## CONTENTS

| A | Abbreviations | i |
| B | List of colour plate | ii |
| C | List of Tables | iii |
| D | List of Figures | v |

### 1 INTRODUCTION

1.1 Biomedical product associated infections

1.2 Surface colonization of microbes on biomedical products

1.3 Clinical management and treatment of device-related infections

1.4 Antimicrobial resistance

1.5 Accepted clinical therapy

1.6 Methods to prevent biomedical product associated infections

1.7 Antimicrobial biomaterials

1.8 Problems associated with antimicrobial biomaterials

1.9 Reason for the present study

1.10 OBJECTIVES

### 2 REVIEW OF LITERATURE

2.1 Biomedical Products

2.2 Different types of implants

2.3 Materials used for manufacturing implants

2.4 Implant-associated infections

2.5 Causative organisms

2.6 Biofilm formation

2.7 Preventing Infection in Surgical Implants

2.8 Risks of Antimicrobial Prophylaxis

2.9 Synergistic drugs

2.10 Studies on antibiotic synergism

2.11 Antimicrobial coatings on implants

2.12 Carriers as drug delivery systems

2.13 Mechanisms of controlled release of drugs

2.14 Fourier Transform Infra-Red (FTIR) analysis of drug loaded materials

2.15 Biocompatibility

2.16 Non-implantable materials – Health care textiles

2.17 Textiles and microbes

2.18 Antimicrobial textiles

2.19 Requirements for Antimicrobial Finishing

2.20 Types of antimicrobial agents used on textiles

2.21 Application of Antimicrobial Agents
3 MATERIALS AND METHODS

Overall methodology of the Research work - flowchart

3.1 Determining the surface colonizing capability of test bacteria on biomedical materials

3.1.1 Preliminary Exit-site challenge test

3.2 Assessing the biofilm forming capability of test bacteria on biomedical materials using two standard confirmatory test methods

3.2.1 Biofilm forming ability of test bacteria by Congo red agar biofilm assay

3.2.2 Confirmatory test on biofilm formation using Microtitre plate biofilm assay

3.3 Determining the synergistic activity of fluoroquinolone and nitroimidazole drugs against test bacteria

3.3.1 Assessing the antimicrobial combinations against test bacteria using standard checker board titration method

3.3.2 Evaluating the synergism between fluoroquinolone and nitroimidazole drugs by fractional inhibitory concentration index (FICI)

3.4 Determining the mode of action of fluoroquinolone and nitroimidazole drugs on bacterial DNA

Implantable Materials

3.5 Preparation of antibacterial drugs for coating the implantable materials

3.6 Coating the implantable materials with prepared antibacterial drugs using the standard two-dip-coating method

3.7 Determining the drug-add on percentage on the implantable materials

3.8 Assessing the qualitative antibacterial activity of dip-coated implantable materials

3.9 Analyzing the drug release profiles from coated implantable materials using the standard in vitro dissolution method

3.9.1 Development of standard calibration curve of antibacterial drugs

3.9.2 Determining the drug release concentrations from (drug coated and drug-carrier coated materials using in vitro dissolution method

3.10 Quantitative antibacterial activity of coated materials using the standard bacterial Adherence test

3.10.1 Statistical analysis of total viable bacteria on coated materials

3.11 Determining the persistence of drugs on coated materials using the standard in vitro challenge test
Non-Implantable Materials

3.12 Different antibacterial finishing methods on non-implantable textile materials

3.12.1 Direct attachment of synergistic drugs onto textile materials using the standard reactive dye method

3.12.2 Determining the durability of drugs using the standard microencapsulation method

3.13 Evaluating the antibacterial activity of treated fabrics using the standard AATCC Test Method 100 – 2004

3.14 Determining the wash durable properties of treated textile materials using the standard wash fastness test (AATCC Test Method 124-2010)

3.14.1 Statistical analysis of wash fastness

3.15 Analysing the physical properties of textile materials after antibacterial finishing

3.15.1 Tensile Strength test (ASTM D 5035-2006 test method)

3.15.2 Abrasion test (AATCC 119-2004 test method)

3.15.3 Resistance to Pilling

3.15.4 Dimensional stability

3.15.4 Air-permeability test (ASTM D 737-96 test method)

3.16 Surface characterization of coated biomedical materials

3.16.1 Determining the presence of drugs and its chemical interventions on coated biomaterials using FTIR analysis

3.16.2 Examining the homogenous coatings on biomaterials using topographic analysis – SEM

3.17 Determining the tissue reactions of coated biomedical materials using the standard HET-CAM test

3.17.1 Direct evaluation of CAM

3.17.2 Histological evaluation of CAM

4 RESULTS AND DISCUSSION

4.1 Determining the surface colonizing capability of test bacteria on biomedical materials - Preliminary Exit-site challenge test

4.2 Assessing the biofilm forming capability of test bacteria on biomedical materials using standard confirmatory test methods

4.2.1 Biofilm forming ability of test bacteria by Congo red agar biofilm assay

4.2.2 Confirmatory test on biofilm formation using Microtitre plate biofilm assay

4.3 Determining the synergistic activity of fluoroquinolone and nitroimidazole drugs against test bacteria

4.4 Determining the mode of action of fluoroquinolone and nitroimidazole drugs on bacterial DNA
Implantable Materials

4.5 Coating the implantable materials with prepared antibacterial drugs using the standard two-dip-coating method

4.6 Determining the drug-add on percentage on the implantable materials.

4.7 Assessing the qualitative antibacterial activity of dip-coated implantable materials

4.8 Analysing the drug release profiles from coated implantable materials using the standard in vitro dissolution method

4.8.1 Quantification analysis of fluoroquinolone and nitroimidazole drug

4.8.2 Determining the drug release concentrations from drug coated (dc) materials using standard in vitro dissolution method

4.8.3 Determining the drug release concentrations from drug-carrier coated (dcc) materials using standard in vitro dissolution method

4.9 Quantitative antibacterial activity of coated materials using the standard bacterial Adherence test

4.9.1 Statistical analysis of total viable bacteria on coated materials

4.10 Determining the persistence of drugs on coated materials using the standard in vitro challenge test

Non-Implantable Materials

4.11 Direct attachment of synergistic drugs onto textile materials using the standard reactive dye method

4.11.1 Evaluating the antibacterial activity of reactive treated fabrics using the standard AATCC 100 – 2004 Test Method

4.12 Determining the durability of drugs using the standard microencapsulation method

4.12.1 Characterization of microcapsules

4.12.2 Evaluating the antibacterial activity of encapsulated drug treated fabrics

4.12.3 Statistical analysis of wash fastness

4.13 Analysing the physical properties of textile materials after antibacterial finishing

4.13.1 Reactive drug treated textile materials

4.13.2 Microencapsulated drug treated textile materials

4.14 Surface characterization of coated biomedical products

4.14.1 Determining the presence of drugs and its chemical interventions on coated biomaterials using FTIR analysis

4.14.2 Examining the homogenous coatings on biomaterials using topographic analysis – SEM

4.15 Determining the tissue reactions of coated biomedical materials using the standard HET-CAM test

4.15.1 Direct evaluation of CAM

4.15.2 Histological evaluation of CAM
### A. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>AATCC</td>
<td>American Association of Textile Chemist and Colorist</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>BCOP</td>
<td>Bovine Corneal Opacity and Permeability</td>
</tr>
<tr>
<td>BMPAIs</td>
<td>Bio Medical Product Associated Infections</td>
</tr>
<tr>
<td>C/P</td>
<td>Cotton blended polyester</td>
</tr>
<tr>
<td>CC</td>
<td>Carrier-coated</td>
</tr>
<tr>
<td>CD</td>
<td>Cyclodextrins</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>DC</td>
<td>Drug coated</td>
</tr>
<tr>
<td>DCC</td>
<td>Drug-carrier coated</td>
</tr>
<tr>
<td>FBAI</td>
<td>Foreign Body Associated Infection</td>
</tr>
<tr>
<td>FIC</td>
<td>Fractional inhibitory concentration</td>
</tr>
<tr>
<td>FICI</td>
<td>Fractional inhibitory concentration index</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infra-red spectroscopy</td>
</tr>
<tr>
<td>GFEC</td>
<td>Gatifloxacin with ethyl cellulose</td>
</tr>
<tr>
<td>GSM</td>
<td>Gram per square meter</td>
</tr>
<tr>
<td>HCWU</td>
<td>Healthcare worker uniform</td>
</tr>
<tr>
<td>HET-CAM</td>
<td>Hens egg test on chorio allantoic membrane</td>
</tr>
<tr>
<td>IS</td>
<td>Irritation score</td>
</tr>
<tr>
<td>IVC</td>
<td>In vitro challenge test</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Bertani</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal inhibitory concentration</td>
</tr>
<tr>
<td>NAC</td>
<td>N-acetylcysteine</td>
</tr>
<tr>
<td>NCTC</td>
<td>National Collection of Type Cultures</td>
</tr>
<tr>
<td>PAH</td>
<td>Poly allylamine hydrochloride</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly lactic acid</td>
</tr>
<tr>
<td>PLC</td>
<td>Poly caprolactone</td>
</tr>
<tr>
<td>PTFE</td>
<td>Poly tetra fluoro ethylene</td>
</tr>
<tr>
<td>SBAs</td>
<td>Serum bactericidal activities</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SSIs</td>
<td>Surgical site infections</td>
</tr>
<tr>
<td>UC</td>
<td>Uncoated</td>
</tr>
</tbody>
</table>
B. LIST OF COLOUR PLATES

1 Surface colonizing ability of test bacteria on test materials
   1.1 Exit-Site test: Implantable materials
   1.2 Exit-Site test: Non-implantable materials
2 Determination of biofilm producing bacteria
   2.1 Synergistic behavior of fluoroquinolone and nitroimidazole drugs based on DNA electrophoresis test
3 Qualitative antibacterial activity
   3.1 Silicone
   3.2 PTFE
   3.3 Polyurethane
   3.4 Polyester
   3.5 Silk suture
4 Bacterial adherence test
5 In vitro challenge test
6 Antibacterial activity of reactive drug treated textile materials (AATCC-100)
   6.1 CFU of the test bacteria at 0th hour and after 18 hour contact time
   7 Wash durability of microencapsulated drug treated textile materials
   7.1 CFU of the test bacteria after 5th and 10th wash of textile materials
   7.2 CFU of the test bacteria after 15th wash of textile materials
8 Microscopic images of microencapsulated drugs
   8.1 Scanning electron microscopic images of biomaterials
9 Tissue response of chick chorio-allantoic membrane to implantable materials
   9.1 Histological examination of CAM
9.2 materials
   9.2 histological examination of CAM
10 Tissue response of chick CAM to non-implantable materials
   10.1 Histological examination of CAM
C. LIST OF TABLES

1. Different types of implants for medical use
2. Antimicrobial Approaches for Preventing Infections Associated with Implants
3. Non-implantable medical textiles
4. Commercially used biocides and their treatment of various fibres
5. Classification of biofilm formation
6. Specification of cotton and C/P fabrics
7. Relationship of scores with category of irritation
8. Screening test bacteria for biofilm formation by MTP method
9. Effect of norfloxacin+metronidazole (D₁) on test organisms
10. Effect of Ofloxacin and Ornidazole (D₂) on test organisms
11. Effect of Ciprofloxacin-Tinidazole (D₃) on test organisms
12. Weight determination of implantable materials
13. Inhibitory effect of drug-carrier coated materials (aerobic)
15. Selected drug combination after qualitative analysis
16. Bacterial adherence test of dcc implantable materials
17. Bacterial adherence test of cc implantable materials
18. Statistical analysis on anti-adherent activity of biomaterials
19. Persistence of antibacterial drugs on implantable materials
20. Reduction (%) of reactive drug (D₁) treated fabrics (S. aureus)
21. Reduction (%) of reactive drug (D₁) treated fabrics (S. epidermidis)
22. Reduction (%) of reactive drug (D₁) treated fabrics (E. coli)
23. Reduction (%) of reactive drug (D₁) treated fabrics (P. mirabilis)
24. Reduction (%) of reactive drug (D₁) treated fabrics (P. aeruginosa)
25. Reduction (%) of reactive drug (D₂) treated fabrics (S. aureus)
26. Reduction (%) of reactive drug (D₂) treated fabrics (S. epidermidis)
27. Reduction (%) of reactive drug (D₂) treated fabrics (E. coli)
28. Reduction (%) of reactive drug (D₂) treated fabrics (P. mirabilis)
29. Reduction (%) of reactive drug (D₂) treated fabrics (P. aeruginosa)
30. Effective drug-carriers selected from reactive dye method
31. Reduction Percentage test of Staphylococcus aureus
32. Reduction Percentage test of Staphylococcus epidermidis
Reduction Percentage test of *Escherichia coli*

Reduction Percentage test of *Proteus mirabilis*

Reduction Percentage test of *Pseudomonas aeruginosa*

Statistical analysis on durability of encapsulated drug treated fabrics

Physical properties of reactive drug treated and untreated Cotton

Physical properties of reactive drug treated and untreated C/P

Physical properties of encapsulated drug treated and untreated Cotton

Physical characteristics of encapsulated drug treated and untreated C/P

FTIR peak assignments and interactions among silicone and antibacterial drugs

FTIR peak assignments and interactions among cotton and antibacterial drugs

Comparative evaluation of irritation scores for test materials, negative control and positive control by HET-CAM test
D. LIST OF FIGURES

1  β-cyclodextrin
2  Checkerboard model to determine synergism of two drugs
3  Simplified Checker Board Method of D_1 for a test bacteria
4  Simplified Checker Board Method of D_2 for a test bacteria
5  Simplified Checker Board Method of D_3 for a test bacteria
6  Standard graph of D_1 (norfloxacin-metronidazole)
7  Standard graph of D_2 (ofloxacin-ornidazole)
8  Standard graph of D_3 (ciprofloxacin-tinidazole)
9  Release profiles of drug-coated silicone
10 Release profiles of drug-coated PTFE
11 Release profiles of drug-coated polyurethane
12 Release profiles of drug-coated Polyester
13 Release profiles of drug-coated Silk suture
14 Release profiles of D_1C_1 coated silicone
15 Release profiles of D_2C_2 coated silicone
16 Release profiles of D_3C_2 coated silicone
17 Release profiles of D_1C_2 coated PTFE
18 Release profiles of D_2C_3 coated PTFE
19 Release profiles of D_3C_2 coated PTFE
20 Release profiles of D_1C_1 coated Polyurethane
21 Release profiles of D_2C_1 coated Polyurethane
22 Release profiles of D_3C_1 coated Polyurethane
23 Release profiles of D_1C_1 coated Polyester
24 Release profiles of D_2C_1 Polyester
25 Release profiles of D_3C_2 coated Polyester
26 Release profiles of D_1C_3 coated Silk Suture
27 Release profiles of D_2C_3 coated Silk Suture
28 Release profiles of D_3C_1 coated Silk Suture
29 FTIR spectrum of implantable material – silicone and dcc silicone
30 FTIR spectrum of implantable material – cotton and dcc cotton