MOLECULAR CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN RESISTANT Staphylococcus aureus ISOLATED FROM CHICKENS AND CATTLE

ABBULKADIR MAGAJI MAGASHI

FPV 2012 2
MOLECULAR CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN RESISTANT *Staphylococcus aureus* ISOLATED FROM CHICKENS AND CATTLE

By

ABBULKADIR MAGAJI MAGASHI

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

JANUARY 2012
DEDICATION

This thesis is dedicated to my late great-grandfather his royal highness late Muhammad Alwali, my grandfather Muhammad Lirwan and my late father Adam Muhammad Alwali.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy
MOLECULAR CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN RESISTANT Staphylococcus aureus ISOLATED FROM CHICKENS AND CATTLE

By

ABDULKADIR MAGAJI MAGASHI

January 2012

Chairman: Assoc. Prof. Zunita Zakaria, PhD

Faculty: Veterinary Medicine

Staphylococcus aureus has been widely recognized as one of the major human pathogen responsible for a wide range of diseases, ranging from minor skin infections to more life-threatening infections of the central nervous system and respiratory, urinary tract infections and infections associated with internal implanted devices. In animals, S. aureus is implicated as a cause of a number of diseases ranging from pneumonia, osteomyelitis, joint infection and septicemia in poultry, mastitis in cattle and small ruminants. The disclosure of MRSA in pets and domestic animals (pigs, horse, poultry and cattle) with the frequent isolation of livestock associated MRSA among animals and human handlers confirmed the fears that animals may serve as reservoirs for MRSA transmission. Staphylococcus aureus has been established as one of the foremost Superantigen producing causative agent, when ingested enterotoxin produced is responsible for food poisoning. Consequently food animals colonized with MRSA pose a substantial public health concern. The initial part of this study was
designed to isolate and characterize *S. aureus* with the aid of conventional and molecular methods. A total of 450 feather samples were collected from chickens in nine designated broiler farms from three states in Peninsular Malaysia, between April 2007 to November, 2008 and 150 nasal swabs from cattle were also collected from three farms.

Conventional phenotypic tests used included gram staining, biochemical tests and DryspotStaphytect plusagglutination kit (DR0M100 UK) to identify *S. aureus*. The identified isolates were inoculated on selective media (mannitol salt agar and oxacillin resistance screening agar) for the detection of MRSA from chickens and cattle samples. Based on the biochemical tests and characteristic growth on the selective media, 153 (34%) and 17 (11.33%) isolates were identified as MRSA from chickens and cattle respectively. Polymerase chain reaction assay for the detection of *meca* and *nucA* genes confirmed 66 as MRSA out of the 170 MRSA identified using conventional test. The overall prevalence of MRSA and MRS from chickens was reported as 13.56% (95%CI 0.0179-0.0503). Low prevalence of MRSA/MRS from cattle farms was observed with the overall prevalence of MRSA from cattle reported as 3.33% (95%CI 0.0080-0.0603) and 5.33% (0.0157-0.0786) for MRS respectively. Statistical analyses were carried out to compare differences between slide coagulase and DryspotStaphytect kit using chisquare test. There was no significant difference between the two tests at p = 0.05, $x^2 = 0.1662$. Nominal logistic regression was used to compare differences between the tests, animal’s species and farms. The
likelihood ratio chi-square showed that slide coagulase \( p = 0.349 \), DryspotStaphytest kit \( p = 0.938 \) and PCR \( p = 0.082 \) had no significant relationship with animal species; whereas farms had a significant relationship with the tests \( p < 0.0001 \).

The second part of the study investigated the *in vitro* antimicrobial susceptibility of the MRSA, MRS and MSSA to 30 antimicrobial agents and the determination of oxacillin MIC using E-test strips (AB Biodisk Sweden). The oxacillin MIC for chicken MRSA ranged from 0.5µg/L to ≥256µg/L. Using the break point for oxacillin resistance by CLSI (2006) ≥4µg/L, 42 MRSA (68.85%) were considered fully resistant and 19 (31.15%) had MIC below the set standard. Comparably 41 MRS were considered resistant based on the CLSI criteria for oxacillin E-test and 20 isolates had lower MIC. The MIC for cattle MRSA isolates ranged from 1-256µg/L based on CLSI cut off 2 isolates were susceptible. The pattern of resistance that is common among the MRSA cut acrossoxacillin, cefoxitin, tetracycline, clindamycin, lincomycin, neomycin, erythromycin, penicillin G, streptomycin and cefuroxime. All the 170 isolates were susceptible to linezolid; furthermore most of the isolates were susceptible to mupirocin and teicoplanin with the exception of two MRSA and two MRS.

The genetic background of some selected isolates using different types of typing methods such as multilocus sequence typing (MLST), spa typing, pulse field gel electrophoresis (PFGE) and pyrogenic toxin genes screening was investigated.
MLST characterized 12 MRSA isolates into 11 sequence types, namely ST9, ST15, ST14, ST537, ST190, ST194, ST795, and ST1279 from chickens while ST59, ST35 and ST573 from cattle. These 12 isolates were grouped into five spa types’ t437, t442, t360, t189 and t5696. The analysis of PFGE macrorestriction patterns percentage of similarity identified from the dendogram at 80% similarity coefficient was used to define pulsotypes. The PFGE analysis identified 22 pulsotypes with nine sub types and the most common cluster is C which appeared to be present in four farms. Cluster B was similar albeit having different spa types. Diversity ensued among the isolates from chickens due to occurrence of more than two pulsotypes, no genetic diversity was observed among the cattle isolates. Thirty staphylococcal isolates (including 27 MRSA and 3 MRS) were screened for the presence of 10 pyrogenic toxin genes. Nine of the 27 (90%) (27/30) MRSA harbored 1 to 5 toxin genes. One organism (ST537, t437) possessed five genes sed + seg + sei + sea + sej, the most predominant toxin genes are seg + sei (20%) (egc cluster). Toxic shock syndrome toxin genes (tsst-1) were found in two (2/30) (6.67%) MRSA and one MRS isolate (1/30) (3.33%). No toxin genes were found in all cattle isolates.

This investigation confirmed the presence of MRSA and MRS from chicken and cattle in Malaysia which was not reported previously. Antibiotic sensitivity tests found more prevalent resistance among the isolates to oxacillin, cefoxitin, erythromycin, cefuroxime, lincomycin, clindamycin, streptomycin, tetracycline and penicillin. Screening of the staphylococcal enterotoxin genes discovered the presence of classical toxin genes (sea, sed and tsst) and preponderance of
newly described toxin genes, which are both implicated in staphylococcal food poisoning. This study highlighted that food animals could serve as a vehicle for the transfer and disseminations of antibiotic resistant bacteria with enterotoxigenic potential to the public thereby making clinical treatment difficult and expensive.
PENCIRIAN MOLEKULAR *Staphylococcus aureus* RENTAN METHICILIN (MRSA) YANG DIPENCILKAN DARIPADA AYAM DAN LEMBU

Oleh

ABDUL KADIR MAGAJI MAGASHI

January 2012

Pengerusi:  Prof. MadyaZunitaZakaria, PhD
Fakulti:  PerubatanVeterinar

*Staphylococcus*

*aureus* telah diakuisecaramelauassebagaisalahsatupatogenutamapadamumanusia yang bertanggungjawabkeatasberbagai penyakit, mulaidarijangkitankulitkepadajangkitan yang lebihmudaratpadasistsarafpusat, pernafasan, jangkitansalurankencingdanjangkitan yang berkaitandengan organ dalaman. Padahaiwan, *S. aureus* menyebabkansejumlahpenyakitbermuladaripadaradangparu-paru, osteomyelitis, jangkitansendipadaunggas, mastitis padalembudanruminankecil. Pendedahan MRSA padahaiwankesayangandanternakan (khinzir, kuda, unggasdanruminan) seringdikaitkandengan MRSA antara haiwandanmanusiamempertingkatkankebimbanganbimbanganbahawahaiwanbolehberfungsisebagaitakunganuntukjangkitan

MRSA. *S. aureus* telah disahkan sebagaisalahsatupatermperingkatkankebimbanganbimbanganbahawahaiwanbolehberfungsisebagaitakunganuntukjangkitan

MRSA. Oleh itu makananberasalhaiwan yang tercemardengan MRSA.
bolehmenimbulkanmasalahkesihatanawam. Bahagianawalkajianinidirekauntuk mengasingandanmengenalpasti S. aureus dengan bantuan kaedah konvensional dan molekul. Empatatus lima puluhsampel buludikumpulkan dan dipadaayam di sembilan ladang ayam di tiga negeri pantaibarat Malaysia, antarapertengahan April 2007 hingga November 2008 dan juga gasaputhidung dikumpulkan dan pada pada 150 lembu di tiga ladang. Kaedah konvensional yang digunakan termasuklah pewarnaan Gram, ujian biokimia, Dryspot Staphytec plus kit (DR0M100 UK) dan media selektif (Mannitol Salt Agar dan Oxacillin Resistance Screening Agar Base) untuk mengesan MRSA pada sampel ayam dan lembu. Berdasarkan ujian biokimia dan ciri-ciri pertumbuhan pada media selektif, 153 (34%) isolat dikenalpasti sebagai S. aureus pada ayam dan terdapat 17 (11,33%) S. aureus pada lembu dari 170 MRSA isolat dikenalpasti secara konvensional. Ujian antitindakan berantai polimerase (PCR) untuk mengesan gen mecA dan nucA turut mengesan 66 sebagai MRSA. Prevalens keseluruhan MRSA dan MSA pada ayam 13.56%. Prevalens MRSA/MRS pada lembu didapati dengan 3.33% MRSA dan 5.33% MRS pada lembu. Bahagian kedua kajian meneliti kerentanan antimikroba secara in-vitro MRSA, MRS dan MSSA 30 agen antimikrobdan penentuan MIC oksasilin mengunakan jalur E-test (AB Biodisk Sweden). MIC oksasilin untuk MRSA berkisar antara 0.5μg / L untuk ≥ 256μg / L. Menggunakan araskerentanan antimikrob secara in-vitro MRSA, MRS dan MSSA dengan MIC oksasilin untuk MRSA berkisar antara 0.5μg / L untuk ≥ 256μg / L. Menggunakan araskerentanan antimikrobditetapkan oleh CLSI, (2006) ≥ 4μg / L, 42 MRSA (68.85%) tahan sepenuhnya dan 19 (31,15%) tahan separuh dengan araskerentanan antimikrobdit tetapkan. Empat puluhsatu (41) Staphylococcus rentan methicillin

ix
berdasarkankriteria CLSI untukoksasilin E-test dan 20 isolatmempunyai MIC lebihrendah.

toksinpirogen. Sembilan dari 27 MRSA mempunyai gen toksin. Satuorganisma (ST537, t437) mempunyailima gen sed + seg + sei + sea + sej, gen toksin paling dominanadalahseg + sei (20%) (kelompokegc). Gen sindromkejutantoksik(tsst-1) ditemuipadaduaisolat (6,67%) MRSA dansatuisolat MRS. Tidakada gen toksin yang ditemuipadaisolatlembu. Penemuaninimempunyaiimplikasikeataskesihatanawam yang memungkinkanrawatanklinikaladalahrumitdanmahal.
ACKNOWLEDGEMENTS

In the name of Allah the most beneficent and most merciful, all praise are bestowed upon him and Prophet Mohammad PBU for granting the successful conclusion of this work. I would like to express my profound gratitude to my chairperson supervisory committee in the name Assoc. Prof. Dr.Zunita Zakaria for her endless support, guidance and patience throughout the period of this study. I would remain indebted to her for grooming a person with no knowledge of molecular biology to this juncture of just beginning to learn. And thanks for guiding me through the art of article writing for publication.

Exceptional gratitude is extended to my co-supervisors, Assoc. Prof. Dr. Goh Yong Meng for exposing me to statistical analysis software’s particularly SPSS and Excel, Prof. Dr. Saleha Abdul Aziz and Prof. Dr. Son Radu for their contributions since the onset of this work during supervisory committee meetings and to painstakingly going through the whole write-up of this thesis despite their tight schedules. I will not forget to thank Dr. Jalila Abu for organizing my sampling trips diligently. Assistance rendered by the staff members of Bacteriology lab is acknowledged with thanks to Mr. Hajaraih, Mr. Hafiz and Ms. Krish.
I wish to express my profound gratitude to Kano State government for sponsoring my study and to Bayero University, Kano for paying my tuition fees through Mac Arthur foundation.

I enjoyed the company of my friends MohdAjiya, Ibrahim Anka, SalisuBuhari, Mustapha Abubakar, Erkyhun, Badlishah, Yitbarek, Hanini and others with whom I discussed and shared information of our respective studies in order to assist one another. Last but not the least I salute the courage of my wife Zahra and my kids for preserving the agony of my absence for the period of this study.
I certify that a Thesis Examination Committee has met on 4th January, 2012 to conduct the final examination of Abdulkadir Magaji Magashi on his thesis entitled “Molecular characterization and Antibiotic susceptibility of Methicillin Resistant *Staphylococcus aureus* isolated from chickens and cattle” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Assoc Prof Dr Siti Khairani Bejo Ph.D.**
Lecturer
Faculty of Veterinary Medicine
(Internal Examiner)

**Assoc Prof Dr Abdul Rahim Matalib Ph.D.**
Lecturer
Faculty of Veterinary Medicine
(Internal Examiner)

**Professor Ian Robertson Ph.D.**
Professor
School of Veterinary and Biomedical Sciences
Murdoch University, Western Australia
(External Examiner)

________________________________________
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies

Universiti Putra Malaysia

Date: 2 March 2012
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of philosophy. The members of the Supervisory Committee were as follows:

**Zunita Zakaria, PhD**  
Associate professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Saleha Abdul Aziz, PhD**  
Professor  
Faculty of veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Goh Yong Meng PhD**  
Associate professor  
Faculty of veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Son Radu, PhD**  
Professor  
Faculty of Food Biotechnology  
Universiti Putra Malaysia  
(Member)

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia  

Date
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

______________________________________________________________

ABDULKADIR MAGAJI MAGASHI
Date: 4th January 2012
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>viii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xiv</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xxi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xxiii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxv</td>
</tr>
</tbody>
</table>

## CHAPTER

1  INTRODUCTION

1.1 Introduction

2  LITERATURE REVIEW

2.1 Introduction

2.2 Taxonomy of *Staphylococcus aureus*

2.3 Emergence of Methicillin Resistant *Staphylococcus aureus*

2.4 Epidemiology of *Staphylococcus aureus* and MRSA in human

2.5 Epidemiology of MRSA in Animals

2.5.1 MRSA in dogs and cats

2.5.2 MRSA in horses

2.5.3 MRSA in cattle, pigs and chickens

2.6 Pathogenesis of *S. aureus* infection

2.6.1 Mechanism of MRSA virulence

2.6.2 Accessory gene regulator (*agr*)

2.7 Evolution of MRSA

2.7.1 Origin of *mec* element
3.2.4 Polymerase Chain Reaction (PCR) 51
   3.2.4.1 DNA extraction 51
   3.2.4.2 Polymerase chain reaction 52
3.3 Results 54
3.4 Morphological and biochemical characteristics of Staphylococcus aureus 54
   3.4.1 Characteristics of the S. aureus isolates on selective media 57
3.5 Detection of meca and nucA genes using Polymerase Chain Reaction (PCR) 68
3.6 Discussion 62
3.7 Conclusion 72

4 ANTIBIOTIC SUSCEPTIBILITY TEST ON STAPHYLOCOCCUS AUREUS ISOLATES
   4.1 Introduction 74
   4.2 Materials and methods 78
      4.2.1 Bacterial isolates 78
      4.2.2 Minimum inhibitory concentration procedure 78
      4.2.3 Disk diffusion procedure 78
   4.3 Results 80
   4.4 Discussion 102
   4.5 Conclusion 113

5 MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS
   5.1 Introduction 114
   5.2 Materials and methods 117
      5.2.1 Pulse field gel electrophoresis 117
         5.2.1.1 Preparation of isolates 117
         5.2.1.2 Preparation of plugs 118
         5.2.1.3 Plug lysis 118
         5.2.1.4 Restriction digestion 119
         5.2.1.5 Electrophoresis 120

xxvii
5.2.2 Multi locus sequence typing (MLST) 120
  5.2.2.1 Genomic DNA extraction 121
  5.2.2.2 Amplification of *S. aureus* housekeeping genes 122
5.2.3 Spa typing 123
5.2.4 Screening for pyrogenic toxin genes 124
5.3 Results 126
5.4 MLST PCR 126
5.5 Spa typing PCR 134
5.1 Pulse field gel electrophoresis (PFGE) 136
5.2 Pyrogenic toxin detection 142
5.3 Discussion 146
5.4 Conclusion 160

6 GENERAL DISCUSSION
  6.1 General discussion and conclusion 162
  6.2 Conclusion 178
  6.3 Future Research 182

REFERENCES 184
APPENDICES 224
BIODATA OF STUDENT 227
LIST OF PUBLICATIONS 228
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.1</td>
<td>Farm identification and locations</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Primers sequences</td>
</tr>
<tr>
<td>3.5.1</td>
<td>The number of the Methicillin Resistant <em>Staphylococcus aureus</em> (MRSA) and Methicillin resistant Staphylococci isolated from broiler chickens and cattle</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Antibiotics used for the disk susceptibility test</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Resistogram profiles of Methicillin Resistant <em>Staphylococcus aureus</em> (MRSA) isolated from chickens</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Resistogram profiles of Methicillin Resistant Staphylococci (MRS) isolated from chickens</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Resistogram Profiles of Methicillin Susceptible <em>Staphylococcus aureus</em> isolated from chickens</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Resistogram Profiles of Methicillin Resistant <em>Staphylococcus aureus</em> isolated from cattle</td>
</tr>
<tr>
<td>4.3.5</td>
<td>Resistogram Profiles of Methicillin Resistant Staphylococci (MRS) isolated from cattle</td>
</tr>
<tr>
<td>4.3.6</td>
<td>Resistogram Profiles of Methicillin Susceptible <em>Staphylococcus aureus</em> (MSSA) isolated from cattle</td>
</tr>
<tr>
<td>4.3.7</td>
<td>E-test for MRSA, MRS and MSSA isolates from chicken and cattle</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Sequence of MLST primers of the seven housekeeping genes</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Staphylococcal toxin primers</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Summary of sequence results for the single locus of <em>S. aureus</em> seven housekeeping genes and the multiple locus – sequence types (STs) obtained from the MLST database.</td>
</tr>
<tr>
<td>5.5.1</td>
<td>The summary of the spa sequencing analysis to determine the repeat numbers and the spa type</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Toxin genes detected in MRSA and MRS isolates in chickens</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6.1</td>
<td>The relationship establishing the agr sensing system</td>
<td>21</td>
</tr>
<tr>
<td>2.7.1</td>
<td>The global distribution of MRSA clones</td>
<td>24</td>
</tr>
<tr>
<td>2.7.2</td>
<td>The variations in the J regions within the same mec-ccr combinations are used for defining SCCmec subtypes and variants</td>
<td>28</td>
</tr>
<tr>
<td>2.8.1</td>
<td>The mechanism of resistance of major classes of antibiotics</td>
<td>33</td>
</tr>
<tr>
<td>2.8.2</td>
<td>The mechanism to glycopeptides resistance pattern</td>
<td>33</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Colony morphology of a <em>Staphylococcus aureus</em> on blood agar</td>
<td>55</td>
</tr>
<tr>
<td>3.4.2</td>
<td><em>Staphylococcus aureus</em> are gram positive cocci, in clusters. Some may appear singly, in pairs or tetrads</td>
<td>55</td>
</tr>
<tr>
<td>3.4.3</td>
<td>Catalase test A and Coagulase test B</td>
<td>56</td>
</tr>
<tr>
<td>3.4.4</td>
<td>Dryspot cartridge for coagulase test</td>
<td>56</td>
</tr>
<tr>
<td>3.4.5</td>
<td><em>Staphylococcus aureus</em> on Oxacillin Resistance Screening Agar Base (ORSAB) plate with intense blue colonies indicating resistance to methicillin</td>
<td>57</td>
</tr>
<tr>
<td>3.4.6</td>
<td>Staphylococcus aureus isolates on Mannitol Salt Agar plate exhibiting yellow colored colonies</td>
<td>57</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Agarose gel electrophoresis of PCR product of mecA and nucA gene from chickens MRSA isolates from a farm in Ayer Hitam, Johor Bahru</td>
<td>58</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Agarose gel electrophoresis of PCR product of mecA and nucA gene from chickens MRSA isolates from a farm in Ayer Hitam, Johor Bahru.</td>
<td>59</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Agarose gel electrophoresis of PCR product of mecA and nucA gene from cattle isolates from three farms</td>
<td>59</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Antibiotic resistance profiles of MRSA from chickens</td>
<td>84</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Antibiotic resistance profiles of MRS from chickens</td>
<td>89</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Antibiotic resistance profiles of MSSA from chickens</td>
<td>92</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Antibiotic resistance profiles of MRSA from cattle</td>
<td>96</td>
</tr>
<tr>
<td>4.3.5</td>
<td>Antibiotic resistance profiles of MRS from cattle</td>
<td>97</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-MRSA</td>
<td>Community associated methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CNS</td>
<td>Coagulase Negative Staphylococci</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxyribonucleotide triphosphate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethyldiaminetetraacetic acid</td>
</tr>
<tr>
<td>EMRSA</td>
<td>European methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GI</td>
<td>genomic Island</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>Hospital associated methicillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MDR</td>
<td>multi drug resistance</td>
</tr>
<tr>
<td>MH</td>
<td>Muller Hinton Agar</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>Min</td>
<td>minute</td>
</tr>
<tr>
<td>ML</td>
<td>Milliliter</td>
</tr>
<tr>
<td>MLST</td>
<td>Multi locus sequence typing</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin Resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>MSA</td>
<td>Mannitol Salt Agar</td>
</tr>
<tr>
<td>MSSA</td>
<td>Methicillin susceptible <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>ORSA</td>
<td>Oxacillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ORSAB</td>
<td>Oxacillin Resistance Screening Agar Base</td>
</tr>
<tr>
<td>PBP</td>
<td>penicillin binding protein</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffer saline</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PVL</td>
<td>Panton ValantineLuekocidin</td>
</tr>
<tr>
<td>S</td>
<td>second(s)</td>
</tr>
<tr>
<td>SE</td>
<td>Staphylococcal enterotoxin</td>
</tr>
<tr>
<td>SFP</td>
<td>Staphylococcal food poisoning</td>
</tr>
<tr>
<td>TBE</td>
<td>tris-borate-EDTA buffer</td>
</tr>
<tr>
<td>TE</td>
<td>tris-EDTA buffer</td>
</tr>
<tr>
<td>V</td>
<td>volt</td>
</tr>
<tr>
<td>VISA</td>
<td>Vancomycin intermediate Staphylococcus aureus</td>
</tr>
<tr>
<td>α</td>
<td>alpha</td>
</tr>
<tr>
<td>β</td>
<td>beta</td>
</tr>
<tr>
<td>λ</td>
<td>lambda</td>
</tr>
<tr>
<td>ψ</td>
<td>pseudo</td>
</tr>
<tr>
<td>%</td>
<td>percentage</td>
</tr>
<tr>
<td>Δ</td>
<td>delta</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µL</td>
<td>Microliter</td>
</tr>
<tr>
<td>bp</td>
<td>Base pairs</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Introduction

*Staphylococcus aureus* has long been recognized as one of the major human pathogens responsible for a wide range of afflictions from minor infections of the skin to wound infections, infections of the central nervous system, respiratory and urinary tracts, and those associated with intravascular devices and foreign bodies (Enright *et al.*, 2000). Most *S. aureus* strains are opportunistic pathogens that can colonize individuals, without symptoms, for either short or extended periods of time, causing disease when the immune system is compromised. They are part of the normal flora on the skin and mucus membrane of healthy animals and humans and sometime is casually found in the environment (Henton, 2004).

In animals, *S. aureus* is implicated with diseases in a wide range of animal species. In poultry it causes pneumonia, osteomyelitis, joint infections and septicemia (Alfonso and Barnes, 2006; Butterworth, 1999; Cervantes *et al.*, 1988), subcutaneous abscess, pododermatitis and mastitis in rabbits (Hermans *et al.*, 2003). In horse, *S. aureus* causes dermatitis and cellulitis, and septicemia in pigs (Devriese, 1990). No doubt *S. aureus* has a
significant pathogenic role as a cause of intramammary infections in cattle and other small ruminants (Wang et al., 2008) leading to substantial economic losses in cattle farming industry (Hughes et al., 2008).

In the early 1940’s, penicillin was discovered and were able to treat all S. aureus derived infections. However, the joy was summarily truncated with the first debut of S. aureus resistant to penicillin in 1942 in hospitals and subsequently acknowledged in the community. This resistance is a result of the acquisition of plasmid that encrypts the formation of penicillin hydrolyzing enzyme “penicillase”(Deurenberg et al., 2007; Lowy, 1998). A concerted effort was made to find an alternative cure for infections caused by recalcitrant S. aureus strains, which leads to the production of semisynthetic penicillin called methicillin. Methicillin was approved for clinical use in 1960. Unfortunately its value as a potent drug was severed a year after due to emergence of methicillin resistant S. aureus (MRSA) and methicillin resistant S. epidermidis in both hospitals and community as a consequence of extensive use of methicillin and other semisynthetic penicillins (Stevens, 2003). Henceforth the acronym methicillin resistant Staphylococcus aureus (MRSA) was used to describe a group of S. aureus that are resistant to methicillin and by extension are resistant to all accessible β-lactam antibiotics including penicillin and cephalosporins (Babel and Decker, 2008). MRSA are strains that have oxacillin minimum inhibitory concentration (MIC) of ≥4μg/mL(Baldoni et al., 2009).
After the discovery of MRSA in UK in 1961, MRSA became pandemic worldwide by mid 1990’s (Loeffler et al., 2005). This defining moment came with the report on the clinical prevalence of MRSA from various European countries which was over 40%, such as Romania (61.4%), Portugal (50%) United Kingdom (43.6%), Greece (42.1%), and Ireland (41.8%) (EARSS, 2005). Some other European countries reported prevalence lower than 40%: Kresken and others, (2004), documented a prevalence of 20.7% in Germany and in Spain 30.5% of MRSA in Spanish hospitals was recorded. A similar SENTRY programme (1997-99) revealed the MRSA incidence in Italy (50.5%), Turkey (37.5%), Greece (34.4%) and Poland (25.5%) (Cuevas et al., 2007; Diekema et al., 2001). Surveillance data for MRSA for year 2004-2005 in USA reported the prevalence of 55.7% among inpatients and 48.7% among the outpatients (Pillar et al., 2008). The national surveillance of MRSA in Trinidad and Tobago documented 12.8% from the three major regional hospitals (Akpa et al., 2006). Among the Asian countries the percentage of MRSA strains vary among countries with 23.6% in Australia, Taiwan (88.2%), China (80%), Korea (70%), Singapore and Japan (83%) each (Aires-de-Sousa et al., 2008; Kim et al., 2003; Voss and Doebbeling, 1995).

During the earlier period of MRSA infections, it was primarily a nosocomial pathogen, but recent surveillance studies have indicated that some MRSA clones are colonizing a significant proportion of healthy individuals in the community, giving rise to community acquired MRSA (CAMRSA), facilitating Comment [C1]: Comments z1,2 & 3 are addressed. Kadir
Comment [u2]: Add – diseases caused by MRSA
disease spread from human to human, and from human to domestic animals through contact (Waller, 2005; Witte et al., 2007b; Wulf et al., 2008). There is increasing information on inter-species transmission of MRSA occurring. Weese and others, (2005) demonstrated the same strain of MRSA in horses and their human counterparts. In another study, isolates from cattle, pigs and chickens were studied using the RAPD and found six isolates were identical to human isolates, thus indicating that the isolates were intimately related to human clones of MRSA, although the actual mode of transmission was not clear (Lee, 2006). In Singapore, ST22 was isolated from pigs, and was also found to be widely spread in Singaporean hospitals, a likely probable pointer to human contamination to the pigs (Sergio et al., 2007). Moreover an investigation in Canada showed that 14% of the MRSA isolated from pigs originated from humans (Khanna et al., 2008). Carriage of MRSA among veterinarians and pig farmers had also been reported in Netherlands (Wulf et al., 2006; Wulf and Voss, 2008). The first MRSA isolation from raw chicken meat was documented in Korea (Lee, 2003), in which the six MRSA isolates in the study were indistinguishable from human isolates. Japan reported two MRSA that were isolated from 292 samples of retail raw chicken meat (Kitai et al., 2005).

Additionally, de novo isolation of MRSA from raw chicken meat, pork and beef has been reported with a high prevalence of multi drug resistant S. aureus (Pesavento et al., 2007). The earliest isolation of MRSA was reported from a cow with mastitis (Devriese et al., 1972). The following decade was greeted with more robust data documented on the isolation of MRSA from
mastitic cattle (Juhász-Kaszanyitzky et al., 2007; Kwon et al., 2005; Lee, 2006). A series of epidemiologic studies have shown that, mastitis had a negative impact on the reproductive performance of dairy cows, apart from reduction in milk production and its quality, which poses a significant loss to farmers (Santos et al., 2004). Apart from milk, MRSA had been reported in raw meat and meat products, in Netherlands, a prevalence rate of 16% of MRSA was reported from chicken meat, beef 10.6%, lamb and mutton 6.2%, Turkey 35.3%, fowl 3.4%, pork 10.7%, veal 15.2% and game 2.2% (de Boer et al., 2008). A similar study in Jordan revealed MRSA suspected of human origin were actually from chicken meat (Quddoumi et al., 2006).

*Staphylococcus aureus* is acknowledged to be responsible for causing food intoxication (Mead et al., 1999) by producing many types of Staphylococcal enterotoxins (Balaban and Rasooly, 2000; Zschöck et al., 2005). The enterotoxins act as superantigens that cause immunosuppression and elicit the proliferation of T-cells coupled with high fever (Rosec et al., 1997). MRSA with potential to produce enterotoxins can likely be found on raw meat or meat products, however in one study enterotoxigenic strains of MRSA were isolated from foods of animal origin in Italy, which showed resistance to at least one of the antibiotics tested (Normanno et al., 2007). Toxin producing MRSA of Brazilian clone lineage was reported from two hospital kitchen workers in Teresina, Brazil (Soares et al., 1997). In that study the overall prevalence of enterotoxigenic *S. aureus* was 32.6%. Data from another study documented a prevalence of 2.5% MRSA ST393 that are toxigenic from raw meat samples in
Netherlands (van Loo et al., 2007). Other investigators characterized food derived oxacillin resistant *S. aureus* and found eight isolates from 132 had MIC 2-4µg/ml and one of the two isolates with MIC 4µg/ml was enterotoxigenic. The borderline resistant isolates were found genetically interrelated to strains associated with human infections (Bystron et al., 2010a).

Currently in Malaysia, researchers have mainly concentrated on human MRSA. The reported prevalence of MRSA in Malaysian hospitals was 19% in 1992 (Cheung et al., 2004) and it rose to 35% in 1998 (Rohani et al., 2000). Sam and others, (2008), revealed the first clinical isolate of CA-MRSA that carry SCCmec IV, principally cause health care associated skin and soft tissue infections in Malaysia. Once again a study documented a new MRSA strain that is not indigenous to Malaysia, ST772 which was originally identified in Bangladesh (Neela et al., 2009b). Limited studies have been carried out on MRSA in the veterinary setting. Nonetheless, Neela and others, (2009a), investigated MRSA in 360 pigs and 90 pig handlers and identified a novel ST9 MRSA that colonized 1% pigs and 5.5% pig handlers. No data exists on the occurrence of MRSA in chickens and cattle.

Food animals that are colonized with MRSA pose a significant public health concern as they serve as reservoirs for the dissemination of MRSA in the community, and moreover during slaughtering may cause contamination of carcasses, the environment and the meat. These animal carcasses would serve as a possible source of human infection as a consequence of eating
contaminated food products from these animals (Kitai et al., 2005; Normanno et al., 2007). The microbiological safety of food must be assured in order to prevent transmission of pathogen or opportunistic microorganisms to consumer, in hazard analysis and critical control point system in food chain. Undoubtedly, MRSA pandemic is now a very serious problem confronting the world in terms of enormous healthcare financial burden and monitoring is one of the best control measures to avert outbreak. The economic consequences of MRSA in New York USA revealed a direct medical cost per patient of $35,000 and $28000 for CA-MRSA and HA-MRSA (Shorr, 2007) and in Canada the recent epidemiological data reported a MRSA financial burden of $82 million in 2004 (Goetghebeur et al., 2007). The financial responsibility of the MRSA scourge in animals has been highlighted; the average cost for the treatment of a single Danish Holstein cow infected with staphylococcal mastitis was estimated at £149 to £570 (Sørensen et al., 2010).

In Malaysia, chicken meat is most popular and a cheap source of protein, largely because there are no religious taboos against the consumption of chicken meat as applied to beef and pork (Ramlah, 1993). Malaysian consumer demands safe and high quality meat/food at affordable price from the poultry industry. The poultry industry is constantly challenged to produce products at reasonable price without compromising the quality (USDA., 2006). Exports of poultry and poultry products have been expanded to Japan, Brunei, Hong Kong, Bangladesh, Philippines and Indonesia, apart from Singapore (http://www.thepoultrysite.com). Given the MRSA prevalence and its clinical
burden and the economic consequences attached to it, it is fundamental to investigate the rate of MRSA occurrence in some food animals including cattle and chickens as they serve as a primary source of livestock associated with MRSA, as well as it has been suggested that they are involved in MRSA transmission.

The objectives of this study are:

a) to isolate and identified MRSA from healthy chicken and cattle.

b) to determine the antibiogram and minimum inhibitory concentrations of MRSA isolates.

c) to detect mecA and nucA gene in MRSA from chicken and cattle.
REFERENCES


Staphylococcus aureus has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. *The Journal of infectious diseases* 192: 801-810.


Brian, M., 2010. Mupirocin-resistant MRSA transmission associated with community hospitals and nursing homes. The Journal of hospital infection 75;141-142


producing *Staphylococcus aureus*. *FEMS immunology and medical microbiology* 51: 220.


fluoroquinolone MICs. *Diagnostic Microbiology & Infectious Disease* 46: 139-145.


staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy* 45: 1323.


in a microepidemiological setting. *Journal of Clinical Microbiology* 40: 3764.


resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *Apmis* 111: 905-914.


