T.R.N.C.

NEAR EAST UNIVERSITY INSTITUTE OF HEALTH SCIENCES

Isolation, Identification and Antimicrobial Sensitivity Pattern of Hospital-Acquired Methicillin Resistant Staphylococcus aureus (HA-MRSA)

HUSSAIN AHMAD

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAMME

MASTER THESIS

NICOSIA 2016

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DEDICATION

I dedicate this Thesis to my family for Nursing me with affection and love and their dedicated partnership for Success in my life

ABSTRACT

Ahmad, H. Isolation, Identification and Antimicrobial Sensitivity Pattern of Hospital-Acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA). Near East University Institute of Health Sciences, M.Sc. Thesis in Medical Microbiology and Clinical Microbiology Programme, Nicosia, North Cyprus 2016. This study was conducted to isolate and analyze antibiotic susceptibility patterns of Methicillin

Resistant *Staphylococcus aureus* (MRSA). A total of 200 different samples were collected from different wards of Near East Hospital Cyprus. S. aureus was isolated and identified by using selective media and biochemical tests including catalase, and coagulase tests. Phoenix BD method was used for antimicrobial susceptibility testing.

MRSA was detected by using methicillin while antimicrobial sensitivity pattern was checked for antibiotics are Ampicillin, Cefazolin, Cefoxitin, Clindamycin, Daptomycin, Erythromycin, Gentamicin, Linezolid, Moxifloxacin, Rifampin, Nitrofurantoin, Norfloxacin, Oxacillin, Levofloxacin, Penicillin G, Quinuprsitin-Dalfopristin, Teicoplanin, Tetracycline, Trimethoprim, Sulfamethoxazole and Vancomycin.

Results showed that out of 200 samples, 32% were male while remaining 68% were female. Highest number of samples was chest disease samples (24%) followed by dermatology (21.3%), cardiology (18.7%), emergency (14.7%) and urology (4%) while other samples were belonging to CV surgical ward (17.3%). S. aureus from the samples possessed a high resistant nature against commonly used antibiotics.

Maximum MRSA positive strains were found among the females than the males (68.0% and 32.0% respectively).

Out of 75 MRSA strains (6.7%) of urine, (29.7%) of blood, (14.7%) of nasal swab, (9.3%) of sputum, (10.7%) of aspiration fluids, (4.0%) of IV catheter, and (25.3%) of wound culture samples were used for screening of MRSA strains.

All the strains of MRSA were found to be 100 % resistant to Ampcilline, Cefazoline, Oxacilline, and Pencilline G while all strains of MRSA were found to be 100% sensitive to Linezolid and Moxifloxacin.

Key Words: MRSA, Cyprus, Phoenix BD, and Antimicrobial Sensitivity.

ÖZET

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The whole Universe, who blessed his countless blessings upon us and enabled me to think, performs and completes this challenge-able task.

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With Regards Hussain Ahmad

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LIST OF ABBREVIATIONS

MRSA	Methicillin-resistant Staphylococcus aureus	
MSSA	Methicillin-sensitive Staphylococcus aureus	
HA-MRSA	Hospital-Acquired Methicillin Resistant Staphylococcus aureus	
VRE	Vancomycin-Resistant Enterococcus	
PCR	Polymerase Chain Reaction	
WHO	World Health Organization	
IV	Intravenous	
TSS	Toxic Shock Syndrome	
SSTIs	Soft tissue infections	
ED	Emergency Department	
 CDC	Center for Disease Control	
BHI	Brain Heart Infusion	
VRSA	Vancomycin Resistance S. aureus	

時に他は現代には人	VISA	Vancomycin Intermediate S. aureus		
	BSI	Compared Blood Stream Infection		
	CoNS	Coagulase Negative Staphylococci		
	PFGE	Pulsed Field Gel Electrophoresis		
	KZN	KwaZulu-Natal		
	NI	Nosocomial Infections		
	CI	Confidence Interval		
	PBP	Penicillin Binding Protein		
	MSA	Mannitol Salt Agar		
	EUCAST	European Committee on Antimicrobial Susceptibility		
	SPSS	Testing Statistical Package for the Social Sciences		
	IQR	Interquartile Range		

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1. INTRODUCTION

1.1 General Information

A great damage to health of animals, plants and humans is endemic in population with high ratio due to infectious diseases caused by vast number and types of bacteria. Some infectious diseases are as fatal as leading to death, reported worldwide (Wilson *et al.*, 2002).

From the list of dangerous pathogens of health-concern, one is a well-known bacterium: *Staphylococcus aureus* (Persoons *et al.*, 2009), which attains bad effects on health of humans and other animals (Milyani and Ashy, 2012). *S. aureus*, when finds its way into an organism (human or other animals) through nasal cavity or skin punctures or other routes, it results in a variety of infections ranging from minor cases (skin infections and abscesses) to life-endangered cases including toxic shock syndrome (TSS), meningitis, pneumonia, septicemia and endocarditis (Enright *et al.*, 2002; Ho *et al.*, 1989).*S. aureus* enters the body of infants, children and young adults with the same pattern as with elders; through their body parts especially dermal layers, nasopharynx and cause infection, while detailed epidemiological results reveals that highest incidence of *S. aureus* infection has been observed in lower ages than in higher ages (Milyani and Ashy, 2012).

Although a variety of infections are caused by *S. aureus*, their resistance pattern against a number of antimicrobial agents also varies. One of such *S. aureus* resistant to methicillin, is called "Methicillin Resistant *Staphylococcus aureus* (MRSA)" while other types include *S. aureus* resistant to other antibiotics as gentamycin, fucidic acid and clindamycin (Shaiet al., 2004). Initially, penicillin was used against *S. aureus* but this bacteriumbecame resistant to penicillin due to the production of β-lactamase soon after using β-lactamase-stable penicillin-derived methicillin for *S. aureus*, rapidly, strains of MRSA emerged (Samiaet al., 2007). Infections caused by resistant *S. aureus* especially methicillin resistant *S. aureus* (MRSA) is a prominent threat to animal's and human's health (Yao et al., 2010; Persoonset al., 2009) because the pathogen becomes resistant to

antibiotics aided with unavailability of controlling or antimicrobial agent (Shaiet al., 2004).

Staphylococcus aureus is not only a threat to human but also to other animal species. The importance of methicillin resistant *S. aureus*(MRSA) in veterinary medicine is not well established (Weeseet al., 2005). However, MRSA outbreaks in horses suggest that this organism might be an emergingproblem in the equine population (Cunyet al., 2006; Weese et al., 2006a). MRSA infection has been reported in different animal species; sheep, goat, cows (Ho et al., 1989), dogs (Bassim and El-Maghraby, 2005) and hospitalized horses (Hartmann et al., 1997) and their transmission between infected horses and veterinary personnel has been documented.

Hospital associated-methicillin resistant *Staphylococcus aureus* (HA-MRSA) and community associated-Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) are two broad categories of MRSA reported till date. Strains of MRSA developed from a community (population or habitat) refers to CA-MRSA while on the other hand if the incidence of strains of MRSA are related to hospital environment it is known as HA-MRSA which also include the development and emergence of MRSA from the patient having current or recent hospitalization, receives dialysis, or resides in a long-term care facility (Milyani and Ashy, 2012). MRSA has been associated with skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritides, endocarditis, osteomyelitis and sepsis (Lowy, 1998).

Since the first European isolate of MRSA, detected in 1960s, MRSA has become a leading cause of nosocomial infections worldwide (Lowy, 1998; Tristan *et al.*, 2007). Sheikh *et al.* (2008) defined nosocomial infection as an infection occurred to a person or a patient within 48 hours after admitted to or discharged from a hospital. After first reported as nosocomial pathogen in 1960s, with the passage of time MRSA got attention as CA-MRSA (Frazee *et al.*, 2005) soon after the introduction of methicillin into clinical practice (Anand*et al.*, 2009).

During the period 1970 to 2010, strains of *S. aureus*resistant to multiple antibiotics including methicillin were increasingly responsible for outbreaks of nosocomial infections in countries around the world, for example, Saudi Arabia (Madani*et al.*, 2001), Austria (Krziwanek*et al.*, 2009), Argentina (Reyes *et al.*, 2009), South Africa (Shittu*et al.*, 2009), Italy (Soavi*et al.*, 2010) and the United States (Boyce, 1990). In many instances, these outbreaks were associated with individual wards, neonatal, intensive care and burns units (Liu *et al.*, 2011). Furthermore, increasing incidence of CA-MRSA has been a growing public health concern and has emerged as the predominant cause of skin infections in the USA (Mandell*et al.*, 2005; Ma *et al.*, 2007).

However, until recently, the majority of such cases were associated with known risk factors for MRSA, particularly recent contact with a health care facility (Chambers, 2001). From an epidemiologic perspective, such cases were believed to represent temporary circulation into the community of a nosocomial strain (Frazee *et al.*, 2005). In the mid1990s, reports began to appear in the United States, particularly among children, of CA-MRSA infection, defined as occurring in patients without identifiable risk factors (Herold*et al.*, 1998; Frank *et al.*, 1999). On the other hand, HA-MRSA also occurs with high ratio in countries around the world (Udo and Jacob, 2000).

MRSA is, by definition, any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. MRSA is capable of resisting Beta-Lactamase resistant Antibiotics via the mecA gene (Sarika Gupta *et al.*, 2015).

1.2 Historical Account

Staphylococci were first observed in human pyogenic lesions by Von Recklinghausen in 1871. Pasteur in 1880 obtained liquid cultures of cocci from pus and produced abscesses by inoculating them into rabbits. But it was Sir Alexander Ogston, a Scottish surgeon in 1880 who established conclusively the causative role of the coccus in abscesses and other suppurative lesions. He also gave the name *Staphylococcus* (Staphyle, in Greek meaning

'bunch of grapes': Kokkos, meaning aberry) due to the typical occurrence of the cocci in grape like clusters in pus and in cultures. Ogston had noticed that non-virulent staphylococci were also present on skin surfaces. Most staphylococcal strains from pyogenic lesions were found to produce golden yellow colonies, and the strains from normal skin, white colonies on solid media. In 1884, Rosenbach named them Staphylococcus aureus and Staphylococcusalbus respectively. Later S. albus was renamed as Staphylococcus epidermidiswhich were coagulase negative, mannitol nonfermenting and usually nonpathogenic strains (Murray et al., 2003). Staphylococci are wide spread in nature although they are mainly found living on the skin, skin glands and mucous membrane of mammals and birds. They may be found in the mouth, blood, mammary glands, intestinal, genitourinary and upper respiratory tracts of these hosts. *Staphylococcus* aureus generally have a benign or symbiotic relationship with their host; however they may develop the lifestyle of a pathogen if they gain entry into the host tissue through trauma of the cutaneous barrier, inoculation by needles or direct implantation of medical devices. Infected tissues of host support large populations of staphylococci and in some situations they persist for long periods. The presence of enterotoxigenic strains of S. aureus in various food products is regarded as a public health hazard because of the ability of these strains to produce intoxication or food poisoning. S. aureus is a major species of primates, although specific ecovars or biotypes can be found occasionally living on different domestic animals or birds (Murray et al., 2003).

1.3 Classification and Cultural characteristics

Staphylococcus aureus is an aerobic or facultative anaerobic, coagulase positive organism which colonises the skin, nasal passage and axillae of humans. It occurs in grape like clusters when viewed through the microscope and has large round golden yellow colonies often with beta hemolysis when grown on blood agar (Murray *et al.*, 2003).

The cocci are spherical, approximately 1µm in diameter, arranged in grape like clusters. They may be found singly, in pairs or in short chains especially in liquid culture. They are non-motile and non-sporing and some strains possess microscopically visible capsules (Anathanarayan 2002). They grow readily on ordinary media within a temperature range of 10-42°C. Optimum temperature is 37°C and pH 7.4-7.6. On nutrient agar a typical 24hr *S.aureus* colonies are pigmented, smooth, entire, slightly raised, translucent and hemolytic on routine blood agar. Small colony variants (SCVs) of *S. aureus* produce colonies that are pin point in size, non hemolytic and non pigmented. In liquid medium, uniform turbidity is produced. Selective media used for isolating *S. aureus* contain 8-10% NaCl like salt-milk agar, ludlam's medium containing lithium chloride and tellurite (Bannerman 2003).

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1.4 Biochemical reactions of Staphylococcus aureus

They ferment sugar producing acid but no gas. Mannitol is fermented anaerobically only by *S. aureus*. They are catalase and urease positive. They reduce nitrates to nitrites, liquefy gelatin and are MR, VP positive but indole negative. They are lipolytic when grown on medium containing egg yolk. They produce phosphatase which can be demonstrated by growing on nutrient agar containing phenolphthalein diphosphate. In a medium containing potassium tellurite, tellurite is reduced and black colonies are produced (Humphreys 2002; Anathanarayan 2002).

Coagulase Production

The ability to clot plasma is generally accepted criterion for the identification of *S. aureus.* Two different coagulase tests are performed: a tube test for detecting free coagulase and slide test for bound coagulase or clumping factor. While the tube test is definitive, the slide test may be used as a rapid screening technique to identify *S. aureus.* Coagulase test is carried out using rabbit plasma containing EDTA (Bannerman 2003).

Heat Stable Nuclease

A heat stable staphylococcal nuclease (thermonuclease (TNase)) that has endo and exonucleolytic properties and can cleave RNA or DNA is produced by most strains of S. *aureus.* TNase can be demonstrated by the ability of boiled cultures to degrade DNA in

an agar diffusion test or detected by using metachromatic agar diffusion procedure and DNasetoludene blue agar.

Acetoin Production

Acetoin production from glucose or pyruvate is a useful alternative characteristic to distinguish *S. aureus*. This is done using a conventional Voges-Proskauer test tube method with an incubation time of 72hrs (Bannerman 2003).

1.5 Antibiotic resistance

At first, penicillin was used to treat S. aureus infections. Soon afterwards, resistance emerged when strains acquired a genetic element coding for β -lactamase production, and today over 80 % of all S. aureus strains are resistant to penicillins. The next drug to be introduced for treating infections with S. aureus was the semisynthetic, penicillinaseresistant penicillin named oxacillin or methicillin, but shortly after its introduction the first isolate with resistance was detected (see text below) (Winn Washington 2006). With the emergence of resistance to the penicillinase-resistant penicillins, the glucopeptide agent vancomycin became the treatment of choice for infections with MRSA, and in 1996 the first isolate with intermediate vancomycin resistance was detected in Japan (Winn Washington 2006). So far, this has not emerged to be a major concern, but the resistance has been detected in different parts of the world and needs to be monitored. Although resistance to methicillin is considered the most important for S. aureus, other types of resistance exist. For example, a fusidic acid-resistant impetigo clone has caused infections around Europe. The antibiotic fusidic acid is used to treat superficial skin infections caused by S. aureus, which include impetigo and atopic dermatitis (Brown and Thomas, 2002), and the substance has been in use since the early 1960s. Despite this, the resistance remained low until the 1990s (Brown and Thomas, 2002). Through the last decade an increase in prevalence of fusidic acid-resistant S. aureus has been seen in northern Europe, and this resistance has been primarily associated with strains causing impetigo bullosa (O'Neill et al., 2004; Osterlundet al., 2002; Tveten et al., 2002). The resistance is a consequence of the recruitment of the fusB gene (O'Neill and Chopra,

2006; O'Neill *et al.*, 2004). Since fusidic acid is the primary treatment for impetigo in many countries, this is likely to be the reason for the success of this clone in causing disease. The management with antibiotic-resistant bacteria of infections suffered by the elderly living in nursing homes is something to take into consideration now and in the future. For example, MRSA has become endemic in hospitals as well as in health care settings globally (Chambers and Deleo, 2009; DeLeo and Chambers, 2009). Many nursing home residents have chronic and multiple diseases, and therefore generally require constant medical care and significant assistance with daily living. This causes the residents to be considered as unintentional vectors disseminating pathogens between hospitals and nursing homes but also the other way around (Bonomo, 2000; Chamchod and Ruan, 2012). So far this does not seem to be the case in Sweden, where the resistance in general is Introduction low and no increase in resistance has been detected for *S. aureus* isolated from nursing home residents (Olofsson *et al.*, 2012; Olofsson *et al.*, 2013).

1.6 Methicillin-resistant Staphylococcus aureus

The massive consumption of antibiotics over the past 50 years has led to the selection of drug-resistance among *S. aureus* strains, and by far the most important is the resistance against methicillin. In 1961, methicillin (celbenin) became available for treatment of penicillin-resistant *S. aureus* strains. Only six months thereafter, the first methicillin-resistant *S. aureus* was detected and nosocomial infections began to increase, and in Sweden efforts to combat the spread were established. In the 1980s the detection of MRSA isolates suddenly increased, and a few strains began to expand worldwide (Chen *et al.*, 2012). MRSA is now a leading cause of nosocomial infections worldwide and has also emerged as a community-associated pathogen (Chambers and Deleo, 2009). MRSA strains are inherently cross-resistant to virtually all beta-lactam antibiotics, the most effective and widely used class of antimicrobials. Moreover, in many countries clinical strains are quite often multi-resistant, which significantly reduces the therapeutic options for treatment of staphylococcal infections (Oliveira and de Lencastre, 2011). The resistance mechanism against methicillin involves the acquisition of the mecA gene, which is a determinant of a unique penicillin binding protein, (PBP) 2a, that has reduced

affinity for β -lactames, including cephalosporins (Hartman and Tomasz, 1981; Song *et al.*, 1987). The expression of PBP2a causes resistance to all β -lactam antibiotics as the protein blocks binding at the active site for β -lactams (Fuda*et al.*, 2005a; Fuda *et al.*, 2005b). mecA is inserted in a large heterologous chromosomal cassette, the SCCmec element (*Ito et al.*, 1999).

In the first international molecular epidemiological study of MRSA, it was discovered that only a few MRSA lineages were responsible for MRSA infections in hospitals located in Europe, the USA and the Far East (Oliveira *et al.*, 2002). Later studies have confirmed these results (Enright *et al.*, 2002). In a recent European study, the prevalence of MRSA, in blood stream infections varied between 0.5 and 30.2 % in the different participating countries (ECDC). This was in contrast to the low prevalence of MRSA in the general healthy population, where the rates did not exceed 2.1 % (den Heijer *et al.*, 2013).

The purpose of this study was to investigate the incidence of MRSA in Near East Hospital, (North Cyprus) according to their specific antibiotic susceptibility profiles. Along with the prevalence of MRSA in the hospital, the main objectives of this work are listed as below.

1.7 Aims and Objectives

Our study was conducted with aims:

- 1. To Isolate and identify *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureu* (MRSA) from the collected samples.
- 2. To analyze and draw inference regarding MRSA and related risks to the patient through which health care can be improved on the basis of data obtained.
- 3. To study the antimicrobial susceptibility pattern of Staphylococcus aureus

2. REVIEW OF LITERATURE

Van-Belkum *et al.* (1995) reported that infections caused by *Staphyloccus aureus* were reported first time by Sir Alexander Ogston in 1880s in which he said that these infections are increasing day by day.

Lowy (1998) and Bhatia and Zahoor (2007) studied that in the treatment of *S. aureus*, it became challenging and very difficult because of the ability of the *S. aureus* to rapidly attain the state of resistance to many drugs. But soon by the end of the 40s decad, half of *S. aureus* strains became resistant to penicillin in USA. But very soon in 1961, methicillin resistant *S. aureus* (MRSA) strains were reported. *S. aureus* can affect any organ or tissue of the human body in which most common includes endocarditis, mastitis, meningitis, osteomylitis, phlebitis (inflammation of veins) and pneumonia.

Greenwood *et al.* (2002) described that pathogenicity of *S. aureus* is enhanced by the presence of extracellular virulence factors which helps the pathogen in improving its pathogenesis and colonization of the host. While in 2002, 90% of *S. aureus* strains isolates found in hospitals worldwide were resistant to penicillin.

Salyers and Whitt (2002) stated that while Staphylococcal scalded skin syndrome (SSSS) and toxic shock syndrome (TSS) are linked to *S. aureus* specific toxins.

Saiman *et al.* (2003) described that infections caused by community-acquired methicillinresistant *Staphylococcus aureus* (CA-MRSA) are being increasingly observed in patients who lack traditional risk factors. They described 8 postpartum women, who developed skin and soft-tissue infections caused by MRSA at a mean time of 23 days (range, 4–73 days) after delivery. Infections included 4 cases of mastitis (3 of which progressed to breast abscess), a postoperative wound infection, cellulitis, and pustulosis. The outbreak strains were compared with the prototype CA-MRSA strain MW2 and found to be indistinguishable by pulsed-field gel electrophoresis. All were *spa* type 131, all contained the staphylococcal chromosomal cassette *mec* type IV, and all expressed Panton-Valentine leukocidin and staphylococcal enterotoxins C and H. The route of transmission was not discovered: the results of surveillance cultures of samples obtained from employees of the hospital, the hospital environment, and newborns were negative for the outbreak strain. They concluded that MW2, which was previously limited to the mid western United States, has spread to the northeastern United States and has become a health care–associated pathogen.

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Wilson *et al.* (2003) reported that pathogenic bacteria utilize a number of mechanisms to cause disease in human hosts. Bacterial pathogens express a wide range of molecules that bind host cell targets to facilitate a variety of different host responses. The molecular strategies used by bacteria to interact with the host can be unique to specific pathogens or conserved across several different species. A key to fighting bacterial disease is the identification and characterization of all these different strategies. The availability of complete genome sequences for several bacterial pathogens coupled with bioinformatics will lead to significant advances toward this goal.

Naimi *et al.* (2003) stated that between 1993 and 2003, novel strains of MRSA that were phenotypically and genotypically distinct from the parent health-care associated MRSA (HA-MRSA) were identified in the community suggesting evolution of the original MRSA. These strains of MRSA became known as community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) (Center for Disease Control (CDC 2003a).

Frazee *et al.* (2005) determined the prevalence of MRSA among emergency department (ED) patients with skin and soft tissue infections, identify demographic and clinical variables associated with MRSA and characterize MRSA by antimicrobial susceptibility and genotype. Out of 137 subjects they studied, 18% were homeless, 28% injected illicit drugs, 63% presented with a deep or superficial abscess, and 26% required admission for the infection. MRSA was present in 51% of infection site cultures. Of 119 *S. aureus* isolates (from infection site and nares), 89 (75%) were MRSA. Antimicrobial susceptibility among MRSA isolates was trimethoprim/sulfamethoxazole 100%, clindamycin 94%, tetracycline 86%, and levofloxacin 57%. Among predictor variables independently associated with MRSA infection, the strongest was infection type being furuncle (odds ratio 28.6). Seventy-six percent of MRSA cases fit the clinical definition of community associated. Ninety-nine percent of MRSA isolates possessed the

SCCmecIV allele (typical of community-associated MRSA), 94.1% possessed Panton-Valentine leukocidin genes, and 87.1% belonged to a single clonal group (ST8: S).

Tiwari and Sen (2006) studied 1681 staphylococcal isolates, consisting of 783 *S. aureus* and 898 coagulase negative staphylococci (CoNS), which were isolated from different clinical specimens from various outpatient departments and wards. All *S. aureus* and 93 CoNS were subjected to MIC testing (against vancomycin, teicolplanin and oxacillin); Brain Heart Infusion (BHI) vancomycin screen agar test; disc diffusion testing, and PCR for *mecA*, *vanA* and *vanB* genes detection. Their results were as follows. Out of 783 *S. aureus* two *S. aureus* strains were found to be vancomycin and teicoplanin resistant (one strain with MIC 32 µg/ml and the other strain with MIC 64 µg/ml); six strains of *S. aureus* have shown to be vancomycin intermediate (two strains with MIC 16 µg/ml and four strains with MIC 8 µg/ml); and two strains with teicoplanin (MIC 32 µg/ml), and two CoNS strain was resistant to vancomycin and teicoplanin (MIC 16 µg/ml). All VRSA, VISA and vancomycin resistant CoNS had shown growth on BHI vancomycin screen agar (vancomycin6 µg/ml) and were *mec A*PCR positive. None of these isolates have demonstrated *vanA*/*vanB* gene by PCR.

Desai *et al.* (2006) stated that prescribed intravenous (IV) glycopeptides usually remain in hospital until completion of this treatment of 211 patients they studied, 62 (29%) could have had a reduced length of stay if they were treated with a suitable oral antibiotic. This would have saved a total of 649 inpatient days (median 5 per patient; range 1–54). A further 31 patients (15%) could have switched to oral therapy as an inpatient thus avoiding IV line use. The patients most likely to be suitable for early discharge were those with skin and soft tissue infection, under the cardiology, cardiothoracic surgery, orthopedics, general medical, plastic surgery and vascular specialties, with no high risk co morbidity and less than five other regularly prescribed drugs.

Popovich *et al.* (2008) concluded that CA-MRSA strains have been reported to cause infections in health-care facilities demonstrating the ecological fitness and emergence of these strains in different clinical settings.

Geddes (2008) the first controlling strategy in 1940s for *S. aureus* was penicillin; a betalactam antibiotic.

Tong *et al.* (2009) compared bloodstream infection (BSI) rates among 18 hospitals in Queensland, Australia for performance measurement, observed rates need to be risk adjusted according to the types of patients cared for by the hospital to identify medical services associated with high or low BSI rates, and to evaluate the services provided by the hospital as indicators that can be used for more objective hospital-level risk adjustment. They identified four risk services for OBSI: AIDS (IRR 2.14, 95% CI 1.20 to 3.82), infectious diseases (IRR 2.72, 95% CI 1.97 to 3.76), oncology (IRR 1.60, 95% CI 1.29 to 1.98) and bone marrow transplants (IRR 1.52, 95% CI 1.14 to 2.03).

El-Moez *et al.* (2009) investigated about the cause of an outbreak in Arabian and foreign breed equine farm with mortality rate 18.82%, the animals showed acute watery diarrhea and colic followed by death. However the animals were treated with multiple broad spectrum antibiotics. Postmortem and histo-pathological findings indicated generalized toxaemia in the form of severe congestion in all vital organs, pneumonia, endocarditis, gastroenteritis and nephritis. Bacteriological examination showed isolation of *S. aureus* from all cases which were tested for their sensitivity toward different antibiotics. They found that all *S. aureus* isolated from infected and dead animals were 100% resistant to all tested antibiotics with an exception for vancomycin which was used to control the progress of cases in the farm. The excessive nonspecific antibiotics treatment leads to propagation of opportunistic multiple drug resistant *S. aureus* which release entero toxins leading to toxic shock syndrome that end fatally after development of signs of toxemia and septicemia leading to increased morbidity and mortality rates.

Shittu *et al.* (2009) reported that epidemiological data based on phenotypic and molecular characterization of MRSA in sub-Saharan Africa are limited. This investigation studied 61 MRSA isolates obtained from 13 health-care institutions in KwaZulu-Natal (KZN) province, South Africa, from March 2001 to August 2003. More than 80% of the isolates were resistant to at least four classes of antibiotics and six isolates were resistant to the aminoglycoside, macrolide-lincosamide and tetracycline groups of antibiotics, heavy

metals and nucleic acid binding compounds. PFGE of SmaI-digested genomic DNA revealed seven types, designated A–G. Type A was the main pulsotype (62.3%) and was identified in 11 of the 13 health-care institutions, suggesting that it represented a major clone in health-care institutions in KZN province. Analysis of representative members of the three major pulsotypes by spa, multilocus sequence typing and SCC mec typing revealed the types t064-ST1173-SCCmec IV and t064-ST1338-SCCmec IV (PFGE type A, single-locus and double-locus variants of ST8), t037-ST239-SCCmec III (PFGE type F) and t045-ST5-SCCmec III (PFGE type G).

Persoons *et al.* (2009) detected MRSA in several species and animal-derived products. To determine whether MRSA is present in poultry, they sampled 50 laying hens and 75 broiler chickens. MRSA was found in some broiler chickens but no laying hens. In all samples, *spa* type t1456 was found.

Anand *et al.*, in 2009 conducted a study to evaluate the efficacy of cefoxitin disc diffusion test to characterize MRSA and compare it with oxacillin agar screening and detection of *mecAgene* by PCR. They used fifty strains of *S. aureus* isolated from clinical samples. Routine antibiotic susceptibility testing was performed including oxacillin disk. Oxacillin screen agar plates with 4% NaCl and 6 μ g/ml of oxacillin were inoculated and interpreted as per standard guidelines. Cefoxitin disc diffusion test was performed using 30 μ g disc and zone sizes were measured. PCR for amplification of the *mec Agene* was performed. Their results showed that out of the 50 isolates, 28 were found to be methicillin resistant by oxacillin disc diffusion test, 30 were resistant by oxacillin screen agar method, and 32 were resistant with cefoxitin disc diffusion. For these 32 isolates *mecA* gene was positive. They concluded on the basis of their study that a result of cefoxitin disc diffusion test is in concordance with the PCR for *mecA* gene. Thus, the test can be an alternative to PCR for detection of MRSA in resource constraint settings.

Yao *et al.* (2010) isolated 111 *S. aureus* (48 CA-MRSA & 63 HA-MRSA) from pus samples of patients with Skin and Soft Tissue Infections (SSTIs) at a teaching hospital in Wenzhou, China. Sixty isolates were confirmed as MRSA harboring *mec* A detected by PCR. A total of 32 PFGE clonal types were obtained by PFGE, with 10 predominant

patterns (types A to J). Twenty-five different STs including ST398 and three novel STs were found among 51 selected isolates. The main STs were ST239, ST1018, ST59, ST7 and ST88. Of 60 MRSA isolates, SCC *mec*II, III, IV and SCC *mec* V were found in three, 50, three and two isolates, respectively. The positive rates of PVL genes in overall isolates, HA-isolates, CA-isolates, MRSA isolates and MSSA isolates were 23.4% (26/111), 20.6% (13/63), 27.1% (13/48), 21.7% (13/60) and 25.5% (13/51), respectively. Eight (33.3%, 8/24) of 24 CA-MRSA isolates and 5 (13.9%, 5/36) of 36 HA-MRSA isolates were positive for PVL genes. ST239-MRSA-SCC*mec*III and ST1018-MRSASCC*mec*III clones were found to be main clones and spread between community and hospital.

Qayyum *et al.* (2010) defined nosocomial infections as the infections which develop within hospital. Their study was based on questionnaire from population including 71 doctors including Consultants, Medical Officers and House Officers. They found that majority of doctors were aware about nosocomial infections (N.I) but have weak knowledge about their routes of transmission and common types of nosocomial infections.

Cummings *et al.* (2010) described that hygiene noncompliance is a major cause of nosocomial infection. To estimate methicillin-resistant *Staphylococcus aureus* (MRSA)-related cost of an incident of hand hygiene noncompliance by a healthcare worker during patient care, they worked on two models. Model 1 involved encounters with patients of unknown MRSA status. Model 2 involved an encounter with an MRSA-colonized patient followed by an encounter with a patient of unknown MRSA status. Results showed that Model 1 was associated with 42 MRSA infections (infection rate, 0.0042%). Mean infection cost was \$47,092 (95% confidence interval [CI], \$26,040–\$68,146); mean cost per noncompliant event was \$1.98 (95% CI, \$0.91–\$3.04). Model 2 was associated with 980 MRSA infections (0.098%). Mean infection cost was \$53,598 (95% CI, \$50,098–\$57,097); mean cost per noncompliant event was \$52.53 (95% CI, \$47.73–\$57.32). A 200-bed hospital incurs \$1,779,283 in annual MRSA infection–related expenses attributable to hand hygiene noncompliance. A 1.0% increase in hand hygiene compliance resulted in annual savings of \$39,650 to a 200-bed hospital.

Sobhy *et al*, (2012) studied the antibiotic resistant profile as well as studied the comparison between Community-Acquired and Hospital-Acquired Methicillin Resistant *Staphylococcus aureus* from samples of Egyptian Population. Out of 50 samples they studied, only 38 (76%) samples contained *Staphylococcus aureus*. Detailed investigations showed that 18 (47.37%) from 38 samples were initially diagnosed as MRSA and were resistant to oxacillin and cefoxitin antibiotics and these were producing penicillin binding protein 2a (PBP2a). These 18 samples were confirmed as MRSA by the detection of *mecA* gene from the samples with the help of real time PCR. On the other hand six (33.33%) strains were PVL positive. Using a sets of primers of nine (50%) out of the 18 CA-MRSA strains were *SCC mec*type V, and one (5.56%) was *SCC mec*type IVc. Then, using the other set of primers out of the eight untypable MRSA strains were found to be *SCCmec*type IV, and six (75%) remained untypable.

Milyani and Ashy (2012) reported that nosocomial and community acquired infections caused by Staphylococcus aureus are increasing day by day. After finding alternative antimicrobial substances from various plant extracts on 70 resistant isolates collected from King Fahad General Hospital and National Guard Hospital, Jeddah, Saudi Arabia, they found the following pattern: 50 isolates were resistant to fusidic acid, 28 isolates were resistant to oxacillin, 20 isolates were resistant to penicillin, 20 isolates were resistant to clindamycin and 21 isolates were resistant to gentamycin. They further found that all the isolates were sensitive to vancomycin. The antimicrobial activity of the aqueous extracts of five medicinal plants namely Canelliasinensis (green tea), Punnicagranatum (pomegranate rind), Psidiumguajava Lim (guava leaves), Cinnamomumverum (cinnamon) and Mourus (raspberry) was tested against the 70 resistant isolates of S. aureususing agar well diffusion assay. Significant difference was noted in the inhibitory effect between most of the tested extracts. Pomegranate rind showed the highest activity, followed by green tea, guava leaves, cinnamon bark and raspberry fruits extract respectively, compared to commercial antibiotics. Interestingly, the inhibitory activity of three combined extracts: green tea, pomegranate rind and guava leaves was found to be higher on 20 clindamycin resistant isolates compared to each extract alone, indicating synergistic interaction.

3. Material and Methods

Study Areas

Northern Cyprus is the Turkish part of the eastern Mediterranean island of Cyprus, divided between Turkey and Greece since the late-20th century. The total population of North Cyprus is 301,988 (2013)

The present study was conducted in North Cyprus from month of January 2015 to month of December 2015.

3.1. Sampling

Total 200 samples for this study were collected from Near East Hospital,Cyprus. These samples included the samples collected from different wards (cardiology, cardiovascular surgery, urology, chest diseases, dermatology and emergency service). These samples included the samples of urine, blood, nasal swab, sputum, aspiration Fluids, IV catheter and wound culture. In these 200 samples 75 samples were MRSA. The samples were labeled accordingly and were subjected for screening of *Staphylococcus aureus* in Microbiology Laboratory at Near East Hospital, North Cyprus.

3.2. Media Preparation and Sterilization

3.2.1 Blood Agar

Blood agar (Merck Millipore) is used for isolation and cultivation of many types of fastidious bacteria. It is also used to differentiate bacteria based on their hemolytic characteristics, so blood agar was used to isolate and identify *Staphylococcus aureus*. 28 g of nutrient agar powder was Suspend in 1 litter of distilled water, Heat this mixture while stirring to fully dissolve all components. After the dissolved mixture was autoclave at 121 degrees Celsius for 15 minutes. Once the nutrient agar has been autoclaved, it was allow cooling but not solidifying. When the agar has cooled to 45-50 °C, Add 5% (vol/vol)

sterile defibrinated blood that has been warmed to room temperature and mix gently but well and avoid air bubbles. Finally dispense into sterile plates while liquid.

3.2.2 Mannitol Salt Agar (MSA)

Mannitol Salt Agar (MSA) (OXIDE Private Limited) was used for inoculation of samples. Media was prepared according to manufacturer's standard protocol. Media along with all required glassware were autoclaved at 121°C for 20 minutes at 15psi. After autoclaving, media plates were prepared by pouring 25ml of each media into sterilized plates (99mm in diameter) under Laminar Flow Hood and were allowed to cool (solidify) for 30 minutes in sterile environment.

3.3. Culturing of Samples on Media

After the media was prepared, first all the samples were inoculated on blood agar for isolation and identification of samples and were incubated at 37°C. Growth was observed after 24 hours of incubation.



As Mannitol Salt Agar (MSA) is used for the isolation of staphylococci, so after that all the samples were inoculated on MSA were incubated at 37°C. Growth was observed after 24 hours of incubation



Colonies from pre-cultured samples were sub-cultured on selective media MSA to obtain pure isolates of *Staphylococcus aureus*.

3.4 Biochemical Tests

3.4.1. Preparation of Cell Suspension

Cell suspension was prepared in saline water (0.85% NaCl Solution) and was compared with McFarland turbidity standard solution (0.5)

3.4.2. Gram Staining

A drop of sterile distilled water was transferred to a sterile clear glass slide. A single colony from fresh culture of *Staphylococcus aureus* was picked with the help of sterile wire-loop and added to normal saline drop on the glass slide. Colony was spread with the help of wire-loop. Smear was prepared with after the suspension was dried and heat fixed on glass slides. Once the smear was prepared, it was flooded with crystal violet and allowed for one minute, then washed with distilled water and flooded with gram iodine

and allowed for one minute. Again it was washed with distilled water followed by decolorizing with 95% Ethyl-Alcohol and again washed with distilled water. After, it was counter stained with Safranin for 45 seconds and finally washed with distilled water. The slide was dried and examined under compound microscope at 100X using oil emulsion.

3.4.3. Catalase Test

Catalase an enzyme, acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism can be tested for catalase production by bringing it into contact with hydrogen peroxide. For this purpose 3.0ml of hydrogen peroxide was taken in sterile test tube. With the help of glass rod, a colony was picked from culture plate and was inoculated in hydrogen peroxide solution. Results were observed for the production of bubbles of oxygen released in the solution which indicates the presence of catalase in a bacterium.



3.4.4. Coagulase Test

This test was performed by adding 0.2ml of blood-plasma to 1.8ml of normal saline (0.9% NaCl Solution) in a test tube. Successively, 1ml of fresh broth culture was added. All these tubes were incubated at 37°C for hours. Gelling of plasma was considered as positive result.



3.5 Susceptibility testing

3.5.1 Phoenix BD 100

The Phoenix[™] Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) is designed for the rapid identification (ID) and antimicrobial susceptibility testing (AST) of clinically significant human bacterial pathogens. In this study, we evaluated the performance and accuracy of the Phoenix against the Microscan Walkaway for ID and AST of staphylococci.

The Phoenix AST method is a broth based microdilution test. The Phoenix system utilizes a redox indicator for the detection of organism growth in the presence of an antimicrobial agent. Continuous measurements of changes to the indicator as well as bacterial turbidity are used in the determination of bacterial growth. Each AST panel configuration contains several antimicrobial agents with a range of two-fold doubling dilution concentrations. Organism identification is used in the interpretation of the MIC values of each antimicrobial agent producing Susceptible, Intermediate, or Resistant (SIR) result classifications.

A maximum of 100 identification and antimicrobial susceptibility tests can be performed in the Phoenix instrument at a time using Phoenix ID/AST combination panels. A sealed and self-inoculating molded polystyrene tray, with 136 micro-wells containing dried reagents, serves as the Phoenix disposable. The combination panel includes an ID side with dried substrates for bacterial identification, an AST side with varying concentrations of antimicrobial agents, and growth and fluorescent controls at appropriate well locations. The Phoenix system utilizes an optimized colorimetric redox indicator for AST, and a variety of colorimetric and fluorometric indicators for ID. The AST Broth is cationadjusted (e.g., Ca⁺⁺ and Mg⁺⁺) to optimize susceptibility testing performance.

3.5.3 Antibiotic drugs

Meticillin-resistant *Staphylococcus aureus* (MRSA) is one of the most frequent causes of antibiotic-resistant healthcare associated infections worldwide. The EU/EEA population-weighted mean MRSA percentage has decreased significantly over the last four years, as a result of a decreasing trend in many individual European countries. Although these observations provide reasons for optimism, MRSA remains a significant public health problem. In 2012, the EU/EEA population-weighted mean MRSA percentage remained high at 18% in 2012, and seven out of 30 reporting countries had MRSA percentages above 25%, mainly in southern and Eastern Europe.

In this study MRSA were identified using different antibiotic disks as Ampicillin, Cefazolin, Cefoxitin, Clindamycin, Daptomycin, Erythromycin, Gentamicin, Lev ofloxacin, Linezolid, Moxifloxacin, Nitrofurantoin, Norfloxacin, Oxacillin, PenicillinG, Quin uprsitin, Dalfopristin, Rifampin, Teicoplanin, Tetracycline, Trimethoprim, Sulfamethoxazole, and Vancomycin.

3.6 Statistical analysis

The data analysis was performed using SPSS software version 22. Data were summarized using percentages. Risk was estimated using the odds ratio (OR) with 95% confidence interval (95% CI). The relative risk with 95% CI was obtained for the case fatality and the adverse outcomes. The associations between various patient characteristics and

MRSA status were studied using the Chi-square test. The median and interquartile range (IQR) was used to summarize the data on duration of treatment and hospital stay. A comparison of the duration of hospital stay and treatment was done using the Mann–Whitney test.

4. Results

S. aureus as a ubiquitous bacterium is the leading cause of superficial infection at the clinical environment for decades and also considered as the third most important cause of reported illnesses in the world. As the result of the widely use of antimicrobials, MRSA caused the increase of hospital-acquired infection. And in the past 20 years, the rising number of infections out of hospital environment caused by CA- MRSA indicated the spreading of MRSA from hospital to our daily life. Since a large number peoples can be colonized with *S. aureus* and the use of antimicrobial agents in medicine is very common, the increase of antimicrobial resistance was observed and MRSA strains were isolated from several patients at Near East Hospital.

In this study samples were taken from different departments of Near East hospital Cyprus. Highest number of samples was chest disease samples (24%) followed by dermatology (21.3%), cardiology (18.7%), emergency (14.7%) and urology (4%) while other samples were belonging to cardiovascular surgical ward (17.3%).

A total of 75 MRSA samples which were collected from the infected persons in the study area including 50 male and 25 females.

This study shows that maximum MRSA positive strains were found among the females than the males (68.0% and 32.0% respectively).

In this study samples types used were urine, blood, nasal swab, sputum, aspiration fluids, IV catheter and wound culture

Out of 75 MRSA strains (6.7%) of urine, (29.7%) of blood, (14.7%) of nasal swab, (9.3%) of sputum, (10.7%) of aspiration fluids, (4.0%) of IV catheter, and (25.3%) of wound culture samples were used for screening of MRSA strains.

The samples were collected from different ages of patients. In this study the patients of age between 50 and 90 show more resistance to antibiotics.

All the strains of MRSA were found to be 100 % resistant to Ampcilline, Cefazoline, Oxacilline, and Pencilline G. Among MRSA, resistance to Cefoxtine were 98.7%,

Clindamycine 74.7%, Daptomycine 4.0%, Erythromycin 90.7%, Gentamicine 5.3%, Levefloxacine 5.3%, Linezolid and Moxifloxacin 0%, Nitrofurantoin 2.7%

Norfloxacine 14.7%, Quinupristin-Dalfopristin 5.3%, Ripamfin5.3%, Tecoplanin5.3%, Tetracycline 89.3%, Sulfamethoxazole 20.0% and Vancomycine were 4.0%.

All strains of MRSA were found to be 100% sensitive to linezolid and moxifloxacin, while ampcilline, cefazoline, oxacillline, and pencilline G were 0% sensitive to MRSA. Cefoxtine 1.3% %, clindamycine 25.3%, daptomycine 96.0%, erythromycin 5.3%, gentamicine 90.7%,levefloxacine 94.7%, nitrofurantoin 97.3% norfloxacine 85.3%, quinupristin-dalfopristin 94.7%, Ripamfin 94.7%, tecoplanin 94.7%, tetracycline 10.7%,trimethoprim- Sulfamethoxazole 80.0% and vancomycine 94.7% were sensitive to MRSA.

Erythromycin and Gentamicine were 4.0% intermediate, and Vancomycine shows 1.3% intermediate to MRSA.



Fig 4.1 Department wise distribution of MRSAStrains

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Fig 4.3 Sample wise distribution of MRSAStrains

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Fig 4.4 Age wise distribution of MRSA Strains

Antimicrobial drugs	MIC(mg/l)	Sensitive	Resistant	Intermediate
Ampicillin	>1	0	75 (100.0%)	
Cefazolin	<=2,>16	0	75 (100.0%)	
Cefoxitin	>8	1 (1.3%)	74 (98.7%)	
Clindamycin	<=0.25, <=0.5,<=2	19(25.3%)	56(74.7%)	
Daptomycin	<=0.25, <=0.5,>1	72(96.0%)	3(4.0%)	
Erythromycin	<=0.5,>1,>=4	4(5.3%)	68(90.7%)	3(4.0%)
Gentamicin	>1,<=2,>8	68(90.7%)	4(5.3%)	3(4.0%)
Levofloxacin	<=0.25,<=2	71(94.7%)	4(5.3%)	
Linezolid	<=2,>=4 ,<=1	75(100.0%)	0	
Moxifloxacin	<=0.25,<=0.5,>1,>16	75(100.0%)	0	· · · · · · · · · · · · · · · · · · ·
Nitrofurantoin	>16	73(97.3%)	2(2.7%)	
Norfloxacin	>8,<=2	64(85.3%)	11(14.7%)	
Oxacillin	<=2	0	75(100.0%)	
Penicillin G	>1	0	75(100%)	
Quinupristin- dalfopristin	<=0.5,>1	71(94.7%)	4(5.3%)	
Rifampin	<=0.5,<=2	71(94.7%)	4(5.3%)	
Teicoplanin	>=4,<=1	71(94.7%)	4(5.3%)	
Tetracycline	>8	8(10.7%)	67(89.3%)	
Sulfamethoxazole	<=19	60(80.0%)	15(20.0%)	
Vancomycin	<=1	71(94.7%)	3(4.0%)	1(1.3%)

Table 4.5Antimicrobial susceptibility pattern of MRSA with MIC

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5. Discussion

Methicillin resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and it now represents a challenge for public health, as community associated infections appear to be on the increase in both adults and children in different regions and countries.

Epidemiological studies suggest that hospitals of all sizes are facing the problem of MRSA. The problem occurs to be increasing regardless of hospital size and control measures for MRSA. Prevalence is constantly mounting in many countries, and in some hospitals, more than 50% of all *Staphylococcus aureus* disease isolates are MRSA.

In the present study *Staphylococcus aureus* was isolated from various types of samples obtained from different wards of a Near East Hospital. Similar studies have been conducted by various researchers at different locations globally.

MRSA are often multidrug-resistant. A study form Tehran, Iran by Vadhani et al., (2004) reported that, from the 90 MRSA isolates, approximately half of them displayed resistance to one or more antimicrobial agents, including Penicillin, Cephalosporins, Tetracycline and Aminoglycosides.

If we look into the Indian literature, it seems the burden of multidrug resistant MRSA is increasing over time for example, 23.2% was reported by Majumder et al., (2001), 32% by Anuparba et al., (2003) and 63.6% by Rajaduraipandi et al., (2006). This is clear, MRSA surveillance and strict drug policy are of more importance or else the threat will increase.

A comparable prevalence rate of 34.7%, 31.0% and 38.5% were also reported from Assam et al., (2006). In Nepal, Sanjana et al., (2010) founded the prevalence of MRSA 39.6%.

Similar study conducted by Siddique et al. (1999) at Sargodha, Pakistan isolated 23% MRSA, Samia et al., (2007) at Karachi, Pakistan, isolated 43% MRSA, and then by Khatoon et al., (2002) at Karachi, Pakistan isolated 38.5% MRSA. Similar study was also conducted in India by Shagufta and Jayaraj (2010) isolated 77.9% MRSA, while Helena

et al., (2010) in Brazil isolate 61% MRSA. This shows an increase in MRSA percentage with time. This increase is due to transfer of resistance genes among bacterial cell and persistence of bacteria in hospital environment due to antibiotic resistance. Another factor which facilitates MRSA to increase in concentration is absences of control program for antibiotics usage pattern (Hacek et al., 1999).

Our findings are in correspondence with Saima et al., (2007) and Hare et al., (2009) who observed methicillin resistant Staphylococcus aureus (MRSA) with high resistance to the various antibiotics including Gentamicin, Ciprofloxacin, Doxycycillin.

MRSA were reported to be resistant to Penicillin, Amoxicillin, Ampicillin, rimethoprim/Sulfamethoxazole, Cephaloxin, Amikacin, and Ciprofloxacin at a resistant rate of 110%, 91.9%, 87.6%, 77%, 55.5%, 19%, and 26.5%, respectively (Hare et al., 2009). Similar results were also reported by Samia et al., (2007) which showed resistance with similar patterns.

In this study I found that all the strains of MRSA were found to be 100 % resistant to Ampcilline, Cefazoline, Oxacilline, and Pencilline G. This corroborates with the finding of Anupurba et al., (2003). Among MRSA, resistance to Cefoxitine were 98.7%, Clindamycine 74.7%, Daptomycine 4.0%, Erythromycin 90.7%, Gentamicine 5.3%, Levofloxacine 5.3%, Norfloxacine 14.7%, Quinupristin-Dalfopristin 5.3%, Ripamfin 89.3%, Sulfamethoxazole 20.0% and 5.3%, Teicoplanin 5.3%, Tetracycline Vancomycine were 4.0% and Nitrofurantoin which was used only for urine samples shows 2.7% . Linezolid and Moxifloxacin seems to be the only antimicrobial agent which showed 100% sensitivity and so may be used as the drug of choice for treating multidrug-resistant MRSA infections as we found it in the susceptibility pattern of MRSA. Moxifloxacin is active against both extracellular and intracellular CA-MRSA if the MIC is low, and is more effective than clindamycin, co-trimoxazole and linezolid (J Antimicrob Chemother, 2011). Linezolid and moxifloxacin have overtaken vancomycin as the most effective drugs, which is a variation from the earlier reports.

Teicoplanin is another glycopeptide antibiotic, structurally related to vancomycin, which is an alternative for the treatment of Gram-positive infections, including MRSA; it has been evaluated in endocarditis, osteomyelitis and septic arthritis (Schaison et al., 2000).

Other antibiotics which are effective in treatment of MRSA are Daptomycine, Gentamicine, Ripamfin, and Levofloxacine.

I reported in my study that 4% of MRSA strains were resistant to Vancomycine which is similar to Vidhani et al., (2004), who reported 7% of isolates were resistant to vancomycin. These reports are an early warning that Staphylococcus aureus strains with full resistance to vancomycin might emerge in future because of widespread use of antibiotics and changing trends of defence acquired by MRSA.

This study revealed that highest percent of MRSA were detected in blood samples (29.7%) while the lowest MRSA ratio was in IV catheter (4.0%) and in urine (6.7%). However, it is crucial to mention that total number of samples may be the cause for such diverse results because it also depends on number of samples. Such a low sample number may not yield a clear depiction about MRSA while increased number of samples may give statistically more valid results.

I reported that highest number of samples was chest disease samples (24%) followed by dermatology (21.3%), cardiology (18.7%), emergency (14.7%) and urology (4%) while other samples were belonging to CV surgical ward (17.3%). According to this study we conclude that chest disease patients have high risk of MRSA and also those people which have skin infection as we know it lives in the nose and on the skin of humans.

The most commonly identified risk groups include elderly patients, long-term care facility residents, patients with chronic skin lesions, patients with a history of recent hospitalization, dialysis patients, patient transferred or released from correctional facilities, or patients with a recent history of antibiotic use (Brumfitt el al., 1989, Harbarth et al., 2006, Haley et al., 2007).

The samples were collected from different ages of patients. The patients of age between 50 and 90 show more resistance to Antibiotics. According to my study as age increase the resistance of MRSA to antimicrobial agent also increases.

Conclusion

Methicillin resistant *Staphylococcus aureus* (MRSA) was isolated in high concentration from hospital environment which showed a high risk to the patients and staff working in the hospital. The over-crowding on hospital, lack of facilities and lack of knowledge about Hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) leads to the presence of nosocomial pathogen in such a high concentration. *Staphylococcus aureus* showed an increase in resistance to different antibiotics. High percentage of ESBL production from gram negative rods and resistance to other antibiotics shows an increasing rate of acquiring antibiotics resistance.

This study suggests implementation of preventive measure in order to minimize the bacterial resistance to antibiotics. An electronic record of antibiotic usage should be made to prevent unnecessary usage of broad spectrum antibiotics and increase recommended usage of antibiotics. All the health care personal should strictly follow the preventive guideline for patient as well as for their own safety. Proper antibiotics susceptibility test should be made for all suspected infection caused by MRSA and ESBL producing bacteria.

The following recommendations are essential in the containment of resistance to antimicrobial agents:

1. Introduce routine MRSA screening of health care workers as part of a suite of infection control measures and continuous surveillance and improvement of hygiene standards should be adopted.

2. Reassess policies in antimicrobial drugs use within and outside the hospital environment.

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