# CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREFACE</td>
<td>i - iv</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>v - iv</td>
</tr>
<tr>
<td>1. REVIEW OF LITERATURE</td>
<td>1-54</td>
</tr>
<tr>
<td>1.1 General characteristics of staphylococci</td>
<td></td>
</tr>
<tr>
<td>1.1.1 Taxonomy</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2 Gram positive cell wall</td>
<td>2</td>
</tr>
<tr>
<td>1.1.3 Basic structure of <em>S. aureus</em> genome</td>
<td>3</td>
</tr>
<tr>
<td>1.2 Pathogenesis of <em>S. aureus</em> infections</td>
<td>4</td>
</tr>
<tr>
<td>1.3 <em>Staphylococcus aureus</em> as a human pathogen</td>
<td>5</td>
</tr>
<tr>
<td>1.4 Colonization and Infection</td>
<td>7</td>
</tr>
<tr>
<td>1.5 Epidemiology</td>
<td>12</td>
</tr>
<tr>
<td>1.6 Nosocomial infections</td>
<td>12</td>
</tr>
<tr>
<td>1.7 Methicillin-resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>13</td>
</tr>
<tr>
<td>1.8 Methicillin-susceptible <em>Staphylococcus aureus</em> (MSSA)</td>
<td>16</td>
</tr>
<tr>
<td>1.9 Risk factors for <em>Staphylococcus aureus</em> infections</td>
<td>17</td>
</tr>
<tr>
<td>1.9.1 <em>Staphylococcus aureus</em> colonization</td>
<td>17</td>
</tr>
<tr>
<td>1.10 MRSA prevalence in hospitals</td>
<td>20</td>
</tr>
<tr>
<td>1.11 Molecular structure of the methicillin resistance gene</td>
<td>20</td>
</tr>
<tr>
<td>1.12 Transmission of MRSA</td>
<td>23</td>
</tr>
<tr>
<td>1.13 Mechanisms of transmission</td>
<td>24</td>
</tr>
<tr>
<td>1.14 Control and preventive measures</td>
<td>24</td>
</tr>
<tr>
<td>1.15 Principles and overview of current typing methods</td>
<td>26</td>
</tr>
<tr>
<td>1.15.1 Phenotypic typing methods</td>
<td>27</td>
</tr>
</tbody>
</table>
1.15.1.1 Biotyping  
1.15.1.2 Antimicrobial susceptibility testing  
(antibiogram-based typing)
1.15.1.3 Serotyping  
1.15.1.4 Phage and bacteriocin typing  
1.15.1.5 SDS-PAGE of cellular and extracellular components  
1.15.1.6 Multilocus enzyme electrophoresis (MLEE)  
1.15.2 Genotypic typing methods  
1.15.2.1 Direct (and reverse) hybridization  
1.15.2.3 Ribotyping  
1.15.2.4 Genome analysis by array hybridization  
1.15.3 Fragment-based methods  
1.15.3.1 Plasmid typing  
1.15.3.2 Among restriction fragment length Polymorphism (RFLP) methods  
1.15.3.3 Amplified fragment length Polymorphism (AFLP) analysis  
1.15.3.4 Multilocus variable number tandem Repeat (VNTR) analysis (MLVA)  
1.15.3.5 Sequence-based methods Single-locus Sequence typing (SLST)  
1.15.3.6 Multi locus sequence typing (MLST)  
1.16 Mechanisms of action of antimicrobials  
1.17 Development of resistance in the hospital ecological
environment 49

1.18 Genetic analysis for Vancomycin resistance 50

2. INTRODUCTION 55-62

3. MATERIALS AND METHODS 63-89

3.1 Chemicals 63

3.2 Study duration and population 63

3.3 Grouping of individuals 64

3.4 Selection and collection of samples 64

3.4.1 Resident flora 64

3.4.1.1 Anterior nares (AN) 64

3.4.2 Transient flora 64

3.4.2.1 Forearm (FA) 65

3.4.2.2 Dorsum of palm (DP) 65

3.5 Microbiological study 65

3.6 Biochemical characterization of *staphylococcus aureus* 66

3.6.1 Catalase test 66

3.6.2 Mannitol fermentation 66

3.6.2.1 Interpretation 67

3.6.3 CoagulaseTest 67

3.6.3.1 Slide coagulation test 67

3.6.3.2 Tube coagulation test 68

3.6.4 DNase test 68

3.7 Phenotypic characterization of *staphylococcus aureus* 69

3.7.1 Oxacillin agar screen test 69

3.7.1.1 Materials 69

3.7.1.2 Procedure 69
### 3.7 Interpretation

#### 3.7.1 Reference strains used

#### 3.7.2 Determination of minimum inhibitory concentration (MIC) of oxacillin

#### 3.7.2.1 Materials

#### 3.7.2.2 Stock solution

#### 3.7.2.3 Procedure

#### 3.7.2.4 Calculations for the preparation of the original dilution

#### 3.7.2.5 Antibacterial susceptibility testing

### 3.8 Kirby-Bauer’s disc diffusion method

#### 3.8.1 Antibiotic discs

#### 3.8.2 Disk diffusion

##### 3.8.2.1 Reagents for the disk diffusion test

##### 3.8.2.2 Preparation of Müeller-Hinton agar

##### 3.8.2.3 Preparation of antibiotic stock solutions

##### 3.8.2.4 Preparation of dried filter paper discs

##### 3.8.2.5 Storage of commercial antimicrobial discs

##### 3.8.2.6 Turbidity standard for inoculum preparation

##### 3.8.2.7 Procedure for performing the disc diffusion test

##### 3.8.2.8 Inoculation of test plates

##### 3.8.2.9 Application of discs to inoculated agar plates

##### 3.8.2.10 Reading plates and interpreting results

### 3.9 Molecular Characterization

#### 3.9.1 Preparation of Genomic DNA
3.9.1.1 Reagents

3.9.2 Procedure of DNA extraction

3.9.3 PCR detection of van A, B and C genes

3.9.3.1 Procedure

3.9.3.2 Electrophoresis of PCR products

3.9.3.2.1 Agarose gel electrophoresis

3.9.3.2.2 Protocol

4. RESULTS

4.1 Sources and collection of samples

4.2 Isolation and characterization of staphylococci.

4.3 Isolation rate of *S. aureus* in clinical samples, hospital personnel and healthy individuals

4.4 Phenotypic characterization of MRSA

4.4.1 Oxacillin disc test

4.4.2 Minimum inhibitory concentration (MIC) of Oxacillin

4.4.3 Minimum inhibitory concentration (MIC) of vancomycin

4.5 Antibiotic Susceptibility Testing of MRSA

4.5.1 Antibiotic susceptibility pattern of MRSA isolates from clinical samples

4.5.2 Antibiotic susceptibility pattern of MRSA isolates from hospital personnel

4.5.3 Antibiotic susceptibility pattern of MRSA isolates from healthy individuals

4.6 Antibiotic susceptibility of MSSA
4.6.1 Antibiotic susceptibility pattern of MSSA isolates from clinical sample 132

4.6.2 Antibiotic susceptibility pattern of MSSA isolates from hospital personnel 132

4.6.3 Antibiotic susceptibility testing for MSSA isolates from healthy individuals 144

4.7 Molecular characterization of *Staphylococcus Aureus* 156

4.7.1 PCR detection of van A, B, C genes 156

5. DISCUSSION 155-175

5.1 Isolation and characterization of staphylococcus 158

5.2 Phenotypic characterization of MRSA 167

5.3 Antibiotic susceptibility test 169

5.4 Molecular characterization of *S. aureus* 174

6. SUMMARY AND CONCLUSION 176-179

7. REFERENCES 180-227

PUBLICATIONS