', and 'supra-sternal notch', is performed through palpation. Examine for the occurrence of an anomalous high-riding innominate artery at the level of the sternal notch. Local infiltration was administered at a concentration of 2%. Lignocaine should be applied at the incision site. A midline horizontal or vertical incision is performed on the anterior neck skin, positioned between the lower border of cricoid cartilage and the sternal indentation. The incision is extended to encompass the skin, subcutaneous tissue, and platysma. In certain instances, the anterior jugular vein may be located in the subcutaneous plane and is either ligated or retracted laterally. In vertical tracheostomy, dissection is limited to the mid-avascular plane within the median raphe. The dissection is advanced to reveal and retract both the superficial and deep stripstrengths, specifically sternohyoidand sternothyroid. The strip of the thyroid secretor, when encountered, is either elevated or depressed and retracted utilizing a cricoid hook. The pre-tracheal fascia has been incised. The location of the trachea is verified by administering 1 ml of 4% lignocaine, which reveals air bubbles upon aspiration, and the patient exhibits a cough upon instillation. 2nd

and 3rdrings of trachea are recognized and incised. Minimizing the initial ring decreases the likelihood of stenosis.

A 'Bjork's flap', which is an inferiorly-based flap of cartilage, was created &then secured to the subcutaneous tissues utilizing non-absorbable suture material such as Mersilk. An appropriately sized TT is inserted, and the flanges are secured laterally with skin sutures, then tied around the neck. Modifications involve the excision of an anterior segment of cartilage, typically comprising 1 or 2 rings, along with implementation of 'vertical anterior incision' spanning one to two rings, as utilized in paediatrics tracheostomy procedures. [96]

BIOFILMS IN ICU IN THE CURRENT ERA :

Intensive care units (ICUs) are particularly vulnerable to infections related to biofilm formation, largely due to the frequent use of invasive medical devices and the weakened immunity of critically ill patients. Common pathogens such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Staphylococcus aureus are frequently isolated in these environments and are strongly linked to conditions like ventilator-associated pneumonia, tracheostomy complications, and catheter-related infections. These organisms are notorious for their resistance to multiple antibiotics.[8]

The conditions in ICUs—including moist surfaces and the presence of foreign bodies like tracheostomy or endotracheal tubes—are ideal for microbial adhesion and biofilm development. Evidence suggests that biofilms can begin forming on these devices within a week of insertion, posing a significant threat to recovery by enabling persistent infections.

The challenge of treating biofilm-related infections is increased by the emergence of multidrug-resistant bacteria. The extracellular matrix that encases biofilms serves as anarmour, preventing antibiotics and immune responses from effectively reaching the embedded microorganisms. This often results in prolonged infections and extended hospital stays.

To address this issue, modern ICU protocols emphasize timely identification of biofilm-producing organisms, cautious use of antibiotics, proper hygiene practices of medical devices, and ongoing research into anti-biofilm technologies. In the context of rising antimicrobial resistance, targeted efforts to manage biofilms are essential for reducing the burden of healthcare-associated infections.[8]

MATERIALS AND METHODS

MATERIALS & METHODS

STUDY DESIGN: A cross-sectional study

STUDY DURATION: April 2023 to July 2024

STUDY SAMPLE: Patients who underwent tracheostomy and were admitted for a minimum period of 2 weeks in R.L. Jalappa Hospital & Research Centre.

ETHICS COMMITTEE APPROVAL: Approval was obtained from the Recognized Ethics Committee of Sri DevarajUrs Medical College (Vide No.DMC/KLR/IEC/99/2023-24) prior to the study. The learning protocol obeyed to the moral principles outlined in the Declaration of Helsinki and the Indian Council of Medical Research (ICMR) guidelines for biomedical research involving human subjects.Written well-versedconsensus was attained from all members after detailed clarification of thestudy measures, potential risks and benefits, and the charitable nature of involvement.

INCLUSION CRITERIA: All patients 18 years and above and underwent tracheostomy and expected to be on tracheostomy tube for 2 weeks or more.

EXCLUSION CRITERIA

- Patients with pre-existing pneumoniae
- Patients with contaminated wounds over the neck
- Patients with fungating tumours of the neck
- Patients with pharyngo-cutaneous fistula

SAMPLE SIZE: In a study conducted in Mangalore, Karnataka in the year 2021 on 35 patients ,NandiniRaveendran et al. reported the prevalence(p) of bacterial biofilm production as

p = Prevalence = 57%

q = (100 - p) = 43%

l = allowable error = 12%

Sample size(n) = $4pq/l^2$

$=4 \times 43 \times 57/(12)$

Estimated sample size $=68.08 = 68$

DATA COLLECTION PROCEDURE: All patients who met the insertion criteria were considered for the current study. Informed consent was obtained as per the standard protocol from the patientsor patient's attenders (if patient is not in a state of giving consent). A detailed history of the patient's indicators, co-morbidities stood elicited, then clinical examination was done. Tracheostomy was performed by a senior consultant. Swabs and scrapings were collected from the tracheostomy tube and tracheostoma soon after tracheostomy, 1 week after tracheostomy, 2 weeks after tracheostomy, and 1 month after tracheostomy in the patients considered for the study. Swabs were collected in strict aseptic conditions and were sent to the microbiology section of CDLS (Central Diagnostic Laboratory Services), RLJH & RC for further microbiological processing.

Figure 5: Collection of secretions from tracheostomy tube & tracheostoma using a sterile swab

BIOFILM DETECTION

Pure cultures from plates were immunized into 10 mL Trypticase soy broth with 1% glucose. Hatched 18 h at 37 °C and diluted 1:100 by fresh medium. Next, 0.2 ml aliquots of the diluted culture were placed in sterile TCP wells. Plain broth was used to test media sterility and non-specific obligatory. The TCP saucers were hatched at 37 °C for 24 hours. After incubation, gently tapping removed well contents. To remove planktonic cells, wells rinsed four times with 0.2 ml phosphate buffer saline. Fixing sessile cells with 2% sodium acetate followed. After discoloration cells with 0.1% crystal violet, plates were rinsed with distilled water and dehydrated. Optical density (OD) of stained adhering biofilms was unrushed at 590 nm by a micro-ELISA auto reader.

PROTOCOL OF TISSUE CULTURE PLATE METHOD TO DETECT BIOFILM PRODUCING STRAINS

Pure culture from plates will be inoculated into Trypticase soy broath with 1% glucose(10ml)

↓

Incubation for 18 hrs at 37°C and dilute at 1 in 100 with fresh medium

↓

Add 0.2ml aliquots of the diluted cultures into sterile TCP wells. And broth only serves as control to check sterility & non-specific binding of media

↓

These TCP wells will be incubated for 24 hrs at 37°C . After incubation contents of wells will be removed by gently tapping

↓

Wash the wells 4 times with 0.2ml of phosphate buffer saline for removal of planktonic cells. Fix the sessile cells in plates with 2% sodium acetate

↓

Stain with 0.1% w/v crystal violet. Wash the plates with distilled water to remove excess stain & allow to dry

↓

Take optical density(OD) of the stained adherent biofilms by using micro ELISA auto reader at 570nm

Figure 6: TCP wells plate after washing off crystal violet

BIOFILM INTERPRETATION:

Standard deviation (SD) =0.012

Optical Density Cutoff value (ODC) = Average OD of negative control + (3 x SD of negative control).

= 0.005 + (3 x 0.012)

=0.0426

Therefore ODC = 0.0426

Table 2: Biofilm interpretation according to optical density value

OD value	**Biofilm formation**
<ODC	Non
ODC < ~ < 2xODC	Weak
2x ODC < ~< 4xODC	Moderate
>4x ODC	Strong

IDENTIFICATION OF BACTERIA

Clinical samples were promptly streaked onto blood jelly and McConkey jelly media plates and hatched at 37 °c for 24-48 hours in the microbiology lab. Plates were checked for microbiological growth. Gram stain, Catalase, Coagulase, Oxidase, Indole, Citrate, Urease,

Mannitol, Motility, and Triple sugar iron assays were performed on the growing bacteria. All isolates were tested for bacteria vulnerability on Muller Hinton agar using Kirby Bauer Disc Diffusion according to CLSI standards.

DATA ANALYSIS

The information was composed and entered into Excel and was assessed by SPSS software version 24. Categorical data was described by frequency and percentage and analysed by the Chi-square test. Quantitative data was analysed by Mean and Standard eccentricity, and analysed by Z or ANOVA test. A p-value less than 0.05 was considered statistically significant.

RESULTS

RESULTS

Overall of 95 patients who underwent tracheostomy , the mean age of the study participants was 57.29 years. There were 44 males and 51 females. The pictorial representation of the same is shown below.

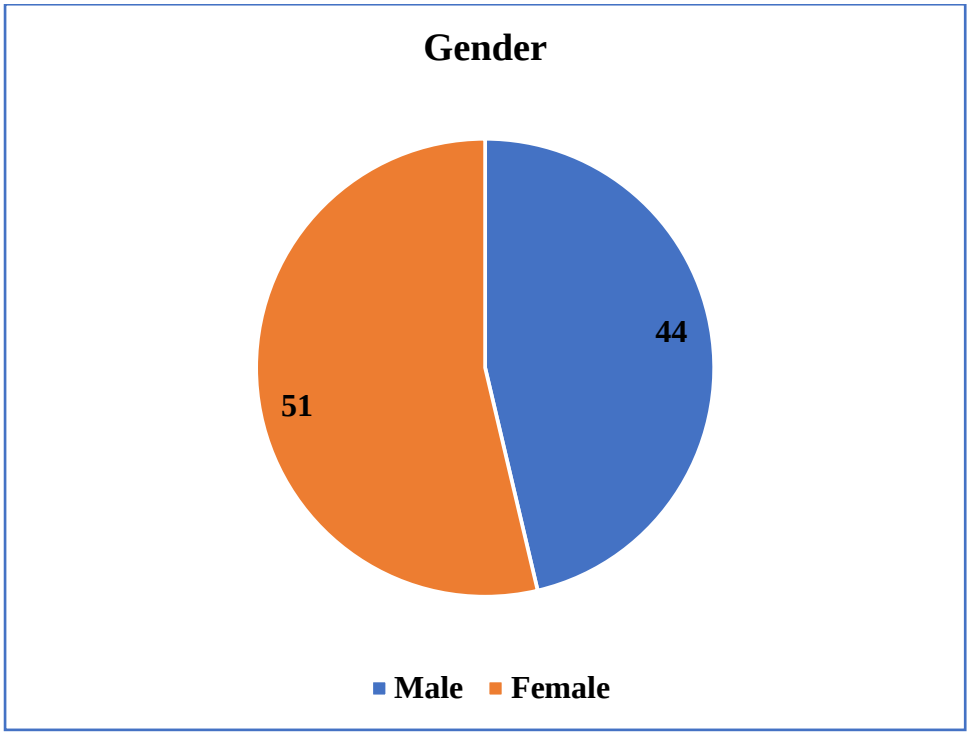


Figure7 : Distribution of Gender

Table 3: Gender wise distribution

Gender	Number (%)
Male	44 (46%)
Female	51 (54%)

There were 46% of males and 54% of females

Table 4: Diagnosis wise distribution of patients who underwent tracheostomyand indication for tracheostomy

Diagnosis	**Indication for tracheostomy**	**Number of patients (Percentage)**
Carcinoma oral cavity Carcinoma larynx	For operating workspace/ expected prolonged ventilation/ preoperative airway protection/ expected postoperative edema/ suspected aspiration risk / tumour obstructing airway	57 (60%) 2 (2.1%)
Traumatic brain injury	Prolonged intubatin	12 (12.6%)
OP poisoning	Prolonged intubatin	5 (5.2%)
Cut throat injury	Airway compromise/ extensive laryngeal or tracheal damage/ airway edema	2 (2.1%)
Deep neck space infections	Airway compromise/ airway edema/ suspected aspiration risk/ expected prolonged ventilation	4 (4.2%)
Face & neck cellulitis	Airway compromise/ airway edema/ suspected aspiration risk/ expected prolonged ventilation	2 (2.1%)
Ludwigs angina	Imminent airway obstruction/ stridor/ difficult or failed intubation / expected	2 (2.1%)

	prolonged ventilation	
encephalitis	Prolonged intubatin	1 (1.05%)
Odontogenickeratocyst of mandible	Imminent airway obstruction/ expected prolonged ventilation	1 (1.05%)
Tracheal stenosis	Stridor	2 (2.1%)
Cerebrovascularaccident (CVA)	Prolonged intubatin	4 (4.2%)
meningitis	Prolonged intubatin	1 (1.05%)

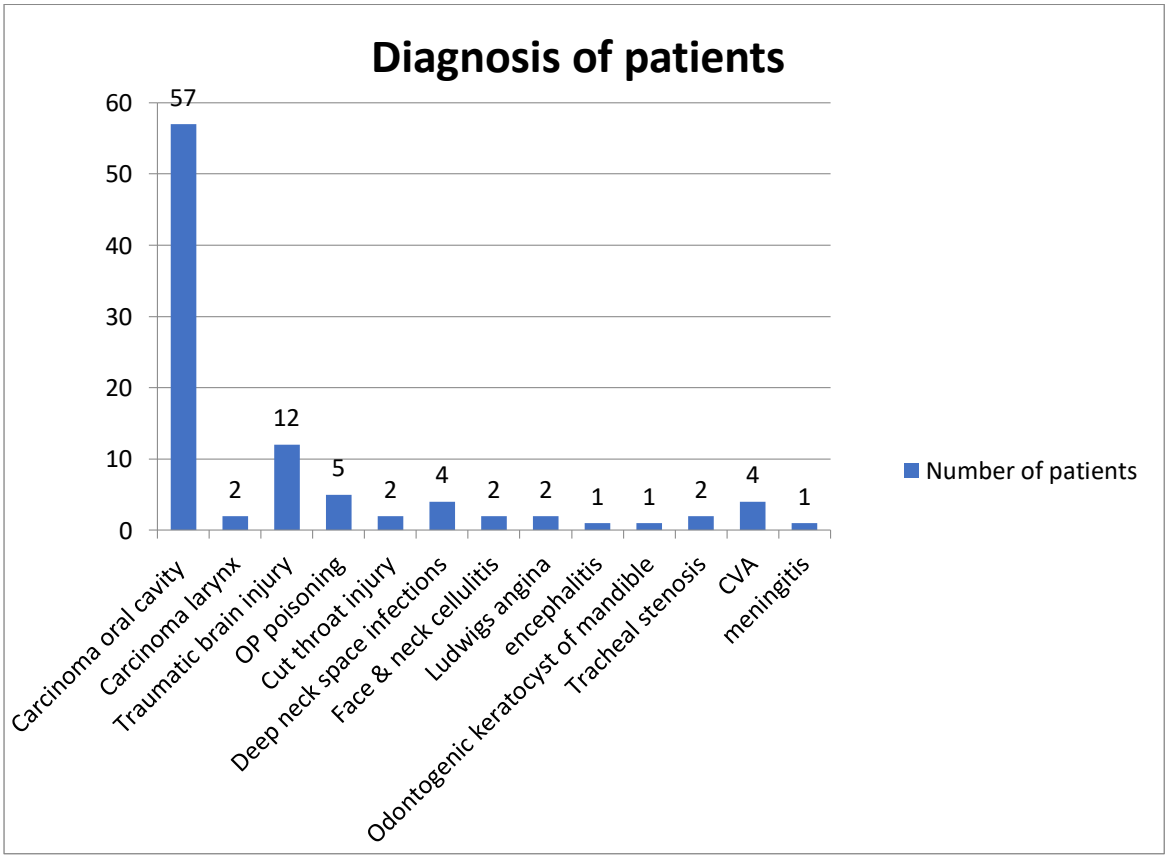


Figure 8: Categorization of subjects based on underlying disorderrequiring tracheostomy

In a total of 95 patients who underwent tracheostomy , 59 (62%) patient's diagnosis isCarcinoma oral cavity / larynx.

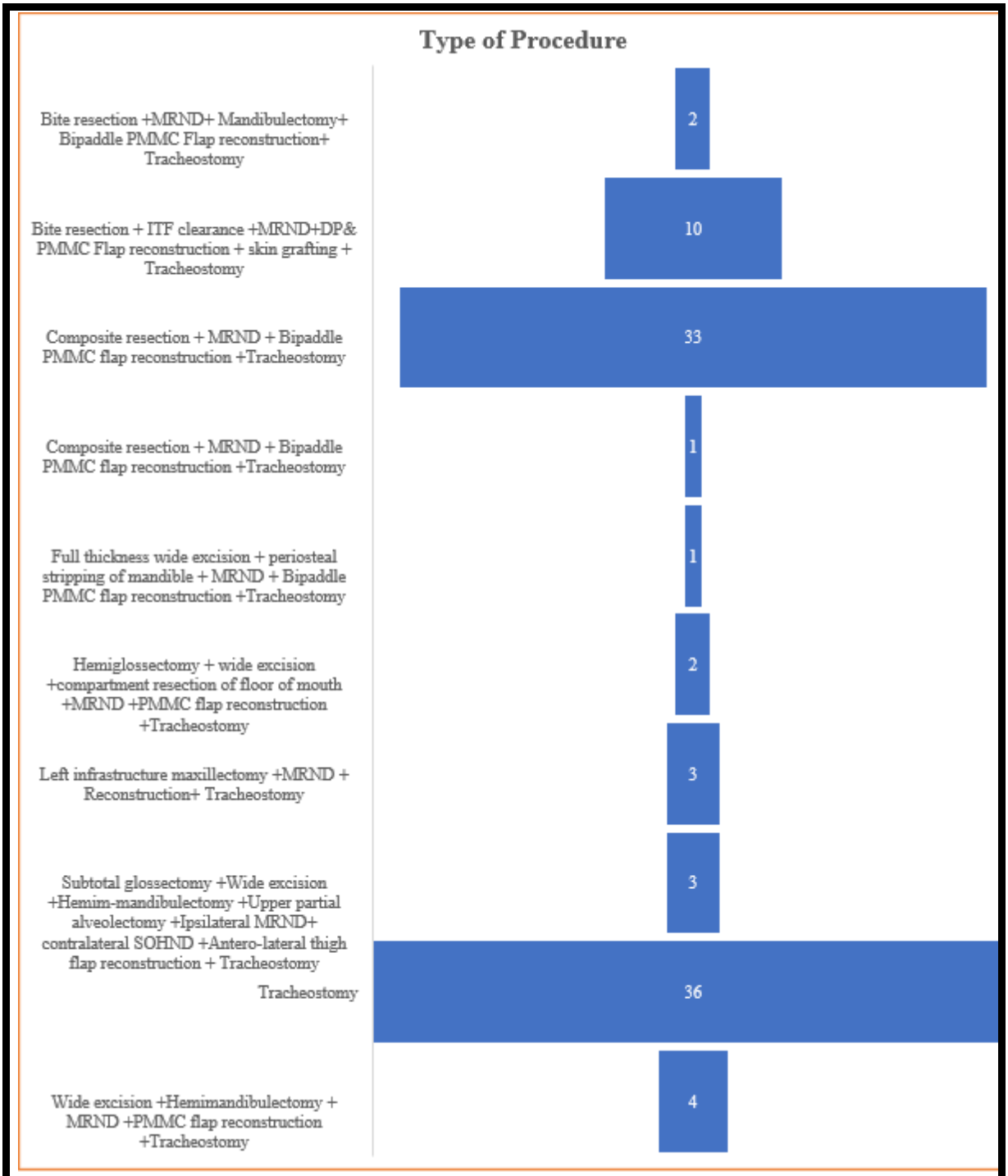


Figure 9: Type of Surgical Procedure

Among the 95 patients, around 36 patits had undergone only tracheostomy, and the rest had a tracheostomy combined with other surgical procedure.

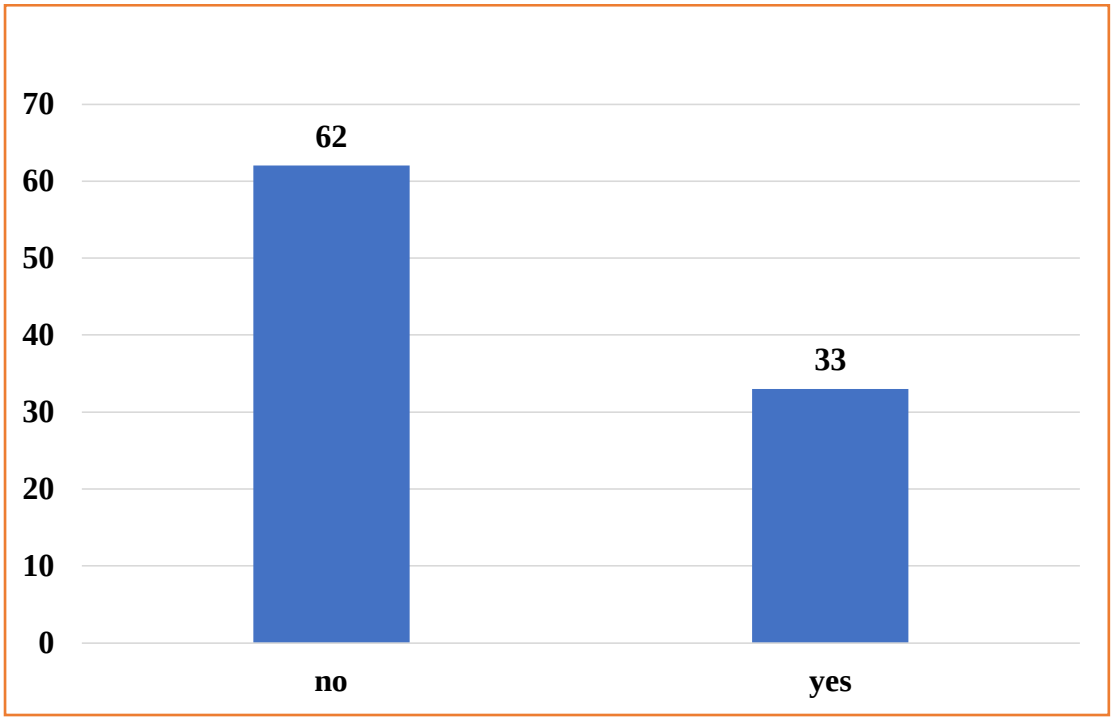


Figure 10: Comorbidities of the study samples

Table 5: Comorbidities

comorbidities	Number	Percentage
No	62	65%
Yes	33	35%

In a total of 95 samples, 65% did not have any comorbidities, and 35% had comorbidities

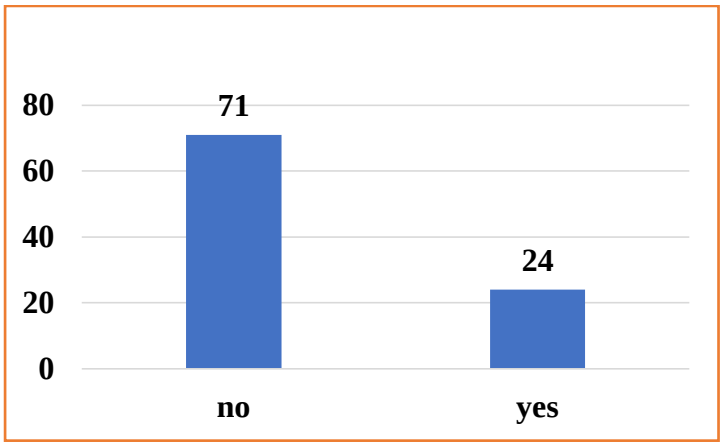


Figure 11: Number of patients with diabetes

Diabetes	Number	Percentage
No	**71**	**75%**
Yes	**24**	**25%**

Table 6: Patients with diabetes

Among 95 patients 75 were non-diabetic and 24 were diabetic.

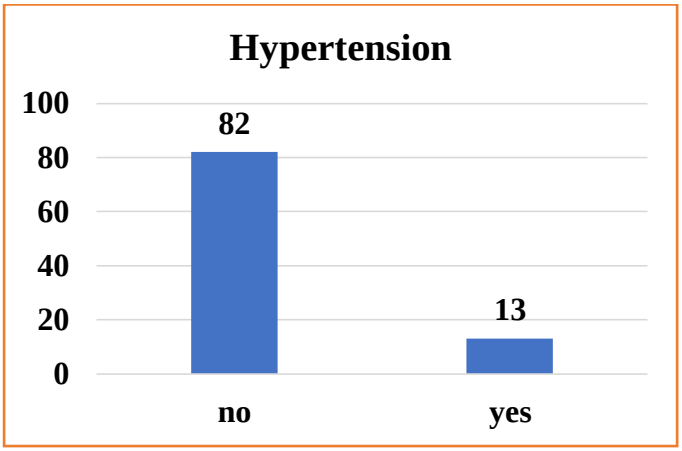


Figure12: Hypertension in study subjects

Hypertension	Number	Percentage
No	82	86%
Yes	13	14%

Table 7: Patients with Hypertension

Among 95 patients, 82 of the patients were non-hypertensive, and 13 were hypertensive

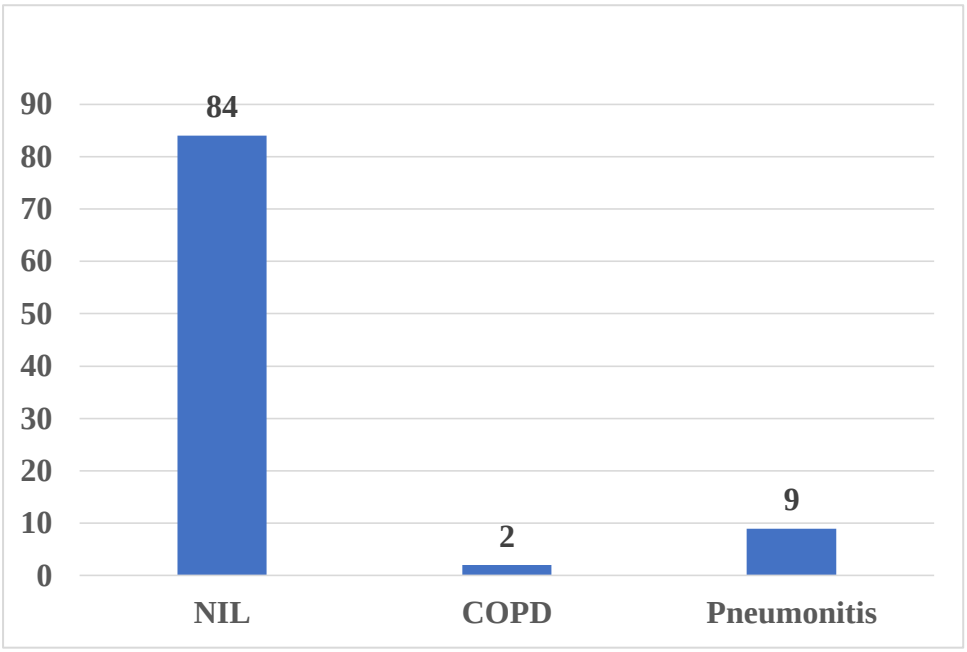


Figure13: Associated respiratory conditions

Table 8: Respiratory conditions

Respiratory condition	Number	Percentage
NIL	84	88%
COPD	2	2%
Pneumonitis	9	9%

- 2% of the patients had COPD
- 9% developed Pneumonitis while in ICU

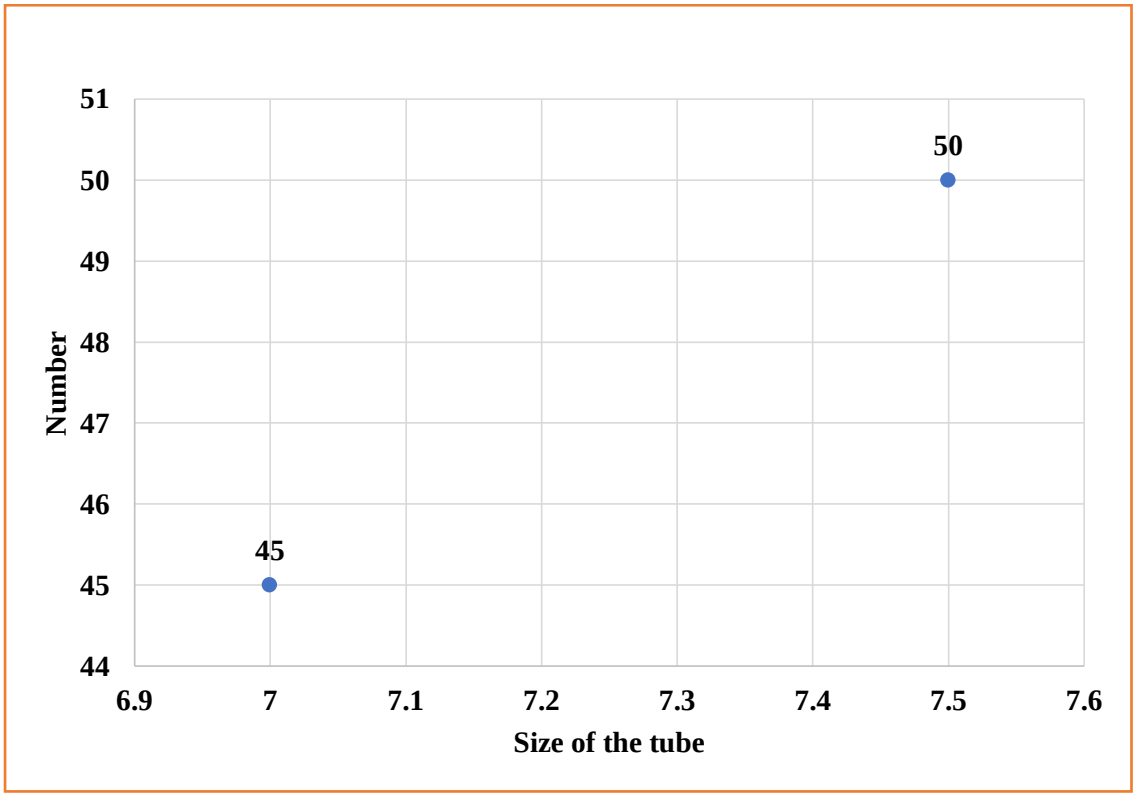


Figure 14: Size of Tube

Table 9: Size of the tube

Size of the tube	Number	Percentage
7	45	47%
7.5	50	52%

47% had tube size as 7 while 52% has 7.5

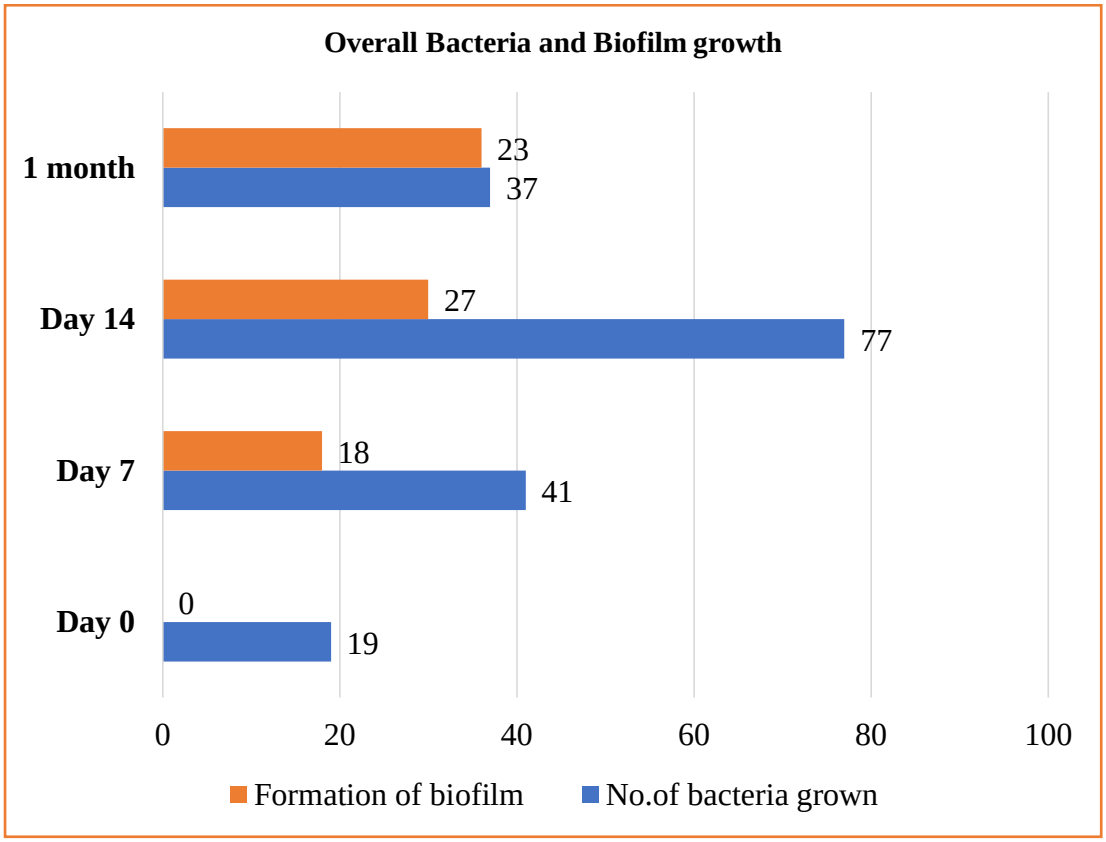


Figure 15: Overall rate of bacteria and biofilm formation

Table 10: Overall growth of bacteria and biofilm

Number of Days	No.of bacteria grown (%)	Biofilm grown
Day 0	19 (20%)	0
Day 7	41(43%)	18
Day 14	77(81%)	27
1 month	37(38%)	23

More bacteria were grown on day 14, i.e, 77, representing 81%.

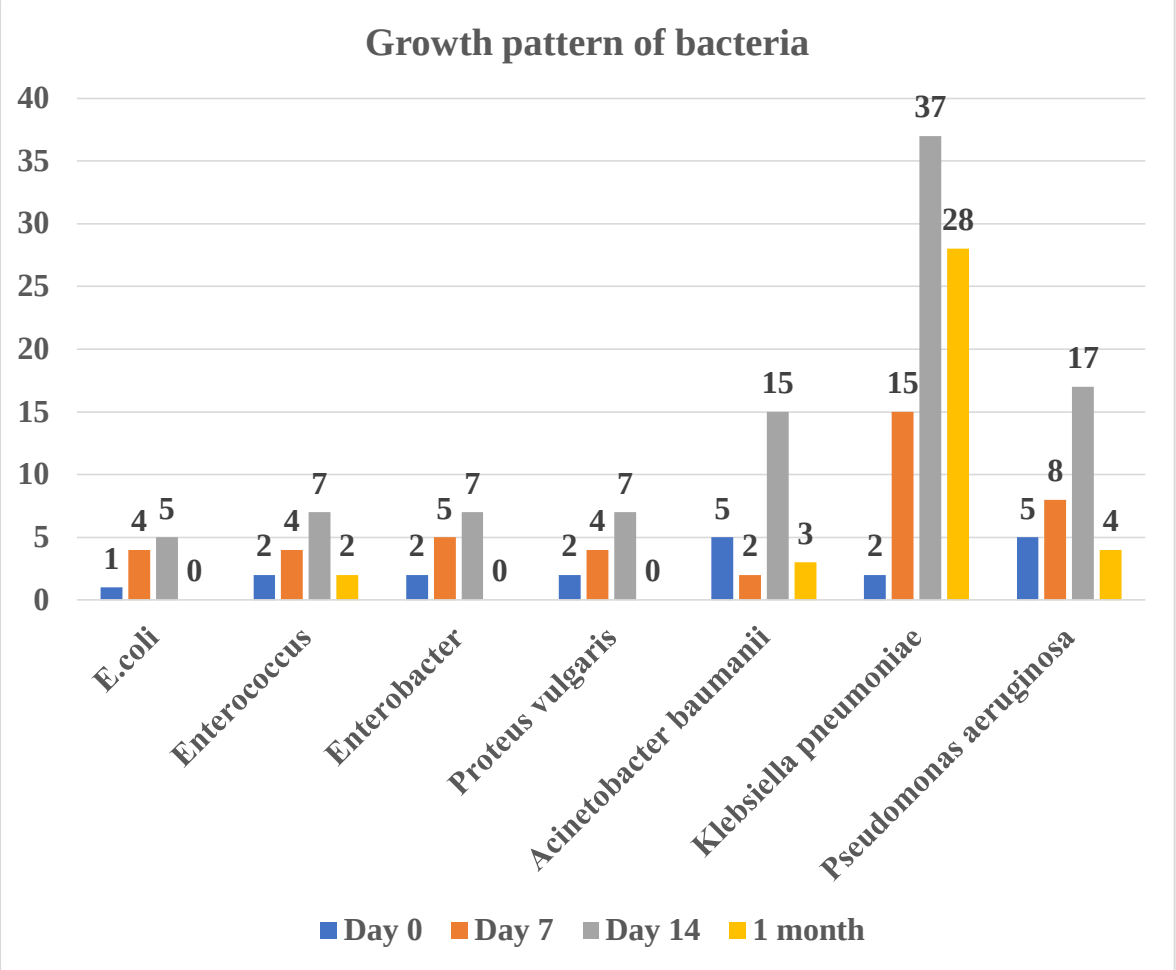


Figure 16: Bacteria growth pattern

Among the bacteria grown, Klebsiella stands as the highest biofilm-forming bacteria, followed by Pseudomonas aeruginosa and Acinetobacterbaumanii

Table 11: Growth of bacteria as per the number of days

Name of the bacteria	Day 0	Day 7	Day 14	Day 21
E.coli	1	4	5	0
Enterococcus	2	4	7	2
Enterobacter	2	5	7	0
Proteus vulgaris	2	4	7	0
Acinetobacterbaumanii	5	2	15	3
Klebsiella pneumoniae	2	15	37	28
Pseudomonas aeruginosa	5	8	17	4

Bacteria were more in day 14 than compared to day 7 and day 0. The number of bacteria after one-month follow-up ere decreased due to the use of a combination of antibiotics.

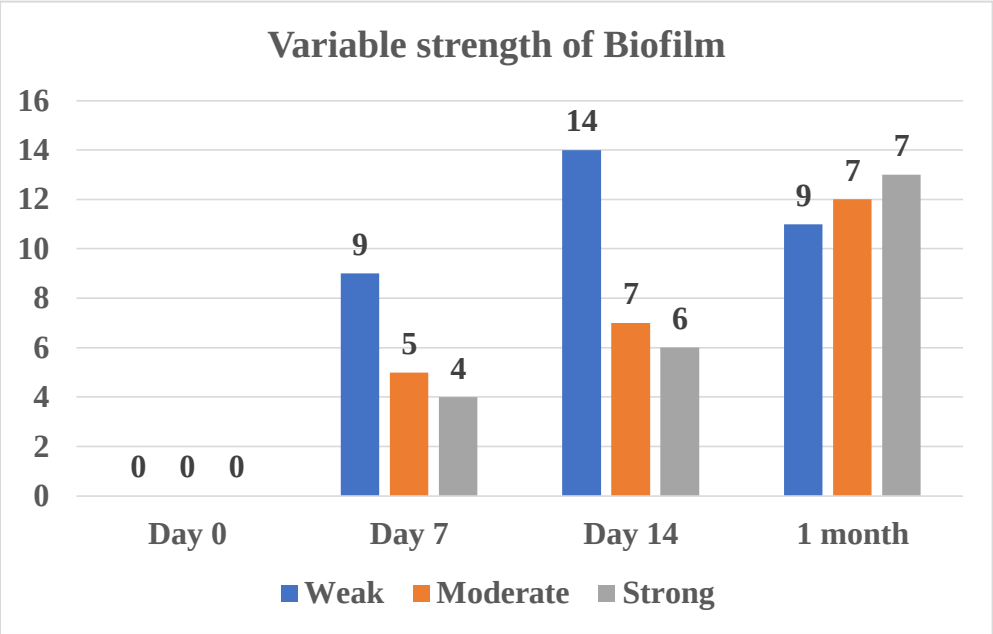


Figure 17: Variable strengths of biofilm formed

Among the compared days, day 14 showedthe highest number of bacteria forming strong biofilm.

Table 12: Variable strength of Biofilm

Biofilm	Day 0	Day 7	Day 14	1 month
Weak	0	9	14	9
Moderate	0	5	7	7
Strong	0	4	6	7

On Day 0, no biofilm is formed

On Day 7, 9 bacteria formed weak biofilm, 5 moderate, and 4 strong

On Day 14, 14 bacteria formed weak, 7 formed moderate, and 6 formed strong biofilms, and on 1-month follow up 9 bacteria showed weak, 7 bacteria displayed moderate, and 7 bacteria depicted strong biofilm formation.

Table 13: Antibiotic usage

Days	Antibiotics used	Number
Day 0	Ceftriaxone, Amoxiclav, Amikacin	27
	Amoxiclav , Metronidazole	68
Day 7	Amikacin, Pitaz, Ceftriaxone	11
	Amoxiclav , Metronidazole	10
	Ceftriaxone, Amoxiclav, Amikacin	9
Day 14	Amikacin + Amoxiclav	19
	Amoxiclac+Piptaz	14
	Colistin + Amoxiclav	20
	Piptaz +Amikacin	24
1-month follow-up	Amikacin + Amoxiclav	13
	Amoxiclac+Piptaz	7
	Colistin + Amoxiclav	9
	Piptaz +Amikacin	8

Correlation of Biofilm and Diabetes

Table 14:Microbial isolates from biofilms& sensitivity pattern on Day 0, 7, 14, 30 following tracheostomy in diabetes patients

Day following tracheostomy	Patients with Positive Culture (n)	Organisms Isolated	Biofilm Formation (n)	Biofilm Strength	Antibiotics Sensitive To	Antibiotic Resistance Observed
Day 0	2	Klebsiella pneumoniae (1) Enterococcus (1)	0	None	Not applicable	Not significant
Day 7	12	Klebsiella pneumoniae (6) Pseudomonas aeruginosa (3) Acinetobacter baumannii (2) Enterococcus (1)	10	5 Weak 3 Moderate 2 Strong	Amikacin Ceftriaxone Piptaz	Resistance to Amoxicillin, some Cephalosporins
Day 14	20	Klebsiella pneumoniae	18	8 Weak	Amikacin Colistin	Klebsiella: MDR

		(10) Pseudomonas aeruginosa (5) Acinetobacter baumannii (3) Enterococcus (2)		6 Moderate 4 Strong	Imipenem	Acinetobacter: β-lactam resistant Pseudomonas: Some fluoroquinolone resistance
Day 30	9	Klebsiella pneumoniae (5) Acinetobacter baumannii (2) Pseudomonas aeruginosa (2)	7	3 Weak 2 Moderate 2 Strong	Amikacin PiptazColistin	Continued MDR in Klebsiella and Acinetobacter

Out of 24 diabetic patients, 20 developed biofilm, showing a statistically significant correlation ($p = 0.001$).

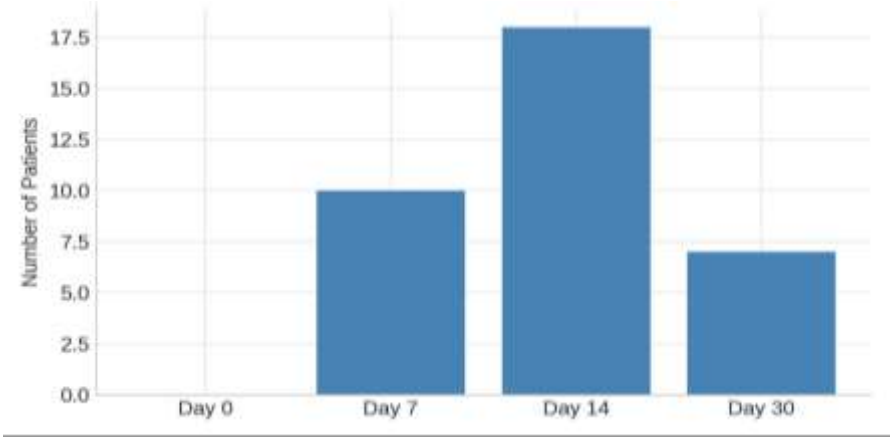


Figure: 18 Biofilm-positive Diabetic patients over time

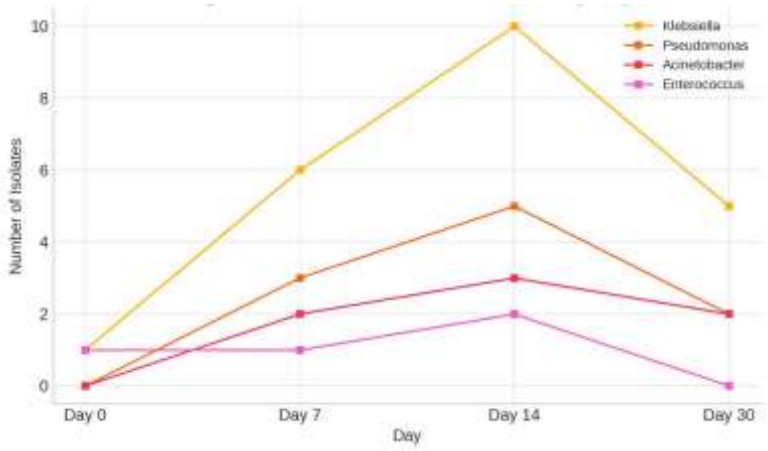


Figure 19: Organisms isolated in diabetic patients by day

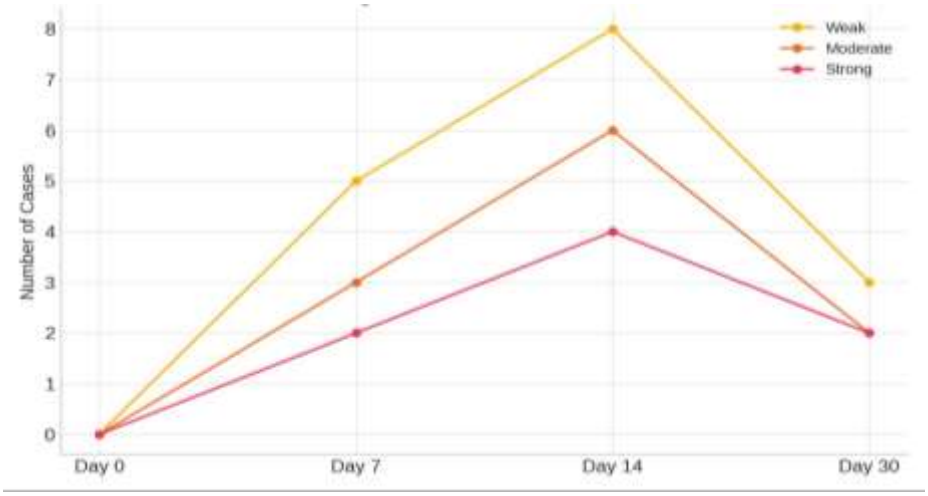


Figure 20: Biofilm strength distribution in diabetic patients

Correlation of Biofilm and HIV

Table 15:Microbial isolates from biofilms & sensitivity pattern in seropositive patients for HIV on Day 0, 7, 14, 30 following tracheostomy (n = number of HIV positive cases, n=2)

Day following tracheostomy	Patients with PositiveCulture (n)	Organisms Isolated	Biofilm Formation (n)	Biofilm Strength	Antibiotics Sensitive To	Antibiotic Resistance Observed
Day 0	1	Klebsiella pneumoniae (1)	0	None	Not applicable	Not applicable
Day 7	2	Klebsiella pneumoniae (1) Acinetobacter baumannii (1)	2	1 Weak 1 Strong	Amikacin Colistin	Klebsiella: Cephalosporin resistance
Day 14	2	Klebsiella pneumoniae (1) Pseudomonas aeruginosa (1)	2	1 Mod 1 Strong	Amikacin Piptaz Imipenem Colistin	Klebsiella: MDR Pseudomonas:Fluoroquinolone resistance
Day 30	2	Acinetobacter aumannii (1)Pseudomonas aeruginosa (1)	2	1 Mod 1 Strong	Amikacin Piptaz Colistin Imipenem	Persistent MDR

Out of 2 HIV-positive patients, both developed biofilm, indicating a statistically significant correlation ($p = 0.04$).

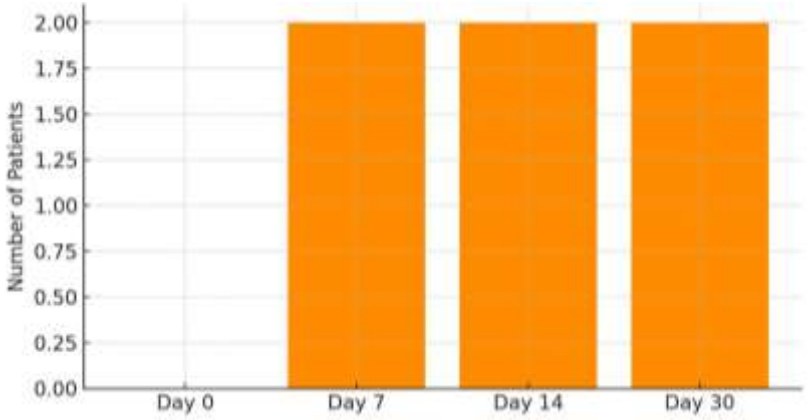


Figure 21: Biofilm positive HIV patients over time

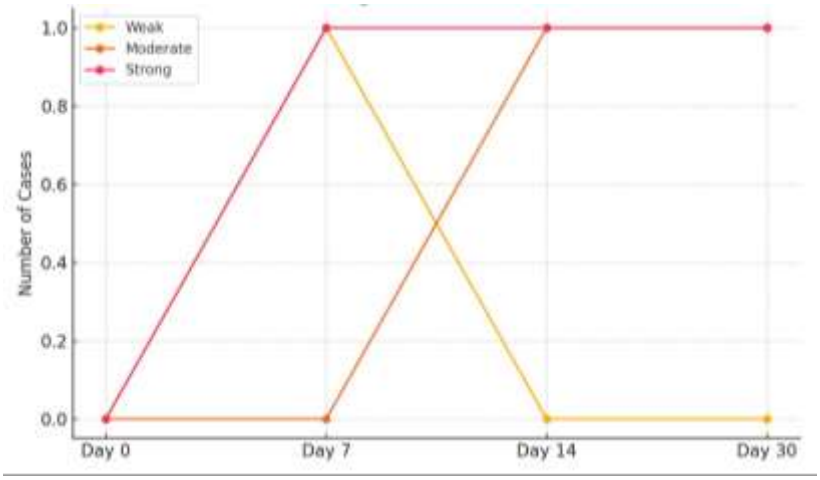


Figure 22: Biofilm strength in HIV-positive patients

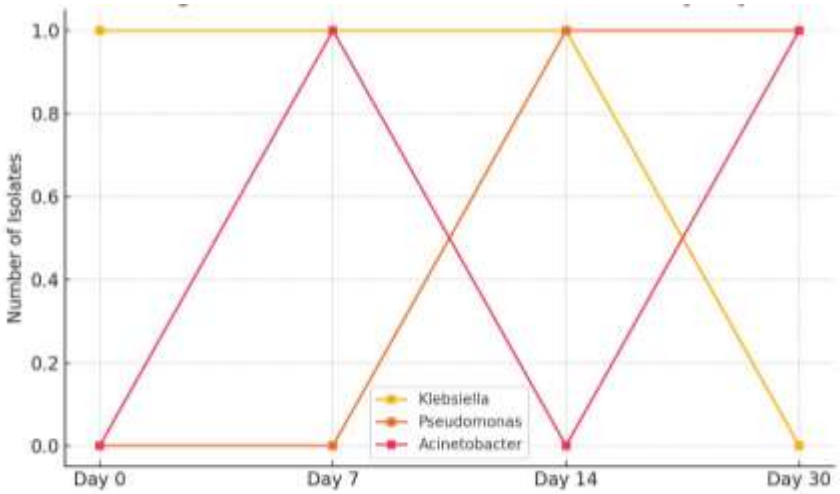


Figure 23: Organisms isolated in HIV-positive patients by day

Correlation of Biofilm Formation and Septicaemia

Table16 :Microbial isolates from biofilms & sensitivity pattern in septicaemia patients on Day 0, 7, 14, 30 following tracheostomy.

Day following tracheostomy	Patientswith Positive Culture (n)	Organisms Isolated	Biofilm Formation (n)	Biofilm Strength	Antibiotics Sensitive To	Antibiotic Resistance Observed
Day 0	1	Klebsiella pneumoniae (1)	0	None	Not applicable	Not applicable
Day 7	2	Klebsiella pneumoniae (1)Pseudomonas aeruginosa (1)	2	1 Weak 1 Mod	Amikacin Piptaz	Klebsiella: Cephalosporin resistance
Day 14	3	Klebsiella pneumoniae (2) Acinetobacter baumannii (1)	3	1 Weak 1 Mod 1 Strong	Piptaz Amikacin Colistin Imipenem	Klebsiella: MDR Acinetobacter: β-lactam resistant
Day 30	3	Klebsiella pneumoniae (1) Pseudomonas aeruginosa (1) Acinetobacter baumannii (1)	3	1 Weak 1 Mod 1 Strong	Piptaz Colistin Imipenem Amikacin	Persistent MDR in Acinetobacter and Pseudomonas

Out of 3 septicaemia-positive patients, all developed biofilm, indicating a statistically significant correlation ($p = 0.03$).

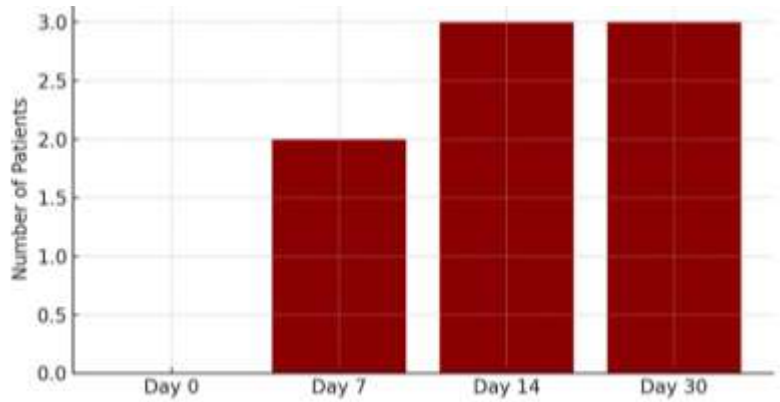


Figure 24: Biofilm positive septicaemia patients over time

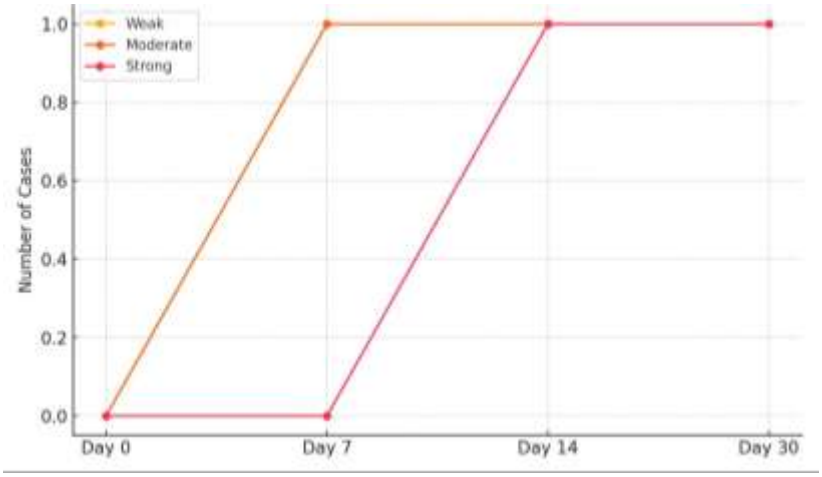


Figure 25: Biofilm strength in septicaemia patients

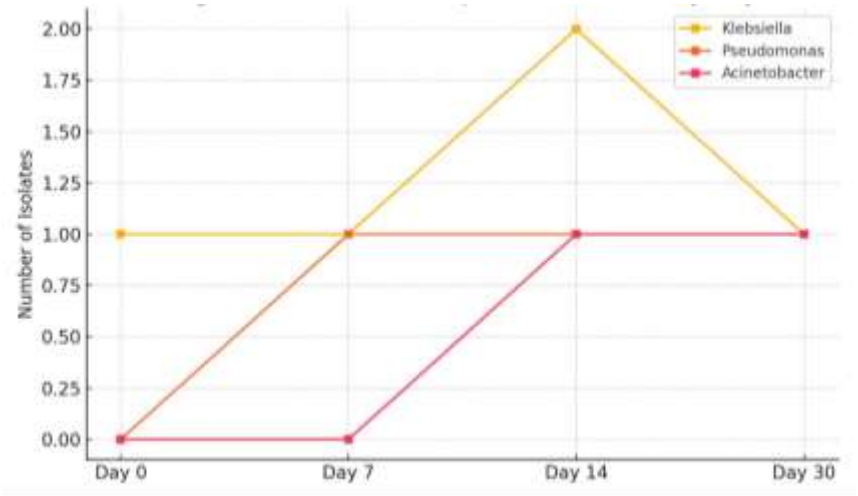


Figure 26: Organisms isolated in septicaemia patients by day

Correlation of microbes in Biofilm & Pneumonitis in ICU Patients

9 ICU patients who developed pneumonitis through the course, underwent tracheostomy due to prolonged intubation

Table 17: Overview of microbial isolates from biofilm, sensitivity pattern in ICU patients who developed pneumonitis

Day following tracheostomy	Biofilm Positive (n)	Biofilm Strength (Weak/Moderate/Strong)	Klebsiella (n)/ Pseudomonas (n)/ Acinetobacter (n)/ Enterococcus (n)	Antibiotics Sensitive To	Antibiotic Resistance Observed
Day 0	0	0/0/0	1/0/0/0	Amikacin, Colistin, Imipenem	Cephalosporins, β-lactams, Fluoroquinolones
Day 7	4	2/1/1	2/1/1/0	Amikacin, Colistin, Imipenem	Cephalosporins, β-lactams, Fluoroquinolones
Day 14	7	3/2/2	3/2/2/1	Amikacin, Colistin, Imipenem	Cephalosporins, β-lactams, Fluoroquinolones
Day 30	6	2/2/2	2/2/2/0	Amikacin, Colistin, Imipenem	Cephalosporins, β-lactams, Fluoroquinolones

Figure 27: Biofilm-Positive Pneumonitis Patients Over Time

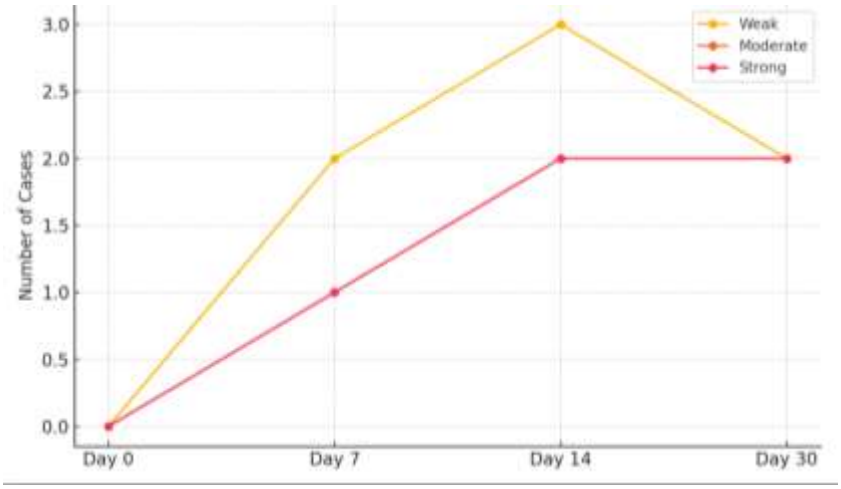


Figure 28: Biofilm Strength in Pneumonitis Patients

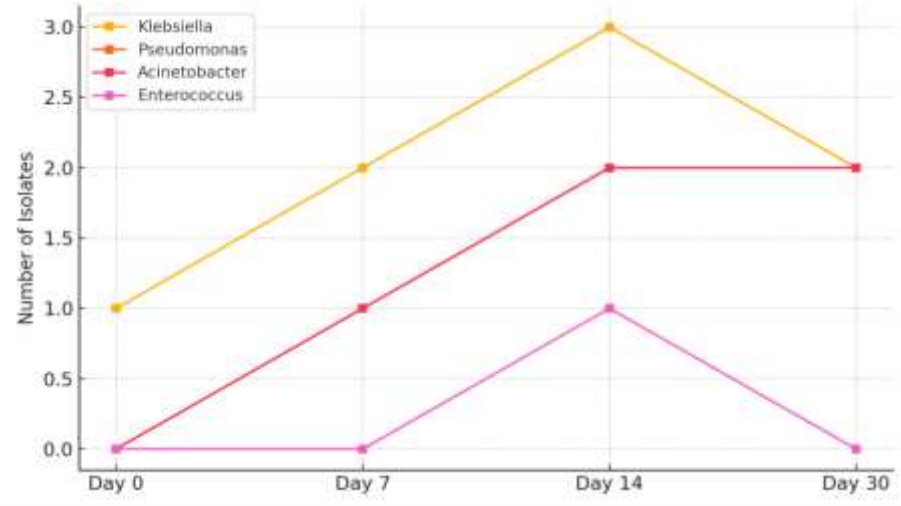


Figure 29: Organisms Isolated in Pneumonitis Patients by Day

Among the 9 ICU patients who developed pneumonitis during their hospital stay, who underwent tracheostomy due to prolonged intubation, progressive increase in biofilm

formation was observed, with 4 patients becoming biofilm-positive by Day 7, peaking at 7 by Day 14, and slightly declining to 6 by Day 30. Biofilm strength evolved from weak to moderate and strong over this period, suggesting increasing microbial adherence and biofilm maturation.

Table 18:Microbial analysis of biofilm in diabetic patients who developed pneumonitis on Day 0, 7, 14, 30 following tracheostomy

Diabetic patient number	Day following tracheostomy	Organism Isolated	Biofilm Strength	Antibiotic Sensitivity	Antibiotic Resistance
D1	Day 0	None	None	NA	NA
D1	Day 7	Klebsiella	Moderate	Amikacin, Colistin	Cephalosporins
D1	Day 14	Klebsiella	Strong	Amikacin, Colistin	Beta-lactams
D1	Day 30	Klebsiella	Strong	Amikacin, Colistin	Fluoroquinolones
D2	Day 0	None	None	NA	NA
D2	Day 7	Klebsiella	Moderate	Amikacin, Colistin	Cephalosporins
D2	Day 14	Klebsiella	Strong	Amikacin, Colistin	Beta-lactams
D2	Day 30	Klebsiella	Strong	Amikacin, Colistin	Fluoroquinolones
D3	Day 0	None	None	NA	NA
D3	Day 7	Acinetobacter	Moderate	Colistin, Imipenem	Beta-lactams
D3	Day 14	Acinetobacter	Strong	Colistin, Imipenem	Beta-lactams
D3	Day 30	Acinetobacter	Strong	Colistin, Imipenem	Fluoroquinolones

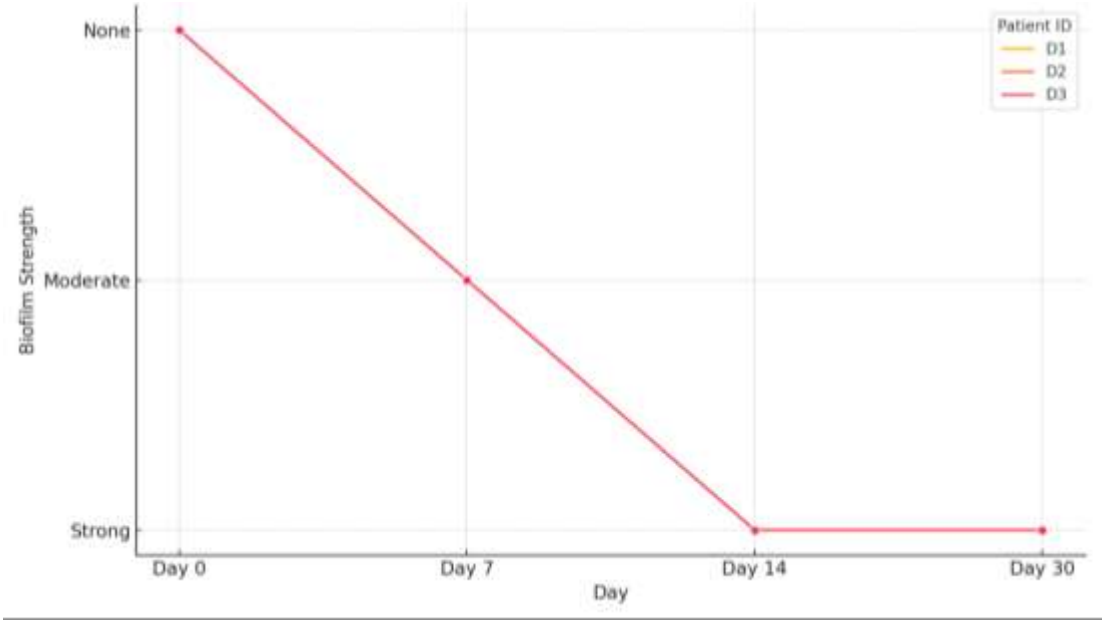


Figure 30: Biofilm Strength progression in Diabetic Pneumonitis Patients

Table 19: Correlation of biofilm & underlying medical condition

Condition	Total Patients	Biofilm-Positive	Biofilm-Negative	P-value
HIV	2	2	0	0.04*
Patients on steroids	11	6	5	0.29
Carcinoma	59	32	27	0.08
Diabetes	24	20	4	0.001*
Hypertension	13	7	6	0.44
Sepsis	3	3	0	0.03*

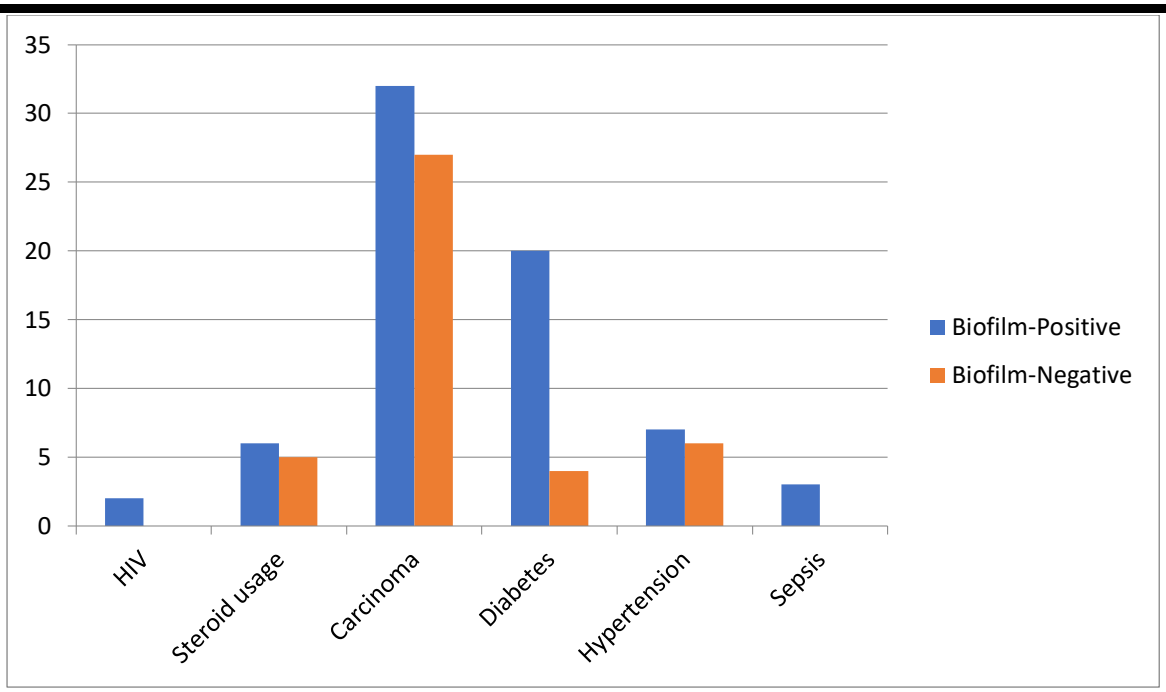


Figure 31: Association between biofilm formation and various conditions

INTERPRETATION:

Among the immunocompromised and comorbid conditions, HIV (P = 0.04), diabetes (P = 0.001), and sepsis (P = 0.03) showed statistically significant association with biofilm formation. Carcinoma (P = 0.08), steroid usage (P = 0.29), and hypertension (P = 0.44) did not show statistically significant correlation. These findings emphasize that patients with diabetes, HIV, or sepsis are at higher risk of biofilm-forming bacterial infections and may require more careful clinical management.

All three diabetic patients with pneumonitis showed increasing biofilm strength—moderate by Day 7 and strong by Days 14 and 30—indicating progressive colonization and biofilm maturation.

2. Dominant Pathogens:

- Klebsiella pneumoniae was isolated in two patients.
- Acinetobacter baumannii was isolated in one patient.

These organisms are known for their high biofilm-forming capability and multidrug resistance.

3. Consistent Antibiotic Sensitivity:

- Klebsiella isolates were uniformly sensitive to Amikacin and Colistin.

- Acinetobacter also showed sensitivity to Colistin and Imipenem.

4. Rising Resistance Pattern:

All isolates demonstrated progressive resistance—from cephalosporins to β-lactams and finally fluoroquinolones—by Day 30.

5. Clinical Implication:

Diabetic ICU patients with pneumonitis are at high risk for developing strong biofilm-forming, multidrug-resistant infections, necessitating early detection, targeted antibiotic use, and proper airway hygiene.

DISCUSSION

DISCUSSION

Despite several breakthroughs in bacterial infection control, biofilms remain a challenge. Biofilms have intricate nutrition and water exchange routes. Due to their resistant exopolysaccharide matrix coating, decreased metabolism, and shared resistance genes, they survive antibiotic treatment and humoral and cellular immune response activation. Biofilm production is easier ontracheostomy tubes due to breach in skin barrier, airway colonization, and exposure to substrates such as respiratory mucus and blood. Synthetic material in tracheostomy tubes provides ideal platform for formation of biofilm & adhesion of muco-polysaccharides[104-105] Patients with tracheostomy tubes may develop granulation tissue, local infections, and tracheostomy tube blockage.

Antimicrobial resistance (AMR) is a growing worldwide health issue that makes traditional treatments ineffective against many bacterial illnesses. Biofilm development is one of the ways bacteria defend against antimicrobials. [119]Biofilms, complex communities of bacteria in a self-produced extracellular matrix, are more resistant to antimicrobials than planktonic bacteria.[120] Biofilm robustness depends on the extracellular polymeric substances (EPS) matrix, which is mostly polysaccharides, proteins, and eDNA. Their constant release creates a physical barrier that prevents antibiotic drugs from reaching bacterial cells, reducing their potency. The biofilm's inherent drug resistance mechanism is formed by the synergy of acquired and adaptive mechanisms that contribute to antibiotic resistance.[97] In the present study as well, multidrug resistance was noted in Klebsiella pneumonia

It is ideal to maintain a clean tracheostomy tube & stoma in all patients requiring long-term tracheostomy tubes. As it has been observed in literature that the longer indwelling tracheostomy tubes undergo significant degradation of material, thereby further predisposing to biofilm formation.So regular change of tracheostomy tube and discarding old tracheostomy tube is mandatory.However, little research has determined how often tracheostomy tubes should be disinfected and should be discarded frequently. Previous

studies found that tracheostomy tubes degraded after 3 months and had higher biofilm than after 1 month.[6]

This study supports previous findings that biofilms are common on implanted medical equipment. Despite our 37% biofilm positive rate, some investigators found higher than 60% bacterial biofilm development. Some investigations revealed 73, 90, and 95% biofilm development on medical prostheses.[5,98,99]

However, K. pneumoniae biofilms on tracheostomy tubes were first reported in 1988. [100] Acinetobacter baumannii (45%) and Klebsiella pneumonia (20%) formed biofilms. [22,101]The most isolated bacteria in 'Gil-PerotinS et al.'s' study were 'Pseudomonas aeruginosa'and'Acinetobacterbaumannii'[100]. 'Radji et al'. and Inglis TJ et al. isolated 'Enterobacteriaceae (E.coli and Klebsiella pneumoniae)'and'Pseudomonas aeruginosa' from the tracheostomy tubes and filters of ventilator [23]

. In the present study, the klebsiella species were representing as the highest biofilm forming bacteria with 38% followed by 17% growth by Pseudomonas aeruginosa and 15% by Acinetobacterbaumanii on the follow day 14.

This study found that Aminoglycosides worked best for isolates. Aminoglycosidesare injectable drugs, making them handy for inpatients and reducing nosocomial infections.[99]

In our study, we observed a significant presence of multidrug-resistant (MDR) organisms, particularly Klebsiella pneumoniae and Acinetobacter baumannii, colonizing tracheostomy tubes. In comparision,a study conducted in Poland by Ścibik et al. (2022) analyzed 45 tracheostomy tubes and found that 50% of Gram-negative isolates were MDR, 8.6% were extensively drug-resistant (XDR), and 5.2% were pandrug-resistant (PDR). Klebsiella pneumoniae was identified in 23.8% of patients, and Acinetobacter baumannii, though less frequent, exhibited resistance to all antibiotics tested except tobramycin .[102]

Similarly, a study by Raveendra et al. (2021) reported that Acinetobacter baumannii (45%) and Klebsiella pneumoniae (28.5%) were the predominant biofilm-forming organisms on tracheostomy tubes, with a high incidence of multidrug resistance .[103]

And it has been seen our study that longer the patient has a tracheostomy tube in hospital environment, longer the chances of having Multidrug resistant Klebsiella forming biofilm.

These findings underscore the global challenge posed by MDR organisms in tracheostomized patients. The consistent emergence of MDR pathogens across different geographical regions emphasizes the need for stringent infection control measures, routine surveillance, and the development of targeted antimicrobial therapies to manage and prevent biofilm-associated infections in tracheostomy care.

Klebsiella Pneumoniae (35.7%), Pseudomonas aeruginosa (15%), and Acinetobacterbaumanii (17%) are extremely drug-resistant, complicating antibiotic use. Similar to Mahendra et al., Acinetobacter species was resistant to most antibiotics except Colistin [22] Radji M et al. found multidrug-resistant Staphylococci, Acinetobacter, and Pseudomonas species [23]However in our study, Staphylococci was not forming biofilms on tracheostomy tubes.

Multiple studies has shown that there is no statistically significant correlation between use of ventilators & biofilm.[100]

Only 18% of biofilm-forming and non-biofilm-forming patients were admitted with infectious aetiology. Biofilms can emerge throughout hospitalization even if infections are not present at admission. Clinicians must take precautions like tracheal tube swabs for biofilms, appropriate sensitivity-based antibiotics, autoclaving tracheostomy tubes at hospital, and washing tubes, maintaining the regular hygienic care of tracheostoma and tracheostomy tube & periodically changing the tracheostomy tube and discarding the earlier tube as it contains MDR organisms in the form of biofilm

Immunocompromised patients like HIV, diabetes, septicaemia were found to be more vulnarable to formation of biofilms multidrug resistant Klebsiella, Acinetobacter &Pseudomonas species.

In a study done by Bhattacharya et al. (2021), vulnerability to biofilm with drug resistant Klebsiella and Pseudomonas species was found to be more common in diabetics.[104]Similarly in our study, statistically significant association was found between diabetes and biofilm formation ($p = 0.001$), with 20 of 24 diabetic patients developing biofilms. The predominant organism was Klebsiella pneumoniae, followed by Pseudomonas aeruginosa and Acinetobacter baumannii. These pathogens showed resistance to multiple antibiotics, particularly beta-lactams, while remaining susceptible to Amikacin and Colistin, as documented in the study by Sharma et al. (2020).[105]

In HIV-positive patients ($n = 2$), both developed biofilm ($p = 0.04$), with moderate to

strong biofilm strength. Klebsiella pneumoniae and Acinetobacter were the primary isolates. Prior research by Singh et al. (2019) demonstrated that immunocompromised patients, particularly those with HIV, are at increased risk of developing biofilm-mediated infections due to impaired immune clearance and frequent hospital exposure.[70]

All septicemia patients (n = 3) were biofilm-positive (p = 0.03), with organisms including Klebsiella, Pseudomonas, and Acinetobacter. These findings align with the results of Mehta et al. (2022), who found high biofilm-forming potential among bloodstream isolates, contributing to therapeutic resistance and prolonged ICU stays.[106]

The consistency of Klebsiella pneumoniae as a dominant biofilm-forming organism across all comorbid groups highlights its virulence. This supports the global consensus described by Donlan and Costerton (2002), where biofilm formation on medical devices contributes significantly to chronic infections and treatment failures.[37]

This study underscores the need for early identification of biofilm in high-risk patients and supports the routine use of combination antimicrobial therapies guided by sensitivity profiles. Strict aseptic protocols and timely tracheostomy tube care may reduce morbidity in such cases.

Further research with larger HIV and septicemia cohorts is needed to validate these findings. Molecular typing of isolates and assessment of biofilm-related genes may also enhance our understanding of resistance mechanisms.

Among the 9 ICU patients who developed pneumonitis,Klebsiella pneumoniae was the most frequently isolated organism, followed by Pseudomonas aeruginosa and Acinetobacter baumannii, while Enterococcus was isolated less commonly. These findings align with the study by Gil-Perotin et al. (2012), who highlighted the role of endotracheal tube biofilms in the pathogenesis of ventilator-associated pneumonia and their frequent association with Gram-negative bacilli[100]

The resistance pattern observed in our cohort also mirrors the findings of Sharma et al. (2020), who reported widespread resistance among ICU isolates, especially to beta-lactams and cephalosporins, with higher sensitivity to Amikacin and Colistin.[105]

Furthermore, the role of device-associated biofilms in chronic infections and treatment failure is well established, as outlined by Donlan and Costerton (2002), reinforcing the clinical implications of our findings.[37]This transition from colonization to

structured biofilm formation likely contributes to the onset and persistence of pneumonitis in ICU patients.

These results support the need for early biofilm detection in tracheostomized patients, targeted antibiotic therapy, and rigorous tracheostomy care protocols to reduce ICU-acquired infections and improve outcomes.

In our subgroup analysis of diabetic patients who developed pneumonitis during ICU stay, all three individuals showed a consistent pattern of progressive biofilm formation, transitioning from moderate (Day 7) to strong biofilm (Days 14 and 30). Klebsiella pneumoniae was the most common isolate in two patients, and Acinetobacter baumannii in one. These organisms were sensitive to Amikacin and Colistin, while demonstrating increasing resistance to cephalosporins, beta-lactams, and fluoroquinolones over time.

This aligns with findings reported by Bhattacharya et al. [128], who observed similar biofilm behavior in diabetic ICU patients, with predominant organisms being Klebsiella and Pseudomonas species, and noted sensitivity to Amikacin and Colistin. Sharma et al. [105] also confirmed these sensitivity patterns among ICU isolates.

In contrast, a study by Mehta et al. [106] reported a higher prevalence of biofilm in Pseudomonas-dominant infections, particularly among non-diabetic immunocompromised patients, indicating a potential influence of host immune status on biofilm-forming bacterial dominance. These variations underscore the need for localized antibiogram protocols and individualized biofilm management strategies in high-risk groups like diabetic ICU patients.

CONCLUSION

- Biofilms on tracheostomy tubes is a well recognized problem which can cause persistant infection of stoma or progress to tracheitis & pneumonia
- These biofilms are resistant to most antibiotics and most of this organisms are nosocomial and multidrug resistant.
- Klebsiellapneumoniae was the most common organism in the biofilms moreso in immuno-compromised patients. The other common organisms were Acinetobacter, and Pseudomonas species.
- In our study, adequate hygienic care of tracheostoma & regular change of tracheostomy tube along with specific antibiotic according to culture sensitivity yielded best control of biofilms.
- Amikacin and Colistin were found to be most effective drugs against the organisms in biofilms in our study.
- Regular inspection of tracheostoma&tracheostomy tube scrapings / swabs to rule out resistant organisms of biofilms is mandatory in care of patients requiring longterm tracheostomy tube. This can minimize complications like pneumonia
- The vulnerable population like patients with uncontrolled diabetis, immunocompromised, malnourished individuals require more care.
- Larger research project preferably multi-institutional studies on this topic will facilitate making definitive guidelines regarding care & precautions against biofilms and ideal treatment in the event of these biofilm

SUMMARY

This research explores the presence and characteristics of microbial communities, specifically biofilms, that form on tracheostomy tubes& tracheostomaand their role in patient infections. Biofilms, formed by microorganisms embedded in a protective matrix, are a well-documented cause of persistent hospital-acquired infections. These communities, particularly on medical devices like tracheostomy tubes, are resistant to standard treatments, making them a significant clinical concern. Biofilms consist of amucopolysaccharides which gives a protective layer and keeps hydrated the micro-organisms.

The Objectives of our study were**:**

- To determine the microbial profile in biofilms on tracheostomy tube and tracheostoma
- To document the antibiogram isolates in biofilms
- To evaluate the response of biofilm to antibiotic treatment

This study was conducted at R.L. Jalappa Hospital & Research Centre, Kolar from April 2023 to July 2024 and included 95 patients who had undergone tracheostomy. Swabs & scrapings from the tracheostomy tube & from tracheostoma were collected at several stages—immediately after the procedure, and at weekly intervals up to one month. These were tested to identify the types of microorganisms present and to assess the extent of biofilm formation. The analysis also involved association of biofilm with underlying immuno compromised conditions like HIV, diabetes & septicaemia and development of pneumonitis in patients with biofilms.

A key observation was that biofilm production notably increased over time, with its peak on the 14th day and by end of 1 month it relatively came down due to combined antibiotic therapy. Among the identified microorganisms, Klebsiella pneumoniae was the most frequent, followed by Pseudomonas aeruginosa and Acinetobacter baumannii. The development of these biofilms ranged in strength from weak to strong, indicating the evolving complexity and resilience of microbial colonies during hospitalization.

Among the immuno compromised and comorbid conditions, HIV (P = 0.04), diabetes (P = 0.001), and sepsis (P = 0.03) showed statistically significant association with biofilm formation. Carcinoma (P = 0.08), steroid usage (P = 0.29), and hypertension (P = 0.44) did not show statistically significant correlation. These findings emphasize that patients with diabetes, HIV, or sepsis are at higher risk of biofilm-forming bacterial infections and may require more careful clinical management

Among the 9 ICU patients who developed pneumonitis during their hospital stay, who underwent tracheostomy due to prolonged intubation, progressive increase in biofilm formation was observed, with 4 patients becoming biofilm-positive by Day 7, peaking at 7 by Day 14, and slightly declining to 6 by Day 30. Biofilm strength evolved from weak to moderate and strong over this period, suggesting increasing microbial adherence and biofilm maturation.

The study also assessed the effectiveness of various antibiotics. Amikacin and Colistin proved to be the most effective against the bacteria, while resistance to drugs like cephalosporins and beta-lactams increased. Patients with immunosuppressive conditions, particularly those with diabetes or HIV, showed a higher likelihood of developing these robust biofilms, with statistically significant results supporting this link.

Critically ill patients, especially those in intensive care units, were more susceptible to such infections. The ICU environment—with prolonged intubation and frequent use of invasive devices—fostered the formation of biofilms. In patients who developed pneumonia during their ICU stay, there was a clear trend of increasing microbial colonization and biofilm strength over time, especially among those with diabetes.

This research mirrors findings from global studies that highlight the resilience and danger of biofilm-related infections. The data underscores the importance of early detection, antibiotic stewardship, and stringent hygiene practices in preventing complications. It also calls attention to the need for customized care plans based on patient risk factors and infection profiles.

Overall, the findings advocate for routine screening of tracheostomy tubes for biofilm formation, particularly in high-risk populations. Preventive care, including regular disinfection, frequent change of tracheostomy tube, tracheostomy tube care and careful selection of antibiotics based on sensitivity testing, could help reduce infection rates and improve patient outcomes. The study highlights need for coordinated care among healthcare providers to effectively manage these complex and often resistant infections.

Future research should involve a larger patient cohort and utilize advanced imaging tools to visualize biofilms directly on medical devices. Such enhancements would help deepen understanding and refine treatment strategies to combat biofilm-associated complications more effectively.

LIMITATIONS

- The lack of a Scanning Electron Microscope (SEM) to directly observe biofilms on tracheostomy tubes limited our work.
- Relatively smaller sample size as it was a dissertation. Larger research project preferably multi-institutional will help.

REFERENCES:

1. Marvasi M, Visscher PT, Casillas Martinez L. Exopolymeric substances (EPS) from Bacillus subtilis: Polymers and genes encoding their synthesis. Vol. 313, FEMS Microbiology Letters. 2010.
2. Hall-Stoodley L, Stoodley P, Kathju S, Høiby N, Moser C, William Costerton J, et al. Towards diagnostic guidelines for biofilm-associated infections. FEMS Immunol Med Microbiol. 2012 Jul;65(2):127–45.
3. Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). Journal of Clinical and Diagnostic Research. 2012;6(9).
4. Wi YM, Patel R. Understanding Biofilms and Novel Approaches to the Diagnosis, Prevention, and Treatment of Medical Device-Associated Infections. Vol. 32, Infectious Disease Clinics of North America. 2018.
5. Solomon DH, Wobb J, Buttaro BA, Truant A, Soliman AMS. Characterization of bacterial biofilms on tracheostomy tubes.Laryngoscope. 2009;119(8).
6. Treter J, Macedo a J. Catheters: a suitable surface for biofilm formation. Science against microbial pathogens: communicating current research and technological advances. 2011;
7. GHELLAI L, HASSAINE H, KLOUCHE N, KHADIR A, AISSAOUI N, NAS F, et al. Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University Hospital of Tlemcen. Journal of Bacteriology Research. 2014;6(1).
8. Cader S.H.A. SFA, NSKGR. Tracheostomy colonization & microbiological isolates of patients in intensive careunits - A retrospectice study. World J Otorhinolaryngol-Head Neck Surg. 2020;6:49–52.
9. Weissbrod PA, Merati AL. Is percutaneous dilational tracheotomy equivalent to traditional open surgical tracheotomy with regard to perioperative and postoperative complications? Vol. 122, Laryngoscope. 2012.

10. Szmuk P, Ezri T, Evron S, Roth Y, Katz J. A brief history of tracheostomy and tracheal intubation, from the Bronze Age to the Space Age.Vol. 34, Intensive Care Medicine. 2008.
11. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-Associated infections, medical devices and biofilms: Risk, tolerance and control. Vol. 64, Journal of Medical Microbiology. 2015.
12. El Cheikh MR, Barbosa JM, Caixêta JAS, Avelino MAG. Microbiology of tracheal secretions: What to expect with children and adolescents with tracheostomies. Int Arch Otorhinolaryngol. 2018;22(1).
13. Rouzé A, Jaillette E, Poissy J, Préau S, Nseir S. Tracheal tube design and ventilator-associated pneumonia. Respir Care. 2017;62(10).
14. Chastre J, Fagon J yves. State of the Art Ventilator-associated Pneumonia. Am J Respir Crit Care Med. 2002;165(23).
15. Lepainteur M, Ogna A, Clair B, Dinh A, Tarragon C, Prigent H, et al. Risk factors for respiratory tract bacterial colonization in adults with neuromuscular or neurological disorders and chronic tracheostomy. Respir Med. 2019;152.
16. McAfee JS, Demarcantonio M, Fine BR, Beydoun H, Derkay CS. Prevalence of ventilation tubes in children with a tracheostomy tube.Int J PediatrOtorhinolaryngol. 2013;77(1).
17. Chung PY. The emerging problems of Klebsiella pneumoniae infections: Carbapenem resistance and biofilm formation. Vol. 363, FEMS Microbiology Letters. 2016.
18. Sottile FD, Marrie TJ, Prough DS, Hobgood CD, Gower DJ, Webb LX, et al. Nosocomial pulmonary infection: Possible etiologic significance of bacterial adhesion to endotracheal tubes. Crit Care Med. 1986;14(4).
19. Jarrett WA, Ribes J, Manaligod JM. Biofilm formation on tracheostomy tubes. Ear Nose Throat J. 2002;81(9).
20. Imane M, Hafida H, Samia B, Meriem L, Ibtissem KT, Ryad D. Biofilm formation by Acinetobacter baumannii isolated from medical devices at the intensive care unit of the University Hospital of Tlemcen (Algeria). Afr J Microbiol Res. 2014;8(3).

21. Wroblewska MM, Sawicka-Grzelak A, Marchel H, Luczak M, Sivan A. Biofilm production by clinical strains of Acinetobacter baumannii isolated from patients hospitalized in two tertiary care hospitals. FEMS Immunol Med Microbiol. 2008;53(1).
22. Mahendra M, Jayaraj BS, Lokesh KS, Chaya SK, Veerapaneni VV, Limaye S, et al. Antibiotic prescription, organisms and its resistance pattern in patients admitted to respiratory ICU with respiratory infection in Mysuru. Indian Journal of Critical Care Medicine. 2018;22(4).
23. Radji M, Fauziah S, Aribinuko N. Antibiotic sensitivity pattern of bacterial pathogens in the intensive care unit of Fatmawati Hospital, Indonesia. Asian Pac J Trop Biomed. 2011;1(1).
24. Bjarnsholt T, Ciofu O, Molin S, Givskov M, Høiby N. Applying insights from biofilm biology to drug development-can a new approach be developed? Vol. 12, Nature Reviews Drug Discovery. 2013.
25. Irie Y, Borlee BR, O'Connor JR, Hill PJ, Harwood CS, Wozniak DJ, et al. Self-produced exopolysaccharide is a signal that stimulates biofilm formation in Pseudomonas aeruginosa. ProcNatlAcadSci U S A. 2012;109(50).
26. Gupta P, Sarkar S, Das B, Bhattacharjee S, Tribedi P. Biofilm, pathogenesis and prevention—a journey to break the wall: a review. Vol. 198, Archives of Microbiology. 2016.
27. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. Sci Am. 1978;238(1).
28. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Vol. 284, Science. 1999.
29. rose J. biofilms: the good and the bad. 2023.
30. Cochrane DMG, Brown MRW, Anwar H, Weller PH, Lam K, Costeron JW. Antibody response to Pseudomonas aeruginosa surface protein antigens in a rat model of chronic lung infection. J Med Microbiol. 1988;27(4).
31. Sutherland IW. Biofilm exopolysaccharides: A strong and sticky framework. Vol. 147, Microbiology. 2001.

32. Singh S, Datta S, Narayanan KB, Rajnish KN. Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. Vol. 19, Journal of Genetic Engineering and Biotechnology. 2021.
33. Limoli DH, Jones CJ, Wozniak DJ. Bacterial Extracellular Polysaccharides in Biofilm Formation and Function.MicrobiolSpectr. 2015;3(3).
34. Vandana, Das S. Structural and mechanical characterization of biofilm-associated bacterial polymer in the emulsification of petroleum hydrocarbon. 3 Biotech. 2021;11(5).
35. Chen MY, Lee DJ, Tay JH, Show KY. Staining of extracellular polymeric substances and cells in bioaggregates.ApplMicrobiolBiotechnol. 2007;75(2).
36. Hussain M, Wilcox MH, White PJ. The slime of coagulase-negative staphylococci: Biochemistry and relation to adherence. FEMS MicrobiolLett. 1993;104(3–4).
37. Donlan RM. Biofilms: Microbial life on surfaces. Vol. 8, Emerging Infectious Diseases. 2002.
38. 38. Costerton JW; LZ; CDE; KDR; LSHM.Microbial biofilms.Annu Rev Microbiol. 1995;49:711–45.
39. Stoodley P, Lewandowski Z, Boyle JD, Lappin-Scott HM. Oscillation characteristics of biofilm streamers in turbulent flowing water as related to drag and pressure drop. BiotechnolBioeng. 1998;57(5).
40. Chen M, Yu Q, Sun H. Novel strategies for the prevention and treatment of biofilm related infections. Vol. 14, International Journal of Molecular Sciences. 2013.
41. Paharik AE, Horswill AR. The Staphylococcal Biofilm: Adhesins, Regulation, and Host Response. MicrobiolSpectr. 2016;4(2).
42. Zaborowska M, Tillander J, Brånemark R, Hagberg L, Thomsen P, Trobos M. Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants. J Biomed Mater Res B ApplBiomater. 2017;105(8).
43. Khan MS, urRehman S, Ali MA, Sultan B, Sultan S. Infection in orthopedic implant surgery, its risk factors and outcome. J Ayub Med Coll Abbottabad. 2008;20(1).

44. Cerca N, Pier GB, Vilanova M, Oliveira R, Azeredo J. Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of Staphylococcus epidermidis. Res Microbiol. 2005;156(4).
45. Rahim MI, Rohde M, Rais B, Seitz JM, Mueller PP. Susceptibility of metallic magnesium implants to bacterial biofilm infections. J Biomed Mater Res A. 2016;104(6).
46. Koseki H, Yonekura A, Shida T, Yoda I, Horiuchi H, Morinaga Y, et al. Early staphylococcal biofilm formation on solid orthopaedic implant materials: In vitro study. PLoS One. 2014;9(10).
47. Glage S, Paret S, Winkel A, Stiesch M, Bleich A, Krauss JK, et al. A new model for biofilm formation and inflammatory tissue reaction: intraoperative infection of a cranial implant with Staphylococcus aureus in rats. ActaNeurochir (Wien). 2017;159(9).
48. Oliveira WF, Silva PMS, Silva RCS, Silva GMM, Machado G, Coelho LCBB, et al. Staphylococcus aureus and Staphylococcus epidermidis infections on implants. Vol. 98, Journal of Hospital Infection. 2018.
49. Zheng Y, He L, Asiamah TK, Otto M. Colonization of medical devices by staphylococci. Vol. 20, Environmental Microbiology. 2018.
50. Kranjec C, Angeles DM, Mårli MT, Fernández L, García P, Kjos M, et al. Staphylococcal biofilms: Challenges and novel therapeutic perspectives. Vol. 10, Antibiotics. 2021.
51. Palmer J, Flint S, Brooks J. Bacterial cell attachment, the beginning of a biofilm. J IndMicrobiolBiotechnol. 2007;34(9).
52. Carniello V, Peterson BW, van der Mei HC, Busscher HJ. Physico-chemistry from initial bacterial adhesion to surface-programmed biofilm growth. Vol. 261, Advances in Colloid and Interface Science. 2018.
53. Yu P, Wang C, Zhou J, Jiang L, Xue J, Li W. Influence of Surface Properties on Adhesion Forces and Attachment of Streptococcus mutans to Zirconia In Vitro. Biomed Res Int. 2016;2016.
54. Tuson HH, Weibel DB. Bacteria-surface interactions.Vol. 9, Soft Matter. 2013.

55. Nourbakhsh F, Namvar AE. Detection of genes involved in biofilm formation in Staphylococcus aureus isolates. GMS Hyg Infect Control. 2016;11.
56. Abraham WR. Going beyond the control of quorum-sensing to combat biofilm infections.Vol. 5, Antibiotics. 2016.
57. Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. Vol. 11, Frontiers in Microbiology. 2020.
58. Costa OYA, Raaijmakers JM, Kuramae EE. Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. Vol. 9, Frontiers in Microbiology. 2018.
59. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: An emergent form of bacterial life. Vol. 14, Nature Reviews Microbiology. 2016.
60. Shen D, Langenheder S, Jürgens K. Dispersal modifies the diversity and composition of active bacterial communities in response to a salinity disturbance. Front Microbiol. 2018;9(SEP).
61. Kaplan JB. Biofilm Dispersal: Mechanisms, Clinical Implications, and Potential Therapeutic Uses. Vol. 89, Journal of Dental Research. 2010.
62. Lee K, Yoon SS. Pseudomonas aeruginosa Biofilm, a Programmed Bacterial Life for Fitness. Vol. 27, Journal of Microbiology and Biotechnology. 2017.
63. Pietrocola G, Campoccia D, Motta C, Montanaro L, Arciola CR, Speziale P. Colonization and Infection of Indwelling Medical Devices by Staphylococcus aureus with an Emphasis on Orthopedic Implants. Vol. 23, International Journal of Molecular Sciences. 2022.
64. Darouiche RO. Device-associated infections: A macroproblem that starts with microadherence. Clinical Infectious Diseases. 2001;33(9).
65. Arciola CR, Campoccia D, Montanaro L. Implant infections: Adhesion, biofilm formation and immune evasion. Vol. 16, Nature Reviews Microbiology. 2018.
66. Solano C, Echeverz M, Lasa I. Biofilm dispersion and quorum sensing. Vol. 18, Current Opinion in Microbiology. 2014.
67. Peschel A, Otto M. Phenol-soluble modulins and staphylococcal infection. Vol. 11, Nature Reviews Microbiology. 2013.

68. Habib A, Le KY, Baddour LM, Friedman PA, Hayes DL, Lohse CM, et al. Predictors of mortality in patients with cardiovascular implantable electronic device infections. American Journal of Cardiology. 2013;111(6).
69. Rabe A, Gesell Salazar M, Michalik S, Kocher T, Below H, Völker U, et al. Impact of different oral treatments on the composition of the supragingival plaque microbiome. J Oral Microbiol. 2022;14(1).
70. Singh N, Mishra S, Mondal A, Sharma D, Jain N, Aseri GK. Potential of Desert Medicinal Plants for Combating Resistant Biofilms in Urinary Tract Infections.Vol. 195, Applied Biochemistry and Biotechnology. 2023.
71. Bair KL, Shafirstein G, Campagnari AA. In vitro Photodynamic Therapy of Polymicrobial Biofilms Commonly Associated With Otitis Media. Front Microbiol. 2020;11.
72. Han J, Poma A. Molecular Targets for Antibody-Based Anti-Biofilm Therapy in Infective Endocarditis. Vol. 14, Polymers. 2022.
73. Klagisa R, Racenis K, Broks R, Balode AO, Kise L, Kroica J. Analysis of Microorganism Colonization, Biofilm Production, and Antibacterial Susceptibility in Recurrent Tonsillitis and Peritonsillar Abscess Patients. Int J Mol Sci. 2022;23(18).
74. Prado MM, Figueiredo N, Pimenta A de L, Miranda TS, Feres M, Figueiredo LC, et al. Recent Updates on Microbial Biofilms in Periodontitis: An Analysis of In Vitro Biofilm Models. In: Advances in Experimental Medicine and Biology. 2022.
75. Grier JT, Arivett BA, Ramírez MS, Chosed RJ, Bigner JA, Ohneck EJ, et al. Two Acinetobacter baumannii Isolates Obtained From a Fatal Necrotizing Fasciitis Infection Display Distinct Genomic and Phenotypic Characteristics in Comparison to Type Strains. Front Cell Infect Microbiol. 2021;11.
76. Hassan NF, Ibrahim MK, El Tablawy SY, Farrag HAA. Characterization of biofilm producer nanobacteria isolated from kidney stones of some egyptian patients. Pakistan Journal of Biological Sciences. 2021;24(9).
77. Wu H, Moser C, Wang HZ, Høiby N, Song ZJ. Strategies for combating bacterial biofilm infections.Vol. 7, International Journal of Oral Science. 2015.

78. Arroyo-Moreno S, Cummings M, Corcoran DB, Coffey A, McCarthy RR. Identification and characterization of novel endolysins targeting Gardnerella vaginalis biofilms to treat bacterial vaginosis.NPJ Biofilms Microbiomes. 2022;8(1).
79. Salmanoglu E, Kim S, Thakur ML. Currently Available Radiopharmaceuticals for Imaging Infection and the Holy Grail. Vol. 48, Seminars in Nuclear Medicine. 2018.
80. 83. Eggleston H. PP. Molecular imaging of bacterial infections in vivo: the discrimination of infection from inflammation. MDPI. 2014;1.
81. Auletta S, Varani M, Horvat R, Galli F, Signore A, Hess S. PET radiopharmaceuticals for specific bacteria imaging: A systematic review. Vol. 8, Journal of Clinical Medicine. 2019.
82. Cruz A, Condinho M, Carvalho B, Arraiano CM, Pobre V, Pinto SN. The two weapons against bacterial biofilms: Detection and treatment. Vol. 10, Antibiotics. 2021.
83. Kujundzic E, Cristina Fonseca A, Evans EA, Peterson M, Greenberg AR, Hernandez M. Ultrasonic monitoring of early-stage biofilm growth on polymeric surfaces. J Microbiol Methods. 2007;68(3).
84. Vaidya K, Osgood R, Ren D, Pichichero ME, Helguera M. Ultrasound Imaging and Characterization of Biofilms Based onWavelet De-noised Radiofrequency Data. Ultrasound Med Biol. 2014;40(3).
85. Calliada F, Campani R, Bottinelli O, Bozzini A, Sommaruga MG. Ultrasound contrast agents: Basic principles. In: European Journal of Radiology. 1998.
86. Unnikrishnan S, Klibanov AL. Microbubbles as ultrasound contrast agents for molecular imaging: Preparation and application. Vol. 199, American Journal of Roentgenology. 2012.
87. Anastasiadis P, Mojica KDA, Allen JS, Matter ML. Detection and quantification of bacterial biofilms combining high-frequency acoustic microscopy and targeted lipid microparticles. J Nanobiotechnology. 2014;12(1).
88. Castro J, Lima Â, Sousa LGV, Rosca AS, Muzny CA, Cerca N. Crystal Violet Staining Alone Is Not Adequate to Assess Synergism or Antagonism in Multi-Species Biofilms of Bacteria Associated With Bacterial Vaginosis. Front Cell Infect Microbiol. 2022;11.
89. Albucasis CPS. The story of Tracheotomy. Brit J Child Dis. 1934;31:1013–116.

90. NELSON TG. Tracheotomy: a clinical and experimental study. II. Am Surg. 1957;23(8).
91. Fockens MM, de Bakker BS, Oostra RJ, Dikkers FG. Development pattern of tracheal cartilage in human embryos.Clinical Anatomy. 2021;34(5).
92. Durkin N, De Coppi P. Anatomy and embryology of tracheo-esophageal fistula. SeminPediatr Surg. 2022;31(6).
93. MC Grath BA. Comprehensive Tracheostomy Care: The national tracheostomy safety project manual. Wiley. 2014;52–61.
94. Higgins KM, Punthakee X. Meta-analysis comparison of open versus percutaneous tracheostomy. Laryngoscope. 2007;117(3).
95. Johnson-Obaseki S, Veljkovic A, Javidnia H. Complication rates of open surgical versus percutaneous tracheostomy in critically ill patients. Laryngoscope. 2016;126(11).
96. Kennedy MM, Abdel-Aty Y, Lott DG. Comparing tracheostomy techniques: Bjork flap vs. tracheal window.American Journal of Otolaryngology - Head and Neck Medicine and Surgery. 2021;42(6).
97. Barnes AMT, Ballering KS, Leibman RS, Wells CL, Dunnya GM. Enterococcus faecalis produces abundant extracellular structures containing DNA in the absence of cell lysis during early biofilm formation. mBio. 2012;3(4).
98. Backman S, Björling G, Johansson UB, Lysdahl M, Markström A, Schedin U, et al. Material wear of Polymeric tracheostomy tubes: A six-month study. Laryngoscope. 2009;119(4).
99. Inglis TJJ, Millar MR, Jones JG, Robinson DA. Tracheal tube biofilm as a source of bacterial colonization of the lung.J ClinMicrobiol. 1989;27(9).
100. Gil-Perotin S, Ramirez P, Marti V, Sahuquillo JM, Gonzalez E, Calleja I, et al. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: A state of concept. Crit Care. 2012;16(3).
101. LeChevallier MW, Cawthon CD, Lee RG. Inactivation of biofilm bacteria.Appl Environ Microbiol. 1988;54(10).

102. Ścibik A GKDKDWKŁ. Microbial colonization and resistance patterns in tracheostomy tubes: a prospective observational study. Anaesthesiol Intensive Ther. 2022;54(2):128–35.
103. Raveendra L PMBKMR. Biofilm formation and multidrug resistance in tracheostomy tube isolates in a tertiary care hospital. J ClinDiagnRes . 2021;15.
104. . Bhattacharya S GADSRPBD. Biofilm-forming multidrug-resistant bacteria in diabetic ICU patients: a concern. . J Infect DevCtries. 2021;15(4):512–9.
105. Sharma A MRCU. Antimicrobial susceptibility patterns of biofilm-forming bacteria isolated from ICU patients. Indian J Crit Care Med. 2020;24(7):556–62.
106. Mehta R BPSR. Role of biofilms in Pseudomonas-dominant infections in immunocompromised patients. BMC Infect Dis. 2022;22(1):367.

ANNEXURE

ANNEXURE-I

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,TAMAKA, KOLAR - 563101.

PATIENT INFORMATION SHEET

STUDY TITLE: MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT - A CROSS- SECTIONAL STUDY

Study location: R L Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

Details: Patients aged 18 years and above undergoing tracheostomy and expected to be on tracheostomy tube for 2 weeks or more at R.L Jalappa Hospital will be included in this study.

Patients in this study will have to undergo routine blood investigations (CBC, RFT. Serum electrolytes, FBS, PPBS, HbA1c, Blood grouping and virology), chest x-ray as a part of surgery.

Patients will be subjected to emergency or elective tracheostomy. Swabs will be collected from tracheostomy tube and tracheostoma soon after tracheostomy, 1 week after tracheostomy, 2 weeks after tracheostomy and 1 month after tracheostomy in patients who still require. The swabs will be collected by strict aseptic precautions and will be sent to microbiology section of CDLS (Central Diagnostic Laboratory Services), RLJH&RC for

further microbiological processing (Gram stain, catalase, coagulase, oxidase, indole, citrate, urease, mannitol motility and triple sugar iron tests), Antibiotic culture sensitivity.

Patient will be explained about the importance of undergoing the above mentioned investigations and treatment procedures, alternative methods of treatment and complications of not undergoing the treatment. The investigator will be bearing the cost.

Please read the following information and discuss with your family members. You can ask any question regarding the study. If you agree to participate in the study, we will collect information from you or the person responsible for you, or both. Relevant history will be taken. This information collected will be used only for dissertation and publication.

For any further clarification you are free to contact the Principal investigator, Dr. Nallagonda Satya Sai SriRam, Mobile Number: 7026835459

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. This study has been reviewed by the Institutional Ethics Committee and you are free to contact the members of the same. There is no compulsion to agree to this study. The care you will get will not change if you do not wish to participate in this study. You will have no financial benefit by being a part of this study, nor will you incur any risk. You are required to sign/provide thumb impression only if you voluntarily agree to participate in this study.

Principal investigator's name: Dr. Nallagonda Satya Sai SriRam (Post graduate)

Department of Otorhinolaryngology

SDUMC, Kolar

Mobile Number: 7026835459

Email Id:sriram21051998@gmail.com

ANNEXURE - II

ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಮತ್ತು ಸಂಶೋಧನೆಯ ಅಕಾಡೆಮಿ, ತಮಕಾ, ಕೋಲಾರ - 563101 .

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ:ಚಿಕಿತ್ಸೆಯ ಮೊದಲು ಮತ್ತು ನಂತರ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ಟ್ಯೂಬ್ ಮತ್ತು ಟ್ರಾಕಿಯೊಸ್ಟೊಮಾದ ಮೇಲೆ ಸೂಕ್ಷ್ಮಜೀವಿಯ ಬಯೋಫಿಲ್ಮ್ - ಒಂದು ಅಡ್ಡ-ವಿಭಾಗದ ಅಧ್ಯಯನ

ಅಧ್ಯಯನ ಸ್ಥಳ:ಆರ್.ಎಲ್.ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರವು ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ತಮಕ, ಕೋಲಾರ.

ವಿವರಗಳು: 18 ವರ್ಷ ಮತ್ತು ಅದಕ್ಕಿಂತ ಹೆಚ್ಚಿನ ವಯಸ್ಸಿನ ರೋಗಿಗಳು ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿಗೆ ಒಳಗಾಗುತ್ತಾರೆ ಮತ್ತು ಆರ್ಎಲ್ ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆಯಲ್ಲಿ 2 ವಾರ ಅಥವಾ ಅದಕ್ಕಿಂತ ಹೆಚ್ಚು ಕಾಲ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ಟ್ಯೂಬ್‌ನಲ್ಲಿ ಇರಬೇಕೆಂದು ನಿರೀಕ್ಷಿಸಲಾಗಿದೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ರೋಗಿಗಳು ಪಾಡಿಕೆಯ ರಕ್ತ ಪರೀಕ್ಷೆಗಳಿಗೆ ಒಳಗಾಗಬೇಕಾಗುತ್ತದೆ (ಸಿ ಬಿ ಸಿ, ಆರ್ ಎಫ್ ಟಿ. ಸೀರಮ್ ಎಲೆಕೋಲ್ಯೆಟ್‌ಗಳು, ಎಫ್ ಬಿ ಎಸ್, ಪಿ ಪಿ ಬಿ ಎಸ್, ಎಚ್ ಬಿ ಎ 1 ಸಿ, ರಕ್ತದ ಗುಂಪು ಮತ್ತು ವೈರಾಲಜಿ). ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ಭಾಗವಾಗಿ ಎದೆಯ ಕ್ಷ-ಕಿರಣ. ರೋಗಿಗಳನ್ನು ತುರ್ತು ಅಥವಾ ಚುನಾಯಿತ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿಗೆ ಒಳಪಡಿಸಲಾಗುತ್ತದೆ. ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ನಂತರ 1 ದಾರ, ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ನಂತರ 2 ವಾರಗಳು ಮತ್ತು ಇನ್ನೂ ಅಗತ್ಯವಿರುವ ರೋಗಿಗಳಲ್ಲಿ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ನಂತರ 1 ತಿಂಗಳ ನಂತರ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ಟ್ಯೂಬ್ ಮತ್ತು ಟ್ರಾಕಿಯೊಸ್ಟೊಮಾದಿಂದ ಸ್ವ್ಯಾಬ್‌ಗಳನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಸ್ವ್ಯಾಬ್‌ಗಳನ್ನು ಕಿಟ್ಟುನಿಟ್ಟಾದ ಅಸೆಪ್ಟಿಕ್ ಮುನ್ನೆಚ್ಚರಿಕೆಗಳಿಂದ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಹೆಚ್ಚಿನ ಸೂಕ್ಷ್ಮ ಜೀವವಿಜ್ಞಾನ ಪ್ರಕ್ರಿಯೆಗಾಗಿ CDLS (ಸೆಂಟ್ರಲ್ ರಯಾಗ್ನೋಸ್ಟಿಕ್ ಲ್ಯಾಬೊರೇಟರಿ ಸೇವೆಗಳು), RLJH & RC ನ ಮೈಕ್ರೋಬಯಾಲಜಿ ವಿಭಾಗಕ್ಕೆ ಕಳುಹಿಸಲಾಗುತ್ತದೆ (ಗ್ರಾಮ್ ಸ್ಟೇನ್, ಕ್ಯಾಟಲೇಸ್, ಕೋಗುಲೇಸ್, ಆಕ್ಸಿಡೇಸ್, ಇಂಡೋಲ್, ಸಿಟ್ರೇಟ್, ಯೂರೇಸ್ ಮತ್ತು ಟ್ರಿಪಲ್ ಮೋಟರಿಟಿ ಸಕ್ಕರೆ ಕಬ್ಬಿಣದ ಪರೀಕ್ಷೆಗಳು). ಪ್ರತಿಜೀವಕ ಸಂಸ್ಕೃತಿಯ ಸೂಕ್ಷ್ಮತೆ.

ಮೇಲೆ ತಿಳಿಸಿದ ತನಿಖೆಗಳು ಮತ್ತು ಚಿಕಿತ್ಸಾ ವಿಧಾನಗಳಿಗೆ ಒಳಗಾಗುವ ಪ್ರಾಮುಖ್ಯತೆ, ಚಿಕಿತ್ಸೆಯ ಪರ್ಯಾಯ ವಿಧಾನಗಳು ಮತ್ತು ಚಿಕಿತ್ಸೆಗೆ ಒಳಗಾಗದಿರುವ ತೊಡಕುಗಳ ಬಗ್ಗೆ ರೋಗಿಯನ್ನು ವಿವರಿಸಲಾಗುವುದು. ತನಿಖಾಧಿಕಾರಿಯೇ ವೆಚ್ಚ ಭರಿಸಲಿದ್ದಾರೆ.

ದಯವಿಟ್ಟು ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬದ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ. ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ನೀವು ಯಾವುದೇ ಪ್ರಶ್ನೆಯನ್ನು ಕೇಳಬಹುದು. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಮ್ಮತಿಸಿದರೆ, ನಾವು ನಿಮ್ಮಿಂದ ಅಥವಾ ನಿಮಗೆ ಜವಾಬ್ದಾರರಾಗಿರುವ ವ್ಯಕ್ತಿಯಿಂದ ಅಥವಾ ಇಬ್ಬರಿಂದಲೂ ಮಾಹಿತಿಯನ್ನು ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಸಂಬಂಧಿತ ಇತಿಹಾಸವನ್ನು ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು. ಸಂಗ್ರಹಿಸಿದ ಈ ಮಾಹಿತಿಯನ್ನು ಪ್ರಬಂಧ ಮತ್ತು ಪ್ರಕಟಣೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ.

ಯಾವುದೇ ಹೆಚ್ಚಿನ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ ಡಾ. ನಲ್ಲಗೊಂಡ ಸತ್ಯ ಸಾಯಿ ಶ್ರೀರಾಮ್ ಅವರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ. ದೊಬ್ಬೈಲ್ ಸಂಖ್ಯೆ: 7026835459

ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಲಾದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನವನ್ನು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯು ಪರಿಶೀಲಿಸಿದೆ ಮತ್ತು ನೀವು ಅದರ ಸದಸ್ಯರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ. ಈ ಅಧ್ಯಯನವನ್ನು ಒಪ್ಪಿಕೊಳ್ಳಲು ಯಾವುದೇ ಒತ್ತಾಯವಿಲ್ಲ. ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ ನೀವು ಪಡೆಯುವ ಕಾಳಜಿಯು ಬದಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದ ಭಾಗವಾಗುವುದರಿಂದ ನಿಮಗೆ ಯಾವುದೇ ಆರ್ಥಿಕ ಪ್ರಯೋಜನವಾಗುವುದಿಲ್ಲ ಅಥವಾ ನೀವು ಯಾವುದೇ ಅಪಾಯಕ್ಕೆ ಒಳಗಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವು ಸಹಿ/ಹೆಬ್ಬೆರಳಿನ ಗುರುತನ್ನು ಒದಗಿಸಬೇಕಾಗುತ್ತದೆ.

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿಯ ಹೆಸರು: ಡಾ. ನಲ್ಲಗೊಂಡ ಸತ್ಯ ಸಾಯಿ ಶ್ರೀರಾಮ್ (ಸ್ನಾತಕೋತ್ತರ ಪದವಿ)

ಓಟೋರಿನೋಲಾರಿಂಗೋಲಜಿ ವಿಭಾಗ

ಎಸ್ ಡಿ ಯು ಎಂ ಸಿ, ಕೋಲಾರ

ಮೊಬೈಲ್ ಸಂಖ್ಯೆ: 7026835459

ಇಮೇಲ್ ಐಡಿ: sriram21051998@gmail.com

ANNEXURE-III

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,

TAMAKA, KOLAR - 563101.

INFORMED CONSENT FORM

STUDY TITLE: MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT - A CROSS- SECTIONAL STUDY

I, _____ aged _____ ,after being explained in a language I know and understand, about the purpose of the study and the risks and complications of the procedure, hereby give my valid written informed consent without any force or prejudice for TRACHEOSTOMY or any other procedure deemed fit, which is a diagnostic & / or therapeutic procedure / biopsy / transfusion / operation to be performed on me under any anaesthesia deemed fit. The nature and risks involved in the procedure (surgical and anaesthetical) have been explained to me to my satisfaction.

I have been explained in detail about the Clinical Research on "**MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT - A CROSS SECTIONAL STUDY**" being conducted. *I have read the patient information sheet and I have had the opportunity to ask any question. Any question that I have asked, has been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.* I hereby give consent to provide my history, undergo physical examination, undergo required investigations and surgical procedure deemed fit and provide its results and documents etc to the doctor / institute etc. I hereby give consent to collect swabs from tracheostomy tube and tracheostoma soon after tracheostomy, 1 week after tracheostomy, 2 weeks after tracheostomy and 1 month after tracheostomy. For academic and scientific purpose the operation / procedure, etc may be video graphed or photographed. All the data may be published or used for any academic

purpose. I will not hold the doctors / institute etc responsible for any untoward consequences during the procedure / study.

A copy of this Informed Consent Form and Patient Information Sheet has been provided to the participant.

Signature & Name of Pt. Attendant Name &Signature/Thumb impression of patient

Relation with patient_____

Witness:----------

Name of Researcher taking the consent_____

Signature of Researcher taking the consent_____

Principal investigator's name: Dr. Nallagonda Satya Sai SriRam , Mobile Number: 7026835459

Email Id:sriram21051998@gmail.com

ANNEXURE-IV

ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಮತ್ತು ಸಂಶೋಧನೆಯ ಅಕಾಡೆಮಿ

ತಮಕಾ, ಕೋಲಾರ-563101

ಮಾಹಿತಿಯ ಒಪ್ಪಿಗೆ ನಮೂನೆ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ:ಚಿಕಿತ್ಸೆಯ ಮೊದಲು ಮತ್ತು ನಂತರ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ಟ್ಯೂಬ್ ಮತ್ತು ಟ್ರಾಕಿಯೊಸ್ಟೊಮಾದ ಮೇಲೆ ಸೂಕ್ಷ್ಮಜೀವಿಯ ಬಯೋಫಿಲ್ಮ್ಸ್ - ಒಂದು ಅಡ್ಡ-ವಿಭಾಗದ ಅಧ್ಯಯನ

ನಾನು, _____, ನಾನು ತಿಳಿದಿರುವ ಮತ್ತು ಅರ್ಥಮಾಡಿಕೊಂಡ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಿದ ನಂತರ, ಅಧ್ಯಯನದ ಉದ್ದೇಶ ಮತ್ತು ಕಾರ್ಯವಿಧಾನದ ಅಪಾಯಗಳು ಮತ್ತು ತೊಡಕುಗಳ ಬಗ್ಗೆ. ಈ ಮೂಲಕ ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ಅಥವಾ ಯಾವುದೇ ಇತರ ಕಾರ್ಯವಿಧಾನಕ್ಕೆ ಯಾವುದೇ ಬಲ ಅಥವಾ ಪೂರ್ವಾಗ್ರಹವಿಲ್ಲದೆ ನನ್ನ ಮಾನ್ಯ ಲಿಖಿತ ತಿಳುವಳಿಕೆಯನ್ನು ನೀಡಿ. ಒಂದು ರೋಗನಿರ್ಣಯ ಮತ್ತು / ಅಥವಾ ಚಿಕಿತ್ಸಕ ವಿಧಾನ / ಬಯಾಪ್ಸಿ / ದರ್ಗಾವಣೆ / ಕಾರ್ಯಾಚರಣೆಯನ್ನು ನನ್ನ ಮೇಲೆ ಯಾವುದೇ ಅರಿವಳಿಕೆ ಅಡಿಯಲ್ಲಿ ನಡೆಸಲಾಗುವುದು. ಕಾರ್ಯವಿಧಾನದಲ್ಲಿ ಒಳಗೊಂಡಿರುವ ಸ್ವಭಾವ ಮತ್ತು ಅಪಾಯಗಳು (ಶಸ್ತ್ರಚಿಕಿತ್ಸೆ ಮತ್ತು ಅರಿವಳಿಕೆ) ನನ್ನ ತೃಪ್ತಿಗೆ ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ.

"ಟ್ರಾಕಿಯೋಸ್ಟೊಮಿ ಟ್ಯೂಬ್ ಮತ್ತು ಟ್ರಾಕಿಯೋಸ್ಟೋಮಾದಲ್ಲಿನ ಮೈಕ್ರೋಬಿಯಲ್ ಬಯೋಫಿಲ್ಮ್‌ಗಳು ಚಿಕಿತ್ಸೆಯ ಮೊದಲು ಮತ್ತು ನಂತರ - ಎ ಕ್ರಾಸ್ ಸೆಕ್ಷನಲ್ ಸ್ಟಡಿ" ಎಂಬ ಕ್ಲಿನಿಕಲ್ ಸಂಶೋಧನೆಯ ಕುರಿತು ನನಗೆ ವಿವರವಾಗಿ ವಿವರಿಸಲಾಗಿದೆ. ನಾನು ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಓದಿದ್ದೇನೆ ಮತ್ತು ಯಾವುದೇ ಪ್ರಶ್ನೆಯನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶವಿದೆ. ನಾನು ಕೇಳಿದ ಯಾವುದೇ ಪ್ರಶ್ನೆಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರಿಸಲಾಗಿದೆ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವವನಾಗಿ ಭಾಗವಹಿಸಲು ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ. ನನ್ನ ಇತಿಹಾಸವನ್ನು ಒದಗಿಸಲು, ದೈಹಿಕ ಪರೀಕ್ಷೆಗೆ ಒಳಗಾಗಲು, ಅಗತ್ಯವಿರುವ ತನಿಖೆಗಳು ಮತ್ತು ಶಸ್ತ್ರಚಿಕಿತ್ಸಾ ವಿಧಾನಗಳಿಗೆ ಒಳಗಾಗಲು ನಾನು ಈ ಮೂಲಕ ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೇನೆ ಮತ್ತು ಅದರ ಫಲಿತಾಂಶಗಳು ಮತ್ತು ದಾಖಲೆಗಳನ್ನು ಇತ್ಯಾದಿಗಳನ್ನು ವೈದ್ಯರು / ಸಂಸ್ಥೆ ಇತ್ಯಾದಿಗಳಿಗೆ ಒದಗಿಸುತ್ತೇನೆ. , ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ನಂತರ 1 ವಾರ, ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ನಂತರ 2 ವಾರಗಳು ಮತ್ತು ಟ್ರಾಕಿಯೊಸ್ಟೊಮಿ ನಂತರ 1 ತಿಂಗಳು. ಶೈಕ್ಷಣಿಕ ಮತ್ತು ವೈಜ್ಞಾನಿಕ ಉದ್ದೇಶಕ್ಕಾಗಿ ಕಾರ್ಯಾಚರಣೆ / ಕಾರ್ಯವಿಧಾನ, ಇತ್ಯಾದಿಗಳನ್ನು ವೀಡಿಯೊ ಗ್ರಾಫ್ ಅಥವಾ ಛಾಯಾಚಿತ್ರ ಮಾಡಬಹುದು. ಎಲ್ಲಾ ದೇಹವನ್ನು ಪ್ರಕಟಿಸಬಹುದು ಅಥವಾ ಯಾವುದೇ ಶೈಕ್ಷಣಿಕ ಉದ್ದೇಶಕ್ಕಾಗಿ ಬಳಸಬಹುದು. ಕಾರ್ಯವಿಧಾನ / ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಯಾವುದೇ ಅಹಿತಕರ ಪರಿಣಾಮಗಳಿಗೆ ನಾನು ವೈದ್ಯರು / ಸಂಸ್ಥೆ ಇತ್ಯಾದಿಗಳನ್ನು ಜವಾಬ್ದಾರರನ್ನಾಗಿ ಮಾಡುವುದಿಲ್ಲ.

ಈ ತಿಳುವಳಿಕೆಯುಳ್ಳ ಒಪ್ಪಿಗೆ ನಮೂನೆಯ ಪ್ರತಿಯನ್ನು ಮತ್ತು ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಭಾಗವಹಿಸುವವರಿಗೆ ಒದಗಿಸಲಾಗಿದೆ.

ವಂ.ನ ಸಹಿ ಮತ್ತು ಹೆಸರು. ಅಟೆಂಡೆಂಡ್ ಹೆಸರು &ಸಹಿ/ರೋಗಿಯ ಹೆಬ್ಬೆರಳಿನ ಗುರುತು

ರೋಗಿಯೊಂದಿಗೆ ಸಂಬಂಧ_____

ಸಾಕ್ಷಿ:----------

ಒಪ್ಪಿಗೆಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ಸಂಶೋಧಕರ ಹೆಸರು_____

ಒಪ್ಪಿಗೆಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ಸಂಶೋಧಕರ ಸಹಿ_____

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿಯ ಹೆಸರು: ಡಾ. ನಲ್ಲಗೊಂಡ ಸತ್ಯ ಸಾಯಿ ಶ್ರೀರಾಮ್.

ಮೊಬೈಲ್ ಸಂಖ್ಯೆ: 7026835459

ANNEXURE-V

STUDY TITLE: MICROBIAL BIOFILMS ON TRACHEOSTOMY TUBE AND TRACHEOSTOMA BEFORE AND AFTER ANTIBIOTIC TREATMENT - A CROSS-SECTIONAL STUDY

PROFORMA

1. BASIC DATA

Name: Age/Sex:

Address: Mobile No:

Date of Admission/OP: UHID NO:

Diagnosis:

Date of Tracheostomy:

Date of Discharge:

1.REASONFOR TRACHEOSTOMY :

2.TYPE OF TRACHEOSTOMY :

- HIGH
- MID
- LOW

3.TYPE/MATERIAL OF TRACHEOSTOMY TUBE USED

- CUFFED
- UN-CUFFED
- METALLIC
- NON METALLIC
- SINGLE LEUMEN
- DOUBLE LEUMEN

4.SIZE OF TRACHEOSTOMY TUBE

- 6.5mm
- 7.0mm
- 7.5mm
- > 7.5mm

5.FREQUENCY OF TUBE CHANGE :

6.PREEXISTING SYTEMIC ILLNESS/CO-MORBIDITIES:

7.OTHER SYSTEMIC ILLNESS:

8.ANTIBIOTICS WHICH PATIENT HAVE RECEIVED:

9.INVESTIGATIONS :

10.NUMBER OF DAYS ON VENTILATOR SUPPORT:

11.NUMBER OF DAYS IN ICU OR SURGICAL ICU:

STATUS OF STOMA:

	IMMEDIATELY AFTER TRACHEOSTOMY	**1 WEEK POST TRACHEOSTOMY**	**2 WEEKS POST TRACHEOSTOMY**	**4 WEEKS POST TRACHEOSTOMY**
GRANULATION				
ULCERATION				
EXCORIATION OF SKIN				
STENOSIS				
MICROBIAL GROWTH				

ANTIBIOTIC SENSITIVITY				
RESPONSE TO TREATMENT				

INTERPRETATION OF BIOFILM FORMATION:

1. **1.** Biofilm formation (Present/Absent) :
2. **2.** If present, Optic density value :
3. If present, Strength of biofilm produced (weak/moderate/strong) :

ANNEXURE-VI

KEY TO MASTER CHART

M-Male

F-Female

GCS- Glasgow Coma Scale

RTA- Road Traffic Accident

SAH- Sub Arachnoid Hemorrhage

GBS-Gingivo Buccal Sulcus

COPD-Chronic Obstructive Pulmonary Disease

FTP-Fronto Temporo Parietal

OP- Organophosphate

MRND- Modified Radical Neck Dissection

PMMC- Pectoralis Major Myocutaneous

ALT-Antro Lateral Thigh

DP-Delto Pectoral

SOHND-Supra Omo Hyoid Neck Dissection

ITF-Infra Temporal Fossa

MASTER CHART

Number	No.of bacteria grown(Day 0)	Biofilm formation (Day 0)	Antibiotics used (Day 0)	No.of bacteria grown (Day 7)	Biofilm formation (Day 7)	Antibiotics used (Day 7)	No.of bacteria grown(Day 14)	Biofilm formation (Day 14)	Combined Antibiotics used (Day 14)	No.of bacteria grown(1 month)	Biofilm formation (1 month)	Combined Antibiotics used (1 month)
1	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
2	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	weak	Amoxiclac+Piptaz	no growth	no	no
3	no growth	no	no	no growth	no	no	Klebsiella pneumoniae	no	Piptaz +Amikacin	no growth	no	no
4	no growth	no	no	Acinteobacter baumanii, Klebsiella Pneumoniae	Weak	Amoxiclav , Metronidazole	no growth	no	Amikacin + Amoxiclav	no growth	no	no
5	no growth	no	no	Escherichia coli	no	Ceftriaxone, Amoxiclav, Amikacin	no growth	no	Colistin + Amoxiclav	no growth	no	no
6	no growth	no	no	Enterococcus sp.	no	Amoxiclav , Metronidazole	Klebsiella pneumoniae	strong	Amoxiclac+Piptaz	no growth	no	no
7	no growth	no	no	Klebsiella Pneumoniae	moderate	Ceftriaxone, Amoxiclav, Amikacin	no growth	no	no	no growth	no	no
8	no growth	no	no	Proteus vulgaris	no	Amoxiclav , Metronidazole	Proteus vulgaris	no	Colistin + Amoxiclav	no growth	no	no
9	no growth	no	no	Enterobacter sp.	no	Amoxiclav , Metronidazole	no growth	no	no	no growth	no	no
10	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
11	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
12	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
13	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
14	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
15	no	no	no	no growth	no	no	no growth	no	no	no	no	no

	growth									growth		
16	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
17	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
18	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
19	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
20	no growth	no	no	no growth	no	no	Klebsiella Pneumonae	moderate	Amikacin + Amoxiclav	Klebsiella Pneumoniae	moderate	Amikacin + Amoxiclav
21	Acinetobacter baumannii	no	Amoxiclav , Metronidazole	no growth	no	no	Escherichia coli	no	Piptaz +Amikacin	no growth	no	no
22	no growth	no	no	no growth	no	no	Enterococcus sp.	no	Colistin + Amoxiclav	no growth	no	no
23	Klebsiella Pneumoniae	no	Ceftriaxone, Amoxiclav, Amikacin	no growth	no	no	Klebsiella pneumoniae	no	Amoxiclac+Piptaz	Klebsiella Pneumoniae	moderate	Amoxiclac+Piptaz
24	no growth	no	no	no growth	no	no	Proteus vulgaris	no	Amikacin + Amoxiclav	no growth	no	no
25	Pseudomonas aeruginosa	no	Ceftriaxone, Amoxiclav, Amikacin	no growth	no	no	Enterobacter sp.	no	Amoxiclac+Piptaz	no growth	no	no
26	no growth	no	no	no growth	no	no	klebsiella pneumoniae	strong	Piptaz +Amikacin	no growth	no	no
27	Escherichia coli	no	Ceftriaxone, Amoxiclav, Amikacin	no growth	no	no	Enterobacter sp.	no	Piptaz +Amikacin	no growth	no	no

28	no growth	no	no	no growth	no	no	Enterococcus sp.	no	Amoxiclac+Piptaz	no growth	no	no
29	Enterococcus	no	Amoxiclav , Metronidazole	Acinteobacter baumanii, Klebsiella Pneumoniae	strong	Amikacin, Pitaz, Ceftriaxone	no growth	no	Colistin + Amoxiclav	no growth	no	no
30	no growth	no	no	Escherichia coli	no	no	Escherichia coli	no	Colistin + Amoxiclav	no growth	no	no
31	no growth	no	no	Enterococcus sp.	no	no	Enterococcus sp.	no	Piptaz +Amikacin	no growth	no	no
32	no growth	no	no	Klebsiella Pneumoniae	moderate	Amoxiclav , Metronidazole	klebsiella pneumoniae	weak	Piptaz +Amikacin	Klebsiella Pneumoniae	weak	Piptaz +Amikacin
33	Enterobacter	no	Amoxiclav , Metronidazole	Proteus vulgaris	no	no	Proteus vulgaris	no	Piptaz +Amikacin	no growth	no	no
34	Proteus vulgaris	no	Amoxiclav , Metronidazole	Enterobacter sp.	no	no	Enterobacter sp.	no	Piptaz +Amikacin	no growth	no	no
35	no growth	no	no	Klebsiella Pneumonae	weak	Amikacin, Pitaz, Ceftriaxone	Klebsiella pneumoniae	strong	Amoxiclac+Piptaz	no growth	no	no
36	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	moderate	Amoxiclac+Piptaz	no growth	no	no
37	no growth	no	no	no growth	no	no	Escherichia coli	no	Colistin + Amoxiclav	no growth	no	no
38	no growth	no	no	no growth	no	no	Enterococcus sp.	no	Colistin + Amoxiclav	no growth	no	no
39	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	weak	Amikacin + Amoxiclav	no growth	no	no
40	no growth	no	no	no growth	no	no	Proteus vulgaris	no	Amikacin + Amoxiclav	no growth	no	no
41	no growth	no	no	no growth	no	no	Enterobacter sp.	no	Piptaz +Amikacin	no growth	no	no
42	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	strong	Amikacin + Amoxiclav	Klebsiella Pneumon	strong	Amikacin +

										iae		Amoxiclav
43	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	moderate	Amikacin + Amoxiclav	Klebsiella Pneumoniae	strong	Amikacin + Amoxiclav
44	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	weak	Amikacin + Amoxiclav	Klebsiella Pneumoniae	strong	Amikacin + Amoxiclav
45	Acinetobacter baumannii	no	Ceftriaxone, Amoxiclav, Amikacin	Klebsiella Pneumoniae	Weak	Ceftriaxone, Amoxiclav, Amikacin	no growth	no	no	no growth	no	no
46	Klebsiella Pneumoniae	no	Amoxiclav , Metronidazole	Klebsiella Pneumoniae	Weak	no	Klebsiella Pneumoniae	weak	Colistin + Amoxiclav	Klebsiella Pneumoniae	weak	Colistin + Amoxiclav
47	Pseudomonas aeruginosa	no	Amoxiclav , Metronidazole	Klebsiella Pneumonae	Weak	Amoxiclav , Metronidazole	Pseudomonas aeruginosa	weak	Colistin + Amoxiclav	no growth	no	no
48	no growth	no	no	Klebsiella Pneumonia	strong	Amikacin, Pitaz, Ceftriaxone	Klebsiella Pneumoniae , Pseudomonas aeruginosa	moderate	Piptaz +Amikacin	Klebsiella Pneumoniae	moderate	Piptaz +Amikacin
49	no growth	no	no	Proteus vulgaris	no	no	Proteus vulgaris	no	Piptaz +Amikacin	no growth	no	no
50	Enterobacter	no	no	Enterobacter sp.	no	Amoxiclav , Metronidazole	Enterobacter sp.	no	Piptaz +Amikacin	no growth	no	no
51	no growth	no	no	Pseudomonas aeruginosa	moderate	no	Pseudomonas aeruginosa	no	Piptaz +Amikacin	no growth	no	no
52	Proteus vulgaris	no	Amoxiclav , Metronidazole	Klebsiella Pneumoniae	Weak	no	Klebsiella Pneumoniae	weak	Amoxiclac+Piptaz	Klebsiella Pneumoniae	weak	Amoxiclac+Piptaz
53	Enteroc	no	Amoxicla	Escherichia coli	no	no	Escherichia coli	no	Amoxiclac+Pipt	no	no	no

	occus		v , Metronidazole						az	growth		
54	no growth	no	no	Enterococcus sp.	no	no	Enterococcus sp.	no	Colistin + Amoxiclav	no growth	no	no
55	no growth	no	no	Klebsiella Pneumoniae	strong	no	Klebsiella Pneumoniae	no	Colistin + Amoxiclav	Klebsiella Pneumoniae	moderate	Colistin + Amoxiclav
56	Acinetobacter baumannii	no	Ceftriaxone, Amoxiclav, Amikacin	Acinetobacter baumannii	no	Ceftriaxone, Amoxiclav, Amikacin	Klebsiella pneumoniae	no	Amikacin + Amoxiclav	no growth	no	no
57	Pseudomonas aeruginosa	no	Ceftriaxone, Amoxiclav, Amikacin	Pseudomonas aeruginosa	no	Ceftriaxone, Amoxiclav, Amikacin	Pseudomonas aeruginosa	no	Amikacin + Amoxiclav	no growth	no	no
58	no growth	no	no	Pseudomonas aeruginosa	Weak	Ceftriaxone, Amoxiclav, Amikacin	Klebsiella pneumoniae	strong	Piptaz +Amikacin	Acinetobacter baumanii	weak	Piptaz +Amikacin
59	no growth	no	no	no growth	no	no	no growth	no	no	no growth	no	no
60	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	weak	Amikacin + Amoxiclav	Klebsiella Pneumoniae	strong	Amikacin + Amoxiclav
61	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	no	Amikacin + Amoxiclav	no growth	no	no
62	no growth	no	no	no growth	no	no	Pseudomonas aeruginosa	no	Colistin + Amoxiclav	no growth	no	no
63	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae , Pseudomonas aeruginosa	strong	Piptaz +Amikacin	Klebsiella Pneumoniae	weak	Piptaz +Amikacin
64	no growth	no	no	no growth	no	no	Proteus vulgaris	no	Piptaz +Amikacin	no growth	no	no

65	no growth	no	no	no growth	no	no	Enterobacter sp.	no	Piptaz +Amikacin	no growth	no	no
66	no growth	no	no	no growth	no	no	Pseudomonas aeruginosa	no	Piptaz +Amikacin	no growth	no	no
67	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	strong	Amoxiclac+Piptaz	Klebsiella Pneumoniae	moderate	Amoxiclac+Piptaz
68	no growth	no	no	no growth	no	no	Escherichia coli	no	Amoxiclac+Piptaz	Enterococcus sp.	no	no
69	no growth	no	no	no growth	no	no	Enterococcus sp.	no	Colistin + Amoxiclav	Enterococcus sp.	no	no
70	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	no	Amoxiclac+Piptaz	Klebsiella Pneumoniae	weak	Amoxiclac+Piptaz
71	no growth	no	no	no growth	no	no	Klebsiella pneumoniae	no	Amoxiclac+Piptaz	Klebsiella Pneumoniae	strong	Amoxiclac+Piptaz
72	no growth	no	no	Klebsiella Pneumoniae	Weak	Amoxiclav , Metronidazole	Acinteobacter baumanii, Klebsiella Pneumoniae	no	Colistin + Amoxiclav	Klebsiella Pneumoniae	moderate	Colistin + Amoxiclav
73	Acinetobacter baumannii	no	Amoxiclav , Metronidazole	klebsiella Pneumoiae	no	Amoxiclav , Metronidazole	Acinetobacter baumannii,klebsiella Pneumoiae	no	Colistin + Amoxiclav	Klebsiella Pneumoniae	weak	Colistin + Amoxiclav
74	Pseudomonas aeruginosa	no	Amoxiclav , Metronidazole	Pseudomonas aeruginosa	no	Ceftriaxone, Amoxiclav, Amikacin	Pseudomonas aeruginosa, Acinetobacter baumanii	weak	Amikacin + Amoxiclav	Klebsiella Pneumoniae	moderate	Amikacin + Amoxiclav
75	Acinetobacter baumannii	no	Amoxiclav , Metronidazole	Acinetobacter baumannii	strong	Amikacin, Pitaz, Ceftriaxone	Klebsiella pneumoniae	no	Amikacin + Amoxiclav	Pseudomonas aeruginosa	weak	Amikacin + Amoxiclav
76	Pseudomonas aeruginosa	no	Amoxiclav , Metronidazole	Pseudomonas aeruginosa	no	Amoxiclav , Metronidazole	Pseudomonas aeruginosa, Acinetobacter baumanii	no	Piptaz +Amikacin	Klebsiella Pneumoniae	strong	Piptaz +Amikacin
77	no	no	no	Enterobacter sp.	no	Amikacin,	Pseudomonas	no	Amikacin +	Pseudom	strong	Amikaci

	growth					Pitaz, Ceftriaxone	aeruginosa		Amoxiclav	onas aeruginosa		n + Amoxiclav
78	no growth	no	no	Pseudomonas aeruginosa , Acinetobacter baumanii	moderate	Ceftriaxone, Amoxiclav, Amikacin	Pseudomonas aeruginosa , Acinetobacter baumanii	moderate	Amikacin + Amoxiclav	Klebsiella Pneumoniae	strong	Amikacin + Amoxiclav
79	no growth	no	no	Acinteobacter baumanii	no	Amikacin, Pitaz, Ceftriaxone	Klebsiella pneumoniae	no	Amikacin + Amoxiclav	Acinetobacter baumanii	weak	Amikacin + Amoxiclav
80	no growth	no	no	Escherichia coli	no	Amikacin, Pitaz, Ceftriaxone	Klebseilla pneumiae	no	Colistin + Amoxiclav	Klebsiella Pneumoniae	moderate	Colistin + Amoxiclav
81	no growth	no	no	Enterococcus sp.	no	Amikacin, Pitaz, Ceftriaxone	Acinetobacter baumanii, Klebsiella Pneumonae	weak	Colistin + Amoxiclav	Klebsiella Pneumoniae	moderate	Colistin + Amoxiclav
82	no growth	no	no	Klebsiella Pneumoniae , Pseudomonas aeruginosa	Weak	Ceftriaxone, Amoxiclav, Amikacin	Klebsiella Pneumoniae , Pseudomonas aeruginosa	weak	Piptaz +Amikacin	Klebsiella Pneumoniae	strong	Piptaz +Amikacin
83	no growth	no	no	Proteus vulgaris	no	Amikacin, Pitaz, Ceftriaxone	Proteus vulgaris	no	Piptaz +Amikacin	no growth	no	no
84	no growth	no	no	Enterobacter sp.	no	Amikacin, Pitaz, Ceftriaxone	Enterobacter sp.	no	Piptaz +Amikacin	no growth	no	no
85	no growth	no	no	Pseudomonas aeruginosa	moderate	Amikacin, Pitaz, Ceftriaxone	Pseudomonas aeruginosa	no	Piptaz +Amikacin	no growth	no	no
86	no growth	no	no	no growth	no	no	Klebsiella Pneumoniae	weak	Amoxiclac+Piptaz	Klebsiella Pneumoniae	weak	Amoxiclac+Piptaz
87	no growth	no	no	no growth	no	no	Acinetobacter baumannii,klebsiella	strong	Amoxiclac+Piptaz	Klebsiella Pneumoniae	moderate	Amoxiclac+Piptaz

							Pneumoiae					
88	no growth	no	no	no growth	no	no	Pseudomonas aeruginosa, Acinetobacter baumanii	strong	Colistin + Amoxiclav	Klebsiella Pneumoniae	strong	Colistin + Amoxiclav
89	no growth	no	no	no growth	no	no	Klebsiella pneumoniae	moderate	Colistin + Amoxiclav	Klebsiella Pneumoniae	strong	Colistin + Amoxiclav
90	no growth	no	no	no growth	no	no	Pseudomonas aeruginosa, Acinetobacter baumanii	weak	Amikacin + Amoxiclav	Pseudomonas aeruginosa	weak	Amikacin + Amoxiclav
91	no growth	no	no	no growth	no	no	Pseudomonas aeruginosa	weak	Piptaz +Amikacin	Klebsiella Pneumoniae	moderate	Piptaz +Amikacin
92	no growth	no	no	no growth	no	no	Pseudomonas aeruginosa	moderate	Amikacin + Amoxiclav	Pseudomonas aeruginosa	strong	Amikacin + Amoxiclav
93	no growth	no	no	no growth	no	no	Klebsiella pneumoniae	no	Amikacin + Amoxiclav	Acinteobacter baumanii	strong	Amikacin + Amoxiclav
94	no growth	no	no	no growth	no	no	Klebseilla pneumiae	no	Colistin + Amoxiclav	Klebseilla pneumiae	moderate	Colistin + Amoxiclav
95	no growth	no	no	no growth	no	no	Enterococcus sp.	no	Colistin + Amoxiclav	no growth	no	no