



A Study of Antibiotic Susceptibility of MRSA in Healthcare Setting: should we really be Worried?

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Submission: August 3, 2016; **Published:** August 17, 2016

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Abstract

Antibiotic susceptibility study of bacteria isolates, in particular *Staphylococcus* and MRSA, were conducted using standard procedure from the samples obtained from hospital settings. The prevalence of MRSA strains were 3.28%. These isolated MRSA strains were resistant to ampicillin (100%) but sensitive against vancomycin, clindamycin and chloramphenicol (100%) with variable zone of inhibition against different other antibiotics. Nosocomial infection has become global health burden with increased morbidity and mortality. Emerging antibiotic resistance has resulted in a significant healthcare expenditure. Therefore necessary steps are warranted to stop the emergence of these resistant strains and provide health education for effective prevention and control of nosocomial infections.

Keywords: Nosocomial infections; *Staphylococcus Aureus*; MRSA; Antibiotic susceptibility; Prevention

Abbreviations: MRSA: Methicillin Resistant *Staphylococcus Aureus*; MSA: Mannitol Salt Agar; PBPs: Penicillin-Binding Proteins; HAI: Hospital-Acquired Infection; NCCLS: National Committee for Clinical Laboratory Standards; MSSA: Methicillin Sensitive *S. Aureus*

Introduction

Hospital provides an ideal environment for hosting different pathogens and is a suitable niche for the transmission of microbial organism. Hospital and hospital setting have a number of patients within an enclosed space, potential of lodging different pathogens. Patients will come in contact with each other and many healthcare workers each day, increasing the likelihood of pathogenic transmission. Direct person-to-person interaction will facilitate the patient and hospital worker to become colonized by organisms adapted to this special environment [1]. Nosocomial infections are infections acquired within health care facilities that commonly include hospitals and nursing home. Nosocomial infections can occur while the patient is in the healthcare setting or after discharge based on incubation period of specific pathogens. Majority of these nosocomial transmissions takes place through direct contact or contamination [2]. Nosocomial infection can constitute surgical infections, with dreadful complications. Clinical sign of wound infection warrants the physician for early recognition and isolation of organism with subsequent management. In general,

patient and pathogenic factor determine the transmission of nosocomial infection. Patient factor incorporates the exposure probability and susceptibility of patient to particular organism.

Similarly pathogenic factor depends on the amount and virulence of the infecting organism [2]. Presence of pathogen with decreased immune status with primary illness makes the patient susceptible to the opportunistic organism. Postoperative nosocomial infection depends on multiple factors such as, wound type; nature, duration of surgery, and duration of hospital stay [3]. All of these primary and secondary factors interplay and increases the chance of acquiring antibiotic-resistant hospital pathogens, like methicillin-resistant *S. aureus* (MRSA). In addition to methicillin, *S. aureus* is also shows resistant to many other antibiotics [4]. Contaminations through infected patients are the primary source of nosocomial infection; however asymptomatic carriers (medical staff, for example) may also transmit infection. Nosocomial infections due to *S. aureus* are very common and can easily be acquired. Around 10% to 15% of healthy people have *S. aureus* in their anterior nares and on some

skin sites that can spread to others when there are no aseptic precautions. Most nosocomial bacterial pathogens are resistant to a number of antibiotics and for the same reason; MRSA infections are increasing in frequency [1]. Present study deals with antibiotic susceptibility of different pathogens isolated from various samples in hospital setting. The study centers around infections caused by *S. Aureus*, in particular MRSA and their antibiotic susceptibility profile.

Staphylococcus aureus and MRSA

S. aureus is gram positive cocci that occur in cluster, like grapes. It contains enzymes such as catalase and coagulase [4]. Catalase enzyme converts hydrogen peroxide to water and molecular oxygen [5]. Different species of *S. aureus* are identified on the basis of the enzyme coagulase, which binds plasma fibrinogen, causing the organism to agglutinate or plasma to clot [2].

S. aureus grows well on blood agar and chocolate agar, producing complete hemolytic appearance on media containing red blood cells [6]. It has a distinctive ability to grow on high salt concentration, making mannitol salt agar (MSA) a differential medium to differentiate other species of *Staphylococci* from MRSA [7]. *Staphylococci* are pyogenic cocci, capable of causing multiple diseases in humans. This can range from simple skin infections to wound infections, abscesses, pneumonia, urinary tract infections, or more deeper infections like septic arthritis, osteomyelitis or even invade blood stream to cause septicemia [8].

Mechanism of antibiotic resistance

MRSA can synthesize an additional membrane protein called penicillin-binding proteins (PBPs), capable of binding to penicillin and are responsible for the final stages of cross-linking of the bacterial cell wall structure. This additional PBP have lower affinity for beta lactams and is therefore able to continue cell wall synthesis when other PBPs are inhibited [3].

In addition they contain *mec A* gene, which codes for the additional PBP is present on the chromosome in all cells of a resistant population [3,9]. *S. aureus* is often problematic in the hospital environment as it is carried by patients, hospital staff members, and visitors as well. Such infections are difficult to treat because bacteria in the hospital environment are exposed to so many antibiotics that they quickly become resistant to them [8].

MRSA in health care setting

MRSA has a very high morbidity and mortality associated with it, most of it relating to emerging antibiotic resistivity. MRSA has a MRSA difficult course and possesses a distinctive challenge for management globally. MRSA forms a major proportion of all hospital-acquired infection (HAI) [10].

MRSA is fairly common in developing countries with significant financial health expenditure [11-13]. Nevertheless it is also a challenge in western world with increasing incidence [14,15]. The carrier rate of MRSA in health workers is another problem to tackle with [16]. Nurses and nurse aide have the highest risk and potential to carry MRSA in the health care setting [17].

MRSA has variable antibiotic susceptibility. MRSA was highly resistant to ciprofloxacin, erythromycin, tetracycline and gentamycin, aminoglycosides, trimethoprim, fusidic acid, chloramphenicol, rifampicin, murpirocin, cadium acetate, mercuric chloride, propamidine isethionate and ethidium bromide but susceptible to vancomycin as elicited by using bacteriophage typing, pulsed-field electrophoresis and antimicrobial susceptibility tests [18]. Genetic studies explained that they carried different levels of plasmid-borne resistance to the antibiotics tested [19]. More than 50% of MRSA has multidrug resistance [20].

Objective

The objective of the present study is to find out the antimicrobial susceptibility pattern of MRSA strains against different antibiotics after isolation and detection of *S. aureus* and MRSA from the collected specimens of surgical patient at Misurata Central Hospital.

Materials and Methods

Samples

Two hundred and seventy four (274) sample swabs were collected from surgical wards at Misurata central hospital during the period from May, 2009 to October, 2009. 135 (45 males, 90 females) samples from pre-operative patients nasal swabs, 74 (27 males, 47 females) samples from post-operative wound pus and exudates, 38 (15 males, 23 females) samples from the anterior nares of medical staff in surgical wards and operation theatre and 27 swabs from different equipments and sites in operation theatre in the surgical wards were taken for study.

Identification and inoculation

All the swabs were labeled properly and cultivated on mannitol salt agar (MSA) (using a sterile loop to confirm the colonies belonging to *Staphylococci*) and on MacConkey agar to support the growth of other bacteria. These samples were incubated aerobically at 37° C for 24 hours. After incubation, colonies were observed and then further identification studies were made by Gram's stain. Grams stain detects the presence of gram-positive cocci in clusters. Gram stain was followed by biochemical test: catalase and coagulase test. Specific biochemical tests for the presumptive identification of *Staph.* was followed by subsequent culture and antibiotic sensitivity.

Culture and sensitivity

Oxacillin susceptibility test: Antibiotic susceptibility test was performed according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) using Muller-Hinton agar. All cultures grown on MSA were swabbed in properly labeled Mueller- Hinton agar plates (4% Sodium Chloride). Oxacillin sensitivity test was done with oxacillin (1µg) impregnated disc and incubated at 35° C for 24 hours. After incubation the sensitivity results were observed and recorded.

Antibiotic sensitivity test (Kirby-Bauer method): Kirby-Bauer method is a standardized filter-paper disc-diffusion agar procedure performed for MRSA isolates by placing filter-paper discs of uniform size impregnated with specified concentrations of different antibiotics on the agar plate that contains the organism to be tested.

The antibiotics selected were: ampicillin 10µg, amoxicillin 25µg, cefuroxime 30µg, cephalexin 30µg, erythromycin 15µg, clindamycin 2µg, chloramphenicol 30µg, cotrimaxazole 30µg and vancomycin 30µg [Oxoid Company, UK]. Each culture grown on MSA was swabbed in properly labeled Mueller-Hinton agar plates. These antibiotic discs were placed on the Mueller-Hinton agar plates (five antibiotics/ plate) using sterile forceps and the discs were gently pressed to make sure that the discs attach to the surface of the agar. All the plates were incubated at 35° C for 24 hours.

Results

247 swabs samples from pre-operative patient, surgical staff members and post-operative patients, and 27 samples from operative theater equipment's were analyzed. Irregular raised colonies observed in MacConkey agar were considered as a contaminant. Yellow colored colonies that have a positive reaction to the catalase and coagulase tests were identified as gram positive cocci, consistent with *S. aureus*.

These isolates of *S. aureus* were further confirmed as methicillin resistant *S. aureus* (MRSA) by showing resistance to the antibiotic oxacillin 1µg, [susceptible >13 mm] (Figure 1). The resistant samples were identified and labeled as methicillin resistance *S. aureus* (MRSA). Coagulase positive *S. aureus* were isolated from 104 (37.95%) samples. 9 (3.28%) MRSA (2-preoperative patients nasal swabs, 2-surgery medical staff nasal swabs and 5-postoperative patients infected wound swabs), 91 (33.21%) MSSA (51-preoperative patients nasal swabs, 5-surgery medical staff nasal swabs and 35-postoperative patients infected wound swabs), 50 (18.24%) coagulase negative Staphylococcus sp. (24-preoperative patients' nasal swabs, 11-surgery medical staff nasal swabs and 15-postoperative patients infected wound swabs), 84 (30.65) other bacterial species (45-preoperative patients' nasal swabs, 20-surgery medical staff nasal swabs and 19-postoperative patients

infected wound swabs), and 13 (4.74%) sterile samples (all from preoperative patient's nasal swabs) were isolated following standard technique (Figure 2).

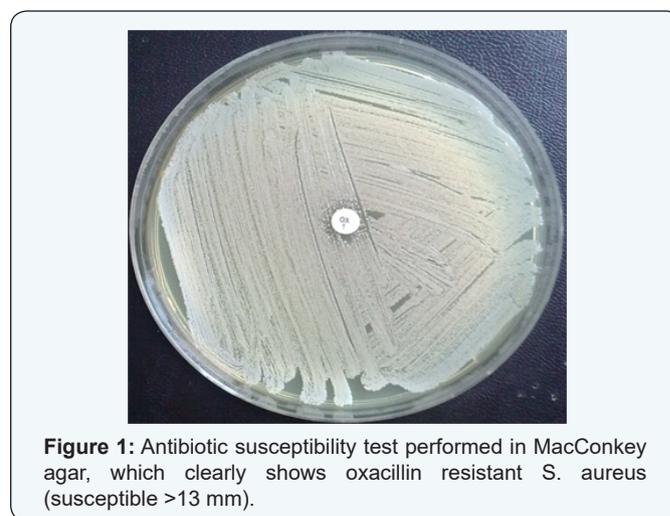


Figure 1: Antibiotic susceptibility test performed in MacConkey agar, which clearly shows oxacillin resistant *S. aureus* (susceptible >13 mm).

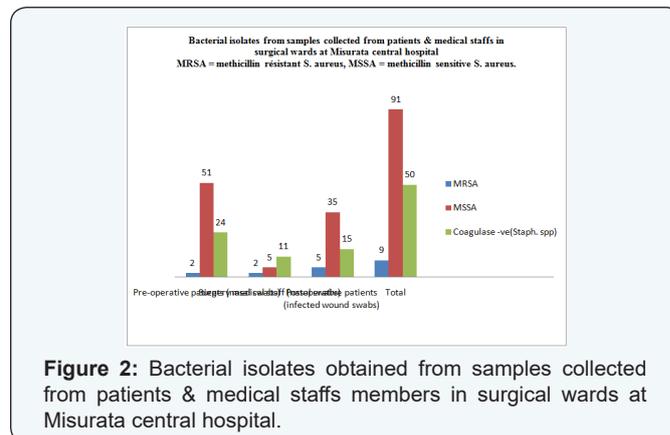


Figure 2: Bacterial isolates obtained from samples collected from patients & medical staff members in surgical wards at Misurata central hospital.

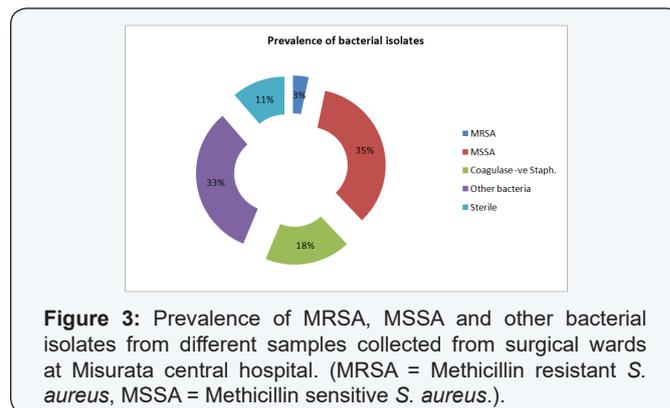


Figure 3: Prevalence of MRSA, MSSA and other bacterial isolates from different samples collected from surgical wards at Misurata central hospital. (MRSA = Methicillin resistant *S. aureus*, MSSA = Methicillin sensitive *S. aureus*).

Similarly, among 27 samples collected from operative theater and utilities, MRSA were isolated from none. But there were 4 (1.45%) samples with MSSA growth, 5 (1.82%) samples with other bacteria isolates and 18 (6.56%) sterile samples without any visible growth. Prevalence of MRSA in our hospital was found to be 3.28% (Figure 3). The findings coincide with the study

done by Koichi et al. [21]. Computerized processing method were utilized to compare and analyzed the data obtained from this study in the preoperative patients, postoperative patients and hospital staff members who carried MRSA (Chi-Sq= 4.579; DF= 1; P-Value= 0.026).Acquiring MRSA in our hospital setting was related to hospital workers (P-Value= 0.026) who had been a daily contact with the patients undergoing surgical operations in the operative theatre, in the surgical intensive unit or in the dressing rooms.

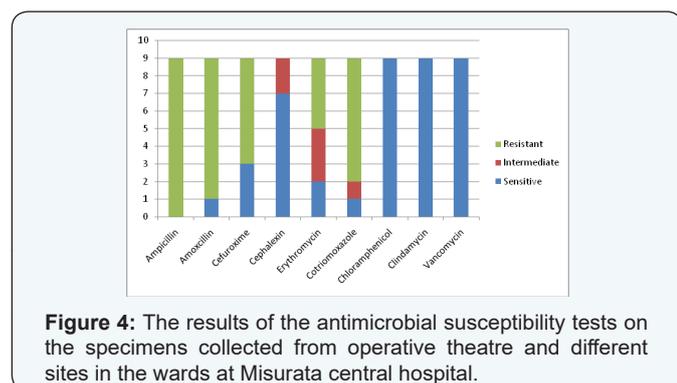


Figure 4: The results of the antimicrobial susceptibility tests on the specimens collected from operative theatre and different sites in the wards at Misurata central hospital.

Discussion

Nosocomial infections have been increasing over the past two to three decades, despite the necessary steps to curb it. As such nosocomial infection has threatened the global quality health care with surplus of health expenditure [26]. Patients own endogenous microbial flora is responsible for majority of nosocomial infections in many cases [27]. Wounds following intra-abdominal surgery can be infected with *Staphylococci* from skin or gram negative rod bacteria from the bowels developing nosocomial infections following cross-colonization with organisms, usually with contact with the hospital environment, and health care workers [28]. Accurate epidemiological typing is of necessary steps to identify MRSA strains and their antibiotic profile. Specific molecular typing methods like southern hybridization with DNA probes and multiplex PCR has been an accurate and rapid method of detection of MRSA strains directly from clinical samples [29].

Surveillance culture can be employed as an important procedure for control and spread of pathogen at hospital setting. But some of the study has doubted this fact and concluded that MRSA rapid screening strategy did not reduce nosocomial MRSA infection in surgical department with endemic MRSA prevalence and is not useful unless applied to high-risk groups [30]. Person to person spread of this pathogen in the health care setting can occur via direct contact aided by droplet, airborne and fecal-oral routes. Blood borne transmission is relatively uncommon. There has been some progress in MRSA control owing to strict aseptic techniques and infection control programs in the hospital setting.

These aseptic techniques include regular hand washing of nursing staffs and doctors after any procedure in order to control the spread of MRSA infections, sterilization of instruments and disinfection of the environments (floors, patient’s beds, etc). Infection control includes and application of chlorhexidine or neomycin cream into nasals of nursing staff and doctors who are MRSA carriers [8]. Precaution and aseptic skin preparation preoperatively by using topical antiseptics while dressing will definitely minimize the number of such pathogen.

Education programs are needed about the multidrug resistance strains of MRSA and to detect and isolate the carriers. A systematic selective screening of patients with high risk of carriage will provide a good applicable barrier and reduce the risk of MRSA transmission. Indeed more attention should be given in every hospital to have clear policy for prevention, management and therapy for MRSA. Hence, it is important that antimicrobial policy should also promote the logical use of antibiotics.

This can be achieved by providing health care education about nosocomial infection to patients and hospital staffs, formulating effective policies and implementing them efficiently (Figure 5).

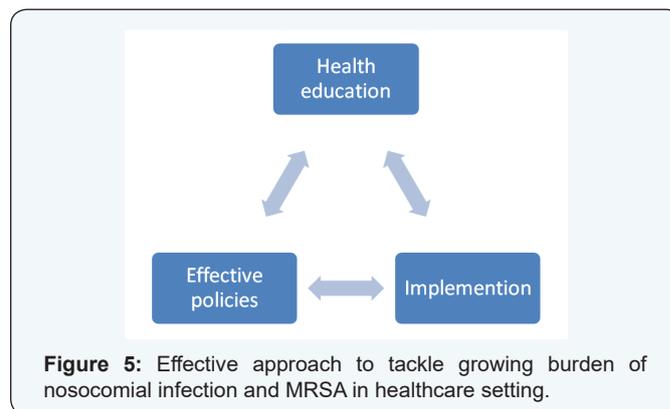


Figure 5: Effective approach to tackle growing burden of nosocomial infection and MRSA in healthcare setting.

Conclusion

Surgical wound infections are common cause of nosocomial infection; second to urinary tract infection in many countries and hospitals. MRSA has significant percentage among other microorganisms causing nosocomial infections because of their high resistance to many antibiotics, which may lead to economic burdens, in addition to social and psychological factors affecting the patients, due to long duration in hospitals.

MRSA strains are usually resistant to multiple antibiotics and it is necessary to study their antibiotic susceptibility and precaution should be taken to stop of antibiotic resistance strains. Hence knowledge of the prevalence of MRSA and their antimicrobial profile is necessary in the effective practical approach of this problem.

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