International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 16, Issue 2, 2024

Original Article

DETECTION OF METHICILLIN RESISTANCE IN CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* IN TERTIARY CARE HOSPITAL, TIRUPATI

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Received: 20 Dec 2023, Revised and Accepted: 24 Jan 2024

ABSTRACT

Objective: The study aimed to estimate the prevalence of methicillin resistance of *Staphylococcus aureus* in various clinical samples received at tertiary care hospital. Initially, the *Staphylococcus aureus* and its antibiotic susceptibility tests is performed in clinical samples which are submitted to the department of Microbiology. And Methicillin Resistance *Staphylococcus Aureus* (MRSA) is determined by using cefoxitin (30 µg) as per CLSI guidelines.

Methods: The prospective study was conducted in department of Microbiology in a tertiary care hospital. All *Staphylococcus aureus* organisms isolated in clinical samples were included in the study and processed as per the standard operating procedure. Methicillin susceptibility was tested by using cefoxitin (30µg) disks on Muller-Hinton agar plates that were inoculated with a suspension (equal to 0.5 McFarland standards) of the *s. aureus*.

Results: In our study, amongst hundred *staphylococcus aureus* isolates, sixty isolates were shown resistance to cefoxitin (30µg), which indicates that percentage of methicillin-resistant *s. aureus* in our study is 60. Majority of *s. aureus* were isolated from blood samples 44% (n= 44) followed by pus samples 32% (n=32). Linezolid resistance reported was 3%. All isolates were sensitive to vancomycin and daptomycin by disc diffusion test as per CLSI guidelines 2021.

Conclusion: To conclude, MRSA plays a significant role and it can be transmitted through endogenous, cross-infection and reinfections. Phenotypic methods like use of cefoxitin disc (30µg) can be considered for detection of methicillin resistance in *S. aureus*, as it consumes less time and easy to perform.

Keywords: Methicillin resistance staphylococcus aureus, Cefoxitin, Staphylococcus aureus, Disc diffusion test

DOI: https://dx.doi.org/10.22159/ijcpr.2024v16i2.4062 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of a medically important Gram-positive bacteria, which first emerged in the year of 1961 [1]. It is established in the African continent in 1978 and appeared in Ethiopia in 1987 [2, 3]. Among the body sites that harbor MRSA, such as throat, perineum, skin, hairline, groin, and axilla, the anterior nares, the most important site for MRSA colonization being nasal cavity [4]. Nasal colonization with *s. aureus* is a vibrant process; a number of factors being responsible for the MRSA carriage. Risk of consequent infection in a person colonized with *s. aureus* as well as with MRSA, upsurges with time and remains insistently increased [5]. The nasal carriage of *s. aureus* is not often the main cause of infection however, it can act as a source for subsequent infections in individuals colonized with this pathogen [6, 7]. MRSA is defined as any strain of *s. aureus* that has established resistance to beta-lactam antibiotics such as Methicillin, Oxacillin, Cefoxitin and Nafcillin [8, 9]. These strains are responsible for a greater number of nosocomial infections which are tough to combat in humans [10].

MATERIALS AND METHODS

The study was conducted in dept of Microbiology in tertiary care hospital. All the samples that were submitted to the microbiology department were processed within 2 h of the collection as per standard operating procedures.

Inclusion criteria

All *Staphylococcus aureus* organisms isolated in clinical samples were included in the study

Exclusion criteria

Staphylococcus species other than *s. aureus* isolated in clinical samples were excluded.

The collected specimens were processed on 5% sheep blood agar, nutrient agar, macconkey agar and incubated at 37 °C for 24 h. *Staphylococcus aureus* isolates were identified based on their colony morphology, catalase test, biochemical reactions, and coagulase test [11]. Susceptibility patterns to different antibiotics, which included cefoxitin (30µg), penicillin (10 IU), clindamycin (2µg), erythromycin (15µg), vancomycin (30µg), co-trimoxazole (25µg), linezolid (30µg), Tigecycline(15µg) and tetracycline (30µg) were determined as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) 2021, using the Kirby-Bauer disk diffusion method [12].

Methicillin susceptibility was tested by using cefoxitin (30µg) disks on Muller-Hinton agar plates that were inoculated with a suspension (equal to 0.5 McFarland standards) of the *s. aureus*. Then positive plates were incubated at 35 °C for 24 h and inhibition zones of diameter were measured using Vernier caliper [13, 14]. The CLSI 2021 criteria was used for interpretation. Cefoxitin zone were measured for *s. aureus* zone of inhibition of ≥ 20 mm were considered as susceptible and ≤19 mm as resistant [15].

Sample size calculation

Based on the published literature and our hospital-based data, prevalence of MRSA is approximately around 50%. Sample size was calculated assuming a clinically relevant percentage of 50%.

Sample size was calculated using the formula.

$$n = \frac{Z^2 P(100-P)}{d^2}$$

Statistical analysis

All the details regarding demographic characteristics and diagnosis were recorded and entered in Microsoft excel sheet and analyzed. The categorical variables were represented as percentage. The data were analyzed using SPSS (version 21) (IBM Corp/Somers NY, USA). Descriptive analysis of culture and antimicrobial susceptibility data were be done.

RESULTS

In our study, amongst hundred *Staphylococcus aureus* isolates, sixty isolates were shown resistance to cefoxitin (30µg), which indicates that percentage of methicillin-resistant *s. aureus* in our study is 60 (60%) (fig. 1).

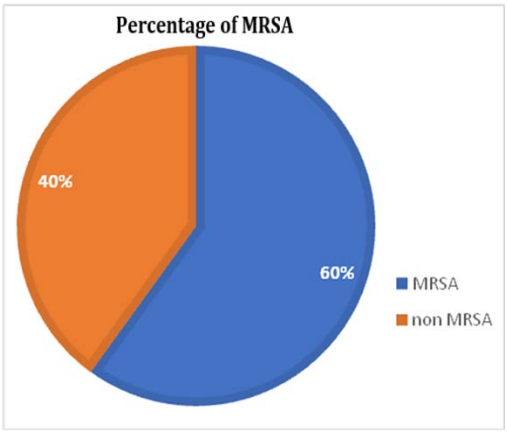

Fig. l: Percentage of MRSA in total samples

High percentage of clinical samples received in age group between 40-60 y and observed male preponderance (n=70, 70%) in all *Staphylococcal* isolates. Majority of *s. aureus* were isolated from blood samples 44% (n= 44) followed by pus samples 32% (n=32). Thirty-five (n=35) of samples were received from the emergency department and remaining samples were received from neurosurgery, surgical gastroenterology, medicine, urology, nephrology, surgical oncology and general surgery wards.

Eighty percent (n=80) of *s. aureus* isolates were resistant to penicillin and ampicillin. Linezolid resistance rated was 3%. Cefoxitin resistant *s. aureus* contributed 60 percent. Eighteen percent of *S. aureus* isolates were resistant to clindamycin and erythromycin. Among 100 *Staphylococcus aureus* isolates sixteen percent [16] were resistant to vancomycin. Tigecycline was used in pus, blood, and sputum samples. Ten percent (n=6) of *s. aureus* were resistant to tigecycline among 56 samples.

Among 60 methicillin-resistant *Staphylococcus aureus* isolates, 55(91.7%) isolates were resistant to ampicillin and penicillin, 53(88.3%) isolates were resistant were ciprofloxacin, 27(45%) isolates were resistant to clindamycin, 31(51.7%) isolates were resistant to cotrimoxazole, 48 isolates were resistant to erythromycin, 18 isolates were resistant to gentamicin, 4 isolates were resistant to linezolid, 19 isolates were resistant to tetracycline and All isolates were sensitive to vancomycin by disc diffusion test as per CLSI guidelines 2021 (fig. 2).

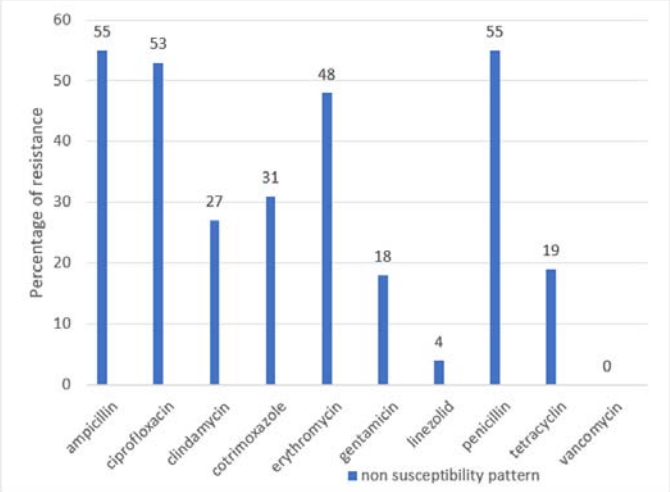

Fig. 2: Percentage of resistance pattern of antibiotics in methicillin resistant *Staphylococcus aureus*

DISCUSSION

Staphylococcus aureus is the most common pathogen and is the important etiological agent of health care-associated infection. Colonization and infection by *staphylococcus aureus* were known to be significantly associated with infection among hospitalized patients. In the era of high prevalence of methicillin resistance among *s. aureus*, a relatively high percentage (11-19%) of MRSA among hospitalized patients on admission will increase the likelihood of MRSA infection during the same episode of hospitalization [16].

Studies on MRSA in intensive care units have also demonstrated that MRSA colonization predisposed to MRSA infection during same hospitalization [17].

In present study, 100 *staphylococcus aureus* isolates were included from various specimens such as blood, pus, synovial fluid, sputum, endotracheal secretions etc. Majority of *s. aureus* isolates were received from various clinical samples like blood 44 (44%) followed by 32 (32%) pus, 12(12%) urine, 6 (6%) sputum etc.

In the present study standard tests were performed to identify *staphylococcus aureus* among clinical samples. Cefoxitin disk (30µg) was used for detection of MRSA by Kirby bauer disc diffusion method. The results were recorded according to the Clinical laboratory and Standard Institution (CLSI) guidelines 2020 as susceptible, intermediate and non-susceptible.

High prevalence of MRSA was observed in blood (65.9%) and pus samples (37.5%) in the present study. Overall percentage of MRSA among blood and pus samples was 54%. This is on par with the study conducted by Manjunath *et al.* (53.9%). A study conducted by Banaras Hindu university where 55% of MRSA isolates were seen in blood and pus samples which is in concordance with our study.

In Hanumanth Appa *et al.*, Baghdady *et al.* and Adhikari *et al.* studies, they have shown lesser prevalence of MRSA 43%, 31%, 35.5% respectively [18-21]. This difference could be attributed to different healthcare setting, sample size of the patient and awareness of infection control measures among healthcare workers.

In a study conducted by Adhikari *et al.* in 2017, inducible clindamycin resistance was reported as 10%. In our study, inducible clindamycin resistance was 18% among all isolates. In both studies, the percentage of inducible clindamycin resistance was different, but the occurrence

of inducible clindamycin resistance was not significantly different among methicillin sensitive *s. aureus* and methicillin resistant *s. aureus* in our study, similar observation was noted in Adhikari *et al.* study. Inducible clindamycin resistance plays a significant role in therapeutic implication of clindamycin. This fact may raise the concern that clindamycin may fail to treat the *s. aureus* infections and it should be avoided as a treatment of choice for patients infected with *s. aureus* when exhibiting *in vitro* inducible resistance.

In the present study, 91.6% of MRSA isolates were shown resistance to penicillin and ampicillin and 88.3% of MRSA isolates were exhibited resistance to ciprofloxacin, which is in agreement with the study conducted by Anupurba *et al.* [20].

In our study Linezolid resistance is noted in 6.6% (n=4) isolates among MRSA and all MSSA isolates were susceptible to linezolid. Percentage of linezolid susceptibility being 93.4% (n=56 amongst 60) of MRSA. The percentage of Linezolid resistance among MRSA in our study is higher when compared with the study conducted by Bing Gu *et al.* [22] where Linezolid resistance were reported as 2% among MRSA. Increased percentage of resistance to Linezolid in our study emphasizes the fact that the emergence of Linezolid resistance in *Staphylococcus aureus* poses a significant challenge to the clinical treatment of infection particularly MRSA in which Linezolid is one of the treatment of choice. This also warns the surge of Linezolid resistance in near future.

In case of cefoxitin sensitive, penicillin or dicloxacin can be considered to treat the infection. In case of MRSA along with multi drug resistant *Staphylococcus aureus*, vancomycin will be the treatment of choice. This conclusion is made based on our study findings and on our hospital antibiotic policy which is updated every 6 mo.

Indiscriminate use of antibiotics may lead to resistance to all the other groups of antibiotics. A very few MRSA resistant to vancomycin have been found in the USA, though our study found 100% sensitivity to vancomycin and daptomycin. In Manjunath *et al.* study, vancomycin resistance was 8.7%. This might be due to inadequate IPC practices, wide use of restricted antimicrobials and prolonged hospital stay in ICUs. Improper and indiscriminate use of antibiotics in future lead to resistant to these drugs globally. So, appropriate judicious administration of antibiotics are recommended to curb multidrug resistance.

CONCLUSION

To conclude, MRSA plays a significant role and it can be transmitted through endogenous, cross infection and reinfections. So, it could be detected bench side either through phenotypic methods or molecular typing methods. However phenotypic methods like use of cefoxitin disc (30µg). It can be considered for detection of methicillin resistance in *s. aureus*, as it consumes less time, easy to perform and less labour intensive. Molecular typing methods are helpful for identification of hospital and community acquired MRSA, but it is more expensive, needs technical expertise and confined to tertiary care hospital.

Vancomycin will be the drug of choice for methicillin resistance *s. aureus* and inducible clindamycin resistance. Presently, VISA and VRSA are emerging pathogens, would impose serious threat in future. So judicious and targeted antibiotic therapy to be used to combat MDR *Staphylococcus aureus.*

LIMITATIONS

Molecular characterization of MRSA could not be done due to financial constraints.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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