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Prevalence and Antimicrobial Sensitivity Pattern of *MRSA* Isolated from Infection Wound, Catheters Swabs and Sewage Water Samples

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Abstract

Nosocomial infection is a major problem in the world today. Methicillin- resistant *Staphylococcus aureus* (MRSA) strains, usually resistant to several antibiotics and also intrinsic resistance to ß- lactam antibiotics, shows a particular ability to spread in hospitals and now present in most of the countries. The present study was carried out to investigate the prevalence of MRSA and their rate of resistance to different anti staphylococcal antibiotics. A total of 100 health care associated (HA) sources such as wound, catheters swabs and sewage water samples were screened for MRSA and their antibiotic resistance pattern was performed. Out of 41 isolated strain of *S.aureus,* 34% were found to be methicillin resistant. There was high prevalence of MRSA in wound (44%), catheter samples (28%) and (22%) in sewage water sample. Almost all MRSA strains were resistant to penicillin followed by cloxacillin and cephalexin, co-trimoxazole. About 60-70% MRSA strains were resistant to erythromycin, ceftazidime, lincomycin, cephalexin, erythromycin and tetracycline. The determination of prevalence and antibiotic sensitivity pattern of MRSA will help the treating clinicians for first line treatment in referral hospitals.

Keywords: MRSA, Wounds, Catheter's swab, Sewage samples, Antibiotic resistance.

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Introduction

The genus *Staphylococcus* includes pathogenic organisms in which *Staphylococcus aureus* is most important.[1] *Staphylococcus aureus*is a versatile, adaptable, ubiquitous bacterium that adjusts to different environmental challenges (including antimicrobial agents) to ensure its survival.[2] *Staphylococcus aureus* is responsible for a broad range of clinical infections, most notable of which are cases of bacteremia, septicemia, endocarditis etc. *Staphylococcus aureus* is found almost everywhere, particularly on the skin and mucous membranes of animals, including humans. *S. aureus* has the ability to colonize humans without causing symptoms until the immune system is unable to control bacterial growth.[3] It is estimated that about 60% of the human population are colonized by *S. aureus* from time to time and as high as 20% of humans are persistent carriers **(**Bob Friendship and Scott Weese, University of Guelph, Guelph, ON N1G 2W1). It has overcome most of the therapeutic agents that have been developed in the recent years and hence the antimicrobial chemotherapy for this species has always been empirical.[4] The most notable example of this phenomenon was the emergence of methicillin resistant *Staphylococcus aureus*(MRSA) which was reported just 1 year after the launch of methicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) were first reported in 1961, and the first hospital outbreak of MRSA was reported in 1963. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant bacterium becoming prevalent throughout the world responsible for several difficult-to-treat infections in humans. It may also be called multidrug-resistant *Staphylococcus aureus*. Since its first identification, methicillin-resistant *Staphylococcus aureus* (MRSA) has established itself as one of the most challenging nosocomial pathogens and is wide spread in the community. In the community it establishes itself by skin to skin contact such as in sports persons, prisoners, boarding schools, sharing the same towels and razors etc. In the media, MRSA is often described as "super bug.[5] Over subsequent years, MRSA has evolved to become resistant to the entire class of penicillin-like antibiotics called beta-lactams: penicillin, amoxicillin, oxacillin, methicillin, and others. Penicillin-resistant strains of *S. aureus* appeared as early as the 1940s, but for many years these remained susceptible to ß-lactamase-stable penicillins.[6] Then, in the mid-1980s, S. aureus strains emerged that were resistant to the ß-lactamase-stable penicillins. These strains were termed "methicillin resistant *S. aureus*" (MRSA), Even though the drug methicillin is no longer the agent of choice for treatment, the acronym MRSA continues to be used. Later use of oxacillin as an alternative to methicillin in susceptibility tests resulted in the term 'oxacillin-resistant *S. aureus*' (ORSA)**.** Currently around two million people acquire bacterial infection every year in U.S hospitals and about 90,000 people die from that infection. This number of death is seven times higher than it was a decade ago as a result of bacteria acquiring drug resistance. In terms of *Staphylococcus aureus,* there are around two billion who harbors the bacteria and about 53 million of them harbor drug resistant form. MRSA was responsible for an estimated 94,000 life threatening infections and 18,650 deaths in 2005. This current trend of antibiotic resistance continues, the prospect of MRSA becoming resistant to all antibiotics becomes more realistic,

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making MRSA an increasingly critical public health issue. The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to hospitals etc. are the possible predisposing factors of MRSA emergence.MRSA only leads to skin or soft tissue infections, but it also can develop into life-threatening infections. MRSA must be combated in hospitals to prevent prophylaxis and treatment for *S. aureus* infections from becoming ineffective. Moreover, since the appearance of strains that are less sensitive or insensitive to glycopeptides, there is a real danger of even greater developing resistance.[7]

Material and Methods

A total of 70 swabs from wounds, catheters and 30 sewage samples were collected over a period from January to May 2021 in Paonta sahib and processed for isolation of bacterial pathogens and MRSA. A total of 35 wound swab samples from postoperative wound from various orthopedics and surgical wards of Puran hospital and Civil hospital, Paonta sahib (H.P) were collected in sterile vials by using sterilized cotton bud dipped in saline water (0.85%). Before taking swab samples, both the hands were thoroughly washed with soap and disinfected with alcohol. The sterile cotton bud was rotated onto the overall surface area of the wound. The cotton bud after swabbing the wound was again kept in the respective sterile vials. A total of 35 catheter samples and 30 sewage samples from the sewage plant of both Puran and Civil hospital were collected.

These collected samples were immediately transported to the microbiology laboratory and inoculated on Mannitol salt agar plates (Hi-Media Laboratories, Mumbai). These plates were incubated at 37^{0}C for 24-48 hrs. Plates were observed for growth and Gram smear was performed from different type of colonies. Gram reaction, colony morphology, pigment formation, catalase, DNAse, coagulase, urease, and oxidase tests were performed and allocated to appropriate genera to the isolates. The golden yellow colored colonies of *S.aureus* on Mannitol salt agar were noted. Forthe identification of the MRSA among the isolates of *Staphylococcus aureus*, the Hi-Media (India) made HIMEDIA Hi Chrome MeReSa Agar Base (M1674) was used. The media was prepared by mixing 41.65 gm of the media into 500 ml of the distilled water. The medium is cooled to around 50-55^{0}C and MeReSa Selective Supplement (FD229) (reconstituted with 5 ml sterile distilled water into each Methicillin vials having 2.0 mg of Methicillin as per the direction of the supplier (Hi Media - India) was added and mixed very thoroughly. *Staphylococcus aureus* strains were streaked onto the Hi Chrome MeReSa agar and incubated at 35^{0}C for 24 hours. The MRSA only grew on this Hi Chrome Me Re Sa agar, while the MSSA was inhibited on the same agar plate. All cultures showing bright blue colored growth were taken as MRSA positive strains, while all others are recorded as MSSA strains.

All the confirmed *S.aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method (1996) using oxacillin discs (1µg) obtained from Hi- Media Laboratories Pvt.Ltd, Mumbai (India). The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less. Further, the antibiotic susceptibility pattern of methicillin resistant *S.aureus* was determined on the day of their isolation by the Kirby-Bauer disc diffusion method 1996 on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials. Finally the data were recorded and analyzed at the completion of the study as per recommendations of the NCCLS (2000).

Results and Discussions

MRSA is a major nosocomial pathogen causing significant morbidity and mortality.[8] The important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage is the predominant mode for patient to patient transmission. In India, the significance of MRSA has been recognized relatively late and epidemic strains of these MRSA strains are usually resistant to several antibiotics. During the last 15 years, the appearance and world wide spread of many such clones have caused major therapeutic problems in many hospitals, as well as diversion of considerable resources to attempts at controlling their spread[9]. In this study, the prevalence and the antibiotic susceptibility pattern of various MRSA isolates obtained from wound, catheters and sewage samples were determined. No. of swabs, isolation of the *S.aureus* and the prevalence rate of MRSA is shown in Table 1 and Figure 1 showing the prevalence of MRSA in wound catheter and sewage samples**.**Swab sample collected from the wound (35), catheters swabs (35), sewage water sample (30) were screened for *S.aureus* employing conventional microbiological methods. A total of 41 *S.aureus* strainswere isolated. Out of these, 14 (34%) were found to be MRSA.

The study showed high prevalence of MRSA in wound samples (44%) then the catheters (28%) and sewage samples (22%).

Thus, the risk of infection is high in individuals occupationally exposed to wounds or wound dressing indicating a need to screen individuals in hospitals for risk exposures and infections, to avoid outbreak and cross infections.[10] The present study indicated high rate of isolation of MRSA (44%) from wound samples but Mehta *et al*., (1998) had reported isolation rate of 33% and Qureshi *et al*., (2004)from Pakistan reported a high isolation rate, up to 83% MRSA from wound swabs.

The drug resistance pattern of MRSA isolates from these sources was found to be highly variable. *Staphylococcus aureus* showing resistance to methicillin is shown in Figure 2 More than

Table 1: No. of swabs, isolation of the *S.aureus* and the prevalence rate of *MRSA*

Type of Sample	*Wound*	*Catheter*	*Sewage water*	*Total*
No. of swabs	35	35	30	100
S.aureus	18(51%)	14(40%)	9(30%)	41(41%)
MRSA	8(44%)	4(28%)	2(22%)	14(34%)

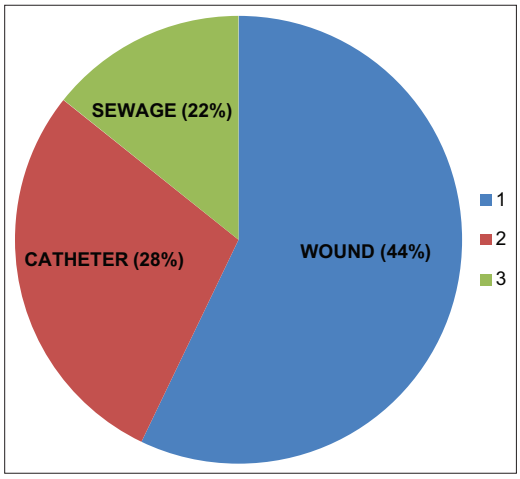

Figure 1: Prevalence of MRSA in wound catheter and sewage samples

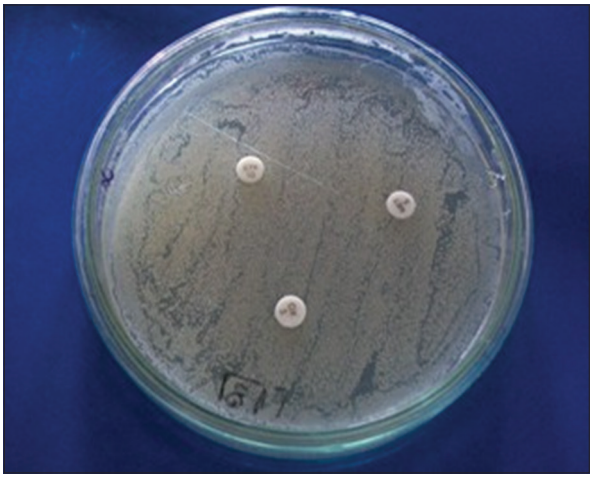

Figure 2: *Staphylococcus aureus* showing resistance to methicillin

70% of the MRSA were found to be resistant to the majority of the antibiotics tested.

In this study, penicillin resistance was observed to be 100% against the organism. This corroborates with the finding of Anupurba *et al*. The resistance for Ceftazidime and Cephotaxime were 72.45% and 62.24% respectively (β-lactam resistance). Resistance to quinolones like Ciprofloxacin and Ofloxacin were 67.35% and 62.24% respectively. This is probably due to the indiscriminate and empirical use of these drugs. Further, quinolones are relatively cheaper and easily available as over the counter drugs in India. Findings of these resistances are relatively less than those of discussed in the paper.[11,12]

Conclusion

The regular surveillance of hospital-associated infections including monitoring of antimicrobial susceptibility pattern of MRSA and formulation of a definite antimicrobial policy may be helpful for reducing the incidence of these infections. Infected or colonized patients may be isolated in a single room or isolation unit to prevent the spread of MRSA. Knowledge about MRSA and carrier status needs to be raised among the health staff of the hospital and control measures need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment. The study showed a high prevalence of MRSA in wound samples, so care must be taken while wound dressing and the used off catheters must be disposed off properly. The hospital sewage plant should be routinely monitored and cleaned, so as to function properly and surveillance of cultures and monitoring of the susceptibility patterns of MRSA may also help in arresting the spread of the infections in this part of India.

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