Emerging Antimicrobial Resistance in Staphylococcus and Spectrum of Oral Oxazolidinone Linezolid in Multidrug Resistant Staphylococcus in a Tertiary Care Hospital

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ABSTRACT

Introduction: *Staphylococcus* species is an asymptomatic colonizer which has exhibited increasing resistance to various antimicrobial agents in the recent times. Although, the organism was naturally susceptible to all the antimicrobial agents, it has acquired multi drug resistance via various mechanisms. In view of rising prevalence antimicrobial resistance, this study was undertaken to study the prevalence of methicillin resistance and the emergence of linezolid resistance amongst the Staphylococcal isolates obtained from various clinical samples in a tertiary care hospital.

Material and Methods: This study was conducted from July 2017 to December 2018 in a tertiary care hospital. Clinical specimens were processed, and Staphylococcal isolates were identified using standard microbiological techniques. Antimicrobial resistance pattern of all the Staphylococcal isolates was determined and interpreted as per the latest CLSI guidelines.

Results: A total of 989 Staphylococcal isolates were obtained amongst which 498 (49.44%) were found to be methicillin resistant. Highest antimicrobial resistance was observed to beta lactam antibiotics followed by azithromycin and fluoroquinolones. 26 (2.628%) strains of Staphylococcal species were found to be resistant to linezolid while they retained sensitivity to Vancomycin.

Conclusion: The emergence of drug resistance in various microorganisms has been a cause of global concern. Rising trend of resistance has been observed to methicillin and linezolid especially in indoor patients of the Intensive care units. This study highlights the high prevalence of Methicillin resistance in both *Staphylococcus aureus* and Coagulase negative *Staphylococcus* species in a tertiary care hospital in Amritsar, India. Although, linezolid resistance is emerging at a slow pace, adequate measures must be undertaken to preserve the therapeutic armoury.

Keywords: Methicillin Resistant *Staphylococcus*, Linezolid Resistant *Staphylococcus*, Multiple Drug Resistance, Antimicrobial Resistance.

INTRODUCTION

Staphylococcus species is an asymptomatic colonizer which has exhibited increasing resistance to various antimicrobial agents in the recent times.[1] Although, the organism was naturally susceptible to all the antimicrobial agents, it has acquired multi drug resistance via various mechanisms including horizontal gene transfer, mutational drug resistance and antimicrobial selection pressure.[2] The mode of transmission can be by direct contact with a colonized or infected individual, although contact with contaminated objects and surfaces also plays a role.[3-6] In 1960, Methicillin-resistant *Staphylococcus species* was first observed, less than one year after the introduction of this second generation beta-lactam antibiotic into clinical practice.[7] Methicillin resistant *Staphylococcus aureus* (MRSA) is currently a major cause of health care and systemic infections.[8-9]

In view of rising prevalence of MRSA and Methicillin resistant coagulase negative *Staphylococcus*, FDA approved Linezolid as the first antibiotic for use from the oxazolidinone class which acts by inhibition of protein synthesis.[10] Currently, emergence of linezolid resistant *Staphylococcus* species has been reported worldwide and is a cause of major concern.[11] The main mechanism of resistance is via the mutations in the V domain of the 23S rRNA.[12] Another mechanism is the modification of ribosomal proteins L3 and L4 encoded by rplC and rplD genes, respectively.[13] The most frequently encountered method of resistance is G2576T point mutation.[14] The acquisition of 23S rRNA methyltransferase gene "cfr" can also provide resistance by modification of A2503 in domain V of the 23S rRNA.[15] This study was undertaken to study the prevalence of methicillin resistance and the emergence of linezolid resistance amongst the Staphylococcal isolates obtained from various clinical samples in a tertiary care hospital.

MATERIAL AND METHODS

This study was conducted from July 2017 to December 2018 in the department of Microbiology, Government Medical College, Amritsar. Clinical specimens received from the patients of various departments of a tertiary care

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Antimicrobial Agent	*Staphylococcus aureus* (n=735)	Coagulase negative Staphylococcal species (n=254)
Penicillin	73.8%(543)	72.44%(184)
Ampicillin	73.8% (543)	72.44%(184)
Amikacin	40.6%(294)	56.22%(143)
Gentamicin	41.49%(305)	54.33%(137)
Norfloxacin/ Ciprofloxacin	48.16%(354)	61.41%(156)
Cephalexin	47.75%(351)	54.33%(138)
Azithromycin (not used for urine samples)	69.99%(286)	63.63% (98)
Amoxicillin Clavulanate	47.75%(351)	54.33%(137)
Linezolid	1.36% (10)	6.299% (16)

Table-1: Antimicrobial resistance pattern of Staphylococcus aureus and Coagulase negative Staphylococcus.

Ward	Methicillin resistant Staphylococcal Isolates obtained. (n=489)
Medical ICU	148
Neonatal and Pediatric ICU	108
Orthopedics ward	66
Surgery ward	52
Gynecology ward	39
Medicine ward	29
Pediatrics ward	23
Others	24

Table-2: Wardwise distribution of Methicillin Resistant Staphylococcal Isolates.

Ward	Sample	Number of Isolates
Neonatal and Pediatric ICU	Blood	38.4% (10)
Medical ICU	Blood	23.07% (6)
Orthopedics	Pus	23.07% (6)
Surgery	Pus	15.38% (4)

Table-3: Wardwise distribution of linezolid resistant Staphylococcal isolates.

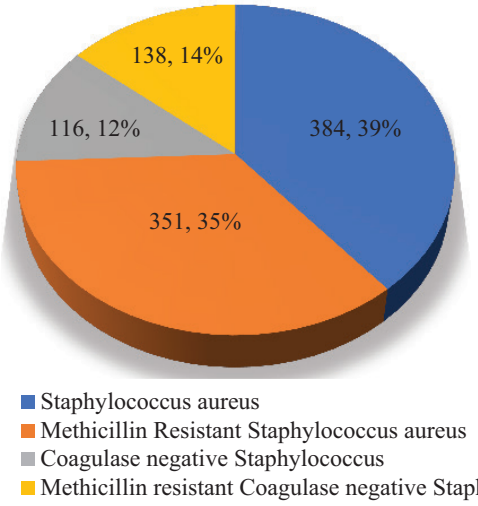

Figure-1: Methicillin resistance in Staphylococcus aureus and Coagulase negative Staphylococcus

hospital were processed and Staphylococcal isolates were identified using standard microbiological techniques.[16] The Antimicrobial Susceptibility pattern of all the Staphylococcal isolates was determined against various antimicrobial agents using Kirby- Bauer disc diffusion method and interpreted as per the latest CLSI guidelines.[17] The antimicrobial discs used were Penicillin, Ampicillin, Cephalexin, Ciprofloxacin/ Norfloxacin, Amikacin, Gentamicin, Azithromycin (not used in urine samples), Linezolid and Amoxicillin Clavulanate. Methicillin resistance was detected by using a disc of Cefoxitin as suggested by CLSI.[17] *Staphylococcus aureus* ATCC 25923 was used as the quality control strain. In the strains which were found to be resistant to linezolid on disc diffusion, MIC was performed by agar dilution for the confirmation of resistance to linezolid. E-test was used to check susceptibility to vancomycin in all the linezolid resistant strains.

RESULTS

A total of 989 non duplicate Staphylococcal isolates were identified. Out of 989 Staphylococcal isolates, 735 (74.31%) *Staphylococcus aureus* and 254(25.68%) coagulase negative *Staphylococcus* were obtained.

Among 735 *Staphylococcus aureus*, 351(47.75%) were found to be Methicillin resistant, while among 254 Coagulase negative *Staphylococcus, 13*8(54.33%) were found to be Methicillin resistant. (Figure-1)

On antimicrobial susceptibility testing, all the methicillin resistant strains were found to be resistant to penicillin and ampicillin. Maximum resistance was observed to beta lactam antibiotics (73.12%) followed by azithromycin (66.81%) and fluoroquinolones (54.785%). 26(2.628%) strains of *Staphylococcus* were found to be resistant to Linezolid. The resistance to linezolid was confirmed by agar dilution as suggested by CLSI. (17) All the isolates which were found to be resistant to linezolid were found to be sensitive to vancomycin on E-Test. (Table-1)

Amongst the 489 Methicillin resistant Staphylococcal isolates, 351(71.77%) were *Staphylococcus aureus* while 138 (28.22%) were Coagulase negative *Staphylococcus* species. It has been observed that Methicillin resistant strains were mostly isolated from indoor patients of Medical (30.26%) and pediatric (22.08%) Intensive care units, followed by Orthopedic wards (13.49%), Surgery wards (10.63%) and Gynecology wards (7.97%). (Table-2)

All the 26 strains of *Staphylococcus* which were found to be resistant to linezolid were obtained from indoor patients. 16 (64%) out of them were isolated from the blood samples obtained from the patients of medical and pediatric intensive care units. (Table-3)

DISCUSSION

The emergence of drug resistance in various microorganisms has been a cause of global concern. After noticing the resistance of *Staphylococcus* to penicillin in 1959, Methicillin was first utilized to treat infections caused by these resistant pathogens. [18] In a period of one year, the Methicillin resistant *Staphylococcus aureus* were reported in United Kingdom followed by other European countries, Japan, Australia and United States making their existence pandemic.[19] The mec A gene responsible for methicillin resistance codes for a methicillin-resistant penicillin-binding protein has been acquired from a distantly related species and is not present in susceptible strains.[18] The *mecA* gene is carried via the mobile element staphylococcal cassette chromosome *mec* (SCC*mec*).[19] The resistance to methicillin, shifted the therapeutic options to further higher antimicrobial agents such as linezolid and vancomycin.

Linezolid was the first oxazolidinone to be effectively used for the treatment of infections caused by various Gram-positive organisms such as multidrug resistant enterococci and methicillin-resistant *Staphylococus aureus*. It has shown remarkable results in various debilitating infections such as endocarditis, bacteraemia, osteomyelitis, joint infections and is even effective when other therapies have failed.[20] But, the last decade has witnessed the emergence of linezolid resistant Staphylococcus species.

In our study, we observed that among 989 isolates of *Staphylococcus*, 47.75%(351) isolates of *Staphylococcus aureus* and 54.33% (138) isolates of coagulase negative *Staphylococcus* were found to be methicillin resistant. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India has reported the prevalence of MRSA to be 42% in a multicentric study involving 17 tertiary care hospitals of India in 2008. [21] Various studies conducted over the past decade have deduced the prevalence of MRSA to be ranging from 30-85% in India. [22] Kock R et al conducted a review of 15 studies and deduced the prevalence of MRSA to be 13- 74% worldwide.[23] MRSA is a primary concern as it not only confers in vivo resistance to methicillin but also to various other widely used antimicrobial agents such as cephalexin and amoxycillin-clavulanate. [18]

In the current therapeutic arena, the emergence of MDR MRSA, which is defined as MRSA showing resistance to ≥3 antimicrobial agents, is furthermore worrisome. The prevalence of MDR MRSA has been reported to be ranging from 23%- 60% in various studies conducted in India.[24] In our study, we observed maximum resistance to Penicillin and ampicillin followed by azithromycin and fluoroquinolones. Least resistance was observed to linezolid while all the strains were sensitive to Vancomycin. This is in concordance with the observation of Bing Gu et al who also reported vancomycin sensitivity in linezolid resistant strains.[25]

Linezolid, an oral drug from the class oxazolidinone possesses a wide spectrum of in vivo activity against many bacteria including *Staphylococcus* species, *Enterococcus* species, *Neisseria gonorrhoeae, Neisseria meningitidis, Mycobacterium tuberculosis,* etc.[26] However, it is ineffective against gram negative bacilli due to their intrinsic resistance by the virtue of efflux pumps. Owing to its wide spectrum of activity, linezolid was approved by FDA for adult use and pediatric use in 2000 and 2005 respectively. It acts by binding with high affinity to catalytic site of 50S at ribosomal peptide-transferase site affecting the position of tRNA.[27] It has a unique target that is not affected by rRna methylases which block binding of macrolides, clindamycin and streptogramins. Linezolid further prevents the synthesis of Staphylococcal virulence factors such as coagulase, hemolysins and protein A.[28]

The past two decades have witnessed the emergence of linezolid resistant strains of *Staphylococcus* in the hospital settings, especially the intensive care units. In 2001, just one year after approval of linezolid by FDA in adults, LRSA was reported in US in a patient after receiving linezolid for a duration of 1 month to treat dialysis associated peritonitis.[29] This was shortly followed by emergence of resistance in North America, South America and Asia. The emergence of linezolid resistance in Coagulase negative *Staphylococcus* is 28 times that of *Staphylococcus aureus*, making it a global concern.[30] The emergence of linezolid resistance can be attributed to over the counter availability of oral formulation of linezolid leading to indiscriminate overuse of the drug. In our study, we obtained 26 (2.628%) linezolid resistant strains, all of them were from the indoor patients. 16 isolates were obtained from the blood samples of the patients from neonatal, pediatric and medical intensive care units. Critically ill patients of the intensive care unit are a high risk group because of low immune status, severe systemic infections, associated organ dysfunctions, underlying debilitating diseases and high prevalence of resistant organisms may aid in hospital acquired infections. Furthermore, invasive procedures make such patients even more susceptible to MDR organisms.

It was observed that linezolid resistant organisms were resistant to many other antimicrobial agents including beta lactam antibiotics, azithromycin, fluoroquinolones and aminoglycosides. All the 26 strains of *Staphylococcus* which were found to be resistant to linezolid on disc diffusion were confirmed by agar dilution as suggested by CLSI.[17] All these strains were subjected to vancomycin susceptibility testing by using vancomycin e-test. Therefore, vancomycin is a reserve drug in the current scenario of antimicrobial resistance and preserves its therapeutic effectivity even in Linezolid resistant Staphylococcal isolates.

CONCLUSION

This study highlights the high prevalence of Methicillin resistance in both *Staphylococcus aureus* and Coagulase negative *Staphylococcus* species in a tertiary care hospital in Amritsar, India. Linezolid serves as an effective treatment option in methicillin resistant *Staphylococcus* species; however, emergence of linezolid resistance is a major set back to the therapeutic armory. Therefore, susceptibility testing must be done prior to the prolonged use of linezolid therapy

for serious infections. Timely and accurate identification of resistant strains and using alternative drug options in such patients along with strict infection control measures, effective surveillance and hand hygiene are the need of the hour. Though the linezolid resistant Staphylococcal isolates remain susceptible to vancomycin, overuse of vancomycin should also be prevented in order to conserve its effectivity. Further studies are needed to explore more therapeutic options for multiple drug resistant linezolid resistant Staphylococcal strains.

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