UNIVERSITI PUTRA MALAYSIA

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

ABBULKADIR MAGAJI MAGASHI

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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 greatgrandfather 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

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ABDULKADIR MAGAJI MAGASHI

January 2012

Chairman: Assoc. Prof. ZunitaZakaria, 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 $p = 0.05$, $x^2 = 0.1662$. Norminal 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, DryspotStaphytect 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 22pulsotypes 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

AbstraktesisinidikemukakankepadaSenatUniversiti Putra Malaysia
sebagaimemenuhikeperluanuntukDoktorFalsafah

PENCIRIAN MOLEKULAR *Staphylococcus aureus*RENTAN METHICIILIN (MRSA) YANG DIPENCILKAN DARIPADA AYAM DAN LEMBU

Oleh

ABDUL KADIR MAGAJI MAGASHI

January 2012

Pengerusi: Prof. MadyaZunitaZakaria, PhD

Fakulti: PerubatanVeterinar

*Staphylococcus aureus*telahdiakuisecarameluassebagaisalahsatupatogenutamapadamanusia yang bertanggungjawabkeatasberbagaipenyakit, mulaidarijangkitankulitkepadajangkitan yang lebihmudaratpadasistemsarafpusat, pernafasan, jangkitansalurankencingdanjangkitan yang berkaitandengan organ dalaman.Padahaiwan, *S. aureus*menyebabkansejumlahpenyakitbermuladaripadaradangparu-paru, osteomyelitis, jangkitansendipadaunggas, mastitis padalembudanruminankecil. Pendedahan MRSA padahaiwankesayangandanternakan (khinzir, kuda, unggasdanruminan) seringdikaitkandengan MRSA antarahaiwandanmanusiamempertingkatkankebimbanganbahawahaiwanbolehberfungsisebagaitakunganuntukjangkitan MRSA.*S.aureus*telahdisahkansebagaisalahsatusuperantigenterkemukadanjikatermakanmenyebabkankeracunanmakanan.Olehitumakananberasalhaiwan yang tercemardengan MRSA

bolehmenimbulkanmasalahkesihatanawam.Bahagianawalkajianinidirekauntukmengasingdanmengenalpasti*S. aureus*denganbantuankaedahkonvensionaldanmolekul.Empatratus lima puluhsampelbuludikumpulkandaripadaayam di sembilanladangayam di tiganegeripantaibarat Malaysia, antarapertengahan April 2007 hingga November 2008 danjugasaputanhidungdikumpulkandaripada 150 lembu di tigaladang. Kaedahkonvensional yang digunakantermasuklahpewarnaan Gram, ujianbiokimia, DryspotStaphytect plus kit (DR0M100 UK) dan media selektif (Mannitol Salt Agar danOxacillin Resistance Screening Agar Base) untukmengesan MRSA padasampelayamdanlembu. Berdasarkanujianbiokimiadanciri-ciripertumbuhanpada media selektif, 153 (34%) isolatdikenalpastisebagai*S. aureus*daripadaayamdanterdapat 17 (11,33%) *S. aureus*daripadalembu,dari 170 MRSA isolatdikenalpastisecarakonvensional. Ujiantindakanberantaipolimerase (PCR) untukmengesan gen *mecA*dan*nucA*turutmengesan 66 sebagai MRSA. Prevalenskeseluruhan MRSA dan MSA padaayam 13.56%.Prevalens MRSA/MRS padalembudicerapdengan 3.33% MRSA dan 5.33% MRS padalembu.Bahagiankeduakajianinimenelitikerentananantimikrobsecara in-vitro MRSA, MRS dan MSSA 30 agenantimikrobdanpenentuan MIC oksasilinmenggunakanjalur E-test (AB Biodisk Sweden). MIC oksasilinuntukayam MRSA berkisarantara 0.5µg / L untuk ≥ 256µg / L. Menggunakanaraskerentananoksasilinditetapkanoleh CLSI, (2006) ≥ 4µg / L, 42 MRSA (68.85%) tahansepenuhnyadan 19 (31,15%) tahanseparadenganaras yang telahditetapkan. Empatpuluhsatu (41) *Staphylococcus* rentan methicillin

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

Terdapatpolakerentanan yang umum di antara MRSA oksasilin, cefoxitin, tetrasiklin, clindamisin, lincomycin, neomisin, eritromisin, penisilin G, streptomisindancefuroxine.Kesemua 170 isolatadalahpekaterhadap linezolid dandenganpengecualiandaripadadua MRSA dandua MRS, yang selebihnyapekamupirocindanteicoplanin.Latarbelakanggenetikbeberapaisolatde nganmenggunakanpelbagaijeniskaedahsepertipenjujukanberbilang loci (multilocus sequence typing (MLST), Spa typing, Elektroforesis gel lapangandedenyut (Pulse Field Gel Electrophoresis(PFGE) denganpengesanantoksinpirogentelahdilakukan. MLST menciri 12 MRSA isolatkedalamsebelas sequence typing ST9, ST15, ST14, ST537, ST190, ST194, ST795, dan ST1279 padaayamdan ST59, ST35 dan ST573 padalembu. Duabelasisolatinidikelompokkankedalamjenis Spa 5 't437, t442 t360, t189 dan t5696.Analisisperatusan PFGE makropembatas, polapersamaandikenalpastidaripadadendogramdandidapatisebanyak 80% persamaandigunakanuntukmendefinisikanpulso-tip.Analisis PFGE mengenalpasti 22 pulso-tipdengansembilanjenis sub dan cluster paling umumadalah cluster C yang munculpadaempatladang. Cluster B adalahserupawalaupunmempunyaijenis Spa yang berbeza. Kepelbagaianterjadi di antaraisolatdariayamkeranaterjadinyalebihdariduapulsotip.Tidakadakepelbagaia ngenetik di antaraisolatlembu.Tigapuluhisolatdipilihuntukkehadiran 10 gen

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

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APPROVAL

I certify that a Thesis Examination Committee has met on **4th January,2012**to conduct the final examination of AbdulkadirMagajiMagashi 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:

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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:

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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.

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ABDULKADIR MAGAJI MAGASHI
Date: 4th January 2012

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LIST OF ABBREVIATIONS

CA-MRSA	Community associated methicillin resistant *Staphylococcus aureus*
CFU	colony forming unit
CNS	Coagulase Negative Staphylococci
dNTP	deoxyribonucleotide triphosphate
EDTA	Ethyldiaminetetraacetic acid
EMRSA	European methicillin resistant *Staphylococcus aureus*
g	gram
GI	genomic Island
HA-MRSA	Hospital associated methicillin resistant *Staphylococcus aureus*
MDR	multi drug resistance
MH	Muller Hinton Agar
MIC	minimum inhibitory concentration
Min	minute
ML	Milliliter
MLST	Multi locus sequence typing
mM	Millimolar
MRSA	Methicillin Resistant Staphylococcus aureus
MSA	Mannitol Salt Agar
MSSA	Methicillin susceptible *Staphylococcus aureus*
MW	molecular weight
^{o}C	degree Celsius
ORSA	Oxacillin resistant *Staphylococcus aureus*

ORSAB	Oxacillin Resistance Screening Agar Base
PBP	penicillin binding protein
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PVL	Panton ValantineLuekocidin
S	second(s)
SE	Staphylococcal enterotoxin
SFP	Staphylococcal food poisoning
TBE	tris-borate-EDTA buffer
TE	tris-EDTA buffer
V	volt
VISA	Vancomycin intermediate Staphylococcus aureus
α	alpha
β	beta
λ	lambda
Ψ	pseudo
%	percentage
Δ	delta
µg	microgram
µL	Microliter
bp	Base pairs

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 (Akpaka *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

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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), inwhich 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 Stapylococcal 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 healthychicken and cattle.

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

c) to detect *mec*A and *nuc*A gene in MRSA from chicken and cattle.

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