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SCREENING AND CONFIRMATION OF MRSA ISOLATES AND A STUDY ON THE MIC VALUES OF VANCOMYCIN AND DAPTOMYCIN USING E-TEST STRIPS

Microbiology

Dr. Ruksana Ahmed MD, Consultant Microbiologist - Care Hospital, Hyderabad

ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is an important nosocomial and community pathogen. The objectives of this study were to determine the prevalence of MRSA in clinical specimens and to detect the sensitivity pattern of these strains against various antibiotics used for treating patients. Strains were identified using standard procedures, and their sensitivity pattern was done on Mueller Hinton agar by Kirby Bauer disc diffusion method. Mean inhibition concentration values of vancomycin and daptomycin were determined.Among 110 isolates of S. aureus, 30(30.90%) were methicillin-resistant. The antibiogram done for 34 MRSA isolates showed that there was 100% resistance to drugs like Penicillin, Oxacillin, Ampicillin, Amoxiclav, Cefoxitin, while the resistance to others was 79.41% to Erythromycin, 64.71% to clindamycin, 52.94% to Ciprofloxacin, 41.17% to Rifampicin, 2.94% to Teicoplanin, and 0% resistance to Linezolid and Tigecycline . 70.59% of the MRSA isolates had an MIC value of between 1 and 2 mcg/ml for Vancomycin. Further, 94% isolates of MRSA showed complete susceptibility to Vancomycin with MIC values below 2mcg/ml. Therefore, Vancomycin is recommended as the first line of treatment for MRSA infections. However, indiscriminate use of Vancomycin has to be avoided as it is leading to emergence and spread of Vancomycin resistance. All the MRSA isolates in the present study are found to be susceptible to Dapto myc in. Therefore, in cases of Vancomycin intermediate Staphylococcus aureus (VISA), it is recommended that Daptomycin Linezolid or Tigecy cline can be used for treatment.

KEYWORDS

INTRODUCTION

Alexander Fleming in his acceptance speech for the Nobel Prize for the discovery of remarkable anti-bacterial property substance Penicillin warned that an inappropriate use of this wonder drug might lead to development of resistance. This became a reality in the year 1950, when resistance to penicillin among strains of Staphylococcus aureus became a major problem. The cause of the resistance was the production of enzyme penicillinase (beta lactamase) that had been described as early as 1940.

Staphylococcus aureus causes a wide range of infections including bacteremia as well as pleuropulmonary, skin and soft tissue infections. This bacterium is also responsible for many types of ocular infections including keratitis, conjunctivitis, endophthalmitis and blepharitis. Some virulence factors such as Panton-valentine leucocidin (PVL) as well as antibiotic resistance have an important role in increasing tissue damage and failure in antibiotic therapy, respectively. There are many reports about ocular infection due to methicillin-resistant S. aureus (MRSA) isolates. In the past decade, infections by MRSA strains have increased in hospital settings and communities. MRSA isolates are commonly resistant to a wide range of antibiotics such as aminoglycosides, erythromycin, tetracycline and fluoroquinolones. The mecA gene is on the staphylococcal cassette chromosome mec (SCCmec) genetic element that causes resistance to β-lactam antibiotics. SCCmec type IV usually is related to community-associated-MRSA (CA-MRSA) and other SCCmec types including SCCmec types I, II and III related to hospital-acquired MRSA (HA-MRSA) isolates. The PVL is a cytotoxin virulence factor produced by some S. aureus commonly related to CA-MRSA isolates and causes cell destruction and tissue necrosis (6). Antibiotics such as erythromycin and tetracycline were used for treatment of ocular infections by S. aureus . Activity of efflux pumps such as msr (A/B) and methylation of the ribosomal drug binding site by methylase enzymes encoded by erm (A, B, C) genes are important resistance mechanisms to macrolide antibiotics.

Early detection of MRSA and formulation of effective antibiotic policy in tertiary care hospitals is very important from the epidemiological point. The antibiogram of MRSA is also important to select appropriate empirical antibiotic therapy in critically ill patients. Hence, this study was conducted to know the prevalence of MRSA in clinical samples and its antibiogram

AIMS AND OBJECTIVES

To screen and confirm the Methicill in Resistant Staphylococcus Aureus in different specimens using Cefoxitin disc diffusion method, with the objective to study the incidence and prevalence of MRSA.

To determine the mean inhibitory concentration values of Vancomycin and Daptomycin using E-Test strips

MATERIALS AND METHODS

The present observational study was conducted for a period of one year whereby a total of 715 samples, 110 samples grew Staphylococcus aureus in pure culture. All age group patients were included. All the samples were processed and inoculated on blood agar, nutrient agar, and Mac Con key agar. S. aureus was diagnosed by their growth characteristics on Mac Con key agar (pink colour colony), on nutrient agar (large, circular, and opaque colony), and on blood agar; (greyish white colony), gram-stain morphology (gram-positive cocci in clusters); positive catalase test; positive coagulase test (slide and tube coagulase test); growth on mannitol salt agar; and pigment characteristics (golden yellow).The 34 MRSA isolates were subjected to antibiotic susceptibility testing by disc diffusion(Kirby-Barer method)and E-Test method (for vancomycin and daptomycin)

RESULTS

110 staphylococcus aureus were isolated in pure culture and processed in the present study.The said 110 coagulase positive S.aureus, when subjected to screening and confirmation test in accordance with CLSI guidelines (M100) using cefoxitin 30 microgram as surrogate marker for oxacillin resistance, 34 isolates showed a cefoxitin zonal diameter of ≤ 21 millimetres (mecA positive) thereby indicating that the said 34 isolates were Methicillin Resistant Staphylococcus Aureus-MRSA .

OVERALL VIEW OF THE CLINICAL SAMPLES OF MRSA

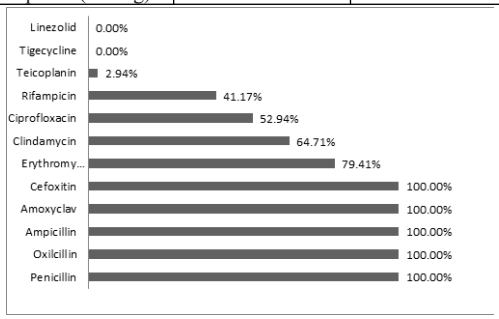
On correlation with the requisition sheet accompanying the specimen samples, it was noticed that 21 samples of MRSA were from pus, 7 from blood, 1 from sputum, 3 from BAL and 2 samples were from ET Secretions.

Samples from different infections	No of S.aureus isolated	No of MRSA Isolated	Percentage
Pus	90	21	23.07%
Blood	12	7	58.3%
BAL	4	3	75%
ET secretion	2	2	100%
Sputum	2	1	50%

Antibiogram of 34 MRSA isolates: The antibiogram done for 34 MRSA isolates showed that there was 100% resistance to drugs like Penicillin, Oxacillin, Ampicillin, Amoxiclav, Cefoxitin, while the resistance to others was 79.41% to Erythromycin, 64.71% to clindamycin, 52.94% to Ciprofloxacin, 41.17% to Rifampicin, and 2.94% to Teicoplanin. Linezolid and Tigecycline 0%.

Antibiotic (disc content)	No of Resistant Isolates	Percentage of Resistance
PenicillinG(10 units)	34	100%

Oxacillin(1mcg)	34	100%
Ampicillin(10mcg)	34	100%
Amoxyclav(30mcg)	34	100%
Cefoxitin(30mcg)	34	100%
Erythromycin(15mcg)	27	79.41%
Clindamycin(2mcg)	22	64.71%
Ciprofloxacin(5mcg)	18	52.94%
Rifampicin(5mcg)	14	41.17%
Linezolid(30mcg)	0	0%
Tigecycline(15mcg)	0	0%
Teicoplanin(30mcg)	1	2.94%



MIC values of Vancomycin using E-test Strips

No. of isolates	MIC values of vancomycin
2	0.5
4	0.75
8	1
5	1.25
8	1.5
5	2
2	4

MIC values of Daptomycin using E-Test strips

Number of Isolates	MIC values of Daptomycin
4	0.125
2	0.19
12	0.25
2	0.38
2	0.50
9	0.75
3	1

In the tables above, with regards to the MIC values of vancomycin and daptomycin it is seen that:

1) The minimum MIC values for vancomycin were 0.5 mcg/mL
2) 70.59% of the isolates had MIC values between 1 and 2(mcg/mL); and 38.24% of the MRSA isolates had an elevated MIC values between 1.5 - 2 mcg/mL.
3) This study also showed 2 isolates of MRSA with MIC of 4 mcg/ml indica ting the intermediate sensitivity pattern. One isolate was from pus and the other isolate from blood.
4) All the 34 isolates were found to be total susceptible to daptomycin with MIC less than or equal to 1.
5) The range included from 0.125 to 1 mcg/mL.The isolates which showed elevated levels of MIC values of vancomycin (equal or more than 2 mcg/mL) also exhibited elevation in the corresponding daptomycin values(i.e.1mcg/mL).
6) The median value of MIC for vancomycin is found to be 1.25 mcg/mL while the medial value for MIC of daptomycin is 0.25 mcg/mL.

DISCUSSION

Prevalence of MRSA :A total number 0f 715 samples from different clinical isolates from different infections were tested in the b The antibiogram done for 34 MRSA isolates showed that there was 100% resistance to drugs like Penicillin, Oxacillin, Ampicillin, Amoxiclav, Cefoxitin, while the resistance to others was 79.41% to Erythromycin, 64.71% to clindamycin, 52.94% to Ciprofloxacin, 41.17% to Rifampicin, and 2.94% to Teicoplanin. Linezolid and Tigecycline 0%.

acteriological Lab during the study period. It was found that 110 were coagulase positive Staphylococcus aureus. The prevalence of MRSA in the present study among the above 110 S.Aureus was 30.90%. This is in accordance with investigators like Vidya pai et al reporting a prevalence of 29.1% and Mehta A et al reporting a prevalence of 32%.In a study done in 2010 in Chennai by Gopalkrishna et al, the prevalence of MRSA was reported as being 40-50%. In another study by DechanTsering et althe prevalence of MRSA was found to be 38%. The present study falls in the lower range of these studies.

Screening and conformation of MRSA : Recent studies indicate that disc diffusion test using cefoxitin is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion. It has been reported as surrogate marker of mecA gene, gives clearer end points, easier to read and is more reproducible than tests with Oxacillin disk diffusion. Thus cefoxitin is accepted method for detecting MRSA with high efficiency and has been used as an alternative to PCR in resource constrained areas. The resistance capability of MRSA isolates to various antimicrobials may be located either on chromosomes, plasmids or transposons.

Vancomycin Susceptibility: In the present study 32 of the isolates of MRSA were found to be susceptible to Vancomycin with a mic less than or equal to 2mcg. The MIC values for vancomycin ranged from 0.5 micrograms/ml to 2 micrograms/ml. 70.59% of the isolates had MIC values between 1 and 2mcg/ml; and 38.24% of the MRSA isolates had an elevated MIC values between 1.5 to 2mcg/ml. This distribution pattern of MIC had a median value of 1.25.Banda Venkata Ramana et al 2012, in a study on vancomycin susceptibility found that 75 out of 80 isolates of MRSA had an MIC value of less than or equal to 2mcg/ml.Dr. Sushmita Bhatacharaya et al 2013, from NRS medical College, Kolkata reported MIC value of vancomycin by E test method in 98.57% strains of MRSA was less than or equal to 2mcg/ml.Chandra Mohan Reddy et al 2012, in a study on vancomycin resistance among MRSA isolates in Rayalseema region reported that in 71 out 84 (84.52%) isolates of MRSA had MIC values of less than or equal to 2mcg/ml.As can be seen from the foregoing, the MIC values of vancomycin in the present study are less than or equal to 2mcg/ml in 94.12% of the MRSA isolates. This broadly corresponds to the MIC values reported in other studies.

Daptomycin Susceptibility: In the present study, all the 34 MRSA isolates were found to be susceptible to daptomycin. The MIC range for daptomycin was 0.125 to 1 micrograms/litre. Maximum number of isolates i.e. 12 (35.29%) had an MIC value of 0.25. Further the median value of this MIC distribution was found to be quite low at 0.25 micrograms/ litre.In the study done by Rajneet Kaur PGIMER, Punjab 2012, on daptomycin susceptibility of MRSA, it was found that daptomycin was highly active against all the 64 isolates with e test method and with a MIC range of 0.065 to 1 micrograms/liter.Padmaja et al 2011 from NIMS, found all the 90 isolates of gram-positive cocci were susceptible to Daptomycin.In a comparative study of antimicrobials involving 727 clinical isolates from seven Indian medical centres, Anand Manoharan et al CMC Vellore 2010, found that all the MRSA isolates were susceptible to daptomycin.Thus from the above it is seen that the 100% susceptibility of MRSA to daptomycin found in the present study confirms to the results reported by various other studies in India. However this drug is contraindicated in pulmonary infection as it reacts with the pulmonary surfactant (Silverman 2005)

CONCLUSIONS

1. 34 MRSA isolates out of a total 110 isolates of Staphylococcus aureus were detected, by using disc diffusion method thereby indicating a prevalence of 30.9%.
2. 70.59% of the MRSA isolates had an MIC value of between 1 and 2 mcg/ml for Vancomycin. Further, 94% isolates of MRSA showed complete susceptibility to Vancomycin with MIC values below 2mcg/ml. Therefore, Vancomycin is recommended as the first line of treatment for MRSA infections. However, indiscriminate use of Vancomycin has to be avoided as it is leading to emergence and spread of Vancomycin resistance.
3. All the MRSA isolates in the present study are found to be susceptible to Daptomycin. Therefore, in cases of Vancomycin intermediate Staphylococcus aureus (VISA), it is recommended that Daptomycin be used for treatment.
4. As Daptomycin reacts with pulmonary surfactant of the lung instead of the bacteria; therefore in cases of VISA involving respiratory tract infection it is recommended that linezolid be used. However, since linezolid has its apparent side effects, it is recommended that its use be only limited to VISA strains.

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