STUDIES ON BACTERIAL DIVERSITY ON FABRICS

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

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Submitted

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Dedicated
To
The Almighty
&
My family

CERTIFICATE

This is to certify that the thesis titled **"Studies on bacterial diversity on fabrics"** being submitted by **"Ms. Swati Varshney"** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of bona fide research work carried out by her under our supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology Delhi, New Delhi, India.

The results presented in this thesis have not been submitted in part or full to any other University for the award of any other degree or diploma.

Date.

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Swati Varshney

ABSTRACT

Mitigation of infectious diseases in developing countries like India is an important healthcare objective. In hospital environments, microorganisms interact with various hospital textiles such as lab coats, bedsheets, and curtains. The spread of infections through fabrics used in the hospital sector is well known. However, there is a lack of systematic studies in this area and the mechanisms involved in spread of nosocomial infections due to textiles are not well understood. Effect of textile properties on microbial adhesion and interactions between fabrics and bacteria have not been studied extensively. Such information is essential to formulate guidelines for the use of textile materials in the healthcare and hospitality sectors, which is currently missing, especially in the Indian context.

The present work focused on assessing factors affecting bacterial adhesion on different types of fibres. Four clinically relevant bacterial genera, viz. *Staphylococcus aureus, Acinetobacter calcoaceticus, Escherichia coli,* and *Pseudomonas aeruginosa* were studied for their adherence to different fabric types, viz. polyester, wool, polypropylene, viscose, silk, and cotton in laboratory conditions. Surface properties of bacteria and fibres were measured. Adhesion between fibres and bacterial strains was assessed by incubating sterile fibres of each type with the pure culture of bacterial species. Bacterial adhesion was correlated with the nano-roughness of the fibres. Scanning electron microscopy was employed for visualization of bacterial adhesion on fibres. Subsequently, the assessment of biofilm formation was performed on seven fabrics (polyester, cotton, polyester-cotton (70:30) blend, silk, wool, viscose, and nylon fabrics) commonly used in hospitals. Fabric characterization was done. Roughness of each fabric was determined at micron and nano-scale level by Kawabata Evaluation System-(KES-FB4) and Atomic Force Microscopy (AFM), respectively**.** Qualitative and quantitative assessment of biofilm formation on different fabrics was performed. Exopolysaccharides (EPS) produced by bacteria were extracted and different functional groups were determined using

Fourier Transform Infrared Spectroscopy (FTIR). The effect of sweat on bacterial abundance on different fabrics (polyester, cotton, polyester-cotton [70:30] blend, silk, wool, viscose, and nylon) was examined. In the first set, the suspension of each bacterial species was inoculated on each type of fabric in the presence of sweat and incubated. In the second set, each bacterial species' suspension was inoculated on fabrics without the addition of sweat suspension. Bacterial count was determined for each set of experiment. The effect of sweat, type of bacteria, and incubation time was examined.

Next, the work was extended to study the bacterial load on hospital fabrics under real-life conditions. Two studies were conducted at a primary health care facility in Delhi, one on nurses' coats and the other on hospital bed sheets in the emergency ward of the facility. Patch test method was used for bacterial sampling in the two studies. In the first case, the effect of fabric type (polyester, cotton and polyester-cotton blend) and temporal variation on bacterial load on nurses' white coats was studied. In the second study, the bacterial load was assessed on patients' bedsheets by employing both culture-dependent and culture-independent approaches. In the culture-dependent approach, seven bacterial species of significance in such settings (*Acinetobacter baumannii*, *S. aureus*, *E. coli*, *Salmonella* spp., *Klebsiella pneumoniae*, *Enterococcus faecalis*, and Group A *Streptococcus*) were enumerated on blend fabrics stitched on patients' bedsheets. Studies were conducted in different seasons (May-November 2019) using group-specific culture media. In the culture-independent approach, DNA was extracted from the patches and specific bacterial phyla (*α-Proteobacteria*, *ß-Proteobacteria*, *Firmicutes* [*Bacillota*], and *Actinobacteria* [*Actinomycetota*]) were quantified by qPCR. Further, amplicon sequencing of the 16S rRNA gene was performed for profiling of the total bacterial community.

Results demonstrated a direct correlation of textile properties on bacterial adhesion and the extent of bacterial adhesion on fibres. Among the four bacteria, the count of *P. aeruginosa* on

fibres was maximum, while *E. coli* count was minimum. Viscose fibre attracted a higher number of bacteria, while silk attracted the least bacterial load. Biofilm formation by bacterial species was found to be highest on wool fabric, and minimum on silk fabric. The presence of different functional groups in EPS of bacteria might affect the adhesion of bacteria on textile surfaces by interaction with functional groups present on fabrics. The nano-roughness of the fabrics was also found to affect bacterial adhesion, with a rougher surface proving more favourable for biofilm formation. The count of *E. coli*, *A. calcoaceticus*, and *P. aeruginosa* was observed to be maximum with sweat on polyester fabrics, while least on viscose fabrics. In the presence of sweat, the abundance of *S. aureus* was maximum on wool, and minimum on viscose. The studies conducted at the healthcare facility indicated that the abundance of bacterial adhesion depends on the type of fabric used, the duration of use, as well as the ambient conditions. In particular, high ambient humidity attracted higher bacterial load on polyester-cotton blended fabrics compared to cotton and polyester fabrics. Several bacterial species (*A. baumannii*, *S. aureus*, *E. coli*, *Salmonella* spp., *K. pneumoniae*, *Enterococcus faecalis*, Group A *Streptococcus*) isolated from fabric patches stitched on patients' bedsheets were found to be resistant to several broad-spectrum drugs. Culture-independent analysis by qPCR showed that the total bacterial abundance was significantly higher (1.0×10^8 copy number cm^{-2}) in the fabrics collected in June. Abundance of various bacterial phyla in the month of June *α-Proteobacteria*, (7.8×106 copy number cm^{-2}), *ß-Proteobacteria* (1.2×102 copy number cm^{-2}), *Firmicutes* (*Bacillota*) (3.0×108 copy number cm^{-2}) and *Actinobacteria* (*Actinomycetota*) (3.0×108 copy number cm^{-2}) were recorded. Bacterial community analysis by amplicon sequencing revealed a dominance of *Firmicutes* (*Bacillota*) (65%) in all samples, followed by *Proteobacteria* (30%) and *Actinobacteria* (*Actinomycetota*) (3%). These results highlight the need for a stricter protocol to minimize contamination in healthcare facilities across the country. Newer strategies like physical and chemical modification of fabrics can be done to

engineer the fabrics to minimise microbial adhesion on fabrics and prevent transfer of infection through fabrics. Specific guidelines could also be formulated based on this data for selecting suitable fabrics which harbour minimal bacterial load.

सार

भारत जैसे विकासशील देशों में संक्रामक रोगों का निवारण एक महत्वपूर्ण उद्देश्य है। अस्पताल के वातावरण में, सूक्ष्मजीव विभिन्न अस्पताल सम्बन्धी वस्त्रों, जैसे लैब कोट, चादरों, और पर्दों के संपर्क में आकर उन्हें संक्रमित करते हैं। अस्पताल के वातावरण में उपयोग किए जाने वाले कपड़ों के माध्यम से फैलने वाले अस्पताल सम्बंधित संक्रमण सर्वविदित है। हालांकि, इस क्षेत्र में व्यवस्थित अध्ययन की कमी है, और वस्त्रों के कारण अस्पताल जैसी जगहों पर होने वाली अस्पताल सम्बंधित संक्रमण (नोसोकोमियल संक्रमण) के प्रसार की क्रियाविधि को अच्छी तरह से समझा नहीं गया है। कपड़ों के साथ सूक्ष्म जीवाणुओं का चिपकना और उसके उपरांत इनके बीच के सम्बन्धों और संक्रमण के प्रभाव का व्यापक अध्ययन बड़े पैमाने पर नहीं किया गया है। स्वास्थ्य सेवा और आतिथ्य क्षेत्रों में कपड़ा सामग्री के उपयोग के लिए दिशानिर्देश तैयार करने के लिए ऐसी जानकारी आवश्यक है, जिसकी वर्तमान समय में कमी है, खासकर भारत के संदर्भ में।

वर्तमान कार्य, विभिन्न प्रकार के कपड़ो के तंतुओं पर जीवाणुओं के आसंजन को प्रभावित करने वाले कारकों का आकलन करने पर केंद्रित है। चिकित्सकीय रूप से प्रासंगिक (clinically-relevant) ऐसे चार जीवाणु प्रजाति, जैसे की: स्टैफाइलोकोकस ऑरियस, एसिनेटोबैक्टर कैल्कोएसेटिकस, एस्चेरिचिया कोलाई और स्यूडोमोनास एरुगिनोसा (*Staphylococcus aureus*, *Acinetobacter calcoaceticus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) के कपड़ों के साथ आसंजन का अध्ययन प्रयोगशाला स्थितियों में विभिन्न प्रकार के कपड़े (जैसे की: पॉलिएस्टर, ऊन, पॉलीप्रोपाइलीन, विस्कोस, रेशम और कपास) पर किया गया था। तंतुओं और जीवाणुओं के सतही गुणों को मापा गया। तंतुओं और जीवाणुओं की प्रजातियों के बीच आसंजन, जीवाणुओं प्रजातियों की शुद्ध कल्चर (pure culture) के साथ प्रत्येक प्रकार के स्टेराइल तंतुओं को इनक्यूबेट (incubate) करके किया गया था। जीवाणुओं का

आसंजन, कपड़ो के तंतुओं के नैनो स्तर तक होने वाले खुरदुरेपन के साथ सहसंबन्धित किया गया था। कपड़ो के तंतुओं पर जीवाणुओं का आसंजन के दृश्य का आकलन करने के लिए स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (Scanning Electron Microscopy) किया गया था। इसके बाद, अस्पतालों में आमतौर पर इस्तेमाल होने वाले सात कपड़ों (पॉलिएस्टर, कपास, पॉलिएस्टर-कपास (७०:३०) मिश्रण, रेशम, ऊन, विस्कोस और नायलॉन कपड़े) (polyester, cotton, polyester-cotton (70:30) blend, silk, wool, viscose, and nylon fabrics) पर बायोफिल्म निर्माण का आकलन किया गया। कपड़ो का कैरेक्टराइजेशन वर्णन किया गया था। प्रत्येक कपड़े की खुरदरापन कावाबाता (Kawabata) मूल्यांकन प्रणाली- (केईएस-एफबी 4) और एएफएम द्वारा माइक्रोन और नैनो-स्केल स्तर पर निर्धारित किया गया था। विभिन्न कपड़ो पर बायोफिल्म निर्माण का गुणात्मक और मात्रात्मक मूल्यांकन किया गया था। जीवाणुओं द्वारा उत्पादित एक्सोपॉलीसेकेराइड (ईपीएस) (exopolysaccharides, EPS) को निकाला गया और विभिन्न कार्यात्मक समूहों को फूरियर ट्रांसफॉर्म इन्फ्रारेड स्पेक्ट्रोस्कोपी (एफटीआईआर) के साथ निर्धारित किया गया। विभिन्न कपड़ों (पॉलिएस्टर, कपास, पॉलिएस्टर-कपास [70:30] मिश्रण, रेशम, ऊन, विस्कोस और नायलॉन) पर जीवाणुओं के वृद्धि पर पसीने के प्रभाव की जांच की गई। प्रत्येक प्रकार के कपड़े के एक तरफ पसीना के घोल को लगाया गया था। पहले सेट में, प्रत्येक जीवाणु प्रजाति के घोल को प्रत्येक प्रकार के कपड़े पर लगाया गया था। दूसरे सेट में, प्रत्येक जीवाणु प्रजाति के घोल को बिना पसीना के घोल कपड़ों पर लगाया गया था। प्रयोग के प्रत्येक सेट पर जीवाणुओं की संख्या निर्धारित की गई थी। पसीने के प्रभाव, जीवाणुओं के प्रकार और ऊष्मायन समय की जांच की गई।

इसके बाद, वास्तविक जीवन की परिस्थितियों में अस्पताल के कपड़ों पर जीवाणु के भार का अध्ययन करने के लिए काम बढ़ाया गया। दिल्ली के एक प्राथमिक स्वास्थ्य देखभाल सुविधाकेंद्र पर दो प्रकार के अध्ययन किए गए, पहला, नर्सों के कोट पर, तथा दूसरा, आपातकालीन वार्ड में अस्पताल की चादरों पर किया गया। उपरोक्त दोनों अध्ययनों में, पैच परीक्षण विधि (patch test method) का उपयोग, जीवाणुओं के नमूनों के लिए किया गया। पहले मामले में, नर्सों के सफेद कोट पर जीवाणुओं के भार पर कपड़े के प्रकार (पॉलिएस्टर, कपास और पॉलिएस्टर-कपास मिश्रण) और वातावरण भिन्नता के प्रभाव का अध्ययन किया गया| दूसरे अध्ययन में, कल्चर-डिपेंडेंट (Culture-dependent) तथा कल्चर-इंडिपेंडेंट (Culture-Independent) दोनों विधियों को नियोजित करके मरीजों के चादरों पर जीवाणु के भार का आकलन किया गया था। कल्चर-डिपेंडेंट विधि में, इस तरह की नियोजन में महत्वपूर्ण पांच जीवाणु प्रजातियां (एसिनेटोबैक्टर बौमानी, स्टैफाइलोकोकस ऑरियस, एस्चेरिचिया कोलाई, साल्मोनेला प्रजाति, क्लेबसिएला निमोनिया, एंटरोकोकस फिकेलिस, ग्रुप ए स्ट्रेप्टोकोकस) (*Acinetobacter baumannii*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumoniae*, *Enterococcus faecalis*, Group A *Streptococcus*) को मरीजों के चादरों पर सिले हुए मिश्रित कपड़ों (blend fabrics) पर लगाया गया था। समूह-विशिष्ट कल्चर मीडिया का उपयोग करके विभिन्न मौसमों (मई-नवंबर 2019) में अध्ययन किए गए। कल्चर-इंडिपेंडेंट विधि में, डीएनए (DNA) को पैच परिक्षण विधि (patch test method) और विशिष्ट जीवाणु समूहों (*α-प्रोटियोबैक्टीरिया, β-प्रोटियोबैक्टीरिया, फर्मिक्यूट्स* [*बेसिलोटा*], और *एक्टिनोबैक्टीरिया* [*ऐक्टीनोमाइसीटोटा*]) (*α-Proteobacteria, ß-Proteobacteria, Firmicutes*

[*Bacillota*], and *Actinobacteria* [*Actinomycetota*]) से निकाला गया था, जो qPCR द्वारा निर्धारित किया गया था| कुल जीवाणु समुदाय की रूपरेखा के लिए 16S rRNA जीन का amplicon sequencing किया गया।

परिणामों में जीवाणु के आसंजन और कपड़ों के तन्तुओं पर जीवाणु के आसंजन की सीमा पर कपड़ों के गुणों का प्रत्यक्ष सहसंबंध स्थापित होता है। चार जीवाणुओं में, तंतुओं पर पी. एरुगिनोसा (*P. aeruginosa*) की संख्या अधिकतम थी, जबकि ई. कोलाई (*E. coli*) की संख्या न्यूनतम थी। विस्कोस तंतुओं ने अधिक संख्या में जीवाणुओं को आकर्षित किया, जबकि रेशम ने कम से कम जीवाणु भार को आकर्षित किया। जीवाणु प्रजातियों द्वारा बायोफिल्म का निर्माण ऊनी कपड़े पर उच्चतम और रेशमी कपड़े पर न्यूनतम पाया गया। जीवाणु के ईपीएस में विभिन्न कार्यात्मक समूहों की उपस्थिति कपड़ों पर मौजूद कार्यात्मक समूहों के साथ सम्पर्क द्वारा कपड़ों की सतहों पर जीवाणु के आसंजन को प्रभावित कर सकती है। कपड़ों की नैनो-क्षमता पर खुरदुरापन भी जीवाणु के आसंजन को प्रभावित करने के लिए पाई गई थी, जिसमें एक खुरदरी सतह बायोफिल्म (biofilm) निर्माण के लिए अधिक अनुकूल साबित हुई। पसीने के साथ कपड़ों पर जीवाणुओं की वृद्धि कपड़े के प्रकार और ऊष्मायन समय के आधार पर भिन्न होती है। ई. कोलाई (*E. coli*), ए. कैल्कोएसेटिकस (*A. calcoaceticus*), और पी. एरुगिनोसा (*P. aeruginosa*) की वृद्धि पॉलिएस्टर कपड़ों पर पसीने के साथ अधिकतम देखी गई, जबकि कम से कम विस्कोस कपड़ों पर । पसीने की उपस्थिति में, ऊनी कपड़ों पर एस. ऑरियस (*S. aureus*) की प्रचुरता अधिकतम थी, और विस्कोस फैब्रिक पर न्यूनतम। स्वास्थ्य

सेवा सुविधा में किए गए अध्ययनों से संकेत मिलता है कि जीवाणुओं का आसंजन की प्रचुरता उपयोग किए गए कपड़े के प्रकार, उपयोग की अवधि, साथ ही अनुकूलन स्थितियों पर निर्भर करती है। विशेष रूप से, पॉलिएस्टर-कपास मिश्रित कपड़े और उच्च परिवेश आर्द्रता कपास (और पॉलिएस्टर कपड़े की तुलना में एक उच्च जीवाणु भार को आकर्षित करती है। कई जीवाणु प्रजातियां एसिनेटोबैक्टर बौमानी, स्टैफाइलोकोकस ऑरियस, एस्चेरिचिया कोलाई, साल्मोनेला प्रजाति, क्लेबसिएला निमोनिया, एंटरोकोकस फिकेलिस, ग्रुप ए स्ट्रेप्टोकोकस) (*Acinetobacter baumannii*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumoniae*, *Enterococcus faecalis*, Group A *Streptococcus*) रोगिओं की चादरों पर सिले कपड़े से पृथक कई व्यापक-स्पेक्ट्रम दवाओं के लिए प्रतिरोधी पाई गईं। क्यूपीसीआर (qPCR) द्वारा कल्चर-इंडिपेंडेंट (Culture-Independent) विश्लेषण से पता चला है कि जून में एकत्र किए गए कपड़ों में कुल जीवाणु बहुतायत काफी अधिक (1.0 × 10^8 copy number cm^{-2}) थी। जून के महीने में विभिन्न जीवाणु फ़ाइला की प्रचुरता *α-प्रोटियोबैक्टीरिया,* (7.8×10^6 copy number cm^{-2}), *ß-प्रोटियोबैक्टीरिया* (1.2×10^2 copy number cm^{-2}), *फर्मिक्यूट्स*, (*बेसिलोटा*) (3.0×10^8 copy number cm^{-2}) और *एक्टिनोबैक्टीरिया* (*ऐक्टीनोमाइसीटोटा*) (3.0×10^8 copy number cm^{-2}) दर्ज की गई थी। एम्प्लिकॉन अनुक्रमण द्वारा जीवाणु समुदाय विश्लेषण से सभी नमूनों में फर्मिक्यूट्स (बैसिलोटा) (65%) के प्रभुत्व का पता चला, इसके बाद प्रोटोबैक्टीरिया (30%) और एक्टिनोबैक्टीरिया (एक्टिनोमाइसिटोटा) (3%) का स्थान रहा। ये परिणाम देश भर में स्वास्थ्य सुविधाओं में संदूषण को कम करने के लिए एक सख्त प्रोटोकॉल की आवश्यकता पर प्रकाश डालते हैं। इन परिणामों के आधार पर नई रणनीतियों (कपड़ों का भौतिक और

रासायनिक संशोधन, कपड़े का कार्यात्मककरण, बहुक्रियाशील रणनीति आदि) को स्वास्थ्य देखभाल नियोजन में अस्पताल सम्बंधित संक्रमण (नोसोकोमियल संक्रमण) के नियंत्रण के लिए कपड़ों की इंजीनियरिंग पर लागू किया जा सकता है। उपयुक्त कपड़ों के चयन के लिए इस डेटा के आधार पर विशिष्ट दिशानिर्देश भी तैयार किए जा सकते हैं जो न्यूनतम जीवाणु भार सुनिश्चित करते हैं।

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ABBREVIATIONS

ATP	Adenosine Triphosphate
AMP	Ampicillin
AST	Antimicrobial Susceptibility Testing
AMR	Antimicrobial Resistance
AFM	Atomic Force Microscopy
ANOVA	Analysis of Variance
bp	Base pair
BPA	Baird Parker Agar
BHI	Brain-Heart Infusion
BLAST	Basic Local Alignment Search Tool
CoNS	Coagulase Negative *Staphylococcus*
cm	Centimeter
$CaCl_2$	Calcium Chloride
CLSI	Clinical and Laboratory Standards Institute
CIP	Ciprofloxacin
C	Chloramphenicol
CX	Cefoxitin
CD	Clindamycin
CAZ	Ceftazidime
CTX	Cefotaxime
CL	Colistin
CDDEP	Centre for Disease Dynamics, Economics, and Policy
CFU	Colony Forming Unit

Ct	Cycle threshold
DVLO	Derjaguin, Verwey, Landau, and Overbeek
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
EPS	Exopolysaccharide
ERY	Erythromycin
EDTA	Ethylene diamine tetra acetic acid
FTIR	Fourier Transform Infrared Spectroscopy
GARP	Global Antibiotic Resistance Partnership
g	Gram
gpl	Gram per litre
h	Hour
IMP	Imipenam
HAI	Healthcare Associated Infections
HCWU	Healthcare Worker's Uniform
ICU	Intensive Care Units
KBr	Potassium Bromide
LZ	Linezolid
LB	Luria Bertani
L	Litre
ºC	Degree centigrade
M MALDI-TOF	Matrix-Assisted Laser Desorption Ionization-Time-Of-Flight
MSSA	Methicillin-Sensitive *S. aureus*
mm	Milimeter

$MgCl_2$	Magnesium Chloride
MRP	Meropenam
MATH	Microbial Adhesion to Hydrocarbons
mV	Millivolt
µm	Micrometer
MRSA	Methicillin-Resistant *Staphylococcus aureus*
min	Minutes
ml	Milliliter
MCT	Microcentrifuge Tube
nm	Nanometer
NaCl	Sodium Chloride
NET	Netlimicin
NIT	Nitrofurantoin
NA	Nutrient Agar
NGS	Next Generation Sequencing
NMDS	Non-Metric Multidimensional Scaling
NTC	No Template Control
OD	Optical Density
OTU	Operational Taxonomic Unit
PEN	Penicillin
PMMA	Polymethyl Methacrylate
PVC	Poly Vinyl Chloride
psi	Pound per square inch
PIT	Piperacillin + Tazobactum

PPE	Personal Protective Equipment
PBS	Phosphate Buffered Saline
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
ppm	Parts per million
qPCR	Real-time quantitative PCR
16S rRNA	16S ribosomal Ribonucleic Acid
RH	Relative Humidity
R^2/r	Coefficient of correlation
RNA	Ribonucleic Acid
rpm	Rotations per minute
SS	Stainless Steel
SMD	Surface Mean Deviation
SEM	Scanning Electron Microscopy
s	Second
TSA	Tryptone Soya Agar
TSB	Tryptone Soya Broth
TET	Tetracycline
3D	Three Dimensional
UPGMA	Unweighted Pair Group Method with Arithmetic
UV	Ultraviolet
VAN	Vancomycin
VRE	Vancomycin-Resistant *Enterococci*

WHO	World Health Organization
α	Alpha
β	Beta
γ	Gamma
δ	Delta
μ	Micro