



**UNIVERSITI PUTRA MALAYSIA**

**MOLECULAR CHARACTERIZATION OF COAGULASE POSITIVE  
STAPHYLOCOCCI ISOLATED FROM DOGS AND CATS**

**OMER HASSAN MOHAMED HASSAN ARABI**

**FPV 2001 3**

**MOLECULAR CHARACTERIZATION OF COAGULASE POSITIVE  
STAPHYLOCOCCI ISOLATED FROM DOGS AND CATS**

**By**

**OMER HASSAN MOHAMED HASSAN ARABI**

**Thesis Submitted in Fulfilment of the Requirement of the Degree of Doctor  
of Philosophy in the Faculty of Veterinary Medicine  
Universiti Putra Malaysia**

**September 2001**



**DEDICATION**

*TO MY LATE PARENTS AND ALL MEMBERS OF MY FAMILY  
TO MY DEAR BROTHER OTHMAN  
TO MY WIFE FIROZE AND MY DAUGHTER TWASUL*



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**MOLECULAR CHARACTERIZATION OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM DOGS AND CATS**

By

**OMER HASSAN MOHAMED**

**September 2001**

**Chairman: Abdul Rahim Abdul Mutalib, DVM, Ph.D.**

**Faculty: Veterinary Medicine**

Isolation studies of coagulase-positive staphylococci were conducted on hospitalised and out patient dogs and cats, on three sites: skin, nose and ear, between January and September 1997. Three tests were used to differentiate between coagulase-positive staphylococci, namely: acetoin production, P agar supplemented with acriflavin and  $\beta$ -galactosidase test. Thirty-six *Staphylococcus aureus* and 90 *Staphylococcus intermedius* isolates were recovered from these animals. *Staphylococcus hyicus* was not isolated, the results indicated that the major coagulase-positive *Staphylococcus* species in dogs was *S. intermedius* and in cats was *S. aureus*.

The antimicrobial typing of *S. aureus* and *S. intermedius* was compared with the molecular typing methods such as: Polyacrylamide-gel Electrophoresis profiles of protein A concentration <sup>and</sup> whole cell proteins, and Polymerase Chain Reaction



(PCR)-based methods that include: Random Amplification of Polymorphic DNA (RAPD-PCR), Enterobacterial Repetitive Intergenic Consensus sequences (ERIC-PCR), Coagulase gene PCR amplification and Restriction Fragment Length Polymorphism (RFLP).

The antimicrobial typing differentiated *S. aureus* and *S. intermedius* isolates into 14 and 28 profiles respectively. Isolates of *S. aureus* and *S. intermedius* containing plasmids were 41.7% and 46% respectively. However, no correlation could be made between plasmid occurrence and antibiotic resistance profiles. The SDS-PAGE profiles of whole cell proteins grouped 24 *S. aureus* and 48 *S. intermedius* strains into 19 and 16 profiles respectively.

In PCR-based methods the isolates were typed using three primers. The combination of three primers for the RAPD gave 33 and 83 profiles of 36 *S. aureus* and 90 *S. intermedius* isolates respectively. ERIC primers grouped 24 *S. aureus* and 47 *S. intermedius* isolates into 19 and 43 profiles respectively. The coagulase gene from 24 *S. aureus* and 47 *S. intermedius* isolates showed limited discriminatory to the other methods and was least useful for the preliminary epidemiological studies. The restriction enzyme analysis of coagulase gene PCR products was very useful to increase the discriminatory power of coagulase gene PCR but required the use of multiple restriction enzymes. It was concluded that RAPD-PCR and ERIC-PCR are the best methods for typing *S. aureus* and *S. intermedius*.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN MOLEKUL STAFILOKOKUS POSITIF KOAGULASE  
DIPENCIL DARIPADA ANJING DAN KUCING**

Oleh

**OMER HASSAN MOHAMED**

**September 2001**

**Pengerusi: Abdul Rahim Abdul Mutalib, DVM, Ph.D.**

**Fakulti: Perubatan Veterinar**

Kajian pemencilan stafilocokus-positif telah dijalankan di antara bulan Januari dan September 1997, terhadap anjing dan kucing pesakit luar dan hospital, pada tiga tapak: kulit, hidung, dan telinga. Tiga ujian telah diguna untuk membeza di antara stafilocokus koagulase-positif iaitu, penghasilan aseton, ujian agar P ditambah akriflavin dan  $\beta$ -galaktosidase. Tiga puluh enam pencilan *Staphylococcus aureus* dan 90 pencilan *Staphylococcus intermedius* telah diperolehi daripada haiwan tersebut. Hasil kajian menunjukkan yang spesies *Staphylococcus* utama dalam anjing ialah *S. intermedius* dan dalam kucing *S. aureus*.

Pengetipan antimikrob *S. aureus* dan *S. intermedius* telah dibandingkan melalui kaedah pengetipan molekul seperti: profil Elektroforesis Poliakrilamida-gel protein dan protein A sel sepenuh, dan kaedah berasas Tindak Balas Rangkaian Polimerase (PCR): Penguatan Rawak DNA Polimorfik (RAPD-PCR), jujukan



## Konsensus Antaragen Berulang Enterobakteria (ERIC-PCR), Penguatan PCR gen Koagulase dan Polimorfisme Panjang Fragmen Pengehadan (RFLP)

Pengetipan antibiogramer dapat membezakan pencilan *S. aureus* dan *S. intermedius* yang mengandungi plasmid masing-masing 41.7% dan 46%. Bagaimanapun tiada sebarang perkaitan berlaku di antara kewujudan plasmid dengan profil ketahanan antibiotik. Profil SDS-PAGE protein sel sepenuh telah mengumpul 24 strain *S. aureus* dan 48 strain *S. intermedius* masing-masing kepada 19 dan 16 profil.

Dalam kaedah berasaskan PCR, strain boleh ditipkan dengan mengguna tiga primer. Gabungan tiga primer untuk RAPD memberikan 33 dan 83 profil masing-masing daripada 36 strain *S. aureus* dan 90 strain *S. intermedius*. Primer ERIC mengumpulkan 24 strain *S. aureus* dan 47 strain *S. intermedius* masing-masing kepada 19 dan 43 profil. Gen koagulase daripada 24 strain *S. aureus* dan 47 strain *S. intermedius* menunjukkan pembezaan terhadap terhadap kaedah lain dan paling kurang kegunaannya dalam kajian epidemiologi awal. Analisis enzim pengehadan hasil PCR gen koagulase adalah paling tinggi kegunaannya untuk meningkatkan kuasa pembezaan PCR gen koagulase, tetapi ia memerlukan penggunaan enzim pengehadan berbilang. Kesimpulannya ialah, RAPD-PCR dan ERIC-PCR merupakan kaedah paling baik untuk mengetip *S. aureus* dan *S. intermedius*.



## ACKNOWLEDGEMENTS

All praise to Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this work would not have been possible.

I would like to express my sincere gratefulness and appreciation to my supervisor, Dr. Abdul Rahim Abdul Mutalib, who has devoted a lot of his time for invaluable guidance, advice, supervision and support throughout the course of this study.

I wish to express my sincere gratitude to the Dean of the faculty and my co-supervisor Professor Dato Dr. Sheikh Omar Abdul Rahman for his invaluable advice, support and continued encouragement towards the completion of this work and being helpful whenever I ran into difficulties.

Sincere thanks also to my co-supervisors, Associate Professor Dr. Saleha Abdul Aziz and Associate Professor Dr. Son Radu who have provided advice and helpful discussion that have enlightened and improved this study.

I would like to express my gratitude to Professor Dr. Abdul Rani Bahaman who had provided facilities of his laboratory during the course of this study.





I would like to thank the staff members of Veterinary Clinic and Hospital, Dr. Habibah Arshad, Dr. Vijayndra Madawar, Pn Halimah Abu Bakar and Pn Hasina Behgam. I wish to thank Mr Shahreer at the Laboratory animal's farm for his kind assistance. I deeply send my "Duaa" to our late helpful man Mr. Basri Kasim, I asked Allah to forgive him and ascend mercies to his grave and enlighten it for him. My deeply indebted thanks to Mr Hajariah Salamah that was very helpful and generous with his time. I am very grateful with Mr Fauzi Che Yosof for his skilled assistance during this study.

Also, I had been very fortunate in receiving assistance from a number of my colleagues and friends. They had to give up some of their valuable time to help me and it would not be possible to name all of them. However, I would like to thank Mr. Omer Hussabo, Dr. Izz Eldeen Babeker, Dr. Esam Mohamed Ali, Mr Elwaleed Awad, Mr. Eltahir Siddig, Mr Tarig Elsharif, Mr Mohamed Ghrihi, Mr Naseer Eldeen Elhadi, Pn Nemita Fefadrah, Mr Samuel Lihan, Pn Zunita Zakaria, Pn Siti Khirani and Ooi Wai Ling.

Lastly, I would like to thank my brother Othman Arabi for his unlimited support to me during my study. Grateful thanks to my supportive wife, Firoze Mustafa Suliman and my daughter Twasul who had to put up with my long absence from them during study, so that I could complete this work.



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

---

**AINI IDERIS, Ph.D.**  
Professor/Dean of Graduate School  
Universiti Putra Malaysia

Date:



## TABLE OF CONTENTS

|  | Page     |
|--|----------|
| DEDICATION   | ii       |
| ABSTRACT   | iii      |
| ABSTRAK  | v        |
| ACKNOWLEDGEMENTS   | vii      |
| APPROVAL SHEETS  | ix       |
| DECLARATION FORM   | xi       |
| LIST OF TABLES   | xvi      |
| LIST OF FIGURES  | xviii    |
| LIST OF ABBREVIATIONS  | xxviii   |
| <b>Chapter</b>   |          |
| <b>1 INTRODUCTION</b>  | <b>1</b> |
| <b>2 LITERATURE REVIEW</b>   | <b>6</b> |
| 2.1 Introduction   | 6        |
| 2.2 Taxonomy   | 7        |
| 2.3 Coagulase-Positive Staphylococcal Virulence                        | 8        |
| 2.3.1 Exoenzymes   | 9        |
| 2.3.2 Toxins   | 11       |
| 2.4 Infections Caused by <i>Staphylococcus</i> species                 | 13       |
| 2.4.1 Coagulase-Negative Staphylococci Infections                      | 14       |
| 2.4.2 Coagulase-Positive Staphylococci Infections in Dogs and Cats     | 14       |
| 2.4.3 Coagulase-Positive Staphylococci Infections in other Animals     | 18       |
| 2.4.4 Coagulase-Positive Staphylococci Infections in Man in Malaysia   | 20       |
| 2.5 Epidemiology of <i>Staphylococcus</i> Species                      | 21       |
| 2.5.1 Hospital and Community Strains                                   | 22       |
| 2.5.2 Nosocomial Infections  | 23       |
| 2.5.3 Nosocomial Infections in Animals                                 | 25       |
| 2.6 Control of Coagulase-Positive Staphylococci                        | 26       |
| 2.6.1 Epidemiological Control  | 27       |
| 2.6.2 Immunologic Control  | 28       |
| 2.7 Isolation and Identification of Coagulase-Positive Staphylococci   | 28       |
| 2.8 Characterisation of Coagulase-Positive Staphylococci               | 29       |
| 2.8.1 Page Typing  | 30       |
| 2.8.2 Molecular Analysis   | 31       |
| 2.8.3 PCR-Based Methods  | 32       |
| 2.8.4 Random Amplification of Polymorphic DNA (RAPD)                   | 33       |
| 2.8.5 Enterobacterial Repetitive Intergenic Consensus Sequences (ERIC) | 35       |
| 2.8.6 Coagulase Gene Polymorphism                                      | 36       |



|  |           |
|--|-----------|
| 2.8.7 Protein Profiles   | 38        |
| 2.8.8 Protein A Assay by IgG Binding   | 39        |
| 2.9 Antibiotic Resistance in staphylococci   | 39        |
| 2.10 Genetic Nature of Antimicrobial Resistance  | 42        |
| 2.10.1 Plasmids  | 43        |
| 2.10.2 Plasmid Analysis of Coagulase-Positive Staphylococci                                | 45        |
| 2.11 Cluster Analysis  | 46        |
| <b>3 ISOLATION AND IDENTIFICATION OF COAGULASE-POSITIVE STAPHYLOCOCCI IN DOGS AND CATS</b> | <b>47</b> |
| 3.1 Introduction   | 47        |
| 3.2 Materials and Methods  | 49        |
| 3.2.1 Culture Media  | 49        |
| 3.2.2 Source of Samples  | 50        |
| 3.2.3 Sites of Samples   | 50        |
| 3.2.4 Isolation of Coagulase-Positive Staphylococci  | 51        |
| 3.2.5 Identification of Coagulase-Positive Staphylococci                                   | 54        |
| 3.2.6 API STAPH  | 57        |
| 3.2.7 The Positive and Negative Control  | 57        |
| 3.2.8 Statistical Analysis   | 57        |
| 3.3 Results  | 59        |
| 3.4 Discussion   | 68        |
| <b>4 ANTIBIOTIC RESISTANCE AND PLASMID PROFILES</b>  | <b>74</b> |
| 4.1 Introduction   | 74        |
| 4.2 Material and Methods   | 76        |
| 4.2.1 Bacterial Isolates Media and Propagation   | 76        |
| 4.2.2 Antimicrobial Susceptibility Tests   | 77        |
| 4.2.3 Preparation of the Inoculum  | 77        |
| 4.2.4 Sensitivity to Antimicrobial Agents  | 78        |
| 4.2.5 The Antimicrobial Agents   | 78        |
| 4.2.6 Multiple Antibiotic Resistance Indexing of Isolates                                  | 78        |
| 4.2.7 Isolation of Plasmids DNA  | 79        |
| 4.2.8 Visualisation of Plasmids DNA  | 80        |
| 4.2.9 The Molecular Size Marker  | 81        |
| 4.2.10 The Positive and Negative Control   | 81        |
| 4.2.11 Statistical Analysis  | 81        |
| 4.3 Results  | 82        |
| 4.3.1 Antibiotic Sensitivity of <i>S. aureus</i> isolates                                  | 82        |
| 4.3.2 Antibiotic Sensitivity of <i>S. intermedius</i> isolates                             | 86        |
| 4.3.3 Plasmid Analysis of <i>S. aureus</i>   | 90        |
| 4.3.4 Plasmid Analysis of <i>S. intermedius</i>  | 92        |
| 4.4 Discussion   | 93        |



|          |   |            |
|----------|---|------------|
| <b>5</b> | <b>CHARACTERIZATION OF COAGULASE-POSITIVE STAPHYLOCOCCI BY THEIR PROTEIN PROFILES, AND CONCENTRATION OF PROTEIN A OF WHOLE CELL PROTEIN</b> | <b>102</b> |
| 5.1      | Introduction  | 102        |
| 5.2      | Material and Methods  | 103        |
| 5.2.1    | Preparation of the Proteins   | 103        |
| 5.2.2    | SDS Polyacrylamide Gel Electrophoresis  | 105        |
| 5.2.3    | Electrophoresis   | 106        |
| 5.2.4    | Enzyme Link Immunosorbent Assay (ELISA)   | 106        |
| 5.2.5    | Quantification of Protein A   | 108        |
| 5.2.6    | Statistical Analysis  | 108        |
| 5.2.7    | The Positive and Negative Control   | 109        |
| 5.3      | Results   | 110        |
| 5.3.1    | Protein Profiles  | 110        |
| 5.3.2    | Protein A Assessment  | 117        |
| 5.3.3    | Cluster Analysis  | 119        |
| 5.3.4    | Cluster Analysis of Protein Profiles  | 119        |
| 5.4      | Discussion  | 122        |
| <br>     |   |            |
| <b>6</b> | <b>CHARACTERIZATION OF COAGULASE-POSITIVE STAPHYLOCOCCI USING POLYMERASE CHAIN REACTION-BASED METHODS</b>                                   | <b>127</b> |
| 6.1      | Introduction  | 127        |
| 6.2      | Materials and Methods   | 130        |
| 6.2.1    | Isolation of DNA  | 130        |
| 6.2.2    | Standardisation of PCR  | 131        |
| 6.2.3    | Determination of DNA concentration and Purity   | 132        |
| 6.2.4    | Primers for RAPD-PCR  | 132        |
| 6.2.5    | RAPD-PCR Conditions   | 133        |
| 6.2.6    | Primers for ERIC-PCR  | 134        |
| 6.2.7    | ERIC-PCR Conditions   | 134        |
| 6.2.8    | Primers for Coagulase gene PCR  | 135        |
| 6.2.9    | Coagulase gene PCR Conditions   | 135        |
| 6.2.10   | Gel Electrophoresis for Amplified Products of RAPD, ERIC, and Coagulase gene-PCR  | 136        |
| 6.2.11   | Visualisation and Photographing of DNA Bands  | 136        |
| 6.2.12   | Restriction Fragment Length Polymorphism (RFLP)   | 136        |
| 6.2.13   | Statistical Analysis of PCR Methods   | 137        |
| 6.3      | Results   | 138        |
| 6.3.1    | RAPD-PCR Analysis   | 138        |
| 6.3.2    | ERIC-PCR Analysis   | 144        |
| 6.3.3    | Coagulase Gene PCR Analysis   | 147        |
| 6.3.4    | Restriction Fragment Length Polymorphism of Coagulase gene  | 151        |
| 6.3.5    | Cluster Analysis  | 155        |



|  |            |
|--|------------|
| 6.3.6 Cluster Analysis of RAPD-PCR         | 155        |
| 6.3.7 Cluster Analysis of ERIC-PCR         | 158        |
| 6.3.8 Cluster Analysis of RFLP             | 161        |
| 6.4 Discussion                             | 168        |
| <b>7 GENERAL DISCUSSION AND CONCLUSION</b> | <b>174</b> |
| <b>BIBLIOGRAPHY</b>                        | <b>182</b> |
| <b>APPENDIX</b>                            | <b>202</b> |
| <b>BIODATA OF THE AUTHOR</b>               | <b>225</b> |



## LIST OF TABLES

| Table |  | Page |
|-------|--|------|
| 2.1   | <i>Staphylococcus</i> species groups.  | 7    |
| 2.2   | Extracellular virulence factors produced by <i>S. aureus</i> and <i>S. intermedius</i>   | 9    |
| 2.3   | The main types, function and some examples of plasmid  | 44   |
| 3.1   | Biochemical tests for the isolation and identification of <i>S. aureus</i> , <i>S. intermedius</i> and <i>S. hyicus</i> using conventional methods | 59   |
| 3.2   | Frequency of staphylococci isolates from dogs and cats   | 60   |
| 3.3   | Frequency of coagulase-positive staphylococci isolates from dogs and cats  | 60   |
| 3.4   | Frequency of <i>S. aureus</i> isolates from dogs and cats  | 61   |
| 3.5   | Frequency of <i>S. intermedius</i> isolates from dogs and cats   | 62   |
| 3.6   | Distribution of staphylococci isolated from three anatomical sites of hospitalised dogs  | 63   |
| 3.7   | Distribution of staphylococci isolated from three anatomical sites of outpatient dogs  | 64   |
| 3.8   | Distribution of staphylococci isolated from three anatomical sites of hospitalised cats  | 65   |
| 3.9   | Distribution of staphylococci isolated from three anatomical sites of outpatient cats  | 67   |
| 4.1   | Antibiotic resistance of <i>S. aureus</i> isolates with and without plasmids DNA   | 83   |
| 4.2   | Multiple antibiotic resistance and occurrence of plasmids in <i>S. aureus</i> isolates, in four groups of animals                                  | 83   |
| 4.3   | Antibiotic resistance of <i>S. aureus</i> isolates from the four groups of animals   | 85   |



|     |   |     |
|-----|---|-----|
| 4.4 | Antibiotic resistance of <i>S. intermedius</i> isolates with and without plasmids DNA   | 86  |
| 4.5 | Multiple antibiotic resistance and occurrence of plasmids in <i>S. intermedius</i> isolates on four groups of animals                   | 87  |
| 4.6 | Antibiotic resistance of <i>S. intermedius</i> isolates from the four groups of animals   | 90  |
| 4.7 | Plasmid DNA occurrence in <i>S. aureus</i> isolates in the four groups of animals   | 91  |
| 4.8 | Plasmids DNA occurrence in <i>S. intermedius</i> isolates in the four groups of animals   | 92  |
| 5.1 | Protein profiles and protein A concentration of <i>S. aureus</i> isolates.  | 115 |
| 5.2 | Protein profiles and protein A concentration of <i>S. intermedius</i> isolates.   | 116 |
| 6.1 | Distribution of <i>S. aureus</i> isolates according to the RAPD PCR, ERIC PCR, Coagulase gene PCR and RFLP fingerprinting profiles      | 164 |
| 6.2 | Distribution of <i>S. intermedius</i> isolates according to the RAPD PCR, ERIC PCR, Coagulase gene PCR and RFLP fingerprinting profiles | 165 |





## LIST OF FIGURES

| Figure |  | Page |
|--------|--|------|
| 3.1    | The steps used for the isolation of coagulase-positive staphylococci from dogs and cats.   | 53   |
| 3.2    | The steps used for the identification of coagulase-positive staphylococci from dogs and cats.  | 58   |
| 4.1    | Agarose gel (0.8%) electrophoresis of plasmid DNA from <i>S. aureus</i> isolates 1-13 in Appendix B3. Lane: M, <i>E. coli</i> strain V517 standard marker plasmid of known molecular weight.   | 91   |
| 5.1    | Standard curve protein by Lowry Method   | 104  |
| 5.2    | Exponential regression analysis of results of ELISA obtained with known concentration of protein A   | 108  |
| 5.3    | Protein profiles of <i>S. aureus</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2 references strain. Lane: 3,1; 4,2; 5,3 6,4; 7,5; 8,6; 9,7; 10,8; 11,9; 12,10; 13,11; 14,12 correspond to isolate numbers given in Table 5.1.     | 111  |
| 5.4    | Protein profiles of <i>S. aureus</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,13; 3,14; 4,15: 5,16; 6,17; 7,18; 8,19; 9,20; 10,21; 11,22; 12,23; 13,24 correspond to isolate numbers given in Table 5.1.                       | 111  |
| 5.5    | Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: M, protein marker (kDa). Lane: 1 references strain Lane: 2,1; 3,2; 4,3; 5,4; 6,5; 7,6; 8,7; 9,8; 10,9; 11,10; 12,11; 13,12 correspond to isolate numbers given in Table 5.2. | 113  |
| 5.6    | Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,13; 3,14; 4,15: 5,16; 6,17; 7,18; 8,19; 9,20 10,21; 11,22; 12,23; 13,24 correspond to isolate numbers given in Table 5.2.                   | 113  |
| 5.7    | Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,25; 3,26; 4,27: 5,28; 6,29; 7,30; 8,31; 9,32 10,33; 11,34; 12,35; 13,36 correspond to isolate numbers given in Table 5.2.                   | 114  |



|      |   |     |
|------|---|-----|
| 5.8  | Protein profiles of <i>S. intermedius</i> obtained with SDS-PAGE. Lane: 1, protein marker (kDa). Lane: 2,37; 3,38; 4,39: 5,40; 6,41; 7,42; 8,43; 9,44 10,45; 11,46; 12,47; 13,48 correspond to isolate numbers given in Table 5.2.  | 114 |
| 5.9  | The protein A concentration of <i>S. aureus</i> and <i>S. intermedius</i>   | 118 |
| 5.10 | The dendrogram generated from matrix data obtained from SDS Page of whole cell protein. The patristic distance showed the genetic dissimilarity among the <i>S. aureus</i> isolates. R, references isolate. Lane numbers correspond to isolate numbers given in Table 5.1.      | 120 |
| 5.11 | The dendrogram generated from matrix data obtained from SDS Page of whole cell protein. The patristic distance showed the genetic dissimilarity among the <i>S. intermedius</i> isolates. R, references isolate. Lane numbers correspond to isolate numbers given in Table 5.2. | 121 |
| 6.1  | RAPD profiles of <i>S. aureus</i> obtained with primer OPA-01 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.1. Lane M, 1kb molecular weight size marker in base pair.  | 138 |
| 6.2  | RAPD profiles of <i>S. aureus</i> obtained with primer OPA-07 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.1. Lane M, 1kb molecular weight size marker in base pair.  | 139 |
| 6.3  | RAPD profiles of <i>S. aureus</i> obtained with primer OPA-08 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.1. Lane M, 1kb molecular weight size marker in base pair.  | 140 |
| 6.4  | RAPD profiles of <i>S. intermedius</i> obtained with primer OPA-01 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.2. Lane M, 1kb molecular weight size marker in base pair.   | 141 |
| 6.5  | RAPD profiles of <i>S. intermedius</i> obtained with primer OPA-07 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.2. Lane M, 1kb molecular weight size marker in base pair.   | 142 |



- 6.6 RAPD profiles of *S. intermedius* obtained with primer OPA-08 electrophoresed on 1% agarose gel. Lane numbers correspond to isolate numbers given in Table 6.2. Lane M, 1kb molecular weight size marker in base pair. 143
- 6.7 ERIC profiles of *S. aureus* isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 2,2; 3,3; 4,4; 5,5; 6,7; 7,8; 8,9; 9,10; 10,11; 11,13; 12,14; 13,15; 14,20; 15,21; 16,22; 17,23; 18,24, correspond to isolate numbers given in Table 6.1. Lane: 1, 1kb molecular weight size marker in base pair. 144
- 6.8 ERIC profiles of *S. aureus* isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 1,25; 2,27; 3,29; 4,31; 5,33; 6,35; 7,36, correspond to isolate numbers given in Table 6.1. Lane: 8, 1kb molecular weight size marker in base pair. 145
- 6.9 ERIC profiles of *S. intermedius* isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lane: 1,1; 2,2; 3,4; 4,5; 5,6; 6,11; 7,14; 8,15; 9,17; 10,19; 11,22; 12,23; 13,25; 14,27; 15,29; 16,30; 17,32 correspond to isolate numbers given in Table 6.2. Lane: 18, negative control. Lane: 19, positive control. Lane: 20, 1kb molecular weight size marker in base pair. 146
- 6.10 ERIC profiles of *S. intermedius* isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 1,33; 2,34; 3,35; 4,36; 5,37; 6,39; 7,41; 8,44; 9,47; 10,49; 11,51; 12,55; 13,56; 14,59; 15,62 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane: 18, 1kb molecular weight size marker in base pair. 146
- 6.11 ERIC profiles of *S. intermedius* isolates using ERIC1R and ERIC2 primers electrophoresed on 1% agarose gel. Lanes: 1, 64; 2, 67. 3,69; 4,72; 5,75; 6,77; 7,78; 8,80; 9,81; 10,82; 11,83; 12,85; 13,86; 14,89; 15,90 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane 18, 1kb molecular weight size marker in base pair. 147



- 6.12 Coagulase gene profiles of *S. aureus* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 2,2; 3,3; 4,4; 5,5; 6,7; 7,8; 8,9; 9,10; 10,11; 11,13; 12,14; 13,15; 14,20; 15,21; 16,22; 17,23; 18,24 correspond to isolate numbers given in Table 6.1. Lane 19 positive control. Lane: 20, negatives control. Lane: 1kb molecular weight size marker in base pair. 148
- 6.13 Coagulase gene profiles of *S. aureus* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 2,25; 3,27; 4,29; 5,31; 6,33; 7,35; 8,36, correspond to isolate numbers given in Table 6.1. Lane: 1, 1kb molecular weight size marker in base pair. 148
- 6.14 Coagulase gene profiles of *S. intermedius* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lane: 1,1; 2,2; 3,4; 4,5; 5,6; 6,11; 7,14; 8,15; 9,17; 10,19; 11,22; 12,23; 13,25; 14,27; 15,29; 16,30; 17,32 correspond to isolate numbers given in Table 6.2. Lane: 18, negative control. Lane 20, 1kb molecular weight size marker in base pair. 149
- 6.15 Coagulase gene profiles of *S. intermedius* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 1,33; 2,34; 3,35; 4,36; 5,37; 6,39; 7,41; 8,44; 9,47; 10,49; 11,51; 12,55; 13,56; 14,59; 15,62 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane: 18, 1kb molecular weight size marker in base pair. 150
- 6.16 Coagulase gene profiles of *S. intermedius* isolates using COAG2 and COAG3 primers electrophoresed on 1% agarose gel. Lanes: 1,64; 2,67; 3,69; 4,72; 5,75; 6,77; 7,78; 8,80; 9,81; 10,82; 11,83; 12,85; 13,86; 14,89; 15,90 correspond to isolate numbers given in Table 6.2. Lane: 16, positive control. Lane: 17, negative control. Lane 18, 1kb molecular weight size marker in base pair. 150
- 6.17 RFLPs generated by restriction endonuclease analysis of coagulase gene of *S. aureus* isolates using *AluI* restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 2,2; 3,3; 4,4; 5,5; 6,7; 7,8; 8,9; 9,10; 10,11; 11,13; 12,14; 13,15; 14,20; 15,21; 16,22; 17,23; 18,24 correspond to isolate numbers given in Table 6.1. Lane 19 positive control. Lane: 20, negative control. Lane: M, 1kb molecular weight size marker in base pair. 151

- 6.18 RFLPs generated by restriction endonuclease analysis of coagulase gene of *S. aureus* isolates using *AluI* restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 2,25; 3,27; 4,29; 5,31; 6,33; 7,35; 8,36 correspond to isolate numbers given in Table 6.1. Lane: 1, 1kb molecular weight size marker in base pair. 152
- 6.19 RFLPs generated by restriction endonuclease analysis of coagulase gene of *S. intermedius* isolates using *AluI* restriction enzyme, electrophoresed on 1% agarose gel. Lane: 1,1; 2,2; 3,4; 4,5; 5,6; 6,11; 7,14; 8,15; 9,17; 10,19; 11,22; 12,23; 13,25; 14,27; 15,29; 16,30; 17,32 correspond to isolate numbers given in Table 6.2. Lane: 18, negative control. Lane: 19, 1kb molecular weight size marker in base pair. 153
- 6.20 RFLPs generated by restriction endonuclease analysis of coagulase gene of *S. intermedius* isolates using *AluI* restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 1,33; 2,34; 3,35; 4,36; 5,37; 6,39; 7,41; 8,44; 9,47; 10,49; 11,51; 12,55; 13,56; 14,59; 15,62 correspond to isolate numbers given in Table 6.2. Lane: 16, negative control. Lane 17, 1kb molecular weight size marker in base pair. 153
- 6.21 RFLPs generated by restriction endonuclease analysis of coagulase gene of *S. intermedius* isolates using *AluI* restriction enzyme, electrophoresed on 1% agarose gel. Lanes: 1,64; 2,67; 3,69; 4,72; 5,75; 6,77; 7,78; 8,80; 9,81; 10,82; 11,83; 12,85; 13,86; 14,89; 15,90 correspond to isolate numbers given in Table 6.2. Lane 16, negatives control. Lane M, 1kb molecular weight size marker in base pair. 154
- 6.22 The dendrogram generated from matrix data obtained from RAPD-PCR. The patristic distance showed the genetic dissimilarity among the *S. aureus* isolates. This is the combined result of the three primers: OPA-01, OPA-07 and OPA-08. Lane numbers correspond to isolate numbers given in Table 6.1. 156
- 6.23 The dendrogram generated from matrix data obtained from RAPD PCR. The patristic distance showed the genetic dissimilarity among the *S. intermedius* isolates. This is the combined result of the three primers: OPA-01, OPA-07 and OPA-08. Lane numbers correspond to isolate numbers given in Table 6.2. 157



- 6.24 The dendrogram generated from matrix data obtained from ERIC-PCR. The patristic distance showed the genetic dissimilarity among the *S. aureus* isolates. Lane numbers correspond to isolate numbers given in Table 6.1. 159
- 6.25 The dendrogram generated from matrix data obtained from ERIC-PCR. The patristic distance showed the genetic dissimilarity among the *S. intermedius* isolates. Lane numbers correspond to isolate numbers given in Table 6.2. 160
- 6.26 The dendrogram generated from matrix data obtained from RFLP. The patristic distance showed the genetic dissimilarity among the *S. aureus* isolates. Lane numbers correspond to isolate numbers given in Table 6.1. 162
- 6.27 The dendrogram generated from matrix data obtained from RFLP. The patristic distance showed the genetic dissimilarity among the *S. intermedius* isolates. Lane numbers correspond to isolate numbers given in Table 6.2. 163



## LIST OF ABBREVIATIONS

|                               |   |
|-------------------------------|---|
| Ab                            | Antibody  |
| AP-PCR                        | Arbitrary primed polymerase chain reaction                |
| Bp                            | Base pair   |
| BSA                           | Bovine serum albumin                                      |
| °C                            | Degree Celcius  |
| cm                            | Centimetre  |
| CNS                           | Central nervous system                                    |
| CO <sub>2</sub>               | Carbon dioxide  |
| D.W                           | Distilled water   |
| D.D                           | De-ionised distilled                                      |
| DNA                           | Deoxyribonucleic acid                                     |
| dNTP                          | Deoxy-nucleotide triphosphate                             |
| Dr.                           | Doctor  |
| e.g.                          | For example   |
| EDTA                          | Ethylene diamine Tetra-acetate                            |
| ELISA                         | Enzyme Link Immunosorbent Assay                           |
| ERIC                          | Enterobacterial repetitive intergenic consensus sequences |
| g                             | Gram  |
| G+C                           | Guanine + Cytisine  |
| H <sub>2</sub> O <sub>2</sub> | Hydrogen Peroxide   |
| h                             | Hour  |
| hrs                           | Hours   |
| HIS                           | Hyperimmune serum   |
| i.e.                          | That is   |
| IgA                           | Immunoglobulin A  |
| IgG                           | Immunoglobulin G  |
| IgM                           | Immunoglobulin M  |
| Kbp                           | Kilobase pairs  |
| KDa                           | Kilodalton  |
| M                             | Molar   |
| Mab                           | Monoclonal antibody                                       |
| Mda                           | Megadalton  |
| M.W.                          | Molecular weight  |
| mg                            | Milligram   |
| Min                           | Minute  |
| Mins                          | Minutes   |
| ml                            | Millilitre  |
| mM                            | Millimole   |
| nm                            | Nanometer   |
| O.D.                          | Optical Density   |
| PAGE                          | Polyacrylamide gel electrophoresis                        |
| PBS                           | Phosphate buffer saline                                   |

