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Original Research

A Cross section study to assess Antibiotic Resistance Pattern of Methicillin Resistant Staphylococcus aureus Isolates in a tertiary care center

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Abstract

Background: Staphylococcus aureus is associated with different infections ranging from skin and soft tissue infections to endocarditis and fatal pneumonia. *S. aureus* is still the most common bacterial species isolated from inpatient specimens and the second most common from outpatient specimens. Today, methicillin resistant *S. aureus* (MRSA) isolates are present in the hospitals of most countries and are often resistant to several antibiotics.

Aims & Objectives: To study the antibiotic resistance pattern of MRSA to commonly used and newer antibiotics.

Materials & Methods: A total of 140 isolates of methicillin resistant S. aureus were collected from various pus, blood, Catheter tips and urine. All isolates were identified at the species level by standard biochemical tests. Susceptibility to eight antibiotics was determined by disc diffusion method. Antibiotic disc were applied within 15 minutes of inoculation and the plates were incubated at 37^0C for 18-24 hours.Zone diameters were measured as per CLSI criteria.

Results: Among a total of 140strains were found to be MRSA isolates. All the isolates showed 100% resistant to Ampicillin, Erythromycin and Ciprofloxacin followed by Cotrimoxazole (62.1%),Clindamycin (53%) and Amikacin (10%). No strain showed resistant to Rifampicin, Vancomycin, Linezolid, Daptomycin, Tigecycline, Teicoplanin,Ceftaroline

Conclusion: The high isolation rates of MRSA from all the age groups of patients, attending all most all the departments of our hospital and their high resistance to commonly used antibiotics is a cause of concern

Keywords: *M*ethicillin resistant *S. aureus*

Introduction

Staphylococcus aureus is the most common cause of nosocomial infections and is one of the most common causes of bacteremia and is a bacterial pathogen that rapidly acquires antibiotic resistance.[1] Microbial infections with antimicrobial resistant strains increased the risk of mortality and increased costs related to treatment compared to infections caused by susceptible strains.[2]The emergence of resistance to antibiotics in Gram-positive pathogens has become a major international problem as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria.[3] The problem of increasing antimicrobial resistance is even more threatening when considering the very limited number of new antimicrobial agents that are in development. As rapidly as new antibiotics are introduced, Staphylococci have developed efficient mechanisms to neutralize them; inevitably this has left fewer effective bactericidal antibiotics to treat these often life-threatening infections.

Multidrug-resistant (MDR) Staphylococci pose a growing problem for human health. The rise of drug-resistant virulent strains of *Staphylococcus aureus*, particularly methicillin-resistant S. aureus (MRSA) is

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a serious problem in the treatment and control of Staphylococcal infections.[4,5]

The true extent of antimicrobial resistance in MRSA in our area is unknown. Thus, this study was carried out to with the aim: To study the antibiotic resistance pattern of MRSA to commonly used and newer antibiotics.

Material & methods

Study area:- Department of Microbiology, Guru Gobind Singh Medical College Faridkot.

Study Design: A cross section study was conducted.

Study period: The study was conducted for a period of 18 months.

Sample size:-Sample size was calculated by considering prevalence of 65% MRSA [6], permissible error of 5% confidence level 95% by using finite population correction formula .

$$S = \frac{n Z^2 p(1-p)}{d^2(n-1)+Z^2 p(1-p)}$$

S= sample size, Z = statistic level of confidence, n = population size, p = prevalence and d = precision level The sample size had came out to be 91 , Design effect=1.5. By applying design effect sample size calculated was 140

Collection of specimens:-

Following specimens were collected Pus: - Two sterile cotton tipped swabs were used for collecting pus from infected wounds ,abscess/boils etc.,Blood: - 5-10 ml of blood was collected aseptically and inoculated in 50-100 ml of brain heart infusion and incubated at 37^0 C, Catheter tips and drains: - Samples were collected using all aseptic condition, Urine.:- Midstream urine sample was collected in a sterile container using aseptic condition.

Processing of specimens

In case of pus swabs, one swab was used for gram staining and the other for culture on Blood agar and MacConkey agar.

In case of blood and urine, the samples were cultured on Blood agar and MacConkey agar

The specimen inoculated on these media was incubated at 37ºC for 24-48 hours

Study of Culture- it was done as follows:

a)Colony Morphology was noted

b) Gram staining - Bacterial colony showing gram positive cocci arranged in clusters would be further subjected to following testsCatalase test and Coagulase test

Antibiotic Sensitivity Test 8

The antibiotic sensitivity pattern of the organisms was tested by modified Kirby Bauer's disc diffusion method against the following antimicrobial agents (Hi Media) as per Clinical and Laboratory Standard Institute guidelines(CLSI)

S. No.	Antibiotic	Disc Content
1	Ampicillin	10 µg/disc
2	Erythromycin	30 µg/disc
3	Cefoxitin	30 µg/disc
4	Ciprofloxacin	25 µg/disc
5	Amikacin	10 µg/disc
6	Cotrimoxazole	5 µg/disc
7	Rifampicin	2 µg/disc
8	Clindamycin	5 µg/disc

Sensitivity Test: Muller Hinton agar plates were over laid within 15 min after the inoculum has been adjusted. Swabs were then streaked over the plates to obtain lawn cultures. Antibiotic disc were applied within 15 minutes of inoculation and the plates were incubated at 37^0C for 18-24 hours.

Zone diameters were measured as per CLSI criteria[8] .In case of cefoxitin, the plates were incubated for full 24 hours. Isolates which yielded zone of inhibition of diameter less than 22mm against cefoxitin(30µg) were considered as MRSA. *S. auerus* ATCC 25923 and MRSA 43300 were used as reference strain[8]

Confirmed MRSA isolates were screened on vancomycin screen agar

Vancomycin screen agar test[9-11]:-Resistance to vancomycin was detected by using a screening medium consisting of brain heart infusion agar (BHI) supplemented with 6 µg/ml vancomycin (BHI-V6). The medium was inoculated with 10 µl of a 0.5 McFarland[9].It was incubated at 37°C for full 24 h. Strains with reduced susceptibility to vancomycin demonstrate growth of one or more colonies after 24 h of incubation.

Confirmed MRSA isolates were further tested for susceptibility to new antibiotics

1	Linezolid	30µg/disc
2	Quinupristin/Delfopristin	15µg/disc
3	Tigecycline	15 µg/disc

The MIC for Vancomycin, Daptomycin, Teicoplanin(Hi media India Pvt .Ltd) and Ceftaroline (biomerieux India)was determined by Epsilometer test(E-Test) following manufactures instructions.

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RESULTS

TABLE 1: Distribution Of 140 Isolates of MRSA According To Age Of The Patients

S.No.	Age (Years)	Number of MRSA Isolates	Percentage (%)
1	0-15	46	32.9
2	16-30	34	24.3
3	31-45	27	19.3
4	46-60	23	16.4
5	61-75	10	7.1
Total		140	100

The maximum strains (32.9%) of MRSA were obtained from patients in age group of 0-1

TABLE 2: Distribution Of 140 Isolates Of MRSA According To Gender Of Patients

S.No.	Gender	Number of MRSA Isolates	Percentage (%)
1	Male*	87	62
2	Female*	53	38
TOTAL		140	100

Among140 MRSA studied, 87(62%) were from Males and 53(38%) were from females. The male to female ratio was (1.6:1) and the results are statistically significant (p=0.001)

TABLE 3: Various Clinical Specimens Showing Distribution Of MRSA (n=140)

S.No	Sample	Number of MRSA Isolates	Percentage(%)
1	Pus*	100	71.25
2	Blood*	40	28.75
3	Urine	Nil	Nil
4	Catheter tips and drains	Nil	Nil
Total		140	100

The 140 isolates of MRSA were obtained from only two clinical specimens, pus and blood. While the isolation rate from pus was (71.25%) and it was (28.75%) from blood and difference was statistically significant (p=0.000) . No MRSA was isolated from urine, catheter tips other body fluids.

TABLE 4: Antimicrobial Resistant Pattern of 140 MRSA Isolates to Commonly Used Antibiotics

S.No	Name of antibiotic	Number of Resistant MRSA isolates	Percentage (%)
1	Ampicilln (10µg)	140	100
2	Erythromycin (30µg)	140	100
3	Clindamycin (5µg)	75	53
4	Ciprofloxacin (25µg)	140	100
5	Amikacin (10µg)	14	10
6	Rifampicin (2µg)	NIL	NIL
7	Cotrimoxazole (25µg)	87	62.1

All the isolates showed 100% resistant to Ampicilin, Erythromycin and Ciprofloxacin followed by Cotrimoxazole (62.1%),Clindamycin (53%) and Amikacin (10%). No strain showed resistant to Rifampicin

TABLE 5: Antibiotic Resistance Of 140 MRSA Isolates To Newly Used Antibiotics

S.No	Name of antibiotic	Number of Resistant isolates	Percentage(%)
1	Vancomycin	Nil	Nil
2.	Teicoplanin	Nil	Nil
3	Daptomycin	Nil	Nil
4	ceftaroline	Nil	Nil
5	Linezolid	Nil	Nil
6	Tigecycline	Nil	Nil
7	Quinupristin	4	2.8

None of the 140 MRSA isolates showed resistant to vancomycin, teicoplanin, linezolid, daptomycin, tigecycline and ceftaroline however 4 (2.8%) isolates showed resistance to Quinupristin/Delfopristin by disc diffusion test.

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Discussion

In our study maximum number(32.9%) of MRSA were isolated from patients below 15 years of age while the least(7.1%) were from age group more than 60 and the difference between these two age groups was statistically significant(p=0.00).This is similar to the findings of AL-Zoubi *et al* who also obtained maximum(12.4%) MRSA isolates from the age group of less than 19 years and minimum(2.5%-6.3%) in the age group of more than 80 years [12]

Male to female ratio was (1.6:1) which was statistically significant(p=0.00). This is in concordance with the findings of Al- Zoudi *et al*[12].

In the present study, the isolates of MRSA were obtained from only two clinical specimens, pus and blood. While the isolation rate was 71.42% from pus it was only 28.75% from blood and no isolate was obtained from urine, other body fluids and catheter tips etc A study from Kathmandu (Nepal) also reported the maximum isolation of MRSA from pus followed by sputum, blood, body fluids and urine specimens [13]

In the present study, maximum resistance (100%) of MRSA was observed against ampicillin, erythromycin, ciprofloxacin followed by Cotrimoxazole (62.1%), clindamycin(53%) and amikacin (10%). No strain showed resistant to rifampicin. This is similar to a study from Maharashtra [14] and a multicentric study conducted by ICMR in 2017 [15].The high resistance to number of these antimicrobial agents is perhaps because of indiscriminate use of these drugs everywhere [15] While resistance to amikacin was observed in 10% of strains in present study it was found to be 100% in study of Kaur *et al*.Many factors contribute to variability in this resistance pattern and important ones are localized difference in antibiotic prescription patterns and the efficacy of the infection control measures employed in various centers.[14]

Conclusion

The high isolation rates of MRSA from all the age groups of patients, and their high resistance to commonly used antibiotics is a cause of concern . Regular surveillance of hospital associated infections, monitoring of antibiotic sensitivity pattern of MRSA and formulation of definite antibiotic policies may be helpful in reducing the incidence of MRSA infections.

Conflict of interest: None

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