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Original Research Paper

Microbiology

BURDEN OF METHICIILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) & METALLO-BETALACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA (MBL-PS.) AMONG ORGANISMS CAUSING SURGICAL SITE INFECTION AT A TERTIARY CARE HOSPITAL IN WESTERN RAJASTHAN

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ABSTRACT

Introduction: Hospital acquired infections (HAI) have always been a menacing problem for healthcare providers; with surgical site infections (SSI) being a major contributor to it. The incidence of MRSA and MBL-Ps. have been steadily increasing over the past few years resulting in limitation of therapeutic options available for patients with SSIs. **Objective:** This study was undertaken to detect MRSA among the Staphylococcus aureus isolates and MBL production among pseudomonas isolates from clinically diagnosed cases of SSI at a tertiary care hospital in Rajasthan. **Materials and Methods:** This study, conducted in Department of Microbiology, JLN Medical College, Ajmer from November 2022 to April 2023, included 246 patients clinically diagnosed wth SSI. Samples were processed as per standard microbiological techniques (CLSI2022 /M100-Ed32). Staphylococcus aureus when isolated was studied for MRSA using cefoxitin disc diffusion test and pseudomonas isolates were screened for MBL production using imipenem-EDTA combined disc test respectively. The study was approved by our Institutional Ethical Committee. Statistical analysis was done using Microsoft Excel, SPSS version 20 Windows software program. **Results:** Among 246 clinically suspected patients from total 1950 registered cases, 220 were culture positive accounting for prevalence 12.61%. MRSA and MBL production was observed in 44.44% of Staphylococcus aureus isolates & 21.87% of all Pseudomonas isolates respectively. **Conclusion:** The prevention of SSI encompasses meticulous operative technique and a variety of preventive measures aimed at neutralizing the threat of bacterial, viral, and fungal contamination posed by operative staff, the operating room environment, and the patient's endogenous skin flora.

KEYWORDS : Hospital acquired infections (HAI) surgical site infections (SSI) ,CLSI

BACKGROUND

Surgeries invariably impair the first line of host defenses between environmental microbes and the host's internal milieu, resulting in increased re-admission rates, morbidity, mortality, length of stay in hospital & treatment cost to the patients. [1] Recent studies reported that SSI rate ranges from 19.4% to 36.5% all over the world, whereas in India it ranges from 3% to 12 %.[2,3]. Since 60% of SSIs are preventable with evidence-based guidelines. SSI is one of the quality metrics frequently used to assess quality of surgical care, & subsequently to performance ranking, patient satisfaction and reimbursement.[4]

According to definition by United States Center For Disease Control And Prevention(CDC), SSI's are defined as an" infection that occur within 30 days after the operative procedure if no implant is left in place, or within 1 year if implant is in place, and the infection appears to be related to the operative procedure."[5]

Infectious agents may cause SSI from endogenous or exogenous sources.[6] The common organisms encountered in post-operative wound infections are Staphylococcus aureus, Coagulase-Negative

Staphylococci, Enterococci, Proteus, Pseudomonas, Escherichia coli and Klebsiella species[7] The incidence of Methicillin-resistant Staphylococcus aureus (MRSA) and metallo-beta lactamases (MBL) producing organisms have been steadily increasing over the past few years resulting in limitation of therapeutic options[7]

Staphylococcus aureus, an important etiological agent of hospital and community acquired infections exhibits Methicillin resistance which is mediated by the *mecA gene* which codes for an additional penicillin binding protein, PBP2 or PBP2a..[8]

Pseudomonas aeruginosa is reported to be amongst the leading cause of nosocomial infection & Carbapenems are drug of choice for severe pseudomonas infections.[9] However, this scenario has changed with the emergence of carbapenem resistant strains of Pseudomonas aeruginosa (mainly due to the production of metallo-beta-lactamases, MBLs) associated with clonal spread. MBLs are broad-spectrum enzymes that hydrolyse most of the beta lactam antibiotics except monobactams and are not inhibited by conventional beta-lactamase inhibitors like clavulanic acid or sulbactam. They belong to the Class b of Ambler Classification of β lactamases wherein Zn (Zn^{+2}) is used to break the amide bond.[10] With this background, this study was undertaken to determine the SSI rates & MRSA among the Staphylococcus aureus isolates and MBL production among pseudomonas isolates from clinically diagnosed cases of SSI, which would help to institute better prophylactic measures and appropriate, timely and accurate therapeutic measures to reduce the cost of treatment and morbidity of the disease.

MATERIALS AND METHODS:

A cross-sectional study, conducted in Department of Microbiology, JLN Medical College, Ajmer from November 2022 to April 2023, included 246 consecutive samples from clinically diagnosed cases of SSI admitted in General Surgery Department of our hospital (after obtaining informed consent). Patients were actively followed up and relevant samples (pus and pus swab) from postoperative SSI cases were collected aseptically. Samples were subjected to direct microscopy by Gram stain, cultured and bacterial pathogen grown were identified by conventional bacteriological methods. [11,12] The data were tabulated and statistically analyzed using Microsoft Excel, SPSS version 20 Windows software program. Pearson's Chi-square test was used to test the strength of association between the variables with $P < 0.05$ taken as statistically significant.

Control strains used :

I. Escherichia coli ATCC 25922

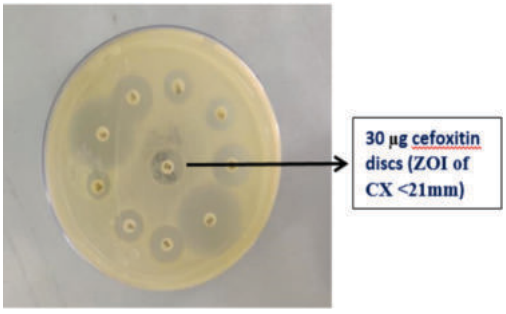
II. Staphylococcus aureus ATCC 25923

Antibiotic susceptibility test

Antibiotic susceptibility test was performed by Kirby-Bauer disk diffusion method & susceptibility was tested against several antibiotics procured commercially from Hi-Media Laboratories Ltd, Mumbai. The diameter of the zone was measured and interpreted according to the CLSI guidelines.[13]

Detection of MRSA isolates:

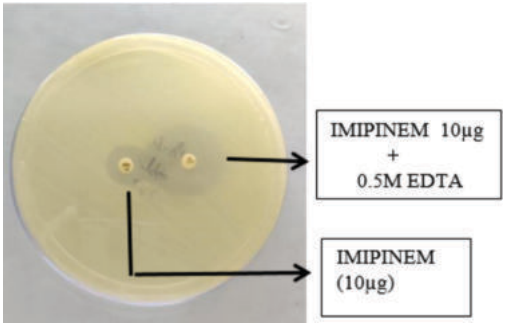
Methicillin resistant *Staphylococcus aureus* detection was done using cefoxitin 30 µg. Those isolates showing zone of inhibition <21 mm considered as MRSA.[13]



MRSA STRAIN

Screening of MBL producing Pseudomonas aeruginosa using Imipenem (IMP)-EDTA combined disc test :

Test organisms were inoculated onto plates with Mueller Hinton agar, as recommended by the CLSI. After allowing it to dry for 5 minutes, two imipenem discs (10µg),one with 0.5 M EDTA and the other plain were placed on the surface of the agar plate approximately 30mm apart. The plates were incubated overnight at 37 °C. An increase in the zone diameter of ≥ 7 mm around imipenem+EDTA disc in comparison to imipenem disc alone indicated production of MBL. [13]



MBL- Imipenem-EDTA Combined Disc Test

OBSERVATIONS & RESULTS:

Demographic & Clinical data

A total of 1950 patients underwent different types of surgeries comprising of elective as well as emergency during this period. The most frequently received sample was pus swab 157(63.83%) followed by pus aspirate 78(31.70%) and purulent discharge from drain 11(4.47%) respectively. Out of these, 1248 were Elective cases & 702 were Emergency cases. 246 surgical site infections were documented and hence, the overall prevalence of surgical site infection rate during the study period was 12.61%(n=246).

Culture characteristics & isolates recovered -

Out of total 251 SSI cases, aerobic growth on culture was observed in 220 cases (89.43%) whereas 23 (10.57%) cases showed no growth on culture. Microscopic studies of the gram stained smear showed isolation of total 228 microbial pathogens. Out of these, 58 (25.44%) were identified as Gram-positive Cocci, 154 (67.54%) as Gram-negative Bacilli, and 16 (7.03%) as Mixed isolates. Among the GPC, *Staphylococcus aureus* 27(11.84%) was the most common isolate while among the GNB, *E.coli 62* (62.22%) was the predominant with 32 (14.03%) Non fermenters.i.e *Pseudomonas aeruginosa*.

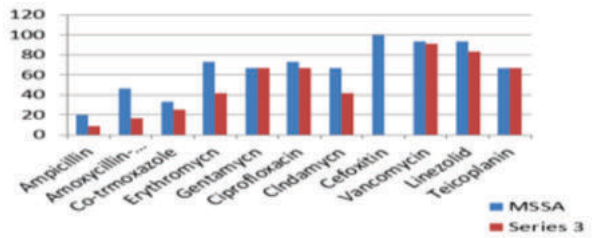
Antibiotic susceptibility pattern of SSI isolates

Antibiotic resistance profiles were reported for the organisms isolated from surgical incision site of infected patients.The antibiogram of Gram Positive Organisms showed maximum susceptibility to vancomycin(90.48 to 93.34%) & linezolid (76.20% to 93.34%), whereas they were highly resistant to ampicillin (80% to 95.23%) and amoxycillin-clavulanic acid (53.34% to 76.19%).

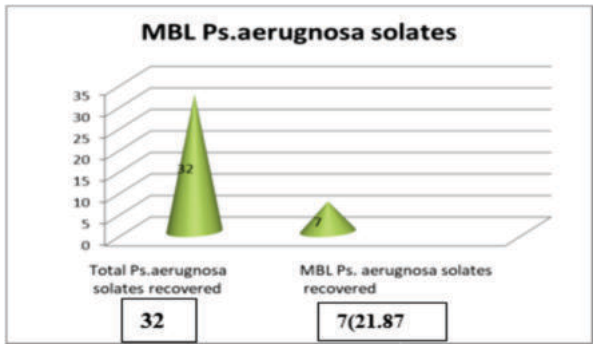
The antibiogram of Gram-negative isolates showed resistance to amoxycillin-clavulanic acid and cephalosporins, moderate susceptibility to fluoroquinolones and aminoglycoside, and good susceptibility to carbapenems.

Prevalence of MRSA & MSSA isolates

Total No. of S.aureus isolates	MRSA		MSSA	
	No.	%	No.	%
27	12	44.44	15	55.56



Antimicrobial Susceptibility of MSSA & MRSA Isolates



Prevalence of MBL *Ps.aeruginosa* isolate

DISCUSSION -

The rate of SSIs varies worldwide from hospital to hospital ranging from 2.5% - 41%, which largely depends on hospital policies and guidelines, other contributory factors being sample size, study design and study period.

The overall SSI incidence rate of 12.61% in our study is in consensus with infection rates observed in earlier Indian studies (Narula H.et al., 2020, *Kumar A et al., 2017*) [1, 3].(Cruse PJE & Frood, 1980; Creamer et al., 2002)[14, 15] SSI rates assessed in these studies over a prolonged period of 10–16 years, indicates that larger groups studied over a longer duration give a better assessment of SSI rates. A higher infection rate in developing countries emphasizes the need of better implementation of infection control practices along with a proper surveillance system for the use of antibiotics.

Taking the host factors into consideration, SSI incidence was maximum among patients of the age 51-60 years (24.0%) in the study. Similar findings have been reported in study by *Kumar A et al., 2017*[3] Increased age is associated with various predisposing factors impairing immunity like diabetes, anemia which could be attributed to this trend of increasing

incidence of SSI with increasing age. However, age, gender, and type of surgery were not statistically significant variables rin the development of SSI ($P > 0.05$).

There was a male preponderance in developing SSIs in our study when compared to the female patients, such a finding has been documented by Kasukurthy R.et al,2020 [16]

Staphylococcus aureus was the most important pathogen among GPC, a major pathogen responsible for various nosocomial infections. A total of 12 (44.44%) MRSA were detected using cefoxitin disc diffusion technique. These results are in accordance with Abbas, *et al* (India) [17] 40.20%, Seifi *et al*. (Iran) [18] 41.7%, and Mohanasoundaram (India) [19] 39.16%, although high prevalence of MRSA have been reported by Venkata *et al*. (India)[20] 75.27%.

This study revealed that sensitivity of MRSA isolates to vancomycin and linezolid 91.67% & 3.34%.respectively. Vancomycin and linezolid were found to be the most sensitive drugs against *S. aureus* in studies by Agarwal, Singh et al. (2015) [21] .Linezolid, a member of the new oxazolidone class of antibiotics is highly active in vitro against MRSA and has excellent oral bioavailability and constitutes the drug of choice against MRSA infection besides vancomycin. Our study supported this.

About 58.33% of MRSA isolates were resistant to erythromycin, 33.33% to ciprofloxacin, 58.33% to clindamycin, 33.33% to gentamicin, 33.33% to co-trimoxazole. Contrary to the reports by Qureshi *et al*. [22] who reported 98.9% resistance to ciprofloxacin and 97.8% to gentamicin, Sharma *et al*. [23] reported 87.3% of MRSA strains were resistant to co-trimoxazole that is much higher when compared to our study. The occurrence of an MBL – positive isolate in a hospital setting poses a therapeutic problem as well as a serious concern for infection control management. Pseudomonas aeruginosa producing MBL was first reported in India in 2002 [24]. Currently there are no recommendations available from Clinical laboratory standards Institute (CLSI) or elsewhere for the detection of MBLs in *Pseudomonas aeruginosa* [13]. A few phenotypic methods have been published for MBL detection; however, the results have shown that no method alone is able to detect all these enzymes, probably due to the genetic variability of these enzymes [25]. PCR gives specific and accurate results; its use is limited to only a few laboratories because of its high cost and because of the different types of MBLs which are present worldwide [26]. In this study we observed MBL production in 21.87% of the *Pseudomonas aeruginosa* isolates. Similar observations were made by Radhika A. et al[25] and Nagaveni *et al* [27] who reported 15% & 20% MBL producting *Pseudomonas aeruginosa* isolates respectively. Contrary observatons were documented by Smita Sood et al with (72.63%) MBL prevalence.[2]

One limitation of the present study resides in the absence of a PCR analysis for the validation of phenotypic methods. Nevertheless, our study shows a good sensitivity with cefoxitin disc diffusion test for MRSA isolates and imipenem-EDTA combined disc test forMBL production in pseudomonas isolates as a screening tests.

CONCLUSION-

There is need of quality surgical care considering all 3 factors, i.e. host, environmental and microorganism. Antibiotic stewardship and tailor-made prophylactic policies based on local susceptibility data should be instituted.

Emergence of MRSA & MBLs producing *Pseudomonas aeruginosa* in our clinical strains is alarming and reflects excessive use of carbapenem. Therefore early detection and prompt instillation of infection control measures is important to prevent spread of MBLs to other gram negative rods. There is no standardized method for MBL detection, though detection by polymerase chain reaction is highly accurate and reliable, but its accessibility is often limited to reference laboratories. Thus, laboratory methods including culture and antimicrobial susceptibility testing with routine screening of S.aureus and Paeruginosa for MRSA & MBL production respectively should be done for proper diagnosis and management of surgical site infections.

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Conflicts of interest

There are no conflicts of interest.

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