Methicillin-Resistant Staphylococcus aureus (MRSA) is a pathogen that causes many complicated infections in humans. MRSA is a type of S. aureus that has produced by process of resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, Oxacillin, etc.) and the cephalosporins. In this study isolation of Methicillin-Resistant Staphylococcus aureus (MRSA) strains was done from various surgical and accidental wounds. A total of 150 Clinical specimens from surgical and accidental wounds were collected from different hospitals of Faisalabad and processed for isolation into Oxacillin broth and sub-cultured on CHROMagar’ MRSA. The isolates through ��-hemolysis on blood agar, clot formation by coagulase test. MRSA isolates were confirmed by latex agglutination test for the presence of Penicillin-Binding Protein 2a (PBP2a). All positive MRSA were molecularly characterize using PCR. The rate of prevalence of MRSA was
found to be 91.7%, higher prevalence of MRSA was found in human males and in 21-40yrs age group. All MRSA isolates showed 100% resistance against Amoxicillin-Clavulanic acid, Ampicillin, Amoxicillin and Ciprofloxacin but sensitive to Vancomycin and Linzolid. Vancomycin (92%) was the most effective drug followed by Linzolid (90%). Present study conclude that high prevalence of MRSA was found in hospitals of Faisalabad, males were more prone to MRSA infection. All MRSA isolates were resistant to commonly used antibiotics only Vancomycin was the drug resistant against MRSA hence considered as the most effective drug.

CHAPTER 1
INTRODUCTION

The Staphylococci are the bacteria which are Gram-positive rounded cells, normally exist in the order of grape like clusters belong to family Staphylococcaceae. Staphylococcus aureus is the most common and ominous staphylococcal bacteria in this family. S. aureus is the cause of many infections but it may also occur as a beneficial bacteria. Tissues can be infectious with S. aureus on rupturing of skin or mucosal barriers. This rupture can cause different types of infections including blisters and abscesses. Due to their pathogenic nature staphylococci often hemolyze blood, coagulate plasma and produce a variety of extracellular enzymes and toxins. The more dangerous forms of S. aureus produce such toxins which are source of host tissue damages, hindering phagocytosis and causing disease symptoms (Lowy, 1998). In systemic infections, it causes osteomyelitis, mastitis, wound infection & occasionally toxic shock syndrome (Mahmood et al., 2010). The main cause of these infections are the toxins produced by staphylococci. For example S. aureus produce enterotoxins which are important source of food poisoning (Salyers and Whitt, 2002).

Post-surgical infections accounts for approximately one-fourth of all hospital acquired infections. Many risk factors for S. aureus infection are linked with patients stay in a surgical intensive care unit (SICU) in hospitals, i.e. care procedure numbers, nearness of infected patients, surgical wounds and foreign bodies presence and long-term antibiotic treatment (Longfield et al., 1985). Throughout the world, particularly in developing countries burn wounds remains an important problem of public health associated with diseases, long-term disability and mortality (Othman and Kendrick, 2010; Othman and Kendrick, 2011). The burned dead moist tissues, along with damaged tissues, provide a nutrient medium which will favor the growth of a many species of bacteria. In a definite part of burns this type of control of bacteria causes septicemia and death (Nakhla and Sanders, 1991).

In the North America almost 20% of all admissions are associated with foot infections which are the source of hospitalization of patients with diabetes (Bild et al., 1989). In diabetic foot ulcers (DFUs) staphylococcus aureus is the most common organism, minor or major amputations of the lower limbs (15'27%) are required by the patients of Diabetes mellitus and infection is the main reason in more than 50% of cases (Mendes et al., 2012). Diabetic foot infections caused by MRSA are linked with lethal results, i.e. slow healing and more repeated amputations, in compared to other pathogens (Lipsky and Stoutenburgh, 2005). In diabetic foot infections MRSA infection is common and is associated with increased time of healing with antibiotic treatment (Tentolouris et al., 1999). In developing countries like Pakistan MRSA infection has become a major ailment in hospitals and they have been a cause of increasing cost, disease rate and deaths associated with surgical operations.

The sensitivity shown by staphylococci against antimicrobial drugs is variable. Staphylococcal resistance falls into many classes. Under the control of plasmid the production of \(i_2\frac{1}{2}i_2\frac{1}{2}\)-lactamase is common, and to many penicillin groups it makes the organisms resistant (penicillin G, ampicillin, ticarcillin, piperacillin, and similar drugs). The production of \(i_2\frac{1}{2}i_2\frac{1}{2}\)-Lactamide does not depends upon methicillin resistance. For methicillin resistance the responsible gene mecA exists on the chromosomes. The resistance mechanism is related to lack of inaccessibility of certain penicillin-binding proteins (PBPs) in the organisms. There is a high attraction for antibiotics having \(i_2\frac{1}{2}i_2\frac{1}{2}\)-lactam shows by PBPs; in MRSA this attraction is decreased which results in resistance to antibiotics. A protein called as PBP2a, possess a less attraction to antibiotics, encoded by a mecA gene on chromosome (Cook, 1998). When the infecting strain is MRSA then there is a poor diagnosis of infections caused by S. aureus (Cosgrove et al., 2003). Increased synthesis of cell wall and modification of cell wall are associated with resistance mechanism and Van genes present in enterococci are
not responsible for this.
Since first reported in 1961 (Jevons, 1961), number of diseases, death rate and treatment expenses associated with infection of skin and soft tissue, pneumonia associated with ventilator, bacteremia and many other nosocomial and community infections has been gradually increasing. Treatment and managerial costs associated with MRSA infections continue to increase whereas the number of effective antibiotics remains relatively constant (Krikland and Adams, 2008). Earlier the MRSA infection was detected in hospitals but now community associated MRSA is increasing also (Chambers, 2001). For example in large hospitals of US about 40% of infections of S. aureus are Methicillin-Resistant acquired (Red Book: American Academy of Pediatrics, 2003).

Transmission of MRSA occurs by direct contact to a colonized carrier. The main source of spread is from patient to patient on the hands of hospital staff. A study conducted in a nursing home where MRSA was found to be endemic in the past, the results indicated 65% patients were negative for MRSA, 25% were found to be colonized even before admission to the nursing home whereas 10% patients were found to acquire MRSA during their stay (Bradley et al., 1991). Health care workers (HCWs) possibly related to principles of hygiene regarding uniforms, equipment sterilization and washing, transmitting the pathogenic organisms on moving from patient to patient (Elmanama et al., 2013). Use of different types of antibiotics especially during long-term treatment and other factors related to MRSA colonization are; long-term hospital stay, burns, surgical wound care (Sanford et al., 1994). In recent US studies, in community the rate of prevalence of MRSA has ranged from 0.2% to 2.8% and prevalence is higher in poor & populated areas where use of injection drug is common (Frazee et al., 2005).

All over the world, increased prevalence and antibiotic resistant bacteria are the issues, faced by the hospitals. For public health and hospitals, antimicrobial resistance is becoming a matter of great concern. In USA, S. aureus is considered to be susceptible to vancomycin (Bhateja et al., 2006). Moreover, numerous reports of completely resistant S. aureus with respect to Vancomycin have also been published (Chang et al., 2003). It was described as ‘Superbugs’ MRSA organisms are generally resistant to many antibiotics including aminoglycosides, chloramphenicol, macrolides, fluoroquinolones, clindamycin, trimethoprim and beta-lactams (Novick et al., 2001). Prevalence of MRSA infection is continuously elevating in many countries and in some hospitals (Voss and Doebbeling, 1995). In the developing countries like Nigeria the antibiotics are used without prescription of doctor and no control measures are adopted having no regulatory policies in this respect has make less effective antibiotics for the treatment of infections of S. aureus. (Onwubiko and Sadiq, 2011).

Keeping in view the importance of the subject, present study has been planned to achieve the following objectives:
1. Isolation, Identification and Molecular characterization of indigenous MRSA strains from accidental and surgical wounds.
2. Determination of Prevalence of MRSA in various kinds of wounds.
3. Study of Antimicrobial Susceptibility pattern of MRSA against commonly used antibiotics.

CHAPTER 2
REVIEW OF LITERATURE
Lesseva and Hadjiiski (1996) studied the burn patients to estimate frequency, features and role of staphylococcal infections in the Sofia Burns Centre. The cause of wound infections and bacteremia in burned patients was studied for a period of 8 years (1987-94). Both in wound samples and blood cultures the prevalence of staphylococci was studied. From 19.4% to 28.0% in 1993-1994 the infections of MRSA have increased during the last year of the study. In 18.8 percent of patients MRSA was the cause of infection. Against gentamicin, and tetracycline more than 70 % of the MRSA strains were resistant and against lincomycin, co-trimoxazole, chloramphenicol and ciprofloxacinabout 1/3rd were resistant. Against Vancomycin all the MRSA strains were sensitive and to rifampicin 71.1 % were sensitive. These results shows the need of quick steps to control the more spread of MRSA infections in burn units.
Majumder et al. (2001) worked in a hospital in Assam-India to analyze the prevalence of methicillin resistant S. aureus infections. Resistance was 15% among coagulase negative staphylococci and 52.9% was among S.
aureus isolates. 23.2% methicillin-resistant and 6.6% methicillin sensitive staphylococci were observed in antimicrobial susceptibility testing. Against most antibiotics the methicillin resistant strains were found highly resistant as compared to isolates which were methicillin sensitive.

Vidhani et al. (2001) worked on selective number of MRSA isolates in patients in burn and orthopedic units. The prevalence of MRSA among S. aureus was found to be 51.6% and isolates were multidrug resistant. The readability of these isolates was obtained 41.8% by the MRSA set of phages. From burn and orthopedic units prevalence of MRSA was observed high, against antibiotics all isolates were found to be highly resistant.

Supriya et al. (2002) studied the prevalence of MRSA: sensitivity pattern of antimicrobials and phage typing. Out of 230 isolates (19.56%) of S. aureus 45 were MRSA. From pus and wound swabs maximum numbers of MRSA (26.9%) were obtained. Beta-lactamase production was shown by all MRSA strains. All MRSA strains were multidrug resistant. Against penicillin (100%), cotrimoxazole (97%) and chloramphenicol maximum strains were resistant. Against gentamicin (6.66%) least resistance was observed. Against Vancomycin all strains were found to sensitive. Against ciprofloxacin 4.86 less resistance was observed while against gentamycin no strains of MSSA were resistant. Out of 44 strains 28 were non-typeable for Oxacillin by phage values less than 1ug/ml. MIC values 4ug/ml were shown by maximum number of isolates. MIC of 12ug/ml was shown by 9 and MIC values of 250 ��g/ml was shown by 2 strains. For phage typing 28 were non-typeable out of 44 strains subjected. 11 strains were from mixed group, while 4 were from the group three indicating hospital strains.

Lipsky and Stoutenburgh (2004) analyzed a set of diabetic patients with an infected ulcer enrolled in two controlled trials of patients with complicated skin and soft-tissue infections (Gram-positive organisms) to compare the effectiveness of dapomycin against penicillins or vancomycin. 103 were important clinically out of 133 patients with a diabetic ulcer infection; dapomycin was received by 47 and 56 received a comparator of dapomycin. Staphylococcus aureus was the prevailing organism and most infections were monomicrobial. Treatment with dapomycin and comparators both had most strict stages of severity, generally well tolerated.

Orrett and Land (2006) studied the isolates at a regional hospital in Trinidad to determine the prevalence of methicillin resistance and found the current resistance pattern of MRSA and MSSA against commonly used antibiotics. Over a period of 6-year 2430 isolates of S. aureus were obtained from various nosocomial and community sources. MRSA prevalence were 60.1%, 15.5% and 6.6% from surgical/burn wounds, urine and pus/abscess. Against erythromycin (86.7%) and clindamycin (75.3%) greatest prevalence of resistance of MRSA was seen. For ampicillin (70%) highest resistance rates were shown by MSSA. Resistance rates were (78.7%) and (73.5%) shown by MRSA and MSSA against tetracycline. There is an increase MRSA prevalence was found in hospital from 12.5% in 1999 to 20.8% in 2004.

Harbarth et al. (2008) designed a study to compare 2 MRSA control conditions between July 2004 and May 2006 from 21754 post-surgical patients and to analyze effect of the finding of detected MRSA on hospital acquired MRSA infection rates in patients. During the interference periods 10193 of 10844 patients (94%) were screened. Out of 515 identified MRSA positive patients 337 were previously unknown carriers of MRSA. In comparing with 76 in the control periods, 93 patients developed nosocomial MRSA infection in the intervention periods. At the time of admission 93 of infected patients, 53 were MRSA-free and during hospitalization MRSA infection was developed.

Tillotson et al. (2008) worked during the years 2005-07 to analyze the rates of antimicrobial susceptibility of S. aureus from skin and wound infections reported from nine regions of the USA. Over 380 000 isolates of S. aureus were tested and reported for the period 2005-07. With little change from 2005 Methicillin resistance was observed in 57.8% in 2007. Against trimethoprim/sulfamethoxazole and gentamicin high activity was observed. Linezolid resistance was rare. No resistance mechanism was shown by less than a third of all isolates. 46% of all resistant strains showed 3 distinct resistance. Overall, there were more highly drug-resistant isolates from the ICU with four, five or six drug-resistant phenotypes accounting for over a third of all strains.

Tiwari et al. (2008) studied the prevalence of multidrug resistant MRSA strains in clinical specimens and sensitivity pattern of these strains against various antibiotics used for treating hospitalized and out patients. Among 783 isolates of S. aureus 301 (38.44%) were Methicillin-Resistant, of which 217 (72.1%) were found to be multidrug-resistant. Almost all MRSA strains were resistant to penicillin, 95.68% were resistant to cotrimoxazole, 92.36% were resistant to chloramphenicol, 90.7% were resistant to norfloxacin, 76.1% were...
resistant to tetracycline, and 75.75% were resistant to ciprofloxacin. Vancomycin was the most effective drug with only 0.33% of MRSA strains being resistant to it.

Thyagarajan et al. (2009) analyzed 440 patients, sequentially admitted to the trauma unit with hip fracture. 5.2% (21/403) were found to be colonized with MRSA out of the 403 who had a swab on admission. Colonization rate of patients with MRSA was as follows; 52 percent of MRSA colonized patients were admitted from their own home, 29% from residential homes and 19% from nursing homes. MRSA colonization was found in 3.6% of patients admitted from their own home, 10.9% of residential home patients, and 17.4% of nursing home patients. The high prevalence of previous hospitalization among people from institutional care may explain the higher rates of MRSA carriage among these individuals a high proportion (80.9%) of colonized patients had been admitted to a hospital within the previous one year.

Shukla et al. (2009) examined patients admitted to the Leicester Royal Infirmary Trauma Unit between January 2004 and June 2006 to find the incidence of infection with Methicillin-Resistant Staphylococcus aureus (MRSA). Using multi-variant analysis the status of MRSA at the time of their admission was examined, together with age, gender and diagnosis. Out of 2473 patients 2394 (96.8%) were MRSA-negative and 79 (3.2%) were MRSA carriers. The chances of developing surgical site infections with MRSA was more in patients those carrying MRSA at the time of admission than non-MRSA carriers. Risk factors of MRSA infections analyzed in the study included hip fracture and increasing age. Increased rate of developing MRSA wound infection is associated with MRSA carriage at admission, age and the pathology.

Wang et al. (2009) analyzed the distribution, drug resistance and epidemiology of pathogenic bacteria in the burn wards of Ruijin Hospital. From January 2004 to December 2006 17 strains of Methicillin resistant staphylococcus aureus (MRSA), 52 strains of Pseudomonas aeruginosa (PA) and 11 strains of Acinetobacter baumannii (AB) isolated from the wound secretion, venous catheters, blood, urine and stool etc. were collected from burn patients. MRSA, PA and AB were the major strains in burn wards in recent years, of which Staphylococcus aureus was the most dominant. During these 3 years, MRSA accounted for 77% (63/82), 85% (63/74), and 75% (74/99), respectively. PA was resistant to Amikacin, Gentamicin, Piperacillin, Ceftazidime, Cefoperazone, Aztreonam and Imipenem; MRSA was resistant to Amikacin, Gentamicin, Erythromycin, Clindamycin and Levofloxacin; AB was resistant to Amikacin, Gentamicin, Piperacillin, Ceftazidime, Imipenem and Ciprofloxacin. In the randomly amplified polymorphic DNA (RAPD) homology analysis three bacteria were found to belong the same type.

Recinos et al. (2009) worked from April 2003 to April 2007 to identify all trauma patients surviving 48 hours or more that had a positive culture result during their SICU stay. Examination of cultures was made and 582 SICU patients with 2860 cultures were assessed for MRSA infection. 36 patients were reported as MRSA positive among 368 cultures (12.9%). Criteria for a CA-MRSA infection was fulfilled by 13 patients. No significant difference in mortality (8.7% vs 15.4%, P = 0.540) or hospital related charges ($364,231 +/- 323,719 vs $242,458 +/- 276,630, P = 0.091) was noted when results were analyzed. Among critically ill trauma patients MRSA constituted an important source of infection.

Delorme et al. (2009) performed a survey on all staphylococcal infections diagnosed by the Ashtabula County Medical Center (Ashtabula, OH) during 2006 and 2007. For the antibiotic resistance 1612 S. aureus were evaluated, number of MRSA isolates were 947. The increase in MRSA infections was noticeable among youth (6'25 years old), middle-aged people (45'50 years old), and elderly people (86'90 years old). MRSA infections increased among nursing home residents by 183%, among inpatients by 58% and among outpatients by 43%. Among healthy people with no apparent risk factors more than 66% of MRSA infections were found. Antibiotic resistance profile showed only 9 profiles were distributed among inpatients, outpatients, and nursing home residents and 88.7% of infections belong to these profiles.

Sisirak et al. (2010) studied the prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) from the surgical wounds (January 2006 to December 2008), MRSA infection in surgical department and antimicrobial susceptibility pattern of MRSA isolates. Conventional methods were used to identify the isolates. Kirby-Bauer disc-diffusion method was used for antimicrobial susceptibility testing. A total of 5755 wound swabs were examined: aseptic swabs were 938 and 4817 (83.7%) were positive. S. aureus was isolated in 1050 (22.0%) swabs. MRSA prevalence was varied in study duration and trend was as follows; from 12.4% samples in 2006, from 6.7% samples in 2007 and from 3.7% samples during 2008. In the department of plastic surgery (24.4%) and in the department of orthopaedic surgery (24.1%) wound infections caused by MRSA were dominant.
73% of MRSA isolates were sensitive only to vancomycin, tetracycline, fucidic acid and trimethoprim/sulfamethoxazole by performing antimicrobial sensitivity test. Antimicrobial susceptibility testing showed that 73% of MRSA isolates were with the same antibiotic sensitivity pattern (antibiotype) sensitive only to vancomycin, tetracycline, fucidic acid and trimethoprim/sulfamethoxazole.

Richard et al. (2010) studied the management of diabetic patients with infected foot wounds in hospital including an evaluation of the outcome 1 year after discharge, the study included 291 patients (73% male; 85% type 2 diabetes; mean age: 64.3±11.7 years). Wounds were located mostly on toes and forefoot, and moderate infection was observed; osteomyelitis was suspected in about 50% of patients. The most frequently isolated microorganisms were Gram-positive cocci and Staphylococcus aureus. During hospitalization, 35% patients undergo lower-limb amputation; in 52%, the wound healed or had a positive outcome. 150 non-amputated patients were examined after one year of discharge this time, 19% had to undergo amputation, whereas without any gap 79% had healed their wounds. Location (toes), severity of the wound and presence of osteomyelitis were the risk factors for amputation.

Xu et al. (2010) studied the surgical upper limb infections in patients with End-stage renal failure, their epidemiology and management. In the study period 47 out of 803 (6%) patients with surgical upper limb infections had end-stage renal failure (ESRF). Most common infections included were abscesses (34%), wet gangrene (26%) and osteomyelitis (11%). Out of all samples collected Methicillin-Resistant Staphylococcus aureus (MRSA) was the common organism (29%) isolated. 18% of single organisms cultured were gram-negative, 29% were multiple organisms. Amputation was needed among 36% of all cases. During treatment 25 percent of patients had a life-threatening event (septic shock).

Motamedi et al. (2010) examined the specimens that have been collected from patients of one of the hospitals of Ahvaz to determine the pattern of antibiotic resistance among Staphylococcus aureus isolates and to identify community-acquired Methicillin-Resistant Staphylococcus aureus (CA-MRSA). S. aureus isolates were separated for antibiotic resistance including methicillin. The MRSA was also treated with ethidium bromide to find the origin of resistance. Among the bacterial isolates, all of 11 S. aureus were resistant to methicillin and cefixime, resistance pattern against other antibiotics was as follows; 2 were resistant to ciprofloxacin, 6 to tetracycline and the remainder were sensitive or intermediate to other antibiotics. The treated isolates were resistant to methicillin and this suggested that the plasmid was not the origin of resistance in these isolates.

Suzuki et al. (2010) studied the prevalence of surgical site infections (SSI) following acetabular fracture open reduction and internal fixation. A total of 326 patients who undergo acetabular fracture surgery were selected. Out of 17 patients (5.2%) who developed a SSI, including 10 deep infections and 7 superficial infections. In 9 patients S. aureus was the most common responsible organism and was Methicillin-Resistant in 3 patients. Enterococcus faecalis was found in 6 patients, Staphylococcus epidermidis in 3 patients, and Pseudomonas aeruginosa and enterobacter cloacae in 2 patients each. Within 4 weeks after the fixation 14 of 17 patients developed their infection.

Mahmood et al. (2010) worked according to NCCL protocol using control strains ATCC 29213 (oxacillin susceptible) and S. aureus ATCC 43300 (oxacillin resistant) and collected 265 MRSA samples from different departments of tertiary care hospital. High prevalence was observed in males 155 (58.5%). Routine antimicrobial sensitivity of MRSA showed 28.7% to Ciprofloxacin, 37.5% to Gentamycin, 35% to Clindamycin, 27.5% to Erythromycin, 18% to fusidic acid, 8% to Penicillin, 87% to Moxi-locacin, 0% to Oxacillin, 100% to Vancomycin, Teicoplanin, Linezolid and Teigecycline. MRSA is more prevalent in ICUs patients. Effective antimicrobials were Vancomycin, Teicoplanin, Linezolid and Teigecycline.

Lin et al. (2011) evaluated the prevalence and susceptibility pattern of Methicillin-Resistant S. aureus (MRSA) in skin and soft tissues infections (SSTIs). Out of 443 SSTI samples included, 40.4% were females and 59.6% were males. Most important cause found was S aureus (53.3%) and 53.0% were MRSA. The major susceptible antimicrobial agents to MRSA were Minocycline (94.4%), trimethoprim/sulfamethoxazole (95.2%), levofloxacin (95.7%), and fusidic acid (98.9%). Susceptibility to clindamycin was found to be 14.4%. 75.6% were community-associated isolates among MRSA infected inpatients. Based on the susceptibility results 15 inpatients with poor clinical response to beta-lactam empirical antimicrobial therapy received minocycline as combination.

Onwubiko and Sadiq (2011) worked on 150 clinical isolates in tertiary care institution in Nigeria to observe the
antibiotic sensitivity pattern of Staphylococcus aureus. Disc diffusion method was used to perform antibiotic sensitivity. Wound infections had the highest frequency of S. aureus isolates (30.7%) while the age group with the highest number of isolates was (0-10) yrs. Males (62.0%) were more infected than females (38.0%). The antibiotics sensitivity pattern of S. aureus was 92.4%, 63.0%, 44.2%, 35.8%, 52.4%, 61.9%, 15.5%, 31.2%, 7.1%, 78.9%, 76.6%, 100%, 71.4%, 30.7% and 100% respectively against following antibiotics; Gentamicin, Amoxycillin/clavulanate, Streptomycin, Cloxacillin, Erythromycin, Chloramphenicol, Cotrimoxazole, Tetracycline, Penicillin, Ciprofloxacine, Ofloxacin, Levofloxacin, Ceftriaxone, Amoxycillin and vancomycin. Levofloxacin 93.7% and Ofloxacin 68.7% were the drugs showed sensitivity by methicillin resistant isolates.

Knapp et al. (2011) studied the treatment of superficial and deep incisional surgical site infections with dapatomycin. Out of 69 selective patients, 60 were determine for efficacy. Abdominal wounds were more among deep SSIs (n = 30), whereas extremity wounds predominated among superficial incisional surgical site infections (n= 30). The overall clinical success rate was 92%, the success rate was 100% in superficial incisional SSI and 83% in deep SSI. Most frequently isolated organism was S. aureus (28/36 Methicillin-Resistant). Out of 10 patients who had fever initially, the median time of suppression was five days and 11.2 days was the mean duration of treatment. Well toleration was shown by dapatomycin.

Agudo et al. (2011) studied the CA-MRSA strains isolated in last three years in the Microbiology Lab of Hospital General La Mancha-Centro to examine the epidemiologic characteristics and resistance to antimicrobial agents by those strains. Out of a total of 97 S. aureus isolates in 2007 (26.8%) the number of CA-MRSA was 26, 40/113 in 2008 (35.4%) and 57/157 in 2009 (36.3%). 63.4% isolates were obtained from purulent skin and soft tissue infections. All strains were susceptible to linezolid, quinupristin/dalfopristin and glycopeptides. The pattern of resistance to antibiotics was fluoroquinolones (94.3%), erythromycin (87.0%), tobramycin (82.9%), and clindamycin (65.3%).

Buzaid et al. (2011) worked in a major tertiary surgical hospital in Benghazi, Libya investigated the prevalence of MRSA isolates and their sensitivity patterns against various antibiotics used for treating hospitalized patients. From different clinical samples they investigated 200 non-duplicate S. aureus isolates. 31% (62/200) were the MRSA from the isolated S. aureus. Samples of burns and surgical wound infections were consisted of high number of MRSA. 62 patients with MRSA showed antibiotic resistance pattern as 17.7%, 33.9%, 41.9%, 38.7% and 46.8% against vancomycin, ciprofloxacin, fusidic acid, chloramphenicol and erythromycin. Kitara et al. (2011) worked in Lacor Hospital (Uganda) to determine the prevalence and antibiotic susceptibility of Staphylococcus aureus in suppurative lesions of the surgical ward and outpatients. The number of Staphylococcus aureus in 122 patients was 59.4% for the surgical inpatients and 48.3% for outpatients giving an average rate of 53.9%. The average antibiotic susceptibility patterns for the 8 antibiotic tested were: Ampicillin (75.0%), Chloramphenicol (34.4%), Ciproflouacin (1.6%), Erythromycin (7.8%), Gentamycin (0%), Methicillin (1.6%), Tetracycline (45.3%) and Co-trimoxazole (50.0%). Surgical inpatients showed higher resistance than outpatients (t=1299, p<0.05).

Liu et al. (2011) studied the reasons of scalp wound infections among craniocerebral trauma patients followed by the 2008 Wenchuan earthquake. A total of 82 patients suffered from scalp trauma, including 52.4% cases (43/82) with wound infections. Isolates of infectious bacteria were 59. The most common organisms isolated was Gram positive bacteria (64.4%), including Staphylococcus aureus (26/59, 44.1%) and Staphylococcus epidermidis (12/59, 20.3%). 35.6% of samples were isolated as Gram-negative bacteria, 22.0% (13/59) were Enterobacter cloacae, 5.1% (3/59) were Klebsiella pneumonia and 8.5% (5/59) were Serratia rubidaea. Duckworth et al. (2012) worked on the patients who were diagnosed with deep infection following hip fracture surgery ant to analyze the predictors of mortality. Over a 3-year period there were 2718 successive operations performed for a fracture of the proximal femur. A deep postoperative infection was diagnosed in fluid and tissue samples in 43 (1.6%) patients. 65% were female and the mean age was 73 years (25-94). The main procedure in 25 (58%) patients was reduction and internal fixation, with 18 (42%) experiencing hemi-arthroplasty out of 43 patients who developed deep infection. The most common responsible organism was Staphylococcus epidermidis (n=13, 30%) with MRSA prevalence was 23% (n=10). The patients with no deep infection (19% vs. 6.5%; p=0.004) had less mortality. On distribution analysis, increasing age, dementia and diabetes were indication of both 30-day and 1-year mortality (all p<0.05).

Belthur at al. (2012) studied the risk factors for pathologic fracture in children with Staphylocococcus aureus osteomyelitis between January 2001 and January 2009 at a tertiary-care pediatric hospital. Seventeen children
who were treated for infective long-bone fracture secondary to Staphylococcus aureus osteomyelitis were compared with a control group matched for age, sex and methicillin susceptibility consisting of 49 children with S. aureus osteomyelitis having no fracture. Two out of 17 patients had methicillin-susceptible Staphylococcus aureus (MSSA) isolates and 15 had Methicillin-Resistant Staphylococcus aureus (MRSA). 72.1 days (range, twenty to 150 days) was the mean time for development of disease to fracture.

Mendes et al. (2012) worked on the bacterial profile on the basis of patient history, diabetic foot characteristics, ulcer duration and antibiotic therapy in diabetic foot infections in Lisbon. In the study 49 were hospitalized patients, and 147 microbial isolates were cultured. The main genus isolated was Staphylococcus, and out of total cases 24.5% were MRSA. 93% of the antibiotic trials were considered not sufficient on the basis of antibiotic susceptibility of clinical samples. 29 days were the average duration of ulcer with any MDR and previous treatment with fluoroquinolones was statistically associated with antibiotic resistance.

Mina et al. (2012) isolated Staphylococcus aureus from burnt patients and checked the antimicrobial susceptibility of Vancomycin and nitrofurantoin in S. aureus. In between May 2008 to December 2011 data was collected from the 2938 hospitalized burn patients. Patients with longer hospital stay (P<0.001) were more likely to have infection as compared to other patients. Vancomycin and nitrofurantoin seem to be the most effective antibiotics for MRSA among all tested antibiotics. With increasing in age the resistance to antibiotics also increased.

Dubey et al. (2013) collected the strains of multidrug resistant S. aureus both of community and hospital acquired by a surveillance over a period of 30 months in a teaching hospital. Of a total of 1507 S. aureus isolates, 485 strains from community and 1022 isolates were from hospital acquired sources; Out of 485 (100%) OPD S. aureus isolates, 390 (80.41%) were MRSA strains. Similarly, from wards and cabins of 564 (100%) isolates, 461 (81.73%) strains were MRSA; whereas 363 (79.25%) strains were MRSA out of 458 (100%) isolates obtained from ICU and NICU. 80 (20.51%) were vancomycin resistant (VRSA) and 173 (44.35%) strains were vancomycin intermediate strains from 390 (100%) MRSA strains isolated from OPD. Out of 461 (100%) MRSA isolates obtained from hospital acquired sources 110 (23.86%) strains were VRSAs and 208 (45.11%) were VISA strains, whereas from ICU and NICU out of 363 MRSA isolates, 164 (45.17%) VISA and 61 (16.8%) VRSAs were found.

Islam et al. (2013) in Trinidad and Tobago examined the microbial profile of diabetic foot infections. At a mean age of 56.9±12.4 years there were 139 patients. 56.8% of cases were included mixed polymicrobial infections. 64.7% were gram- negative aerobes, 1% was gram-positive aerobes and 3.2% were obligate anaerobes out of 221 organisms isolated. 25.9% of cases were of multidrug resistant organisms and included extended spectrum β-lactamase (ESBL) producers (11.3%), MRSA (4.5%) and VRE (1.4%). Against gram-negative and gram-positive pathogens both ciprofloxacin and ceftazidime had good overall anti-microbial activity. Due to institutional constraints obligate anaerobes were uncommonly isolated.

Udobi et al. (2013) worked in the orthopedic ward of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria- Nigeria the prevalence and antibiotic resistance pattern of MRSA. Out of 217 samples taken from the orthopedic wards of the hospital 185 isolates of Staphylococcus aureus were confirmed. Out of these 185 isolates, 44 (23.8%) were from the wounds and 70 (37.8%) from the skin. Beds and the atmospheric air comprised of remaining 65 (35.1%) and 6 (3.2%), respectively. The prevalence of MRSA out of these were found to be 33 (75%), 36 (51.4%), and 48 (73.8%) from wounds, skin, and bed, respectively. From the atmosphere no MRSA isolate was detected. The level of resistance against ampicillin was found to be 100% in all the three sites, pefloxacin 90.9%, 72.2%, 66.7%, ceftriaxone 69.7%, 72.2%, 70.8%, gentamicin 54.5%, 52.8%, 37.5%, and ciprofloxacin 51.5%, 47.2%, 35.4% by performing antimicrobial susceptibility test.

Yali et al. (2013) compared pathogens and their antibiotic resistances of burn patients from burn intensive care unit (ICU) or common burn ward. Of 2395 clinical samples from 63 patients in burn ICU, pathogens were detected in 1621 isolates of Staphylococcus aureus were confirmed. Out of these 185 isolates, 44 (23.8%) were from the wounds and 70 (37.8%) from the skin. Beds and the atmospheric air comprised of remaining 65 (35.1%) and 6 (3.2%), respectively. The prevalence of MRSA out of these were found to be 33 (75%), 36 (51.4%), and 48 (73.8%) from wounds, skin, and bed, respectively. From the atmosphere no MRSA isolate was detected. The level of resistance against ampicillin was found to be 100% in all the three sites, pefloxacin 90.9%, 72.2%, 66.7%, ceftriaxone 69.7%, 72.2%, 70.8%, gentamicin 54.5%, 52.8%, 37.5%, and ciprofloxacin 51.5%, 47.2%, 35.4% by performing antimicrobial susceptibility test.
unit patients. Clinical samples of burn patients from ICU showed antibiotic resistance significantly higher than those from common units.

Kahsay et al. (2014) worked in an Ethiopian hospital to analyze the prevalence, antimicrobial susceptibility patterns and associated risk factors of S. aureus in patients with surgical site infections. S. aureus was isolated from 73 (39.7%) cases from 184 surgical patients who had developed surgical site infection and 36 (49.7%) were MRSA. The clinical isolates showed <50% level of resistance was observed against clindamycin, oxacillin, tetracycline and vancomycin whereas >80% level of resistance to ampicillin, amoxicillin, penicillin G, erythromycin, gentamicin and cotrimoxazole. The resistance showed by MRSA strains was ranging from 5.6% (vancomycin) to 100% (cotrimoxazole). The identified risk factors for infection by S. aureus included sex, age, pus consistency, duration of operation, type of surgery, ward and hospital stay, laparotomy and type of surgery.

Shibabaw et al. (2014) examined the healthy hospital staff members for the antimicrobial susceptibility pattern of S. aureus isolates, the prevalence of MRSA, and the nasal carriage rate. There were 118 Health Care Workers (HCWs), 34 had S. aureus and 15 had MRSA with a positive rate of 28.8% and 12.7%, respectively. The sensitivity to penicillin by S. aureus was found to be 0%. Against commonly available antibiotics MRSA isolates were resistant. From the nasal isolates only two (13.3%) were vancomycin- resistant.

Radji et al. (2014) studied the antibiotic susceptibility patterns and microbiology of diabetic foot infections. A total of 288 of diabetic patients were admitted to hospital, and 35 patients had diabetic foot infections during January to December 2012. In 37.1% of patients diabetic control care was carried out and in 62.9% of patients surgical intervention was carried out. A total of 59 pathogens were isolated. Staphylococcus aureus (47.5%) was the most common infecting microorganism isolated on pus followed by Pseudomonas spp (16.9%), E. coli (10.2%), Streptococcus spp. (8.5%), Enterobacter spp. (7.0%), Proteus spp. (6.7%), and Acinetobacter spp. (3.2%). A single microorganism caused 37.2% of the diabetic foot infection and 62.8% had polymicrobial infections. Ceftriaxone (40.0%), ciprofloxacin (11.4%) and meropenem (8.6%) were the most frequently administered antibiotics.

Islam et al. (2014) carried out a study in a tertiary care hospital in Dhaka, Bangladesh to check the prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA), vancomycin-resistant S. aureus (VRSA), and Panton-Valentine leukocidin (PVL)-positive S. aureus. Between July 2011 and June 2012 S. aureus strains were isolated from 200 postoperative wound swab samples. Out of 44 isolates of S. aureus 15 were MRSA (2 of them were VRSA) and 29 were MSSA. The resistance to Oxacillin (MIC ‘ 256 mg/mL) by all MRSA isolates was found to be very high. The sensitivity and specificity of the Oxacillin disc diffusion method were 93.33% and 100% when compared with polymerase chain reaction (PCR); both the sensitivity and specificity were 100% for the cefoxitin disc diffusion method and minimum inhibitory concentration of Oxacillin. Four (26.67%) MRSA isolates were mecA genes positive which PVL positive were also. The MRSA strains were highly resistant to ciprofloxacin (93.33%), ceftriaxone (86.63%), azithromycin (73.33%), gentamycin (73.33%), and amoxiclav (66.67%). 86.67% sensitivity was shown by Vancomycin and 100% by Linzolid by all MRSA strains.

Haleem et al. (2014) studied the consistency between nasal and diabetic foot ulcer (DFU) Staphylococcus aureus carriage. 29 (36.7%) subjects had DFU colonization with Staphylococcus aureus and 25 (31.6%) had nares colonization with Staphylococcus aureus. 7 (8.8%) subjects had DFU colonization with MRSA and 7 (8.8%) had nares colonization with MRSA. The MRSA presence (P = 0.01) was associated with duration of ulcer. 41% and 74% was the sensitivity and specificity of positive nasal S. aureus colonization with positive DFU colonization. The results were found dissimilar with S. aureus strains infecting DFU and the nasal cavity. Staphylococcus aureus colonization of a DFU by Staphylococcus aureus strains can’t be supposed due to poor positive isolates of S. aureus in a DFU of nasal carriers.

CHAPTER 3
MATERIALS & METHODS
3.1. Isolation and Identification of Methicillin-Resistant Staphylococcus aureus (MRSA)
3.1.1. Sample collection
The samples were collected from three busy and tertiary care hospitals in Faisalabad namely Allied Hospital, District Headquarter Hospital (DHQ) and National Hospital. A total of 150 swab samples were collected from different types of accidental & surgical wounds, burn wounds and diabetic foot with pus discharge and
purulent discharge as shown in plate 1 & plate 2. Under aseptic conditions, samples were collected from wound depth using a clean cotton swab and transferred to Microbiology Laboratory of Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, for further processing.

3.1.2. Processing of samples for bacterial isolation and identification
Swab samples collected from different wounds were processed according to procedures described by Wolk et al. (2009). For isolation of S. aureus, swabs samples taken from humans were inoculated individually into Oxacillin broth (sodium chloride, mannitol, tryptone, yeast extract and Oxacillin) and incubated at 37°C for 24 hours. Following incubation, a loopful of broth was streaked on CHROMagar MRSA (CrA; CHROMagar, Paris, France) plates (90mm plastic Petri-plates) and incubated at 37°C for 24 hours as shown in plate 3.

Plate.1. A swab sample being collected from a patient with diabetic foot
Plate. 2. A swab sample being collected from a patient with burn wound

3.1.3. Identification of isolates of MRSA
Following incubation, colonies on CHROMagar MRSA were observed for identification of Staphylococci through specific colony characters; mauve colored, round colonies on CrA. For further identification of staphylococci, isolates were subjected to Gram staining reaction (Kahsay et al., 2014).

Protocol:
' With the help of sterile loop, smear of bacterial colony was prepared.
' Smear was heat fixed by passing the slide through burner 2-3 times.
' Then it was flooded with crystal violet (principle stain) for 1 minute, rinsed off with distilled water.
' Then flooded with Gram's iodine for 1 minute and rinsed off.
' Slide was decolorized with 95% ethyl alcohol drop by drop for 5-10 seconds, immediately rinsed with water.
' Smear was flooded with Safranine (counter stain) gently and left for 1 minute, then rinsed with water.
' Slide was viewed using light microscope under oil-immersion lens.
' Shape, arrangements and staining character of bacteria were observed.

3.1.4. Identification on the basis of Hemolysis
All previously identified isolates including MRSA on CHROMagar MRSA were sub-cultured on plates of blood agar and incubated at 37°C for 24 hours for further identification through colony morphology, hemolysis (α-hemolysis) and pigment production (golden yellow) (Schuenck et al. 2006).

Plate.3. Culturing of samples on CHROMagar MRSA

3.1.5. Identification through Tube Coagulase test
All hemolytic isolates from blood agar plates were tested for presence of free/extracellular coagulase using rabbit plasma. Staphylococcus aureus produce coagulase (clumping factor) which can clot plasma into gel in tubes and this property makes it different from coagulase-negative staphylococci. The detail protocol is given below:

Protocol:
' All tubes for test were marked as 1, 2, 3’n, according to the hemolytic isolates on blood agar plates.
' 1 ml rabbit plasma was poured in all marked coagulase tubes.
' A loop full of suspected S. aureus was picked up from blood agar plate and mixed with rabbit plasma.
' All cultured tubes were incubated at 37°C for 4 hours.
' The clot formation was observed and results were recorded as coagulase positive or coagulase negative.

3.1.6. Identification of the S. aureus on the basis of Latex Agglutination test (Capsular Polysaccharide)
Staphytect is a test to differentiate S. aureus by the detection of clumping factor, Protein A and capsular polysaccharides found in MRSA from those staphylococci that do not possess these properties. Hemolytic isolates from blood agar were tested for Staphytect latex agglutination test. The detail protocol is given below:

Protocol:
' First of all, circles of test card were labeled for each colony sample to be tested.
Reagent was dispensed onto the circles on the test card.
With the help of sterile wire loop, 4-5 average sized suspected staphylococcal colonies from culture media plate were picked up and mixed onto the circle with test latex.
Card was rocked/rotated for up to 20 seconds and observed for agglutination.
Results of the test were recorded as +ve and ‘ve.

3.2. Confirmation & Molecular Characterization of Methicillin-Resistant S. aureus (MRSA)

3.2.1. Confirmation of Methicillin-Resistant Staphylococcus aureus (MRSA)
Isolates of S. aureus were checked by latex agglutination test (PBP2a test kit; Oxoid Ltd., Basingstoke, UK) for the presence of penicillin binding protein 2a. MRSA latex agglutination test was carried out as per manufacturer’s instructions (Shore et al., 2011).

Protocol:
4 drops of Extraction Reagent#1 (0.1 M NaOH) were added into centrifuge tube.
With the help of sterile 1μl loop, a loopful of growth was removed from blood agar plate and suspended in Extraction reagent 1.
Tubes were placed in water bath at 95°C for 3 minutes.
Tubes were removed from water bath and allowed to cool to room temperature.
1 drop of Extraction Reagent #2 (0.5 N KH2PO4) was added into the tubes and mixed well.
For 5 minutes tubes were centrifuged at 1500 g.
On test card 50 μL of the supernatant was added and mixed with 1 drop of test latex (anti-PBP2a monoclonal antibody sensitized latex particles) for 3 minutes.
Agglutination was observed and recorded as strong positive, moderately positive or negative.

3.2.2. Molecular Characterization of Methicillin-Resistant S. aureus (MRSA)

3.2.2.1. Detection of nuc and mecA genes by Polymerase Chain Reaction (PCR)
The isolates were tested for the chromosomal genes ‘nuc’ encoding (thermo-nuclease specific for S. aureus) and mecA (methicillin resistant specific gene) (Brakstad et al., 1992) using commercially available multiplex PCR kit (BactReady’ multiplex PCR system; Genescript, USA). Sequence of primers used and size of PCR product is mentioned in the Table 1. All primers were purchased from GeneLink’ (USA) through local vendor (The Worldwide Scientific, Lahore, Pakistan).

3.2.2.2. Preparation of Genomic DNA
For PCR, genomic DNA was prepared using extraction components (solution) available with BactReady’ (Genescript, USA) the composition was mentioned in table 2. All preparations were made according to instruction manual. Briefly, single, isolated, 24-hour old colony from blood agar plate was picked up and thoroughly suspended in 25 microliter of DNAse free water and 1 microliter of cell suspension was mixed with 20 microliter of cell lysis buffer (BR-A buffer supplied with BactReady) and lysate was spun down by short centrifugation at 2000 rpm for 1 minute. The supernatant containing genomic DNA was used directly for amplification.

3.2.2.3. PCR amplification
The reaction was performed using BactReady’ multiplex PCR system (Genescript, USA). The reaction mixture (20 μl) was prepared in thin walled with flat cape, DNase-RNase free 0.2 mL tubes Thermo-Tubes, (Thermo-scientific, UK) with 1 μl of template DNA. Amplifications were performed using a micro-processed controlled Swift’ Maxi Thermal Cycler Block (ESCO Technologies Inc. France) under the following conditions: activation of Script’ DNA polymerase at 94°C for 15 minutes followed by 35 cycles of denaturation (95°C for 1 min), annealing (55°C for 1 min), extension (72°C for 1 min), and a final extension step of 72°C for 3 minutes. The composition of reaction mixture is given in the Table 2.
The analysis of amplicons were made by agarose gel electrophoresis using a horizontal mini agarose gel electrophoresis system (ENDURO’ Lab net International Inc., Woodbridge, NJ, USA). A mixture of undiluted PCR products (5 μl) and 5X loading dye (1 μl; Fermentas Thermo Fischer Scientific Inc., UK) was loaded to 1.2% Agrose gel (multipurpose agarose, low EEO, multipurpose, Fischer Scientific Ltd, Loughborough, UK) containing ethidium bromide (0.5 μg/mL; Fischer Scientific Ltd, Loughborough, UK) for DNA staining. The gels were run in 1X TAE buffer (50X TAE Buffer, Fischer Scientific Ltd, Loughborough, UK) at 80 volts (80mA) for 1 hour. The amplicons were visualized on a trans-illuminator (Vilbert Lourmart,
Cedex France) and saved using gel documentation systems (DP-CF-011, France). The size of the products was measured using a ready to use 100 bp molecular markers (O gene-Ruler 100bp DNA ladder, Fermentas, Thermo-scientific, UK).

Table 1. Sequence of Primers used and PCR product size

<table>
<thead>
<tr>
<th>Targeted genes</th>
<th>Sequences</th>
<th>Product Size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA forward</td>
<td>MecA1: 5′-GTA GAA ATG ACT GAA CGT CCG ATA A-3′</td>
<td>310bp</td>
<td>Jonas et al., 2001</td>
</tr>
<tr>
<td></td>
<td>MecA2: 5′-CCA ATT CCA CAT TGT TTC GGT CTA A-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nuc forward</td>
<td>Nuc: 5′-GCG ATT GAT GGT GAT ACG GTT- 3′</td>
<td>279bp</td>
<td>Brakstad et al., 1992</td>
</tr>
<tr>
<td></td>
<td>Nuc: 5′-AGC CAA GCC TTG ACG AAC TAA AGC-3′</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Composition of PCR reaction mixture used for amplification of nuc (S. aureus specific) and mecA (methicillin resistance) genes

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Volume (μl)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR grade (DNAse free) water</td>
<td>7</td>
<td>——</td>
</tr>
<tr>
<td>Forward primer 1</td>
<td>200nM</td>
<td></td>
</tr>
<tr>
<td>Reverse primer 1</td>
<td>200nM</td>
<td></td>
</tr>
<tr>
<td>DNA solution 1</td>
<td>——</td>
<td></td>
</tr>
<tr>
<td>PCR premix 10</td>
<td>——</td>
<td></td>
</tr>
<tr>
<td>Total volume 20</td>
<td>——</td>
<td></td>
</tr>
</tbody>
</table>

3.3. Antimicrobial Susceptibility Testing

In-vitro antimicrobial susceptibility testing was performed on Mueller-Hinton agar by disc diffusion method (Udobi et al., 2013). After incubation, around discs measurement of zones of inhibition was made in mm and the strains were classified as sensitive, intermediate and resistant according to the guidelines of Clinical and Laboratory Standards Institute (CLSI 2005).

3.3.1. Disc Diffusion Method

In-vitro susceptibility of MRSA was determined to following antimicrobials: Imipenem, Fusidic acid, Doxycycline, Amoxicillin, Gentamycin, Ciprofloxacin, Linezolid, Pipracillin-Tazobactum, Vancomycin, Amoxicillin-Clavulanic acid, Clindamycin, Clarithromycin, Sulfamethoxazole-Trimethoprim, Ampicillin, Levofloxacin and Oxacillin.

Protocol:
- The susceptibility testing was performed on Mueller-Hinton agar plates, prepared following the manufacturer instructions (Oxoid, U. K).
- The turbidity of each test inoculum was equivalent to 0.5 McFarland standards that was prepared in normal saline by dissolving 2-3 freshly grown isolated colonies.
- The inoculums dipped cotton swabs were inoculated over whole surface of Mueller-Hinton agar plates.
- Following inoculation, antibiotic discs were placed evenly on the surface of plates.
- The plates were then incubated at 37°C for 24 hours.
- Following incubation, zones of inhibition were measured with antibacterial zone guage.
- Results were recorded as sensitive, intermediately resistant or resistant.
RESULTS & DISCUSSION

4.1. Isolation and identification of Staphylococcus aureus and MRSA
All swab samples of wounds from hospitalized patients were cultured on CHROMagarTMMRSA, following incubation mauve coloured round colonies were found on CHROMagar'MRSA as shown in the plate 7. All isolates were identified through Gram staining reaction, purple coloured bunches of staphylococci were observed as shown in the plate 4. The S. aureus isolates showed \( \delta \frac{\lambda}{\gamma} \)-Hemolysis on blood agar as shown in the plate 8, clot formation using tube coagulase test as shown in the plate 5 and agglutination by Staphytect test. All MRSA isolates were confirmed by latex agglutination test for the presence of penicillin binding protein 2a (PBP2a) as shown in the plate 6 and amplified bands of nuc and mecA gene by polymerase chain reaction (PCR) as shown in the plate 9 & plate 10.

4.2. Occurrence and distribution of Staphylococcus aureus and MRSA
A total of 150 randomly collected samples of human patients from two Government and one Private hospital in Faisalabad city i.e. Allied Hospital (n=97), DHQ (n=43) and National hospital (n=10) were analyzed for the presence of Methicillin-Resistant S. aureus (MRSA) as mentioned in fig. 1. Of these 150 samples, number of S. aureus was 73 (48.6%) and of these positive 67 (91.7%) were MRSA (Fig. 2).

4.2.1. Sample site based distribution of S. aureus and MRSA
On the basis of sample source distribution, it was found that major proportion of MRSA (n=67) from three hospitals was recovered from burn wounds (53.7%) followed by the accidental and surgical wounds (41.8%) and diabetic foot (4.4%) as shown in table 3 and fig. 3.

4.2.2. Gender based distribution of S. aureus and MRSA
Of the 150 total human patients, 94 (62.66%) were males and 56 (37.33%) samples were taken from females. Of a total of 73 S. aureus positive samples from 150 human patients, 48 (65.75%) were from males and 25 (34.24%) from females. Gender based analyses showed that the prevalence of S. aureus was more among male patients than females. The prevalence of MRSA was found to be 47% in males as compared to 41% in females as shown in table 4 and fig. 4.

Plate 4. Gram staining showing clusters of S. aureus
Plate 5. Tube Coagulase test showing clot formation
Plate 6. Penicillin Binding Protein 2 (PBP2a) test for confirmation of MRSA
Plate 7. Mauve colored round colonies on MRSA CHROMagar medium
Plate 8. \( \delta \frac{\lambda}{\gamma} \)-Hemolysis on blood agar plate
Fig. 1. Distribution of samples from patients attended Allied, DHQ, and National Hospital, Faisalabad
Fig. 2. Distribution of S. aureus and MRSA among hospitalized patients
Table 3. Sampling site based distribution of S. aureus and MRSA

<table>
<thead>
<tr>
<th>Type of Isolates</th>
<th>Burn wounds</th>
<th>Accidental &amp; surgical wounds</th>
<th>Diabetic foot</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>39 (53.4%)</td>
<td>31 (42.4%)</td>
<td>3 (4.1%)</td>
<td>73</td>
</tr>
<tr>
<td>MRSA</td>
<td>36 (53.7%)</td>
<td>28 (41.8%)</td>
<td>3 (4.4%)</td>
<td>67</td>
</tr>
</tbody>
</table>

Fig. 3. Sampling site based distribution of MRSA recovered from hospitalized patients

Table 4. Total number and percentage of S. aureus and MRSA recovered from male and female
Gender Total samples Total positive samples (S. aureus) Total MRSA samples
Male 94 (62.66%) 48 (65.75%) 44 (47%)
Female 56 (37.33%) 25 (34.24%) 23 (41%)
Total 150 73 (49%) 67 (92%)

Fig. 4. Gender based distribution of S. aureus and MRSA

4.2.3. Age based distribution of S. aureus and MRSA
Of the 73 S. aureus isolates collected from males and females, 67 were MRSA. Age based analyses revealed that the distribution of MRSA was 15% among patients aged 1-20 years. Similarly the order of distribution of MRSA was 55% among patients aged 21-40 years, 30% in 41-60 years age group and 4.47% in 61-80 years age group. This data showed that patients aged between 21-60 years were more prone to get MRSA infection as shown in table 5 & fig. 5.

4.2.4. Hospital wise distribution of S. aureus and MRSA
On the basis of hospital wise distribution, it was observed that the highest proportion of MRSA isolates (95%) was obtained from DHQ Hospital, followed by 91% from Allied Hospital and 86% from National Hospital, respectively as shown in table 6 and fig. 6.

Table 5. Age based distribution of S. aureus and MRSA

<table>
<thead>
<tr>
<th>Isolates</th>
<th>1-20 Year</th>
<th>21-40 Year</th>
<th>41-60 Year</th>
<th>61-80 Year</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>12 (16%)</td>
<td>37 (51%)</td>
<td>21 (29%)</td>
<td>3 (4%)</td>
<td>73</td>
</tr>
<tr>
<td>MRSA</td>
<td>10 (15%)</td>
<td>34 (55%)</td>
<td>20 (30%)</td>
<td>3 (4%)</td>
<td>67</td>
</tr>
</tbody>
</table>

Fig. 5. Age based distribution of S. aureus and MRSA

Table 6. Hospital based distribution of S. aureus and MRSA

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Total S. aureus</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Hospital</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>DHQ Hospital</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td>Allied Hospital</td>
<td>97</td>
<td>44</td>
</tr>
</tbody>
</table>

Fig. 6. Distribution of S. aureus and MRSA among three hospitals

4.3. Molecular characterization of S. aureus and Methicillin-Resistant S. aureus (MRSA)
Using PCR the presence of nuc gene and mecA gene was confirmed by getting amplified bands of genes comparable with gene markers for molecular characterization of S. aureus and Methicillin-Resistant S. aureus (MRSA) as shown in plate 9 & plate 10.

4.4. Antimicrobial Susceptibility profiles of MRSA isolates
Of 67 MRSA isolates, 50 were selected on sample site basis for antimicrobial susceptibility on Mueller-Hinton agar; accidental & surgical wounds 20, burn wounds 28 and diabetic foot 2 (plate 11). All isolates were resistant to Amoxicillin-Clavulanic acid, Ampicillin, Amoxicillin and Ciprofloxacin but sensitive to Vancomycin. The resistance pattern among 50 Methicillin-Resistant S. aureus strains was as follows; Imipenem 41 (82%), Fusidic acid 13 (26%), Doxycycline 32 (64%), Amoxicillin 50 (100%), Gentamycin 47 (94%), Ciprofloxacin 50 (100%), Linezolid 5 (10%), Pipracillin-Tazobactum 48 (96%), Vancomycin 0 (0%), Amoxicillin-Clavulanic acid 50 (100%), Clindamycin 6 (12%), Clarithromycin 40 (80%), Sulfamethoxazole-Trimethoprim 31 (62%), Ampicillin 50 (100%), Levofloxacin 42 (84%) and Oxacillin 48 (96%). The pattern of intermediate resistance was as follows; Doxycycline 6 (12%), Levofloxacin 6 (12%), Vancomycin 4 (8%) and Oxacillin 2 (4%). Table 7 shows the antimicrobial resistance pattern among MRSA strains identified by disc diffusion method and figure 7 & fig 8 shows graphical representation of antimicrobial susceptibility profile.
Plate.9. PCR amplicons of nuc gene encoding S. aureus

Plate.10. PCR amplicon of mecA gene for MRSA

Plate.11. Zones of inhibition on Mueller-Hinton agar

Table.7. Antimicrobial Susceptibility pattern among MRSA isolates through disc diffusion method

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive</th>
<th>Intermediately resistant</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>%age</td>
<td>Number</td>
<td>%age</td>
</tr>
<tr>
<td>Imipenem</td>
<td>9 8%</td>
<td>41 82%</td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>3 4%</td>
<td>13 26%</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>12 4%</td>
<td>6 12% 32 64%</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0 0%</td>
<td>50 100%</td>
<td></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>3 6%</td>
<td>47 94%</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 0%</td>
<td>50 100%</td>
<td></td>
</tr>
<tr>
<td>Linezolid</td>
<td>45 0%</td>
<td>5 10%</td>
<td></td>
</tr>
<tr>
<td>Piperacillin- tazobactam</td>
<td>2 4%</td>
<td>48 96%</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>44 8%</td>
<td>6 12%</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>46 9%</td>
<td>4 8% 0 0%</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>10 0%</td>
<td>40 80%</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim</td>
<td>19 8%</td>
<td>31 62%</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0 0%</td>
<td>50 100%</td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>2 4% 6 12%</td>
<td>42 84%</td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0 2 4%</td>
<td>48 96%</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin Clavulanic acid</td>
<td>0 0%</td>
<td>50 100%</td>
<td></td>
</tr>
</tbody>
</table>

Fig.7. Antimicrobial Susceptibility profile of 50 MRSA isolates

Fig. 8. Percent in vitro Resistance to 16 antibiotics in 50 MRSA isolates

Table.8. Prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) among S. aureus isolates in Pakistan

<table>
<thead>
<tr>
<th>Studies Conducted in Pakistan</th>
<th>(Year) Study Period</th>
<th>(Year)</th>
<th>MRSA Prevalence (%)</th>
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Faisalabad
DISCUSSION
Methicillin resistant Staphylococcus aureus (MRSA) remains a main and persistent health problem all over the world. The present study also shows the high rates of MRSA in three Hospitals of Faisalabad. Keeping in view the importance of MRSA, the present study was planned to find the prevalence of MRSA in various kinds of wounds i.e. accidental & surgical, burns and diabetic foot. This study would be among the first few studies that report the prevalence rates of MRSA in humans in District Faisalabad and will helpful to treat and control the infections related to MRSA.

S. aureus is the cause of many infections but it may also occur as a beneficial bacteria. Tissues can be infectious with S. aureus on rupturing of skin or mucosal barriers. This rupture can cause different types of infections including blisters and abscesses. The present study was designed with three main objectives; 1st was to isolate, identify & characterize MRSA strains on molecular basis from accidental & surgical wounds, 2nd was to find the prevalence of MRSA and 3rd one to study the antimicrobial susceptibility profile of MRSA against commonly used antibiotics. The isolation was done on CHROMagr’ MRSA as described by Wolk et al. (2009), identification as described by Kahsay et al. (2014), confirmation of MRSA as described by Shore et al. (2011) and molecular characterization as described by Brakstad et al. (1992). The antimicrobial susceptibility testing was performed on Mueller-Hinton agar as described by Udobi et al. (2013).

Isolation of MRSA strains was performed on CHROMagr’ MRSA. For this purpose the 150 swab samples from human wounds were individually inoculated into Oxacillin broth a loopful of broth was streaked on CHROMagr’ MRSA plates and mauve coloured colonies were found. Same procedure was used by Wolk et al. (2009) in which all wound samples were inoculated into nutrient broth and sub-cultured on CHROMagr’ MRSA and CHROMagr’ SA. Presumptive identification was done by Gram staining reaction and purple coloured bunches of Staphylococci were observed this procedure is in accordance with Kahsay et al. (2014), α-Hemolysis on blood agar Schuenck et al. (2006), clot formation by tube coagulase test (Sibabaw et al. 2014)) and latex agglutination for S. aureus identification by Staphytect test. Confirmation of MRSA strains was done by latex agglutination test for the presence of penicillin binding protein 2a (PBP2a) and same procedure was used by Shore et al., (2011). Molecular characterization was done using Polymerase Chain Reaction (PCR) for the occurrence of nuc gene (thermos nuclease specific for S. aureus) and mecA gene (methicillin resistant), for this purpose mecA forward & reverse primers with 310 bp size and nuc forward & reverse primers with 279 bp size were used, Brakstad et al. (1992) used the same procedure in their study.

In hospitals, number of cases of MRSA varies noticeably from an area to another and in same city but the data on prevalence of MRSA in Pakistan is limited. In some reports the MRSA accounts for only <10% whereas more than 65% in some other as documented by Safder et al. (2003). Variable rates of prevalence of MRSA have documented by Pakistani workers in Pakistani hospitals but all studies clearly shows increase in trend of MRSA prevalence rate. The results of present study were found to be very high with respect to the prevalence rate of 91.7%. In Karachi the prevalence rate of MRSA was 5% reported by Aashiq and Tareen (1989), 43% by Perwaiz et al. (2007) and 39.0% was reported by Sabir et al. (2014). Similar work with 41.9% prevalence was documented by Hafiz et al. (2002). In Peshawar the prevalence rate of MRSA was 5.26% documented by Khan et al. (2014) and 31.5% by Naeem et al. (2013). In Rawalpindi the prevalence rate of MRSA was 53.3% documented by Khan et al. (2014) and 60.40% by Perveen et al. (2013). In Lahore the trend of MRSA prevalence was 34.76% documented by Siddiqi et al. (2009) and 58.88% by Mahmood et al. (2010). In Faisalabad, the prevalence rate of MRSA was 52.64% documented by Kanwal et al. (2014). The high percentage of MRSA in the present study as compared to the studies reported earlier could be due to the rising levels of resistance among S. aureus isolates in Pakistani hospitals. A comparison on prevalence rate of MRSA among S. aureus isolates was made with similar studies conducted in Pakistan shown in the Table 8. The results of present study are in agreement with work of Wolk et al. (2009) with prevalence rate of 97%. Similarly prevalence rate of 88.23% described by Belthur et al. (2012) and 84.1% by Maina et al. (2012) are also comparable with present study. Dubey et al. (2013) reported 80% prevalence of MRSA who worked on 1507 nosocomial and community associated isolates of S. aureus, out of 485 OPD isolates of S. aureus 80.41% were MRSA. Likewise, out of 564 from wards and cabins 81.73% were MRSA, while out of 458 isolates obtained from ICU and NICU 79.25% were MRSA. The prevalence rate of MRSA in other studies was low in comparison to the present study as 66.3% reported by Udobi et al. (2013), 57.8% by Tillotson et al. (2008), 50% by Kahsay et al. (2014), 39.8% by Prasanna and Thomas (1998) and 38.56% by Mohanty et al. (2004). Similar results were
found as 38.44% documented by Tiwari et al. (2008) and 31% by Buzaid et al. (2011). On the basis of sample site, a higher portion of MRSA was isolated from burn wounds (53.7%), followed by accidental & surgical wounds (41.8%) and diabetic foot (4.4%). Orrett and Land (2006) found 60.1% MRSA isolates from surgical/burn wounds and 6.6% from pus/abscess samples. The result in present study showed 53.7% from burn wounds and 41.8% from surgical & accidental wound samples. These results are in accordance with Orrett and Land as his study contained collective burn & surgical wound samples. Perwaiz et al. (2007) reported 32% MRSA isolates from pus samples and 36% MRSA positive from surgical wound samples. The high rates of MRSA from pus samples could be due to large pus sample size used in the study of Perwaiz and coworkers. The results in this study are also in accordance with that described by Kanwal et al. (2014). The overall prevalence of MRSA was higher (95%) in DHQ Hospital followed by (91%) Allied Hospital and (86%) National Hospital. This difference could be due to hospital management, cleanliness and other factors associated with nosocomial infections.

On the basis of Gender, MRSA prevalence in Faisalabad was recorded higher in males (47%) than females (41%). Similar prevalence rate was found in other study of Richard et al. (2010). The prevalence of MRSA in all sample sites in various age groups in this study was found to be highest in 21-40yrs (55%) age group followed by 41-60yrs (30%), 1-20yrs (15%) and 61-80yrs (4%). The results are in agreement with the results of Rahman et al. (2011) where major age group at risk was 20-29yrs with male predominance. The results in this study are proportionate to that described by Anwar et al. (2004) where the prevalence of MRSA was described as 0-5yrs (25.5%) age group, 6-14yrs group (13.2%), 15-29yrs (38.3%) group, 30-59yrs (19.4) age group and above (3.7%) age group. Anwar et al. (2004) reported higher prevalence of MRSA in urban population as compared to rural populations but in present study we did not distributed the population on these bases. The main reason for high prevalence rate of MRSA in males could be due to the fact that in countries like Pakistan, mostly male population is involved in outside work and thus exposed to environmental hazards as compared to females.

Antibiotic resistance to all beta-lactam antibiotics is due to presence of mecA gene and was confirmed by polymerase chain reaction (PCR). In the present study 50% MRSA isolates were selected on hospital bases for molecular characterization. 20 MRSA isolates were selected from Allied hospital, 11 from DHQ hospital and 3 from National hospital. The mecA gene was detected among all selective isolates. Fridkin et al. (2005) also found mecA gene using PCR for confirmation of MRSA. Different microbiological methods have been used for recovery of MRSA from samples and confirmation, but using PCR detection of mecA gene is considered as gold standard method. The study of Kuzucu et al. (2002) is in accordance with present study as MRSA isolates were confirmed using PCR to detect mecA gene.

On Mueller-Hinton agar plates by disc diffusion technique the antimicrobial susceptibility testing was performed. For this turbidity of each test inoculum was adjusted according to 0.5 McFarland standards. The inoculum dipped cotton swabs were inoculated over entire surface of agar plates, antibiotic discs were placed on the surface of agar plates then incubated at 37°C for 24 hrs. Zones of inhibition were measured and classified as sensitive, intermediate and resistant according to the guidelines of CLSI. Vancomycin remains an effective treatment but reports regarding resistant MRSA isolates against vancomycin have been published (Rybak and LaPlante, 2005). The drug resistance pattern of MRSA isolated from clinical samples was found to be highly variable. All 67 MRSA isolates were resistant to Amoxicillin (100%), Ciprofloxacin (100%), Ampicillin (100%) and Amoxicillin-Clavulanic acid (100%) and no resistance against vancomycin (0%). These results are comparable to the results of Zulfiqar et al. (2007) where Ciprofloxacin showed 100% resistance and 0% resistance to Vancomycin. In the present study only 2 samples showed sensitivity against Pipracillin-Tazobactum while remaining all 65 samples found resistant to it. All MRSA isolates showed 92% sensitivity against Vancomycin and 90% sensitivity against Linezolid. The intermediate resistance was also found against 4 antibiotics as follows; Doxycycline 6 (12%), Levofoxacin 6 (12%), Vancomycin 4 (8%) and Oxacillin 2 (4%). Linezolid showed 90% sensitivity as only 5 MRSA isolates were found resistant against Linezolid. The results of antibiotic sensitivity in this study are proportionate to the study of Liu et al. (2011) which described 100% resistance by MRSA against Ampicillin and Penicillin and 100% sensitivity against Vancomycin.

In the present study Vancomycin (92%) was found to be most effective drug against MRSA followed by Linezolid (90%) which are comparable to results of Mahmood et al. (2010) in which Vancomycin, Linzolid and Teicoplanin were the most effective drugs with 100% sensitivity. Sensitivity against Clindamycin was found to
be 88% and against Fusidic acid was 74% which are comparable to study of Nizamuddin et al. (2011) where resistance of MRSA isolates against Fusidic acid was found to be 85% and against Clindamycin 75.5% as reported by Hamid. Et al. (2011). Gentamycin showed 94% resistance in the present study and this is in agreement with study of Kahsay et al. (2014). MRSA isolates showed resistance against Imipenem and Levofloxacin as 82% and 84%, respectively in the present study which is comparable to the study of Perveen et al. (2013) where resistance against Imipenem was found to be 77% and 80.40% against Levofloxacin. The antibiotic resistance of MRSA isolates against commonly used antibiotics becomes widespread in Pakistan and this situation is alarming. Vancomycin was considered as drug of choice against MRSA but there are some reports indicating emerging resistance against Vancomycin as well. Thati et al. (2011) reported that MRSA isolates showed intermediate resistance against Vancomycin. In this study 8% intermediate resistance against Vancomycin was found whereas 4% VRSA was found in the study of Bukhari et al. (2004). The percentage of VRSA in this study is higher and this is alarming and there is need to find out a good alternate of Vancomycin. According to the results of present study, Vancomycin was the most effective drug followed by Linzolid and Clindamycin whereas Amoxicillin, Ciprofloxacin, Ampicillin and Amoxicillin-Clavulanic acid were the least effective drugs.

Based on overall results, it was concluded that MRSA infections were found in all three studied hospitals of Faisalabad City. The highest rate of prevalence was found in DHQ hospital, Faisalabad. Among all kinds of wounds, the highest rate of MRSA infection was reported in burn wounds. MRSA infections were found highest in human males as compared to females. Among all age groups, the highest numbers of MRSA isolates were found in 21-40yrs age group. All isolates possess nuc gene & mecA gene and it was confirmed by PCR and it is the most accurate method for detection of mecA gene. All MRSA isolates showed high sensitivity to Vancomycin and Linzolid. Amoxicillin, Ciprofloxacin, Ampicillin and Amoxicillin-Clavulanic acid were least effective as all MRSA isolates were resistant to these drugs. According to present study, Vancomycin and Linzolid are the most effective drugs against MRSA infections but these drugs have not yet been introduced in these hospitals for treatment of such patients.

Based on results of present study some points for future recommendations include; there is a need to control potential responsible factors for MRSA infection and transmission in human population in Faisalabad. Proper hygienic measures and management regarding hospitals should be adopted. A comprehensive approach is required to prevent and treat MRSA infections in hospitalized humans. Use of antibiotics for shortest limited time period and only on the prescription of physician. Avoid self-medication.

CHAPTER 5
SUMMARY

Methicillin-Resistant Staphylococcus aureus (MRSA) is a pathogen that causes many complicated infections in humans. MRSA is a variant of S. aureus that has produced by of process of resistance to beta-lactam antibiotics, which include the penicillins (methicillin, Oxacillin, etc.) and the cephalosporins. Resistance to methicillin is independent of $i_1i_2i_3$-$\beta$-Lactamase production. In various countries the prevalence of MRSA has varied from hospital to hospital. All over the world, increased prevalence and antibiotic resistant bacteria are the emerging issues, faced by the hospitals.

MRSA infections have become a reason of important diseases with sometimes lethal results. There are number of factors responsible for increasing trends in MRSA infections in developing countries like Pakistan including unawareness, lack of surveillance systems, poverty, self-medication and long list of medicines prescribed by doctors to patients. Transmission of MRSA occurs by direct contact to a colonized carrier. The main source of spread is from patient to patient on the hands of hospital staff. Post-surgical infections accounts for approximately one-fourth of all hospital acquired infections. MRSA prevalence rate from burn wounds have also increased worldwide.

The present study was planned to isolate, identify and characterize the MRSA isolates on molecular basis and to determine their prevalence rate. For isolation and identification of MRSA from human patients, collection of 150 swab samples was made from different kinds of wounds including accidental & surgical wounds, burn wounds and diabetic foot. The samples were processed on CHROMagar MRSA following incubation mauve colored colonies were obtained and identified by Gram staining, tube coagulase test, $i_1i_2i_3$-$\beta$-Hemolysis on blood agar and Staphytect latex agglutination test. MRSA isolates were confirmed by latex agglutination test.
for the presence of penicillin binding protein 2a, molecular detection of nuc and mecA genes by PCR.
The overall prevalence of S. aureus in samples obtained from human wounds was found to be 48.6%, the
prevalence of MRSA was found to be 91.7%. The prevalence rate of MRSA was found to be higher in human
males (47%) as compared to the females (41%) the reason for this could be due to high exposure of males to
environment for working purpose. Higher proportion of MRSA was obtained from the patients with age group
21-40yrs (55%). Highest rate of MRSA prevalence was isolated from District Head Quarter hospital (DHQ),
Faisalabad. High prevalence of MRSA was collected from burn wounds.
All MRSA contains mecA gene which are responsible for resistance to beta-lactam antibiotics. All MRSA
isolates confirmed by latex agglutination by having penicillin binding protein 2a (PBP2a) were molecularly
characterize to detect mecA gene using polymerase chain reaction (PCR). The isolates were tested for the
chromosomal genes ‘nuc’ encoding (thermo-nuclease specific for S. aureus) and mecA (methicillin resistant
specific gene). All isolates were found positive as mecA gene and nuc gene was found in all MRSA isolates.
Antimicrobial susceptibility profile was performed on Mueller-Hinton agar plates against commonly used
antibiotics. All MRSA isolates showed complete resistance against Amoxicillin, Ciprofloxacin, Ampicillin and
Amodicillin-Clavulanic acid. Intermediate resistance was found against Vancomycin (8%), Doxycycline (12%),
Oxacillin (4%) and Levofloxacin (12%). Higher level of sensitivity was observed against Vancomycin and
Linzolid. Less sensitivity was observed against Clindamycin. Vancomycin was considered as the drug of choice
against MRSA infections but resistance against this drug have been developed, still it is the effective drug as
Vancomycin is not commonly used in our hospitals. The percentage of VRSA in this study is higher and this is
alarming and there is need to find out a good alternate of Vancomycin.
In conclusion, high prevalence of MRSA was found in human patients in hospitals of Faisalabad. The
proportion of MRSA was high in human males as compared to females and in 21-40yrs of age group. Multi-
drug resistance is an important finding in this study. Multiple factors are responsible for increased rate of
MRSA infections including self-medication, unawareness, non-surveillance system, treatment trend from
quacks, carelessness by doctors regarding prescription of antibiotics and poverty. According to present study
Vancomycin is the most effective drug against MRSA infections followed by Linzolid.

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