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CASE STUDY

STUDY OF MUPIROCIN RESISTANCE IN CLINICAL ISOLATES OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS CAUSING WOUND INFECTION IN A TERTIARY CARE RURAL HOSPITAL

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ABSTRACT

MRSAis one of the commonest causes of wound infections and is significant pathogen because of its multi-drug resistance nature. Mupirocin is an effective topical antibacterial agent that is used for the management of skin infections. There are two mupirocin resistance phenotypes; low level and high level. Mupirocin-resistant MRSA has also been associated with an increase in in-hospital mortality, compared to the level associated with mupirocin-susceptible MRSA. So the present study was undertaken with the aim to know the prevalence of the low-level and the high-level mupirocin resistances among MRSA causing Wound Infection

Materials & Methods: A total of 65 non-duplicate MRSA isolates were then tested for mupirocin resistance by the concomitant use of 5µg and 200µg mupirocin discs.

Discussion: Among 65 MRSA isolates, mupirocin resistance was found in 9 (13.85%) isolates. In our study, of 65 MRSA isolates, High-level Mupirocin resistance was observed in 2 (3.08%) and Low-level resistance in 7 (10.77%). The 7 low-level mupirocin resistance isolates were from Skin 1(1.54%); Medicine 2 (3.08%) and maximum were from obgy 4 (6.15%). Two High-level mupirocin resistances were from NICU (1.54%) & Medicine (1.54%). There is a strong association between previous mupirocin exposure and both low-level and high-level mupirocin resistance.

Conclusion: The prevalence of High-level & low-level mupirocin resistances among MRSA in the patients with wound infections is a matter of concern. High-level mupirocin resistance (mupA carriage) is also linked to MDR. Hence, we conclude that all MRSA isolates from wounds should be routinely screened for mupirocin resistance.

INTRODUCTION

Wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues. When bacteria breach this barrier, infection can result. Most wound infections can be classified into two major categories: skin and soft tissue infections, although they often overlap as a consequence of disease progression.[1]Wound infections can be caused by different groups of microorganisms, most commonly isolated aerobic microorganisms includes S.aureus, CoNS, Enterococci, E.coli, P.aeurginosa, Klebsiellapneumoniae, Enterobacter, Pr.mirabilis, Streptococci, Candida, Acinetobacter.[2] The overall incidence of wound sepsis in India is from 10-33 %. [3]Among the Gram-positive pathogens, S. aureus continues to cause skin and soft tissue infections (SSTI) in the community as well as invasive infections in the hospitalized patients and is a frequent cause of bacterial infections in both developed and developing countries.[4,5] It is a highly versatile and adaptable pathogen, causing a range of infections of varying severity affecting the skin, soft tissue, respiratory system, bone, joints and endovascular tissues.[4] The soft-tissue infections are common, they are generally of mild to modest severity, and they are easily treated with a variety of agents. Mupirocin derived from Pseudomonas fluorescens is a topical antibiotic that has been extensively used for treating MRSA associated skin and soft-tissue infections, decreasing certain types of surgical site infections and eliminating nasal colonization of MRSA among patients and medical staff.[6] Mupirocin(pseudomonic acid A) acts by binding specifically to the bacterial isoleucyl- tRNAsynthetase enzyme and inhibits its protein synthesis. Mupirocin was first introduced in the UK in, within two years after its introduction, mupirocin resistance among MRSA isolates emerged worldwide. [8, 6] Although no performance standards or interpretive criteria have been published for mupirocin susceptibility testing, two mupirocin

resistance phenotypes namely low level (MuL) and high level (MuH) mupirocin resistance are defined in Staphylococci. Low-level resistance (MICs, 8 to 256 µg/ml) is usually associated with point mutations in the chromosomally encoded **ileS**gene which is typically stable and nontransferrable. As mupirocin is frequently delivered directly to the site of infection as a topical agent, low level resistance is typically not clinically relevant. Whereas high-level resistance (MICs, ≥512 µg/ml) is generally due to a plasmid-mediated gene, **mupA**(also referred to as **ileS2***)*, which encodes an additional modified isoleucyl-tRNAsynthetase. [9] and is typically located on mobile genetic elements, which likely facilitates the dissemination of this resistance mechanism. The plasmid carrying the *mupA*gene may also carry resistance determinants to other systemic antimicrobial agents, raising concern that mupirocin use could select not only for mupirocin resistance, but also for increasing antimicrobial resistance overall. The mupAgene is typically plasmid mediated and some of these plasmids are conjugative**.** MupB is a new high level mupirocin resistance mechanism in Staphylococcus aureus.[10] Mupirocin-resistant MRSA has also been associated with an increase in in-hospital mortality, compared to the level associated with mupirocin-susceptible MRSA. [11]

Detection and differentiation of both types has important clinical implications. The presence of high-level mupirocin resistance (MuH) excludes its clinical use, however low-level mupirocin resistance (MuL) can be overcome by recommending higher than usual dosage. In a study by Jacob S. Hogue, prevalence of mupirocin resistance among MRSA isolates has cited mostly in hospitalized adult and elderly patients ranging from 0 to 65% of isolates. They also mentioned rates of mupirocin resistance correlate with increased use in closed inpatient settings. [12]Mupirocin resistances have been reported widely in various countries, so the present study was undertaken with the aim to the prevalence's of the low-level and the high-level mupirocin resistances among Methicillin resistant Staphylococcus aureus causing Wound Infection

MATERIALS AND METHODS

The prospective study was carried out in the department of Microbiology from the period of July 2014 to December 2015.Wound aspirates and Pus sample were collected from Orthopedics, ICU, medicine, pediatric, Surgery and Gynecology. The swabs were immediately placed in test tubes for further processing in the laboratory. All the samples were subjected to gram staining, culturing on blood agar (BA) for 24 h at 37°C. Identification of Staphylococcus aureus was done by standard biochemical techniques.[13]All the confirmed Staphylococcus aureus isolates were subsequently tested for oxacillin resistance using cefoxitin disc (30µg).The Isolates were considered methicillin-resistant if the zone of inhibition was 21mm or less.[14]A total of 65 non-duplicate MRSA isolates were then tested for mupirocin resistance by the concomitant use of 5µg and 200µg mupirocin discs to determine low and high level resistance respectively. [15] Criteria of zone diameter breakpoints for susceptible and resistant isolates were set at > 14mm and < 13mm respectively**.** 3 different phenotypes are:[15]

- Mupirocin susceptible: A zone diameter of greater than or equal to 14 mm for both 5 and 200 µg discs
- Low-level resistance (MuL): Isolates that showed zone diameters less than 14 mm in the 5 µg disc but more than or equal to 14 mm in the 200 µg disc.
- High-level resistance (MuH): Isolates with zone diameters less than 14 mm for both 5 and 200 µg

Discs were procured from Hi-media Laboratories, Mumbai, India&Mast group, UK.

DISCUSSION

Wound of the patients have the potential to be colonized and infected more readily than other patients due to deprivation of mechanical barrier provided by the skin and mucous membrane as well as the depression of immunological response. Pathogens infecting wound can be part of normal flora or it can be acquired from the hospital environment or other infected patients. Staphylococcus aureus, being the normal microbial flora of the skin, is one of the commonest causes of wound infections. Methicillin resistant Staphylococcus aureus (MRSA) is emerging as a common human pathogen causing several nosocomial infections and is generally recognized as the most significant pathogen because of the burden of serious diseases it causes and its multi-drug resistance nature. [16]

Several Studies have reported the prevalence of MRSA in wound to the tune of 29.0% to 45%.[17-20] Sangeeta Joshi *et al*, INSAR observed the prevalence of MRSA isolated from skin and soft tissue infections (36% in 2008 and 40% in 2009)[21] Infectious Diseases Society of America2005 guidelines infections recommend mupirocin as the first line agent on the diagnosis and management of skin and soft tissue.Mupirocin is an effective topical antibacterial agent that is used for the management of skin infections and for the colonization with MRSA in both patients and health care workers. As per the surveillance program reports[22]of 1995 to 1999, the proportions of the MRSA strains with high and low-level mupirocin resistances were 1.6% and 6.4%, respectively, whereas as per those of 2000 to 2004, the resistant rates were 7.0% and 10% respectively, which showed that there was a considerable increase in the percentage of the resistance upon the usage of mupirocin.

In our study, of 65 MRSA isolates, High-level Mupirocin resistance was observed in 2 (3.08%) and Low-level resistance in 7 (10.77%). This is comparable to reports in the literature of 1-13% for low-level and 2.4-14% for high-level resistance. [6] Our study is similar to the finding of Jayakumar S *et al* who reported 2.2% High level mupirocin resistance among the 46 MRSA isolates & Hee-Jeong Yun *et al* who reported 4.7% High level mupirocin resistance.[18, 23] In our study, about 7 isolates (10.77%) showed Low-level mupirocin resistance, this is in contrast to the findings of Hee-Jeong Yun *et al*, Jayakumar S *et al* who did not found any Low-level mupirocin resistance MRSA isolates.[23,18] In a study by Nizamuddin S *et al*, among 156 MRSA isolates, one (0.7%) showed a low-level resistance and none of the isolates showed high level mupirocin resistances.[24] whereas Singh A *et al* in their study found MuH (1.4%) and none of their isolates showed low level resistance. [25] In our study among 65 MRSA isolates, mupirocin resistance were found in 9 (13.85%) isolates . similar were the findings of ParulChaturvedi *et al* 18.3% [26] The reason for higher prevalence of mupirocin resistance may be an increased use of mupirocin ointment for skin and soft tissue infections.

Some workers have reported Mupirocin resistance as high as 81%.[27]

Prolonged, widespread or uncontrolled use and multiple courses of mupirocin are all associated with the development of mupirocin resistance. [8]Another reason could be increase use of mupirocin in the preoperative eradication of the *Staphylococcus aureus* colonization as a strategy for preventing postsurgical infections. [28] Various studies suggest that during mupirocin prophylaxis transfer of mupAgene from normal commensal flora of skin such as Staphylococcus epidermidisto MRSA is responsible for emergence of mupirocin resistance.[29] In a study carried out on 267 clinical isolates, from pus High-level mupirocin resistances and low-level mupirocin resistances were reported to the tune of 2.99% (8 isolates) & 7.87% (21 isolates)[30] The 7 low-level mupirocin resistance isolates were from Skin 1(1.54%); Medicine 2 (3.08%) and maximum were from obgy 4 (6.15%).

Two High-level mupirocin resistances were from NICU (1.54%) & Medicine (1.54%). There is a strong association between previous mupirocin exposure and both low-level and high-level mupirocin resistance. The "gold standard" method for detection of mupirocin resistance is MIC determination by the agar dilution method. In our study we used disc diffusion method for detection of low and high level mupirocin resistance. The sensitivity and specificity of this method has already been evaluated by Malaviolle *et al* previously.[31] This makes the disc diffusion susceptibility test a cheaper and simple alternative method for its routine use. Genotypic method like pulsed-field gel electophoresis, is used to know the specific MRSA clones as Mupirocin resistance differs among specific clones. But the lack of a confirmatory test is the weaknesses of our study. The limitation of our study was no repeat MRSA isolates from the same patient was included in the study. Another limitation is the lack of data regarding outpatient use of mupirocin or routine practice of our institution to screen patients for MRSA preoperatively or to attempt to decolonize patients known to be MRSA carriers.

CONCLUSION

The prevalence of High-level & low-level mupirocin resistances among MRSA in the patients with wound infections is a matter of concern. High-level mupirocin resistance (mupA carriage) is also linked to MDR. Hence, we conclude that all MRSA isolates from wounds should be routinely screened for mupirocin resistance; and so, one must make judicious use of mupirocin.

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