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Prevalence of Methicillin Resistant *Staphylococcus aureus* in Pus samples in a tertiary care hospital of Eastern India

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Abstract:
Background: *-Methicillin Resistant Staphylococcus aureus (MRSA) are emerging multidrug resistant bacterial strains which are posing great therapeutic challenges worldwide. Aim of the study was to find the prevalence of Staphylococcus aureus and MRSA in pus samples and also the drug susceptibility of these MRSA strains.*
Materials & Methods*: - 179 pus samples were collected between September 2019 and January 2020 in the Department of Microbiology, Burdwan Medical College, Burdwan, West Bengal. They were inoculated in Nutrient agar and Blood agar and incubated for 24 hours at 37[0] C. Standard conventional methods were used for isolation and identification of Staphylococcus aureus. Methicillin Resistant Staphylococcus aureus (MRSA) were identified by the cefoxitin (30µ) disk method. Antibiotic susceptibility test was done using the Kirby-Bauer disk diffusion method and interpretation of results was done based on the Clinical and Laboratory Standards Institute guidelines.*
Result: *- Out of the 179 processed wound swab, Staphylococcus was isolated from 134 samples (74.86%). Of these, 39 (29.10%) were identified as methicillin-resistant Staphylococcus aureus and 95 (70.90%) were methicillin-sensitive Staphylococcus aureus. Out of the total 39 methicillin-resistant S. aureus, all showed multidrug resistance showing resistance to—Amikacin (100%), Erythromycin (100%), cotrimoxazole (71.5%), Cephalexin (54.15%), Ciprofloxacin (30.5%) and gentamicin (88.1%). They were susceptible to Linezolid (96.6%) and were 100% susceptible to Tigecycline, Teicoplanin, and Vancomycin.*
Conclusion: *- Judicious use of antimicrobials, especially in OPD patients, could prevent the emergence of the MRSA strains and guidelines for evaluation, treatment of MRSA isolates should be followed appropriately to ensure their resolution, prevention and control.*
Key words: *- multi drug resistance, Methicillin resistant Staphylococcus aureus, Kirby-Bauer disk diffusion method, Clinical and Laboratory Standards Institute guidelines.*

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I. Introduction

Staphylococcus aureus is the most important pathogen belonging to the genus *Staphylococcus.* [1] It is Gram positive cocci and is an important bacterium forming a part of the normal flora of our body. It is present in the anterior nares, nasopharynx, perineal area and skin. [2] It is slowly emerging as an important pathogen in hospitals and community worldwide. [3] *Staphylococcus aureus* is responsible for a variety of infections like skin infections, joint infections, urinary tract infections, pneumonia and even septicaemia. [4]

It is present as a commensal in many healthy individuals including health care providers which gets easily transmitted to hospitalized patients causing serious infections. [5] Previously all the strains of *Staphylococcus aureus* were susceptible to the beta lactam group of antibiotics but with the emergence of *MRSA (Methicillin Resistant Staphylococcus aureus)* treatment of these infections has become a serious challenge. [6]

Methicillin Resistant Staphylococcus aureus (MRSA) an emerging multidrug resistant strain worldwide, prevalence (4.6% to 54.4%). [7] It is of 2 types--HA *MRSA (Healthcare associated methicillin resistant Staphylococcus aureus)* acquired due to prolonged or frequent hospitalizations from infected patients/staff and *CA MRSA (Community acquired methicillin resistant Staphylococcus aureus)* affecting healthy people of the community. [8]

Infected patients in the hospitals spread the *MRSA* with the staff of the hospitals being the reservoir of the strain leading to many cases of endemics and epidemics due to *MRSA.* [9] The probable predisposing causes of this increased spread of this strain is indiscriminate use of antibiotics leading to emergence of resistant strains, lack of awareness, intravenous drug abuse, indwelling devices and repeated hospitalisation.[10]

MRSA has caused outbreaks in many countries like USA, Europe, Australia, Japan [11] and its transmission in hospitals is due to 2 genes--mecR1 and mecI gene responsible for production of a penicillin binding protein which has low affinity to beta lactam antibiotics.[12,13]

MRSA strains are multi drug resistant and are difficult to eradicate responding only to the glycopeptides making them the drugs of choice.[14] But recently resistance to even these drugs have been reported from various parts of the country.[15,16]

As *Staphylococcus aureus i*s the most commonly isolated bacterium in pus samples, we selected it for our study. The aim of the study is to find the prevalence of methicillin resistant Staphylococcus aureus in pus samples and to know their susceptibility to the commonly used antibiotics.

II. Material and Methods

The cross-sectional study was carried out from September 2019 and January 2020 in 179 pus samples collected from the patients admitted in the different wards of our hospital. The pus samples were collected aseptically from infected wound surfaces, labelled and taken to the Laboratory immediately for culture and antimicrobial susceptibility testing within 1 hour. In the laboratory, the pus samples were inoculated in Nutrient Agar, Blood agar and incubated overnight at 37^0 C for 24 h.

Staphylococcus aureus was identified as Gram Positive Cocci (GPC) colonies in clusters, β-hemolytic colonies on blood agar, catalase and coagulase production, and yellow colony surrounded by yellow zone in Nutrient Agar. It was confirmed by slide and tube coagulase test and mannitol fermentation. The S. aureus strains were then tested for methicillin resistance by Kirby-Bauer Disc Diffusion technique on Muller Hinton Agar (MHA) using cefoxitin (30µg) disk. The Isolates showing a zone of inhibition of 10mm or less were declared as methicillin-resistant and >20mm was considered as methicillin sensitive.

For antibiotic susceptibility, antibiotics tested were Amikacin (30 µg), Erythromycin(15 µg) , Cephalexin (30 µg),Gentamicin (10 µg), Teicoplanin (30 µg), Linezolid(30 µg), Vancomycin(30 µg), Ciprofloxacillin (5 µg),Tigecycline(15 µg), and (Cotrimoxazole (25 µg).

Susceptibility patterns were interpreted by comparing the zones of inhibition according to the Clinical and Laboratory Standards Institute (CLSI, 2014) guidelines. A standard strain of S. aureus (ATCC29213) was used as control.

III. Result

The total number of swabs collected between September 2019 and January 2020 was 179. Out of these, 179 pus samples, 134 samples (74.86%) showed the presence of *Staphylococcus aureus.* Of these, 39 (29.10%) were identified as *methicillin-resistant S. aureus* and 95 (70.90%) were *methicillin-sensitive S. aureus.*

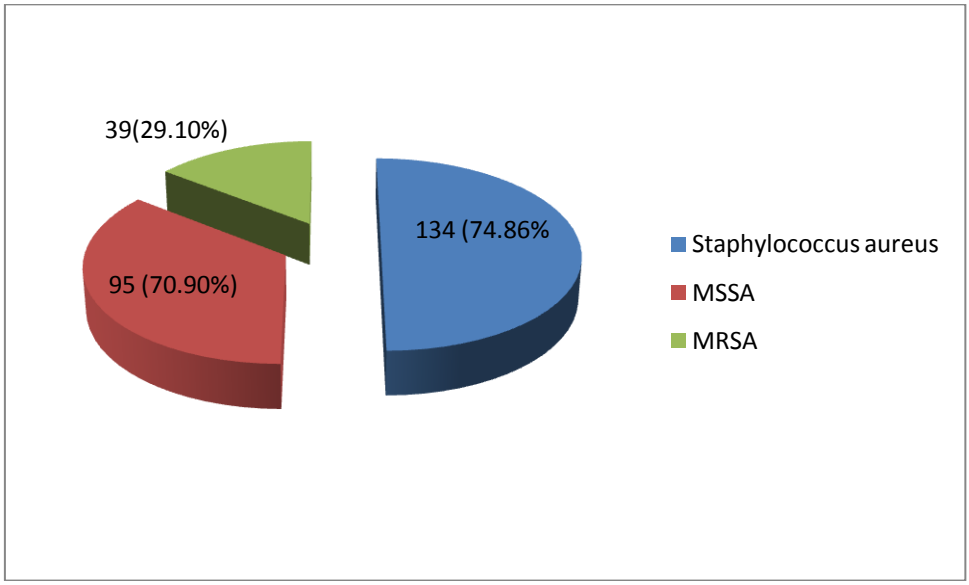

Table 1. Distribution of Staphylococcus aureus and MRSA in the pus samples.

The 39 *methicillin-resistant S. Aureus* showed resistance to—Amikacin (100%), Erythromycin (100%), Cotrimoxazole (71.5%), Cephalexin (54.15%), Ciprofloxacin (30.5%) and Gentamicin (88.1%). They were susceptible to Linezolid (96.6%) and were 100% susceptible to Tigecycline, Teicoplanin and Vancomycin.

RESISTANCE PATTERN OF DIFFERENT DRUGS

DRUGS	% OF RESISTANCE
Amikacin	100%
Erythromycin	100%
Gentamicin	88.1%
Co-trimoxazole	71.5%
Cephalexin	54.15%
Ciprofloxacin	30.5%
Linezolid	3.40%
Tigecycline	0%
Teicoplanin	0%
Vancomycin	0%

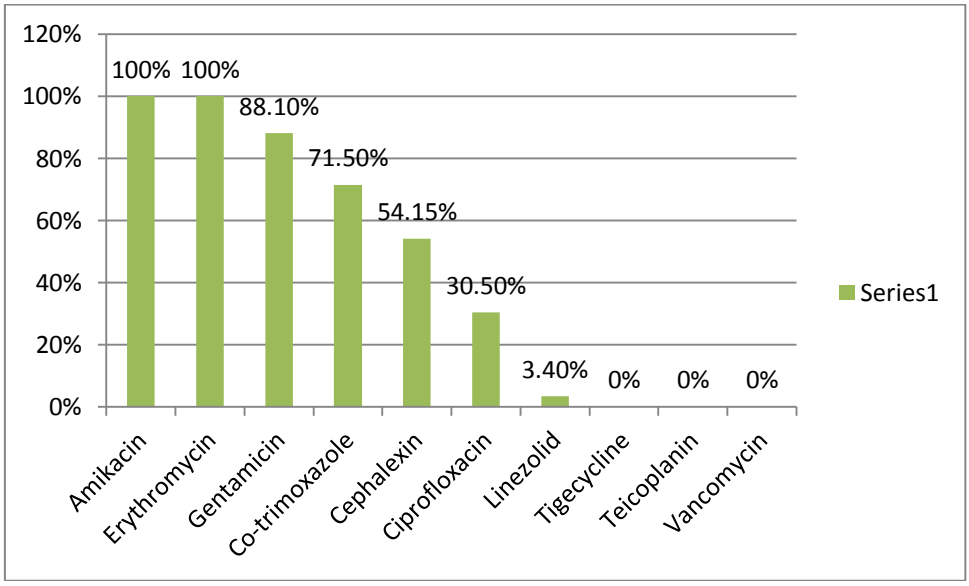

Table 2. Sensitivity pattern of Staphylococcus aureus to different drugs

IV. Discussion

MRSA is a major nosocomial pathogen responsible for significant patient morbidity and morbidity showing high prevalence. [17] In our study, from 179 pus samples, we isolated *Staphylococcus* from 134 samples of which *MRSA* was found in only 39 isolates (29.10%). This is very similar to a study conducted by Uma devi et al [2] and Sangram Singh et al.[18] Uma devi et al [4] reported 59 isolates of (43.3%) *Staphylococcus aureus* and 17 (28.8%) *MRSA* from 136 pus samples. Sangram Singh et al [18] isolated 80 (40%) *Staphylococcus aureus* from 200 samples; *MRSA* was identified in 28 isolates.

Arti Tyagi et al [19] between December, 2001 and March, 2002, conducted a study on 2,080 pus samples collected from surgical wounds at AIIMS, New Delhi. They reported that the *MRSA* prevalence rate was 44% amongst all the S. aureus isolates. All the *MRSA* isolates were found to be sensitive to Vancomycin and Teicoplanin.

A similar study in Ethiopia in 1360 samples isolated 194 (14.3%) isolates of *Staphylococcus aureus*; of which 34 (17.5%) were *MRSA. Staphylococcus aureus* was isolated highest from pus (118/194—55.4%) but none from CSF and urethral discharges. [20]

The observed high prevalence of *MRSA* in our study and other studies is probably due to misuse of antibiotics.

MRSA wound infections were significantly found to be associated in patients with poor immunity status and the admitted patients. This may be due to resistant bacteria strain cross-contamination in hospitals. Healthy people may carry *MRSA* asymptomatically for long periods of time, but patients with compromised immune system are at a significantly greater risk of symptomatic infections [21, 22].

The current study showed that the *MRSA* isolates showed 100% resistance to Amikacin and Erythromycin and high resistance to Gentamicin (88.1%), cotrimoxazole (71.5%), Cephalexin (54.15%), (Ciprofloxacin (30.5%). But these isolates showed 100% susceptibility to Tigecycline, Teicoplanin and Vancomycin. This variation in drug resistance may be attributed to availability and indiscriminate use of certain drugs.

A similar study conducted by Mohammed Fareed Khan et al [23] in Bastar, Central India found 631 isolates of *Staphylococcus aureus* from 2074 pus samples—of this, 215 isolates were *MRSA*. These *MRSA*

isolates showed varied resistance to different antibiotics—82.8% to cotrimoxazole; 77.2% to tetracyclines; 68.8% to gentamicin; 64.2% to ciprofloxacin; 1% to Vancomycin and none (0%) to Linezolid.

V. Conclusion

Thus, high prevalence of multidrug resistance predisposes patients to infection with *MRSA* isolates, emphasizing the need for improved infection control practices and guidelines for the use of antibiotics. Guidelines thus need to be formulated for prevention and control of *MRSA* isolates in different hospitals.

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