Incidence, Molecular Characterization and Antibiotic Resistance Profile of Methicillin Resistant *Staphylococcus aureus* Isolated from Clinical Origin and Associated Surroundings in Hospital

By

Faria Marha
15136021

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Biotechnology

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Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

FARIA MARHA
Student ID: 15136021

Approval

The thesis titled "Incidence, molecular characterization and antibiotic profile of methicillin-resistant *Staphylococcus aureus* isolated from the clinical origin and associated surrounding in hospital" submitted by

1. Faria Marha (15136021)

of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology on 3rd October 2019.

Examining Committee:

Supervisor:
(Member)

Dr. Mahboob Hossain
Professor, Mathematics and Natural Sciences
Brac University

Co-supervisor:
(Member)

Dr. Zahid Hayat Mahmud
Associate Scientist, Project Coordinator and Head
Laboratory of Environmental Health
Laboratory Sciences and Services Division
icddr,b

Program Coordinator:
(Member)

Iftekhar Bin Naser
Assistant Professor, Mathematics and Natural Sciences
Brac University

Departmental Head:
(Chair)

Prof. A. F. M Yusuf Haider
Chairperson, Mathematics and Natural Sciences
Brac University

Ethics Statement

This study has been conducted with samples from the Swab study performed by International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) and the consent was taken in agreement to use the samples for thesis purpose.

Abstract/ Executive Summary

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is notorious for its resistance to Penicillin derivative antibiotics and is also resistant to multiple drugs. In Bangladesh, the prevalence of MRSA is a staggering 72%. This study takes an approach to investigate the incidence, molecular characterization and antibiotic profiling of MRSA from clinical as well as associated surroundings in hospitals. Initial screening was done using selective and differential media and then confirmed by PCR against *nuc, mecA* and *femA* gene. The nuc^+ isolates were tested against various antibiotic classes to produce an antibiotic profile. Isolates taken from hand swabs were tested for their susceptibility against hand sanitizers. 68.9% of the isolates in this study were proved to be MRSA through detection of *nuc*, *femA* and *mecA* and subsequent disk diffusion test revealed that 90.9% of those isolates were non-susceptible to multiple drugs and surprisingly a 100% of the isolate found on hand were unresponsive to hand sanitizers. Currently, we are at the cusp of a time when to combat the emergence of such a Hospital Associated Infection (HAI) causative superbug extensive research into its cultural along with molecular machinery is imperative. This thesis aims to investigate the incidence of MRSA in hospitalized patients and their immediate surroundings and their characterization.

Keywords: HA-MRSA; MDR-MRSA; hand sanitizers; *mecA*; *femA*; *nuc*; HAI; PCR.

Dedication

Dedicated to Abbu, Ammu, Arup Bhaiya and Laaz Apu.

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List of Acronyms

DNA	Deoxyribonucleic Acid
HA-MRSA	Extremely drug-resistant Healthcare Associated Methicillin Resistant *Staphylococcus aureus*
HAI	Hospital Associated Infection
MRSA	Methicillin Resistant *Staphylococcus aureus*
MDR-MRSA	Multiple Drug Resistant Methicillin Resistant *Staphylococcus aureus*
PBP2a	Penicillin Binding Protein 2a
RNA	Ribonucleic Acid

Chapter1
Introduction

1.1 Basic characteristics and morphology

Staphylococcus aureus, or as affectionately called staph, pertains to the population of the microbiota living in the human body. Its taxonomy is as follows:

Domain: Bacteria
Phylum: Firmicutes
Class: Cocci
Order: Bacillales
Family: Staphylococcaceae
Genus: *Staphylococcus*
Species: *S. aureus*

From the nomenclature (Becker, Heilmann, & Peters, 2014), its morphology can be inferred as coccus and under a microscope; they appear in grape-like clusters of cocci. Asexual reproduction via binary fission where autolysin is not present or is specifically inhibited prevents daughter cells from diverging; thereby, conferring to a cluster-like morphology (Varrone et al., 2014). Moreover, it is known to be non-motile and incapable of forming spores (Namvar et al., 2014; Pollitt & Diggle, 2017).

S. aureus is a facultative anaerobe, surviving in the absence of oxygen by fermentation of glucose to produce lactate, also, it can reduce nitrate to nitrite (Fuchs, Pané-Farré, Kohler, Hecker, & Engelmann, 2007). Also, ferment mannitol which allows staph to be distinguishable from its Genus (Kenny et al., 2013). The Gram-positive result of Gram staining for staph indicates to thick peptidoglycan that retains crystal violet dye post decolourization via alcohol. (Murdoch & Greenlees, 2004). *S. aureus* produces catalase enzyme than can disintegrate deleterious hydrogen peroxide to effervesce oxygen gas and water. Subsequently, it is termed catalase-positive (de la Fuente, Díez, Domínguez-Bernal, Orden, & Martínez-Pulgarín, 2010). Usually, staph is coagulase secreting bacteria. This enzyme coagulates or clots plasma through initiating the conversion of fibrinogen to fibrin (McDevitt, Vaudaux, & Foster, 1992). The genome of *S. aureus* is armed with a DNA and RNA extracellular endonuclease. It is called TNase; an

endonuclease with thermostable quality encoded by the *nuc* gene whose performance sustains a temperature of 100°C for an hour. Using TNase encoding gene, *nuc*, as a characteristic defining quality of *S. aureus*, bacterial identification can be performed via Polymerase Chain Reaction (PCR) amplification (Brakstad, Aasbakk, & Maeland, 1992).

1.2 Epidemiology

1.2.1 Carriage

An astounding array of about 10^{14} microbes reside on a human body and those bacteria are referred to as normal microbial flora which is present on the skin and mucosal surfaces, post-birth and persists till death. *S. aureus* is a perennial inhabitant of the nose, perineum (10-40%) and vulva skin (60%). Moreover, the incidence of staph on dermatological disease-ridden skin is as high as 80 to 100 percent (Davis, 1996). *S. aureus* resides on roughly three-tenth of the *Homo sapiens* population as a commensal organism, although they are capable of infecting the body when an opportunity arises. Besides, this pathogen is a prime determinant in bacteremia and infective endocarditis (IE) along with osteoarticular; skin and soft tissue; pleuropulmonary; and device-related infections (Davis, 1996).

1.2.2 Transmission

S. aureus can be transmitted from person to person as well as through fomites and unwashed hands (McLaws, Pantle, Fitzpatrick, & Hughes, 2009). As the organisms are transmitted, it may become established as the recipients' normal flora and later be introduced to a sterile site. Person to person spread of *S. aureus* infections in hospital as well as in the community presents challenges. Community-based infections are increasing and it is the transmission of these organisms that have acquired antibiotic resistance that is of most concern (Munckhof et al., 2009).

1.2.3 Risk Factors

As aforementioned, staph usually are not pathogenic but are opportunistic organisms thereby, any individual can be affected; more so if they already are immuno-compromised or have surgical incisions, puncture wounds among another form of injury

that could provide a potential point of entry for the pathogen. Furthermore, in healthcare, the risk of more serious staph infection is higher for patients in intensive care units (ICUs), patients who have undergone certain types of surgeries and patients with medical devices inserted in their bodies. Infections can be inflicted by different types of *S. aureus,* including Methicillin-resistant *Staphylococcus aureus* (MRSA) (Sievert et al., 2013).

1.3 Emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA)

In 1959, S. aureus had become resistant to penicillin by deactivating it employing penicillinase enzyme henceforth, a semi-synthetic drug called Methicillin was introduced to abate the infection. Only two years later, in the United Kingdom, cases of Methicillin-resistant Staphylococcus aureus (MRSA) prevailed (Jevons, 1961). The prevalence rate of MRSA documented as a nosocomial pathogen is alarming and it has established itself as a serious concern for public health. More importantly, it has been stated to be in an endemic phase (Johnson et al., 2001). Prescription of Methicillin as a treatment for S. aureus infection a long been ceased, it was the first penicillinase-resistant penicillin to be used in the 1960s. Hence, resistant strains were termed 'methicillin-resistant S. aureus' (MRSA) (Henry F Chambers & DeLeo, 2009).

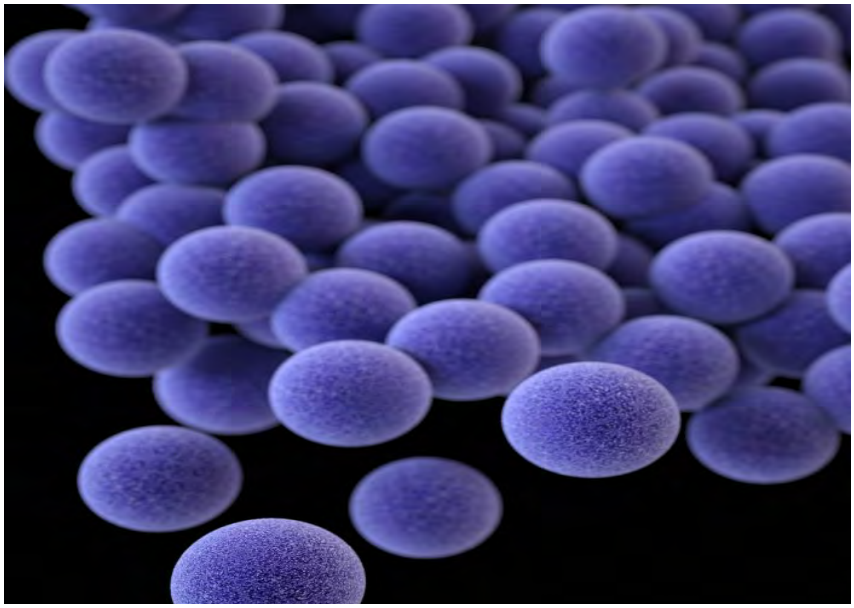


***Figure 1: This illustration depicts a three-dimensional (3D) computer-generated image of a group of methicillin-resistant, Staphylococcus aureus (MRSA) bacteria, which were arranged in a cluster* (Rosario-Cruz et al., 2019).**

1.4 Genomic design of MRSA conferring resistance

The mobile genetic element staphylococcal cassette chromosome *mec* (SSC*mec*) on the genome carries methicillin resistance gene (*mecA*) is supposedly attained from distantly related species which has been horizontally acquired from distantly related species (Hiramatsu, Cui, Kuroda, & Ito, 2001). The penicillin-binding protein PBP2a which is coded by the *mecA* gene is inherently unresponsive to methicillin along with all beta-lactams, even isoxazolyl penicillins, oxacillin. The horizontally attained *mecA* gene provided *S. aureus* protection against all broad-spectrum beta-lactams including cephalosporins although they were initially targeted against Gram-negative organisms (H F Chambers, 1997). SCC*mec* housing the *mecA* gene is inserted into a gene of an unknown function named *orfX.* MRSA strains with varying SCC*mec* allotypes have been discovered and classified as SCC*mec*I–SCC*mec*VIII based on the insertion of *mecA* and cassette chromosome recombinase (*ccr*) and its removal from of the entire cassette that resides in *orfX*. There are also other differences among the various SCC*mec* allotypes, particularly in terms of insertion sequences and antimicrobial resistance genes (Henry F Chambers & DeLeo, 2009; Lee et al., 2018).

The variance in the status of resistance suggests that there might be other factors involved in bestowing high resistive capacity to antibiotics aside from PBP2a. The *femAB* operon entails two proteins contributing to the pentaglycine interpeptide bridge in the cross-linkage of peptidoglycan (Hegde & Shrader, 2001). The encoded protein by the gene *femA* is a 48-kDa protein which is vital for the addition of glycine 2 and 3. Inactive FemA protein attributed to low-level methicillin resistance thus, a strong positive correlation has been observed in the possession of *femA* gene and exhibiting a high level of methicillin resistance. However, FemA protein does not affect the assimilation of PBP2a protein(Kobayashi et al., 1994). Adopting mechanisms to become resistant to an antibiotic is a well-endowed quality possessed by *S. aureus* (Henry F Chambers & DeLeo, 2009). Ultimately, this poses challenges in the prescription of antibiotics while treating diseases where staph is the causative agent.

1.5 Virulence factors

To evade the host's immune system and interfere with its response, the sneaky *S. aureus* displays and secretes a congregation of structural and soluble factors. These structural factors include surface proteins which elevate the affinity of its anchorage to damaged tissue. Furthermore, they bind to blood proteins to prevent being identified by the antibody-mediated immune responses and promote iron uptake (Foster & Höök, 1998; Mazmanian et al., 2003).

During infection, this organism secretes membrane damaging toxins inducing rupture of red blood cells in addition to leukocidin that damage leukocyte membrane. On top of that, systematized secretion of the alpha-toxin invokes septic shock. Similarly, enterotoxins as well as, toxic shock syndrome toxin- 1 (TSST-1) will bring about toxic shock in the affected individual (Foster, 2004). MRSA has adopted a multitude of methods for escaping the host's immune system which encompasses both the polymorphonuclear leukocytes mediated innate immunity along with induced immunity invoked via B and T cell (Bohach, 2006; de Haas et al., 2004; Goodyear & Silverman, 2003). Besides, the pathogenic aptitude of MRSA stems from mobile genetic elements referred to as pathogenicity islands which are responsible for TSST-1, in addition to other enterotoxins (Novick, 2003). Another virulence factor confers to genes expressed by a lysogenic bacteriophage, Panton-Valentine leucocidin (PVL). PVL is a two-component, the beta-pore forming toxin that fuses with cellular membranes of leukocytes and can lead to their lysis (Henry F Chambers & DeLeo, 2009). The chromosomal cassette genome also retains genes capable of production of key components in inhibition of the innate immune system such as chemotaxis inhibitory protein and staphylokinas (de Haas et al., 2004).

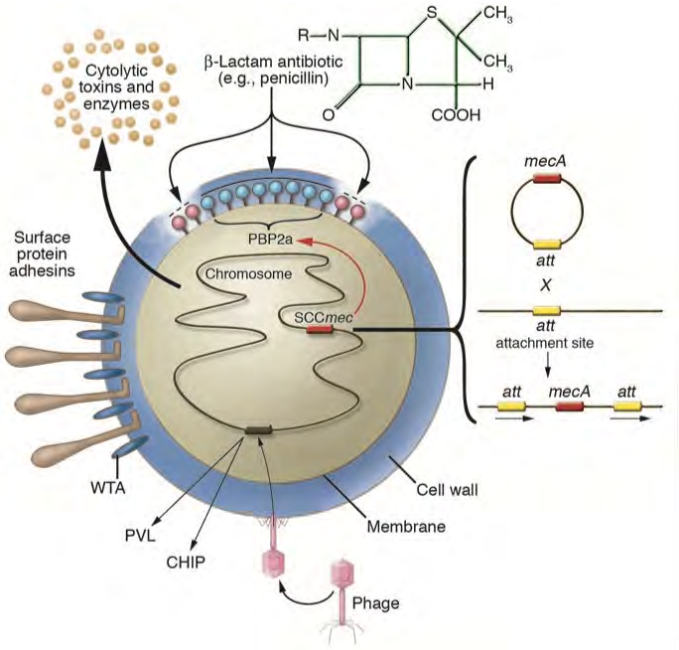


***Figure 2: Illustration of MRSA exhibiting methicillin resistance and expressing virulence factors* (Foster, 2004).**

A depiction of how *S. aureus* exhibits its virulence factors along with its resistance to methicillin is shown in *Figure 2*. The chromosomal gene activation induces the production of many toxins and enzymes. Also, the bacteria expression of surface protein adhesins and cell wall anchored wall teichoic acid (WTA) may colonize in nasal route and skin. Methicillin resistance is obtained by internalization of a horizontally transferred mobile genetic element called SCC*mec*. PBP2a encoded by *mecA* gene is a beta-lactam insensitive penicillin-binding protein, which continues to synthesize new cell wall peptidoglycan even when the normal penicillin-binding proteins are inhibited. Some virulence factors, such as PVL and the chemotaxis inhibitory protein, CHIP, are encoded by genes located on lysogenic bacteriophages (Foster, 2004).

1.6 Impact of MRSA

To make matter severe, carriers of MRSA and infected persons are susceptible to auxiliary infections. Also, they are reservoirs of the pathogen that disperse pathogens into the community. When these individuals gather in enclosed parameters, like hospitals where antibiotics are prevalently used for treatment, it poses a selection pressure on the bacteria and facilitates their dispersal among the already compromised immune system. MRSA has become an epidemic in health care facilities (Lee et al., 2018). Combating the spread of MRSA in health-care associated settings and a community is a public health

concern. MRSA infections are harder to treat and often have worse outcomes. MRSA infections are associated with the increased morbidity and mortality when compared to methicillin-susceptible strains of S. aureus (Cosgrove et al., 2003; Shorr et al., 2006).

1.7 Healthcare-associated MRSA (HA-MRSA)

The infestation of MRSA in individuals who have been hospitalized for more than 48 hours with no prior record of MRSA infection is said to have Healthcare-associated MRSA(HA-MRSA) infection (Parvez, Ferdous, Rahman, & Islam, 2018). Bangladesh is at great risk of HA-MRSA epidemic with an astounding 63% prevalence rate in clinical samples whereas, in the United States of America the prevalence rate is 3% lower (Haq et al., 2005; Rice, 2006). A study deduced that only in Dhaka the occurrence of MRSA is 72% and individuals in their 50s along with women are more inclined towards infection (Parvez et al., 2018).

The dissemination of MRSA within hospitals must be contained. Therefore, upholding the hygiene and cleanliness of the surroundings as well as the medical staff should be emphasized (Dettenkofer et al., 2004; Rutala & Weber, 2004). Surrounding surfaces, like bed rails, that are in most contacts with the patients' hands are essential (Asoh et al., 2005). One study concluded that the presence of MRSA was frequent in areas within the hospital environment which could transmit themselves to the skin of staff members. MRSA was detected in surroundings (bed rails) of non-MRSA patients. Furthermore, MRSA may be prevalent in such settings; However, they have not labeled a major source (Kurashige, Oie, & Furukawa, 2016).

1.8 Multiple drug-resistant (MDR) *S. aureus*

Organisms attaining resistance to at least one antibiotic from each class of antibiotics can be referred to as a multi drug resistant organism (MDRO). More defining terms have been structed to appropriately describe the varying resistance pattern shown by pathogens responsible for nosocomial infections. A study proposed a well-defined parameter especially for categorizing *S. aureus* (Magiorakos et al., 2012). XDR and PDR are commonly used term for classifying the resistance pattern.

XDR has a few elaborations including ‘extreme drug resistance’; ‘extensive drug-resistance’; ‘extremely drug-resistant’ and ‘extensively drug-resistant’ (Falagas & Karageorgopoulos, 2008; Park et al., 2009). Pandrug resistant (PDR) is only applicable for organisms which are resistant to almost all antimicrobial agent that are available commercially (Hsueh et al., 2002). MRSA in healthcare settings is more likely to attain resistance to multiple drugs (Barrett, 2005).

Table 1: MDR and its classification in S. aureus **(Magiorakos et al., 2012).**

Bacterium	MDR	XDR	PDR
Staphylococcus aureus	The isolate is non-susceptible to at least 1 agent in ≥3 antimicrobial categories.	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories.	Non-susceptibility to all agents in all for each bacterium.

Chapter2
Methods and Materials

2.1 Standard laboratory practice

Glassware such as test-tube, conical flask, beakers was washed once with tap water followed by second time wash with distilled water. Culture media (both agar-based and broth), pipette tips, centrifuge tubes, empty test-tube for double-layer agar method were autoclaved at 121°C at 15 psi for 15 minutes before use (except for Chrome MRSA) and stored culture media at 4°C and autoclaved equipment in aseptic condition. While performing experiments, clean lab coat was worn and hand gloves were used and the experiments were performed inside a Biosafety cabinet or laminar flow cabinets which in prior was cleaned with 0.5 per cent Hypochlorite solution and 70 per cent ethanol to avoid contamination.

2.2 Media and solution preparation

A chromogenic medium was used to isolate and differentiate Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Methicillin Susceptible *Staphylococcus aureus* (MSSA). By following the manual provided by the manufacturer, for 1L of media 82.5g of powdered media is dissolved in 1L of distilled water. Later, it was heated over a Bunsen burner flame to autoclaved at 110°C for 5 minutes and allowed it to reach a temperature of 45-50°C before adding 1ml of CHROMagar^{TM} MRSA supplement (CHROMagar^{TM}, France). Later on, the sterilized media was poured into disposable, sterile Petri dishes in the horizontal laminar hood and allowed to cool. They were labelled and stored at 4°C refrigerator for storage.

Mannitol Salt Agar (MSA), a selective and differential medium, is utilized for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens. The label on the container instructs to add 111g of media powder to 1L of deionized, distilled water and mix thoroughly. It is heated while being stirred later, autoclaved at 121°C for 15 minutes. Afterwards, the sterilized media was poured into disposable, sterile Petri dishes in the horizontal laminar hood and allowed to cool. They were labelled and stored at 4°C refrigerator for storage.

Kirby-Bauer tests require Mueller Hinton Agar (MHA) as the media base. As per Difco™'s instruction 38g of MHA powder was suspended in 1L of distilled water and gently heated until it dissolved completely. Later it was autoclaved at 121°C for 15 minutes for sterilization. Later on, the sterilized media was poured into disposable, sterile Petri dishes in the horizontal laminar hood and allowed to cool. They were labelled and stored at 4°C refrigerator for storage.

2.3 Sample Collection

Place of study: The study was conducted in the Laboratory of Environmental Health, icddr,b, Mohakhali, Dhaka, whilst the samples were collected from Faridpur Medical College Hospital, Rajshahi Medical College Hospital and Rangpur Medical College Hospital during a period of 3 months; March to May 2019.

The study targeted patients and their respective surroundings to investigate the presence of MRSA. For sampling, the surfaces of the bed curtains, pillow, and bedside rails were wiped using cotton swab moistened with sterile physiological saline. The cotton swab was transported in 10ml Falcon tubes holding 5ml Phosphate Buffer Saline (PBS) contained within temperature-controlled cool box to the laboratory for further processing which was carried out within 24 hours of collection.

Table 2: Unique sample identification nomenclature.

Parameters	Elaboration with abbreviation
Medical College Name	Rajshahi Medical College: RS
	Rangpur Medical College: RP
	Faridpur Medical College: FP
Ward Name	Female Medicine: FM
	Male Medicine: MM
	Male Surgery: MS
	Neonatal: NN
	Pediatric: PD
Bed Patient	B
Type of Clinical Sample	Nasal throat: NT
	Pus Sample: PS
Clinical sample	CL
Types of Environmental Sample	Patient's Hands: PH
	Family Care Giver's Hands: FH
	Bed Rail: BP
	Bed Pillow: BP
	Floor: FL

2.4 Sample processing

2.4.1 Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolation and identification

The samples were spread onto MSA and Chromogenic MRSA plate. For each media plate, 200 µl of the sample was taken and spread using glass beads. The spread plate technique was adopted from a study and was optimized in regards to the sample handled (Prusokas, Hawkins, Nieduszynski, & Retkute, 2018). All bacterial culture, media and broth pertaining to inoculation of MRSA were handled in Biosafety cabinet for self-protection as well as prevention of media contamination. Before spread plating, the MSA and Chromogenic MRSA media plates were well dried and labelled. For each media, 200 µl of the sample was pipetted all over the surface of the media in drops. An average of ten to twelve pre-autoclaved and dried glass beads per plate were introduced to the petri dish then, covered with lid before inducing agitation. The plates were moved

in an ‘L’ shaped loops for uniform coverage for six to seven times till the beads stuck to the media creating slight indentation (Prusokas et al., 2018) this occurrence is an indication of proper absorption of the inoculum into the media afterwards, the beads were discarded into a 0.5 per cent bleach solution by tilting the plate over the discard. The plates were kept for 18-24 hours in an incubator at 37°C. This technique yields single colonies originating from individual cells onto the media’s surface contained among the population of cells in PBS after overnight incubation. The subsequent individual colonies were enumerated.

Single colonies suspected of being MRSA from Chromogenic MRSA plates were re-streaked onto fresh media of MSA and Chromogenic MRSA. Identical colonies were streaked both on MSA and Chromogenic MRSA plates for ensuring isolation of singular colonies which were primarily MRSA. Isolated colonies were stocked in LB broth with 30% glycerol and stored in -80°C for further assessment.

2.5 Chromosomal DNA extraction

The preserved stock was re-streaked onto MSA plates for DNA extraction via heat and cold shock method. The utilization of heat to disintegrate the cell wall and cell membrane prior to providing cold shock has been in practice for its simplicity and relative pure yield of DNA which can consequently be used for molecular testing. Submerging of heat-treated DNA into ice can further damage the cell wall and membrane in addition to breaking down cytoplasmic structures by promoting ice crystal formation. Low temperatures were also observed to destroy cell walls and membranes (Brahmadathan & Jose, 2006; Tell, Foley, Needham, & Walker, 2003). More importantly, a routine heat and cold exposure can aid in the isolation of bacterial DNA (Tell et al., 2003).In brief, four to five colonies from MSA agar plate is suspended in a microcentrifuge tube containing 600 µl distilled water and vortexed to form a homogenous mixture. It is heat-treated for 10 minutes at 100°C heating block and immersed into ice straight away. It received the cold shock for another 10 minutes. Tubes were centrifuged at 13000 rpm for 8 minutes. 100 µl of the supernatant was pipetted into a new, labelled microcentrifuge tube. For preservation, they were stored at -20°C refrigerator in labelled refrigerator boxes.

2. 6 Molecular confirmation of *S. aureus* using nuc gene

As stated previously, the *nuc* gene is a golden standard for *S. aureus* which has been employed as an identification marker for the sample set that were amplified during PCR. Pre-designed primers were ordered that are complementary to regions within the 447-bp nuclease A coding *nuc*A gene (Brakstad et al., 1992).

Table 3: Primer details of nuc gene **(Brakstad et al., 1992).**

Primer name	**Primer sequence (5'-3')**	**Band size**
Forward primer	GCGATTGATGGTGATACGGTT	279bp
Reverse primer	AGCCAAGCCTTGACGAACTAAAGC	

2.7 Molecular confirmation of Methicillin Resistant *S. aureus* using *mecA* and *fem*A genes

Although the *mecA* gene reflects staph's ability to resist the antimicrobial effect of methicillin, the presences of *femA* gene consolidate its high resistive properties. Moreover, the simultaneous existence of two of these genes confirms the presence of MRSA (Menezes-Martins et al., 2005). Consequently, both *mecA* and *femA* genes were amplified using simplex PCR method for an individual gene. Again, already designed primers were utilized.

Table 4: Primer details of mecA gene **(Omar, Ali, Harfoush, & El Khayat, 2014).**

Primer name	**Primer sequence (5'-3')**	Band size
Forward primer/ M1	TGGCTATCGTGTCACAATCG	310 bp
Reverse primer/ M2	CTGGAACTTGTTGAGCAGAG	

Table 5: Primer details of femA gene **(Menezes-Martins et al., 2005).**

Primer name	**Primer sequence (5'-3')**	Band size
femA-F	CTTACTTACTGGCTGTACCTG	686 bp
femA-FR	ATGTCGCTTGTTATGTGC	

2.7 Polymerase Chain Reaction (PCR)

The extracted DNA samples were used to perform molecular assessment via Polymerase Chain Reaction (PCR) to observe the presence of particular genes. PCR is a key technique in molecular genetics that permits the analysis of any short sequence of DNA through amplification of a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Table 6: Composition of PCR mixture used for each of the simplex PCRs performed for the detection of nuc, mecA and femA genes.

Serial No.	**Reagents**	**Volume (µL)**
1	2x DreamTaq™ Green PCR Master mix	12.5
2	Forward primer	1
3	Reverse primer	1
4	Template DNA	1
5	Nuclease free water	9
	Total Volume	25

All the reaction mixtures were prepared in a laminar flow cabinet to provide contamination-free environment. The PCR tubes contained 25µl of the reaction mixture and the composition is as stated in Table 5. Each of the simplex PCRs performed for the detection of *nuc*, *mecA* and *femA* genes were conducted with different parameters and cycle numbers and every time the thermal cycler was programmed accordingly.

2.7.1 PCR programs followed:

Table 7: PCR program set for the detection of nuc gene **(Brakstad et al., 1992).**

PCR Steps	Temperature	Time
Initial denaturation	94^oC	4 min
37 Cycles	94^oC	1min
	55^oC	0.5 min
	72^oC	1.5 min
Final Extension	72^oC	3.5 min

Table 8: PCR program set for the detection of mecA gene **(Tiwari & Sen, 2006).**

PCR Steps	Temperature	Time
Initial denaturation	92^oC	3 min
30 Cycles	92^oC	1min
	56^oC	1 min
	72^oC	1 min
Final Extension	72^oC	3 min

Table 9: PCR program set for the detection of femA gene.

Steps	Temperature	Time
Initial denaturation	92^oC	3 min
30 Cycles	92^oC	1min
	56^oC	1 min
	72^oC	1 min
Final Extension	72^oC	3 min

2.8 Gel electrophoresis using 1 per cent agarose gel

0.8g of agarose powder was dissolved into 80 ml of 0.5X TBE buffer over a gentle heat. When the agarose cooled slightly, 4 µl of Midori Green Advance -a nucleic acid stain- was used. Then, liquid agarose was poured into a casting try with the comb pre-installed. Afterwards, the solidified gel was immersed in 0.5X TBE buffer in a horizontal gel electrophoresis machine and the 7µl of PCR products were loaded into the wells with positive control, a negative control as well as 5µl of 100 bp ladder. The tray was then connected to a current generator and run at 94 volts, 48 amps till the dye reaches the end of the gel. For gel visualization, gel doc was utilized and UV ray exposed pictures of the gels showcasing the bands were obtained.

2.9 Antimicrobial Susceptibility Testing

To observe the translation of the genes into methicillin resistance, the disk diffusion test or Kirby–Bauer test was carried out. This is a simple method of assessing the antibiotic sensitivity of bacteria. A standardized method of antibiotic susceptibility testing has been established which is quite pragmatic (Jorgensen & Turnidge, 2015; The Clinical and Laboratory Standards Institute, 2016). This method required the bacterial culture in Muller-Hinton broth of approximately $1–2\times10^8$CFU/mL to be inoculated over a dried Mueller-Hinton agar plate. Commercially available antibiotic disks (*Table 10*) were strategically placed over each plate using sterile needles. The plates were incubated for 16–24 h at 37°C prior to the determination of results. The zones of growth inhibition (ZOI) around each of the antibiotic disks were measured to the nearest millimetre. The diameter of the zone of inhibition is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS).

Table 10: List of antibiotics disks and their classes.

Carbapenem	**Tetracycline**	**Aminoglycosides**	**Cephalosporin**		**Quinolones**	**Chloramphenicol**	**Penicillin**	
Meropenem (MEM) 10µg	Doxycycline(DO) 30µg	Gentamycin (CN) 10µg	2° Cefoxitin (FOX) 30µg	2° Cefaclor (CEC) 30µg	2° Ciprofloxacin (CIP) 5µg	Chloramphenicol (C) 30µg	Ampicillin (AMP) 10µg	2° Oxacillin 1µg

2.10 Efficacy of hand sanitizer

2.10.1 Agar well diffusion

Well diffusion method investigates the effect of hand sanitizers on bacterial isolates found on patient's hands or family care giver's hands. This strategy is usually employed to evaluate the antimicrobial activity of plants or microbial extracts (Balouiri, Sadiki, & Ibnsouda, 2016). Similarly to the procedure used in the disk-diffusion method, the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire Mueller- Hinton agar surface. Then, five wells with a diameter of 6 mm were bored aseptically with a sterile cork-borer, and a 50µL of the hand sanitizer of two different companies, Dettol and Sepnil, were introduced into the well. On the fifth well, 50µL of distilled water was kept as a control. Then, agar plates were incubated at 37°C for 24 hours; consequent zone of inhibition (ZOI), if any, was measured.

Table 11: Hand sanitizers and their ingredients as written on their labels.

Name	Composition
Sepnil	Ethanol, Carbomer, Glycerin, Polyethylene Glycol TEA, Aqua and perfume
Dettol	Denatured Alcohol- 69.4% w/w, Water PEG/PPG-17/6 copolymer, Propylene glycol, Acrylate /C10-30 alkyl acrylate, cross polymer, Tetrahydroxpropyl ethylenediamine, Perfume.

2.10.2 Droplet method

This method is implemented to attain enumeration of bacterial count after being treated with hand sanitizer infused Mueller-Hinton agar plates. 1 ml of each of the hand sanitizer was evenly spread over the agar plates and kept overnight for proper absorption in media. Bacterial suspension in normal saline was serially diluted five times, 50 µL diluted samples (10^{-3} and $10^{-4)}$ were pipetted as droplets on to sanitizer infused plates and a regular Mueller-Hinton agar plate was used for control.

Chapter3
Results

The mauve or pink colonies on chromogenic MRSA suspected of being MRSA, produced yellow colonies on MSA media as portrayed in *Figure 3*.

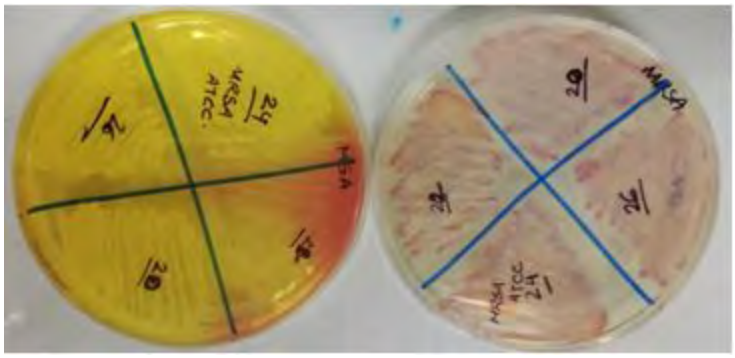


Figure 3: Suspected MRSA isolates streaked onto MSA and chromogenic MRSA media.

3.1 PCR Analysis for *nuc, mecA and femA* genes

Out of the 54 suspected MRSA, 35 isolates produced bands at 279 bp which indicates the presence of a *nuc* gene thus, were confirmed as *S. aureus*. These *S. aureus* isolates were put under further scrutiny when those 35 samples were amplified with primers specifically designed for *mecA* gene. Aside from one isolate, all the other isolates showed the presence of *mecA* gene by giving a band at 310bp in agarose gel electrophoresis. Afterwards, to confirm that the isolates were indeed MRSA, another PCR was performed for *femA* gene where 22 isolates exhibited bands at 686bp. For isolates to be MRSA positive, they must have all of the three- *nuc*, *mecA* and *femA* genes. 22 isolates fulfilled this criteria and were considered as Methicillin resistant *S. aureus*. Only one isolate had neither *mecA* nor *femA* gene. T*able 10* contains the summarized results for the PCR carried out for individual genes.

Table 12: Summarized results of the detection of nuc, mecA and femA genes.[1]

Cap ID	Sample ID	Envn. Type	Nuc (279)	MecA (310)	FemA (686)
2	RSFMBNT-12	BP-1	+	+	+
3	RSFMBNT-12	BP-2	+	+	-
5	RSMMBNT-12	BP-1	+	+	+
8	RSMMBNT-13	CL-1	+	+	+
9	RSMMBNT-13	CL-2	+	+	+
10	RSMMBNT-12	PH-1	+	+	+
11	RSMMBNT-12	CL-2	+	+	+
12	RSMMBNT-12	CL-1	+	+	+
13	RSMMBNT-12	PH-2	+	+	+
15	RSNNBNT-13	Cl-1	+	+	+
16	RSNNBNT-13	CL-2	+	+	+
19	RSNNBNT-16	BP-1	+	+	+
20	RSNNBNT-15	FL-1	+	+	+
22	RSNNBNT-16	FL-1	+	+	-
23	RSFMBNT-11	FL	+	+	+
25	RSNNBNT-13	FH-2	+	+	-
26	RSNNBNT-13	FL-1	+	+	+
28	RSPDBNT-11	BP-1	+	+	-
30	RSPDBNT-15	CL-1	+	+	+
31	RSPDBNT-15	CL-2	+	+	-
32	RSPDBNT-15	FL-1	+	+	-
33	RSPDBNT-15	FL-2	+	+	+
34	RSFMBNT-13	PH-1	+	+	-
35	RSFMBNT-13	PH-2	+	+	+
36	RSFMBNT-13	FH-1	+	+	+
37	RSFMBNT-13	FH-2	+	+	+
38	RSPDBNT-15	FH-1	+	+	+
39	RSPDBNT-15	BP-1	+	+	-
40	RSPDBNT-12	FL-1	+	+	-
51	RSPDBNT-11	FL-1	+	+	+
52	RSPDBNT-11	FL-2	+	+	+
56	RSPDBNT-14	BP-1	+	+	-
61	RSMSBPS-13	BP-2	+	-	-
63	RSMSBPS-13	PH-2	+	+	-
69	RPPDBNT-11	FL-2	+	+	-
24	Positive control for MRSA		+	+	+

[1] '+' indicates presence of the gene and '-' signifies its absence.

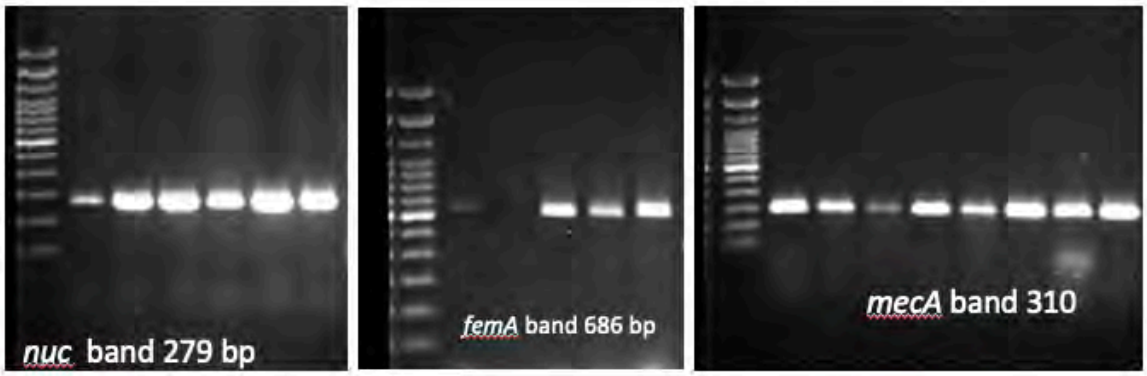


Figure 4: PCR bands of nuc, mecA and femA.

3.2 Antibiotic susceptibility of *S. aureus*

The zone of inhibition of the isolates for each antibiotic disk was noted and the susceptibility pattern was mapped in line with the CLSI guidelines (Table-11). All the isolates were resistant to ampicillin. They were mainly resistant to cephalosporins: 74.29% resistant to cefaclor and 88.57% resistant to cefoxitin (Figure 4). The tested isolates were mostly susceptible to carbapenem, aminoglycosides and tetracycline class of antibiotics. One oxacillin (1μg) susceptible strain was isolated however, it was resistant to ampicillin.

Table 13: Summarized results of antibiotic susceptibility test.

			Carbapenem	Tetracycline	Aminoglycosides	Cephalosporin	Cephalosporin	Quinolones	Chloramphenicol	Penicillin	Penicillin
Cap ID	Sample ID	nvironment Ty	Meropenem (MEM) 10µg	Doxycylin(DO) 30µg	Gentamycin (CN) 10µg	2ª Cefoxitin (FOX) 30µg	2ª Cefaclor (CEC) 30µg	2ª Ciprofloxacin (CIP) 5µg	Chloramphenicol (C) 30µg	Amipicilin (AMP) 10µg	2ª Oxacilin 1µg
2	RSFMBNT-12	BP-1	S	S	S	R	R	S	I	R	R
3	RSFMBNT-12	BP-2	R	R	S	R	R	R	S	R	R
5	RSMMBNT-12	BP-1	S	S	S	R	R	R	S	R	R
8	RSMMBNT-13	CL-1	S	S	S	R	R	S	S	R	R
9	RSMMBNT-13	CL-2	S	S	S	R	R	S	S	R	I
10	RSMMBNT-12	PH-1	S	S	S	R	R	R	S	R	I
11	RSMMBNT-12	CL-2	S	S	S	R	R	R	I	R	R
12	RSMMBNT-12	CL-1	S	S	S	R	R	R	S	R	I
13	RSMMBNT-12	PH-2	S	S	S	R	R	S	S	R	R
15	RSNNBNT-13	CI-1	S	S	S	R	R	R	S	R	R
16	RSNNBNT-13	CL-2	S	S	S	R	R	S	R	R	R
19	RSNNBNT-16	BP-1	S	S	S	R	R	S	S	R	R
20	RSNNBNT-15	FL-1	S	I	S	R	R	R	R	R	R
22	RSNNBNT-16	FL-1	S	S	S	R	I	S	S	R	R
23	RSFMBNT-11	FL	S	S	S	R	I	S	S	R	I
25	RSNNBNT-13	FH-2	R	S	R	R	R	R	I	R	R
26	RSNNBNT-13	FL-1	S	S	S	R	R	I	R	R	R
28	RSPDBNT-11	BP-1	S	S	S	R	S	S	S	R	I
30	RSPDBNT-15	CL-1	S	S	S	R	I	S	S	R	R
31	RSPDBNT-15	CL-2	S	I	I	R	R	I	R	R	I
32	RSPDBNT-15	FL-1	S	S	S	S	R	I	R	R	R
33	RSPDBNT-15	FL-2	I	S	S	R	R	I	R	R	R
34	RSFMBNT-13	PH-1	S	S	S	R	S	S	S	R	R
35	RSFMBNT-13	PH-2	S	I	S	R	R	S	S	R	R
36	RSFMBNT-13	FH-1	S	S	I	R	R	S	S	R	I
37	RSFMBNT-13	FH-2	S	S	S	S	R	R	S	R	S
38	RSPDBNT-15	FH-1	S	S	S	R	R	S	R	R	R
39	RSPDBNT-15	BP-1	S	S	S	R	S	S	S	R	R
40	RSPDBNT-12	FL-1	S	S	S	R	R	S	S	R	R
51	RSPDBNT-11	FL-1	S	R	S	S	S	R	S	R	I
52	RSPDBNT-11	FL-2	S	S	S	S	S	R	S	R	S
56	RSPDBNT-14	BP-1	S	S	S	R	R	I	S	R	R
61	RSMSBPS-13	BP-2	S	S	S	R	S	S	I	R	I
63	RSMSBPS-13	PH-2	R	S	S	R	R	R	S	R	I
69	RPPDBNT-11	FL-2	I	S	S	R	R	R	S	R	R
24	Postive control for MRSA		R	S	S	R	R	R	S	R	R

3.2.1 Multidrug resistance analysis

In line with the definition of MDR provided in the previous chapter, most of the isolates in this study were multiple drugs resistant. Most importantly, isolates with the highest number of antibiotics to which it is non-susceptible are *nuc*$^{+}$, *mecA*$^{+}$, *femA*$^{-}$ isolate found on family caregivers' hands (RSNNBNT-13 FH) and *mecA*$^{+}$, *femA*$^{-}$ *S. aureus* found on a bed (RSFMBNT-12 BP). RSNNBNT-13 FH was resistant to carbapenem (meropenem); aminoglycosides (gentamycin); cephalosporin (cefoxitin and cefaclor); quinolones (ciprofloxacin) and penicillin (ampicillin and axacillin). RSFMBNT-12 BP and RSNNBNT-13 FH without *femA gene* were found to be resistant to Carbapenem (Meropenem); Tetracycline (Doxycylin); Cephalosporin (Cefoxitin and Cefaclor); Quinolones (Ciprofloxacin) and Penicillin (Ampicillin and Oxacillin). Both these isolates were susceptible to Phenicol class of antibiotic namely Chloramphenicol. An MRSA isolate, RSNNBNT 15 FL, was resistant to 5 classes of antibiotics which include Cephalosporin (Cefoxitin and Cefaclor); Quinolones (Ciprofloxacin) and Penicillin (Ampicillin and Oxacillin) and Phenicol (Chloramphenicol). Since RSNNBNT 15 FL is MRSA and resistant to 5 classes of antibiotics it can be called an MDR-MRSA. Other than that, 20 isolates (Cap ID: 2, 5, 8, 10, 11, 12, 13, 15, 16, 19, 20, 23, 26, 28, 33, 35, 37, 38, 51 and 52) were MDR-MRSA as they were resistant to at least one antibiotic in three or more classes of antibiotics (*Figure 5).*

Figure 5: Antibiotics susceptibility pattern S. aureus.

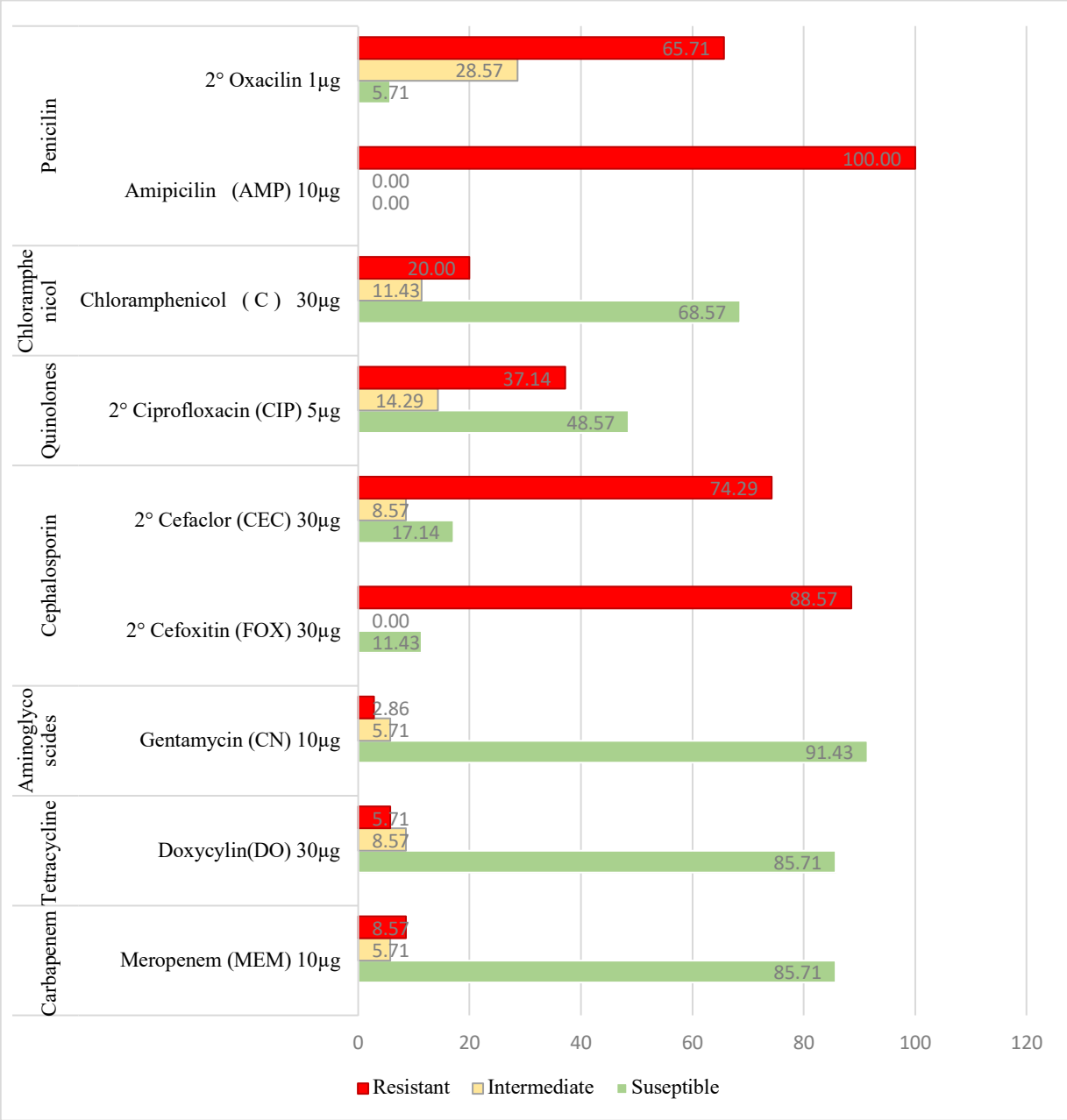
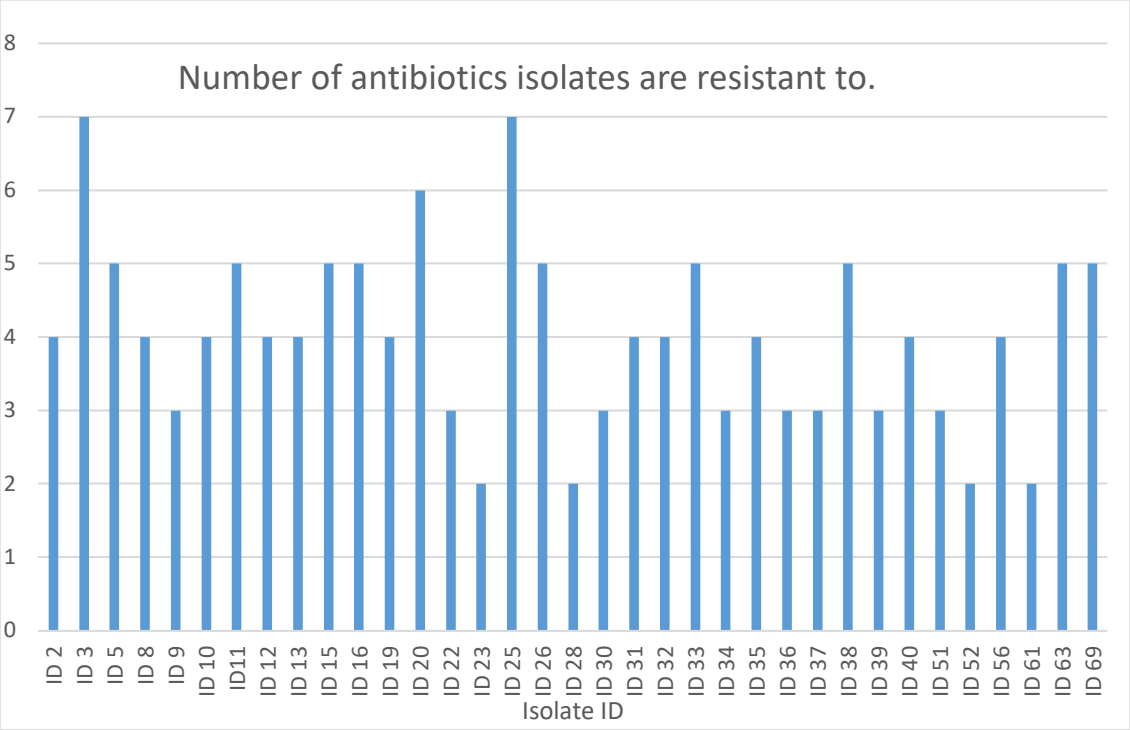


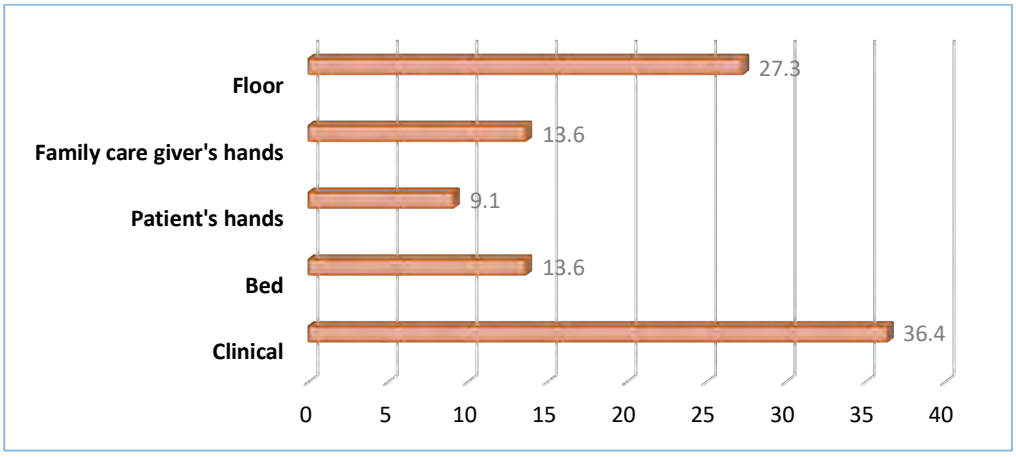
Figure 6: Enumeration of antibiotics each isolate is resistant to.



3.2.2 Distribution of MRSA isolates based on source

The incidence of MRSA in clinical samples was the highest (36.4%). Among the patients' surroundings, 27.3% of MRSA was detected from floor swabs and 22.7% of MRSA were found on hands (9.1% Patients' hands; 13.6% Family caregivers' hands) (*Figure 6)*.

Figure 7: Distribution of MRSA isolates from clinical and environmental swab samples.



3.3 Efficacy of hand sanitizers against bacterial isolates from patients' and family caregivers' hands' swabs.

No zone of inhibition was observed for either of the hand sanitizers for any of the isolates found on hand. Thereby, MRSA is unaffected by the application of hand sanitizers.

Table 14: Efficacy of hand sanitizers against bacterial isolates from patients' and family caregivers' hands' swabs.[2]

Cap ID	Sample ID	Environment type	Isolate Number	Hand Sanitizer	
				Dettol	Sepnil
13	RSMMBNT-12	PH	2	Nil	Nil
25	RSNNBNT-13	FH	2	Nil	Nil
34	RSFMBNT-13	PH	1	Nil	Nil
35	RSFMBNT-13	PH	2	Nil	Nil
36	RSFMBNT-13	FH	1	Nil	Nil
37	RSFMBNT-13	FH	2	Nil	Nil
38	RSPDBNT-15	FH	1	Nil	Nil
63	RSMSBPS-13	PH	2	Nil	Nil

[2] Highlighted samples contain *nuc* and *mecA* gene but not *femA* gene, the rest are MRSA with all the three gene present.

Chapter4
Discussion

In accordance with Centres for Disease Control and Prevention (CDC)'s 2015 HAI Hospital Prevalence Survey, patients admitted to hospitals acquiring one or more Hospital-Associated Infection (HAI) were recorded to be 3%. Their report states a staggering 72,000 deaths of HAI infected patients during hospitalization (Arnold et al., n.d.). An opportunistic strain of the *Staphylococcus aureus*, a Gram-Positive bacteria that comprise the general microbiota on human skin takes lead in causing HAI; namely Methicillin-Resistant *Staphylococcus aureus* (MRSA). MSRA is notorious for its resistance to penicillin derivative antibiotics, mainly against methicillin and oxacillin and for acquiring resistance to other classes of antibiotic. Currently, we are at the cusp of a time when to combat the emergence of such a HAI causative multidrug resistant organism extensive research into its cultural along with molecular machinery is imperative.

The isolates from the clinical samples and surroundings of a patient represent the bacterial population the patient is most inclined to be infected by when they are already at a vulnerable state. MRSA can persist in surrounding inanimate object for a few months (Wagenvoort, Sluijsmans, & Penders, 2000). MRSA contamination is prevalent on floor, bed linens, patient's gown, overbed tables and pressure cuffs (Boyce, Potter-Bynoe, Chenevert, & King, 1997). MRSA colonized individuals can transmit pathogen on to surfaces they are in contact with. Subsequently, MRSA may transfer onto the hands of staff members when they handle those contaminated surfaces (Boyce et al., 1997). With this perspective, the environmental sampling sites were selected to be bed: bed pillow, bed rail; floor; hands: patients' hands and caretakers' hands.

Isolated bacterial colonies suspected of being MRSA gave mauve or pink colonies on CHROMagar™ MRSA media plates and yellow colonies on MSA plates. It has been evaluated that CHROMagar MRSA medium can be utilized for dependable isolation of HA-MRSA (Nahimana, Francioli, & Blanc, 2006). Post initial screening using selective and differential media, molecular typing was carried out to confirm the presence of MRSA. A study employs primers against the *nuc* and *mecA* genes for rapid detection of methicillin resistance and S. aureus species, whereas a group of researchers detected

MRSA by the presence of *mecA* and *femA* genes (Costa, Kay, & Palladino, 2005; Francois et al., 2003). Therefore, the suspected MRSA isolates were evaluated using primers for all the three genes: *nuc, mecA* and *femA.* Isolates with all the three genes present were considered MRSA positive. To investigate their resistance to penicillin: oxacillin and ampicillin and other classes of antibiotics, disk diffusion test was performed.

In this study, 68.9% of the isolates were proved to be MRSA through detection of *nuc*, *femA* and *mecA* and subsequent disk diffusion test revealed that 90.9 % of those isolates were non-susceptible to multiple drugs. Among the samples, an incidence of oxacillin susceptible, *mecA*$^{+}$ *Staphylococcus aureus* was observed, although only two isolates were susceptible to oxacillin yet resistant to ampicillin. Similarly, another investigation of *S.aureus* revealed the bacterial isolates with the presence of *mecA* gene, all the while exhibiting non-resistance to oxacillin (Hososaka et al., 2007). A research team in Saudi Arabia found that 13 out of 15 isolates exhibiting methicillin resistance had *mecA* gene and the other two isolates although expressed methicillin resistance but had no *mecA* gene (Al-Ruaily, & Khalil, 2011). This study also isolated one *mecA*$^{-}$ *S. aureus* that showed resistance to ampicillin but had an intermediate response to oxacillin. On the other hand, all the isolates deemed resistant to ampicillin (10μg). The majority of the isolates (91.43%) were observed to be sensitive to gentamycin. A survey in France concludes that in recent times there has been a rise in the incidence of gentamycin susceptible MRSA (Aubry-Damon et al., 1997). A different research concluded that 79.5% of their *S. aureus* isolates were resistant to ampicillin (Bernardo, Boriollo, Gonçalves, & Höfling, 2005). 85.71% of isolates were susceptible to meropenem and doxycycline which is comparable to the 80% susceptibility of isolates to meropenem in a similar study (Al-Ruaily, & Khalil, 2011). About 74% of the isolates were resistant to cefaclor and an even higher percentage of isolates (88.57%) were non-susceptible to cefoxitin. A small percentage of isolates (11.43%) were susceptible to cefoxitin whereas 17.14% showed susceptibility to cefaclor. Although the disk diffusion test yields cephalosporin susceptible isolates, cefoxitin and cefaclor of the cephalosporin class can give misleading results against oxacillin or methicillin-resistant *S. aureus*. This is because the *mecA* gene coding PBP2a gives resistance to all lactam drugs (Marshall & Blair, 1999). Innovative fifth-generation cephalosporins are being produced which are effective against MRSA (Duplessis & Crum-Cianflone, 2011).

The infestation of MRSA in Male Medicine was the most at 31.8% and the other wards examined: Neonatal, Pediatric and Female Medicine had an equal distribution of MRSA presence (22.7%). According to a study, females are at most risk, which is a concern as 22.7% of the MRSA in this study were found in the ward for female patients (Parvez et al., 2018). An astounding 45.4% of the MRSA originated from either Neonatal or Pediatrics' ward. The spread of S. aureus in neonatal units transpired with the help of the health care workers' hands, nasal carriage and the contamination in the surroundings also aggravated it. (Otter, Klein, Watts, Kearns, & French, 2007). Exposure to such daunting opportunistic pathogens puts the infants and children at a disadvantage.

Contaminated hands of individuals working at healthcare facilities are on the top of the list for being an origin of HAI and germ dispersal (Carboneau, Benge, Jaco, & Robinson, 2010). MDR-MRSA was found to be present in hand swab samples collected from the hospital, which were at least resistant to 3 classes of antibiotics. All samples found on hand with *nuc, mecA* genes were investigated against hand sanitizers. Surprisingly, neither the MRSA nor the nuc^{+}, $mecA^{+}$ isolates were susceptible to the sanitizers. Diverse collection of hand sanitizers are commercially available which have varying efficacy against microorganisms (Jain, Karibasappa, Dodamani, Prashanth, & Mali, 2016). Researchers found that alcohol-based hand rubs were most effect against MRSA compare to hand soaps (Kocak Tufan et al., 2012).

Good hand hygiene is imperative in combatting the dispersal of MRSA among the inhabitant of the hospital. MRSA isolates found on hands of family caregivers have a high chance of being released into the community. Infection control committees and healthcare providers must keep MRSA colonization in check and take appropriate measurements to control the dispersal of such pathogens.

4.1 Limitations and future aspect

For this thesis to be a study of the prevalence of MRSA in hospitals surrounding more samples were needed to be collected. So far, only a report of incidence based upon the sample size could be deduced.

Few classes of antibiotics mainly glycopeptide, linezolid could have been included to observe their susceptibility pattern with newer antibiotics. However, as the CLSI guidelines state that disk diffusion method for glycopeptide is not reliable for detection of glycopeptide- resistant MRSA, e-test or molecular screening could be employed.

In addition to the genes that were detected, another gene that could give some insight into their pathogenicity is PVL. Its molecular detection via PCR could determine which isolates could be inclined towards infecting individuals than others. Protein A or spa typing could aid in classifying the types of *mecA* gene present in the $mecA^{+}$ isolates.

Resistance to hand sanitizers evaluation in this study was a binary assessment method. Minimum inhibitory concentration could be determined for each isolate. Although, an attempt at establishing a new protocol of assessing the efficacy of hand sanitizer was made by the droplet method. For Dettol, 10^{-4} dilution showed an observable decline in count for one isolate. This protocol cannot yet be utilized to produce a compelling data of the efficacy of hand sanitizers against MRSA. An in-depth study of how the hand sanitizer affects the MDR-MRSA in healthcare facilities and their mechanism could be investigated.

Chapter5
Conclusion

This thesis investigated the occurrence of MRSA in clinical samples along with environmental samples, namely hands; bed and floor. To include a diverse sample set, the sampling was carried out in male medicine ward; male surgery ward; female medicine ward; pediatric ward and neonatal ward of hospitals. Incidence of MRSA is quite high and they are concentrated in floor samples and hand samples. Furthermore, these MRSA are resistant to multiple drugs which have been observed by the disk diffusion test. Presence of such multiple drug-resistant S. aureus is an opportunistic pathogen is hospitals is an alarming issue and more so as these are non- susceptible to hand sanitizer in use. Unwarranted intake of broad-spectrum antibiotics in the medical the concerned authorities; patients and their caretakers along with hospital staff must be vigilant in maintaining good hygiene practices to avoid transmission of MRSA within the hospital and into the community and controlled used of antibiotics.

References

Al-Ruaily, M.A., & Khalil, O.M. (2011). Detection of (mecA)gene in methicillin resistant Staphylococcus aureus (MRSA) at Prince A / Rhman Sidery Hospital, Al-Jouf, Saudi Arabia.

Arnold, K. E., Avery, L., Bennett, R., Brinsley-Rainisch, K., Boyter, M., Coffin, N., … Zimmerman, K. (n.d.). *National and state healthcare-associated infections progress report* (N. C. for E. and Z. I. D. (U. S.). D. of H. Q. Promotion., Ed.). Retrieved from https://stacks.cdc.gov/view/cdc/22160

Asoh, N., Masaki, H., Watanabe, H., Watanabe, K., Mitsusima, H., Matsumoto, K., … Nagatake, T. (2005). Molecular Characterization of the Transmission between the Colonization of Methicillin-resistant Staphylococcus aureus to Human and Environmental Contamination in Geriatric Long-term Care Wards. *Internal Medicine*, *44*(1), 41–45. Retrieved from http://ci.nii.ac.jp/naid/10014318759/en/

Aubry-Damon, H., Legrand, P., Brun-Buisson, C., Astier, A., Soussy, C.-J., & Leclercq, R. (1997). Reemergence of Gentamicin-Susceptible Strains of Methicillin-Resistant Staphylococcus aureus: Roles of an Infection Control Program and Changes in Aminoglycoside Use. *Clinical Infectious Diseases*, *25*(3), 647–653. https://doi.org/10.1086/513749

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/J.JPHA.2015.11.005

Barrett, J. F. (2005). MRSA - What is it, and how do we deal with the problem? *Expert Opinion on Therapeutic Targets*. https://doi.org/10.1517/14728222.9.2.253

Becker, K., Heilmann, C., & Peters, G. (2014). Coagulase-Negative Staphylococci. *Clinical Microbiology Reviews*, *27*(4), 870 LP – 926. https://doi.org/10.1128/CMR.00109-13

Bernardo, W. L. de C., Boriollo, M. F. G., Gonçalves, R. B., & Höfling, J. F. (2005). Staphylococcus aureus ampicillin-resistant from the odontological clinic environment. *Revista Do Instituto de Medicina Tropical de São Paulo*, *47*(1), 19–24. https://doi.org/10.1590/S0036-46652005000100004

Bohach, G. A. (2006). Staphylococcus aureus Exotoxins. In *Gram-Positive Pathogens, Second Edition* (pp. 464–477). Retrieved from https://www.asmscience.org/content/book/10.1128/9781555816513.chap38

Boyce, J. M., Potter-Bynoe, G., Chenevert, C., & King, T. (1997). Environmental Contamination Due to Methicillin-Resistant Staphylococcus aureus: Possible Infection Control Implications. *Infection Control and Hospital Epidemiology*, *18*(9), 622–627. https://doi.org/10.2307/30141488

Brahmadathan, K., & Jose, J. (2006). Evaluation of simplified dna extraction methods for *EMM* typing of group a streptococci. *Indian Journal of Medical Microbiology*, *24*(2), 127. https://doi.org/10.4103/0255-0857.25200

Brakstad, O. G., Aasbakk, K., & Maeland, J. A. (1992). Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. *Journal of Clinical Microbiology*, *30*(7), 1654 LP – 1660. Retrieved from http://jcm.asm.org/content/30/7/1654.abstract

Carboneau, C., Benge, E., Jaco, M. T., & Robinson, M. (2010). A Lean Six Sigma Team Increases Hand Hygiene Compliance and Reduces Hospital-Acquired MRSA Infections by 51%. *Journal for Healthcare Quality*, *32*(4), 61–70. https://doi.org/10.1111/j.1945-1474.2009.00074.x

Chambers, H F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clinical Microbiology Reviews*, *10*(4), 781 LP – 791. https://doi.org/10.1128/CMR.10.4.781

Chambers, Henry F, & DeLeo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nature Reviews Microbiology*, *7*(9), 629–641. https://doi.org/10.1038/nrmicro2200

Cosgrove, S. E., Sakoulas, G., Perencevich, E. N., Schwaber, M. J., Karchmer, A. W., & Carmeli, Y. (2003). Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Meta-analysis. *Clinical Infectious Diseases*, *36*(1), 53–59. https://doi.org/10.1086/345476

Costa, A.-M., Kay, I., & Palladino, S. (2005). Rapid detection of mecA and nuc genes in staphylococci by real-time multiplex polymerase chain reaction. *Diagnostic Microbiology and Infectious Disease*, *51*(1), 13–17. https://doi.org/10.1016/J.DIAGMICROBIO.2004.08.014

Davis, C. P. (1996). *Normal Flora* (S. Baron, Ed.). Galveston (TX).

de Haas, C. J. C., Veldkamp, K. E., Peschel, A., Weerkamp, F., Van Wamel, W. J. B., Heezius, E. C. J. M., … van Strijp, J. A. G. (2004). Chemotaxis Inhibitory Protein of Staphylococcus aureus, a Bacterial Antiinflammatory Agent. *The Journal of Experimental Medicine*, *199*(5), 687 LP – 695. https://doi.org/10.1084/jem.20031636

de la Fuente, R., Díez, R. M., Domínguez-Bernal, G., Orden, J. A., & Martínez-Pulgarín, S. (2010). Restoring catalase activity in Staphylococcus aureus subsp. anaerobius leads to loss of pathogenicity for lambs. *Veterinary Research*, *41*(4), 41. https://doi.org/10.1051/vetres/2010013

Dettenkofer, M., Wenzler, S., Amthor, S., Antes, G., Motschall, E., & Daschner, F. D. (2004). Does disinfection of environmental surfaces influence nosocomial infection rates? A systematic review. *American Journal of Infection Control*, *32*(2), 84–89. https://doi.org/10.1016/j.ajic.2003.07.006

Duplessis, C., & Crum-Cianflone, N. F. (2011). Ceftaroline: A New Cephalosporin with Activity against Methicillin-Resistant Staphylococcus aureus (MRSA). *Clinical Medicine Reviews in Therapeutics*, *3*, a2466. https://doi.org/10.4137/CMRT.S1637

Falagas, M. E., & Karageorgopoulos, D. E. (2008). Pandrug Resistance (PDR), Extensive Drug Resistance (XDR), and Multidrug Resistance (MDR) among Gram-Negative Bacilli: Need for International Harmonization in Terminology. *Clinical Infectious Diseases*, *46*(7), 1121–1122. https://doi.org/10.1086/528867

Foster, T. J. (2004). The Staphylococcus aureus "superbug." *The Journal of Clinical Investigation*, *114*(12), 1693–1696. https://doi.org/10.1172/JCI23825

Foster, T. J., & Höök, M. (1998). Surface protein adhesins of Staphylococcus aureus. *Trends in Microbiology*, *6*(12), 484–488. https://doi.org/10.1016/S0966-842X(98)01400-0

Francois, P., Pittet, D., Bento, M., Pepey, B., Vaudaux, P., Lew, D., & Schrenzel, J. (2003). Rapid Detection of Methicillin-Resistant Staphylococcus aureus Directly from Sterile or Nonsterile Clinical Samples by a New Molecular Assay. *Journal of Clinical Microbiology*, *41*(1), 254 LP – 260. https://doi.org/10.1128/JCM.41.1.254-260.2003

Fuchs, S., Pané-Farré, J., Kohler, C., Hecker, M., & Engelmann, S. (2007). Anaerobic gene expression in Staphylococcus aureus. *Journal of Bacteriology*, *189*(11), 4275–4289. https://doi.org/10.1128/JB.00081-07

Goodyear, C. S., & Silverman, G. J. (2003). Death by a B Cell Superantigen. *The*

Journal of Experimental Medicine, *197*(9), 1125 LP – 1139. https://doi.org/10.1084/jem.20020552

Haq, J. A., Rahman, M. M., Asna, S. M. Z. H., Hossain, M. A., Ahmed, I., Haq, T., & Morshed, M. A. H. G. (2005). Methicillin-resistant Staphylococcus aureus in Bangladesh--a multicentre study. *International Journal of Antimicrobial Agents*, *25*(3), 276—277. https://doi.org/10.1016/j.ijantimicag.2005.01.004

Hegde, S. S., & Shrader, T. E. (2001). FemABX Family Members Are Novel Nonribosomal Peptidyltransferases and Important Pathogen-specific Drug Targets. *Journal of Biological Chemistry*, *276*(10), 6998–7003. https://doi.org/10.1074/JBC.M008591200

Hiramatsu, K., Cui, L., Kuroda, M., & Ito, T. (2001, October 1). The emergence and evolution of methicillin-resistant Staphylococcus aureus. *Trends in Microbiology*, Vol. 9, pp. 486–493. https://doi.org/10.1016/S0966-842X(01)02175-8

Hososaka, Y., Hanaki, H., Endo, H., Suzuki, Y., Nagasawa, Z., Otsuka, Y., … Sunakawa, K. (2007). Characterization of oxacillin-susceptible mecA-positive Staphylococcus aureus: a new type of MRSA. *Journal of Infection and Chemotherapy*, *13*(2), 79. https://doi.org/10.1007/s10156-006-0502-7

Hsueh, P. R., Teng, L. J., Chen, C. Y., Chen, W. H., Yu, C. J., Ho, S. W., & Luh, K. T. (2002). Pandrug-resistant Acinetobacter baumannii causing nosocomial infections in a University Hospital, Taiwan. *Emerging Infectious Diseases*.

Jain, V. M., Karibasappa, G. N., Dodamani, A. S., Prashanth, V. K., & Mali, G. V. (2016). Comparative assessment of antimicrobial efficacy of different hand sanitizers: An in vitro study. *Dental Research Journal*, *13*(5), 424–431. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27857768

Jevons, M. P. (1961). "Celbenin" - resistant Staphylococci. *British Medical Journal*, *1*(5219), 124–125. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1952888/

Johnson, A. P., Aucken, H. M., Cavendish, S., Ganner, M., Wale, M. C. J., Warner, M., … UK EARSS participants, the. (2001). Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *Journal of Antimicrobial Chemotherapy*, *48*(1), 143–144. https://doi.org/10.1093/jac/48.1.143

Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility Test Methods: Dilution and Disk Diffusion Methods*. In *Manual of Clinical Microbiology, 11th Edition*. https://doi.org/10.1128/9781555817381.ch71

Kenny, J. G., Moran, J., Kolar, S. L., Ulanov, A., Li, Z., Shaw, L. N., … Horsburgh, M. J. (2013). Mannitol Utilisation is Required for Protection of Staphylococcus aureus from Human Skin Antimicrobial Fatty Acids. *PLoS ONE*, *8*(7), e67698. https://doi.org/10.1371/journal.pone.0067698

Kobayashi, N., Wu, H., Kojima, K., Taniguchi, K., Urasawa, S., Uehara, N., … Kurokawa, I. (1994). Detection of *mecA, femA* , and *femB* genes in clinical strains of staphylococci using polymerase chain reaction. *Epidemiology and Infection*, *113*(2), 259–266. https://doi.org/10.1017/S0950268800051682

Kocak Tufan, Z., Irmak, H., Bulut, C., Cesur, S., Kinikli, S., & Demiroz, A. P. (2012). [The effectiveness of hand hygiene products on MRSA colonization of health care workers by using CHROMagar MRSA]. *Mikrobiyoloji bulteni*, *46*(2), 236–246.

Kurashige, E. J. O., Oie, S., & Furukawa, H. (2016). Contamination of environmental surfaces by methicillin-resistant Staphylococcus aureus (MRSA) in rooms of inpatients with MRSA-positive body sites. *Brazilian Journal of Microbiology :*

[Publication of the Brazilian Society for Microbiology], *47*(3), 703–705. https://doi.org/10.1016/j.bjm.2016.04.002

Lee, A. S., de Lencastre, H., Garau, J., Kluytmans, J., Malhotra-Kumar, S., Peschel, A., & Harbarth, S. (2018). Methicillin-resistant Staphylococcus aureus. *Nature Reviews Disease Primers*, *4*, 18033. Retrieved from https://doi.org/10.1038/nrdp.2018.33

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., … Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268–281. https://doi.org/10.1111/J.1469-0691.2011.03570.X

Marshall, W. F., & Blair, J. E. (1999). The Cephalosporins. *Mayo Clinic Proceedings*, *74*(2), 187–195. https://doi.org/10.4065/74.2.187

Mazmanian, S. K., Skaar, E. P., Gaspar, A. H., Humayun, M., Gornicki, P., Jelenska, J., … Schneewind, O. (2003). Passage of Heme-Iron Across the Envelope of Staphylococcus aureus *Science*, *299*(5608), 906 LP – 909. https://doi.org/10.1126/science.1081147

McDevitt, D., Vaudaux, P., & Foster, T. J. (1992). Genetic evidence that bound coagulase of Staphylococcus aureus is not clumping factor. *Infection and Immunity*, *60*(4), 1514 LP – 1523. Retrieved from http://iai.asm.org/content/60/4/1514.abstract

McLaws, M.-L., Pantle, A. C., Fitzpatrick, K. R., & Hughes, C. F. (2009). More than hand hygiene is needed to affect methicillin-resistant Staphylococcus aureus clinical indicator rates: clean hands save lives, part IV. *The Medical Journal of Australia*, *191*(8 Suppl), S26-31.

Menezes-Martins, L. F., Menezes-Martins, J. J., Michaelsen, V. S., Aguiar, B. B., Ermel, T., & Machado, D. C. (2005). Diagnosis of parapneumonic pleural effusion by polymerase chain reaction in children. *Journal of Pediatric Surgery*, *40*(7), 1106–1110. https://doi.org/10.1016/J.JPEDSURG.2005.03.057

Munckhof, W. J., Nimmo, G. R., Schooneveldt, J. M., Schlebusch, S., Stephens, A. J., Williams, G., … Giffard, P. (2009). Nasal carriage of Staphylococcus aureus, including community-associated methicillin-resistant strains, in Queensland adults. *Clinical Microbiology and Infection*, *15*(2), 149–155. https://doi.org/10.1111/j.1469-0691.2008.02652.x

Murdoch, D. R., & Greenlees, R. L. (2004). Rapid identification of Staphylococcus aureus from BacT/ALERT blood culture bottles by direct Gram stain characteristics. *Journal of Clinical Pathology*, *57*(2), 199–201. https://doi.org/10.1136/jcp.2003.10538

Nahimana, I., Francioli, P., & Blanc, D. S. (2006). Evaluation of three chromogenic media (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant Staphylococcus aureus. *Clinical Microbiology and Infection*, *12*(12), 1168–1174. https://doi.org/10.1111/j.1469-0691.2006.01534.x

Namvar, A. E., Bastarahang, S., Abbasi, N., Ghehi, G. S., Farhadbakhtiarian, S., Arezi, P., … Chermahin, S. G. (2014). Clinical characteristics of Staphylococcus epidermidis: a systematic review. *GMS Hygiene and Infection Control*, *9*(3), Doc23–Doc23. https://doi.org/10.3205/dgkh000243

Novick, R. P. (2003). Mobile genetic elements and bacterial toxinoses: The superantigen-encoding pathogenicity islands of Staphylococcus aureus. *Plasmid*, Vol. 49, pp. 93–105. https://doi.org/10.1016/S0147-619X(02)00157-9

Omar, N. Y., Ali, H. A. S., Harfoush, R. A. H., & El Khayat, E. H. (2014). Molecular typing of methicillin resistant staphylococcus aureus clinical isolates on the basis of protein a and coagulase gene polymorphisms. *International Journal of Microbiology*. https://doi.org/10.1155/2014/650328

Otter, J. A., Klein, J. L., Watts, T. L., Kearns, A. M., & French, G. L. (2007). Identification and control of an outbreak of ciprofloxacin-susceptible EMRSA-15 on a neonatal unit. *Journal of Hospital Infection*, *67*(3), 232–239. https://doi.org/10.1016/J.JHIN.2007.07.024

Park, Y. K., Peck, K. R., Cheong, H. S., Chung, D. R., Song, J. H., & Ko, K. S. (2009). Extreme drug resistance in Acinetobacter baumannii infections in intensive care units, South Korea. *Emerging Infectious Diseases*, *15*(8), 1325–1327. https://doi.org/10.3201/eid1508.080772

Parvez, M. A. K., Ferdous, R. N., Rahman, M. S., & Islam, S. (2018). Healthcare-associated (HA) and community-associated (CA) methicillin resistant Staphylococcus aureus (MRSA) in Bangladesh - Source, diagnosis and treatment. *Journal, Genetic Engineering & Biotechnology*, *16*(2), 473–478. https://doi.org/10.1016/j.jgeb.2018.05.004

Pollitt, E. J. G., & Diggle, S. P. (2017). Defining motility in the Staphylococci. *Cellular and Molecular Life Sciences : CMLS*, *74*(16), 2943–2958. https://doi.org/10.1007/s00018-017-2507-z

Prusokas, A., Hawkins, M., Nieduszynski, C. A., & Retkute, R. (2018). The effectiveness of glass beads for plating cell cultures. *BioRxiv*, 241752. https://doi.org/10.1101/241752

Rice, L. B. (2006). Antimicrobial resistance in gram-positive bacteria. *American Journal of Infection Control*, *34*(5), S11–S19. https://doi.org/10.1016/j.ajic.2006.05.220

Rosario-Cruz, Z., Eletsky, A., Daigham, N. S., Al-Tameemi, H., Swapna, G. V. T., Kahn, P. C., … Boyd, J. M. (2019). The *copBL* operon protects *Staphylococcus aureus* from copper toxicity: CopL is an extracellular membrane–associated copper-binding protein. *Journal of Biological Chemistry*, *294*(11), 4027–4044. https://doi.org/10.1074/jbc.RA118.004723

Rutala, W. A., & Weber, D. J. (2004). The benefits of surface disinfection. *American Journal of Infection Control*, *32*(4), 226–231. https://doi.org/10.1016/j.ajic.2004.04.197

Shorr, A. F., Tabak, Y. P., Gupta, V., Johannes, R. S., Liu, L. Z., & Kollef, M. H. (2006). Morbidity and cost burden of methicillin-resistant Staphylococcus aureus in early onset ventilator-associated pneumonia. *Critical Care*, *10*(3), R97. https://doi.org/10.1186/cc4934

Sievert, D. M., Ricks, P., Edwards, J. R., Schneider, A., Patel, J., Srinivasan, A., … Fridkin, S. (2013). Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. *Infection Control & Hospital Epidemiology*, *34*(1), 1–14. https://doi.org/10.1086/668770

Tell, L. A., Foley, J., Needham, M. L., & Walker, R. L. (2003). Comparison of Four Rapid DNA Extraction Techniques for Conventional Polymerase Chain Reaction Testing of Three Mycobacterium spp. that Affect Birds. *Avian Diseases*, *47*(4), 1486–1490. https://doi.org/10.1637/7070

The Clinical and Laboratory Standards Institute. (2016). Performance Standards for Antimicrobial Susceptibility Testing CLSI supplement M100S. In *Clinical and*

Laboratory Standards Institute, Wayne, PA.
Tiwari, H. K., & Sen, M. R. (2006). Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. *BMC Infectious Diseases*, *6*, 156. https://doi.org/10.1186/1471-2334-6-156
Varrone, J. J., de Mesy Bentley, K. L., Bello-Irizarry, S. N., Nishitani, K., Mack, S., Hunter, J. G., … Schwarz, E. M. (2014). Passive immunization with anti-glucosaminidase monoclonal antibodies protects mice from implant-associated osteomyelitis by mediating opsonophagocytosis of Staphylococcus aureus megaclusters. *Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society*, *32*(10), 1389–1396. https://doi.org/10.1002/jor.22672
Wagenvoort, J. H. T., Sluijsmans, W., & Penders, R. J. R. (2000). Better environmental survival of outbreak vs. sporadic MRSA isolates. *Journal of Hospital Infection*, *45*(3), 231–234. https://doi.org/10.1053/JHIN.2000.0757