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EPIDEMIOLOGY OF MRSA IN SURGICAL SITE INFECTIONS A STUDY ON MICROBIAL DISTRIBUTION AND ANTIBIOTIC RESISTANCE

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ABSTRACT

Background: Surgical site infections [SSIs] are represents a significant challenge in health care, and methicillin-resistant Staphylococcus aureus [MRSA] has become a major issue. The current research is, therefore, aimed to investigate the epidemiology of microbes associated with SSIs, with a major focus on the prevalence of MRSA and its accompanying resistance phenotypes. A sample size of 130 SSI specimens was taken to outline the microbial profile. Detection of MRSA was done through conventional phenotypic techniques, which were then confirmed by polymerase chain reaction [PCR], and the antibiotic resistance patterns also evaluated. Staphylococci species were the most commonly isolated microorganisms [52.3%], then Escherichia coli [20.0%], and Klebsiella spp. [16.1%]. Among the 61 isolates of Staphylococcus aureus, 45 [73.7%] isolates were found to be MRSA. Susceptibility testing showed marked resistance rates of erythromycin [66.7%] and clindamycin [51.1%] with better susceptibility to linezolid which was 97.8. The gene of mecA was identified in all MRSA isolates. These results highlight the need to have effective diagnostic modalities and excellent antibiotic stewardship. Continued surveillance and custom-designed infection control interventions play a crucial role in ensuring the successful management of the antibiotic-resistant pathogens. The research provides critical information on SSI epidemiology and the prevalence of MRSA, confirming the importance of molecular diagnostics and the need to be alert on the practice of infection control in the health care setting.

Keywords: Staphylococci; Methicillin-resistant Staphylococcus aureus; Surgical site infections ; Antibiotic resistance and susceptibility

INTRODUCTION

Surgical site infections [SSIs] are significant issue in the contemporary healthcare facilities, as they have the potential to compromise the health and well-being of patients and outcomes [1]. Methicillin-resistant Staphylococcus aureus [MRSA] is one of the most dangerous opponents due to its enormous resistance to many antibiotic classes, making the treatment a difficult task and raising morbidity and mortality [2]. The comprehensive understanding of the prevalence of MRSA in SSIs possess a major role for development of effective infection control strategies, optimization of antibiotic stewardship programmes, and eventually improving patient outcomes [3].

MRSA, a remote resistant branch of S. aureus, has developed the mechanisms that render resistant to β-lactam antibiotics, such as methicillin, making it less responsive to the conventional antimicrobial methods [4]. Consequently MRSA-induced SSIs are characterized by a high rate of therapeutic failures; prolong hospitalization, expensive, healthcare and mortality compared to infections that can be attributed to methicillin-sensitive Staphylococcus aureus [MSSA] or other pathogens. Furthermore, MRSA presents a clinical impact to the overall health of any population due to its ability to community level and causing epidemics and intensifying the global burden of antimicrobial resistance [5, 6].

The epidemiological landscape of the MRSA in SSIs is highly heterogeneous both in terms of geographic locations, healthcare settings, and patient groups [7]. Ghia et al. have outlined different rates of prevalence of MRSA in SSIs, emphasizing the role of patient demographics, surgical workup, antimicrobial use, infection control, and microbial characteristics [8]. However, in spite of the fact that MRSA has, at all times, been related to the healthcare-associated infection [HAIs], the designation of community-associated MRSA [CA-MRSA] as an independent entity has underscored its potential to arise in individual with the absence of the classic HAI-related risk factors. Such a subtle approach to the issue of MRSA epidemiology in SSIs is essential to develop specific prevention and containment strategies that would be properly tailored to the unique healthcare environment [8].

Multiple predisposing factors contribute MRSA related SSIs. These include patient-related variables such as age, comorbidity, and immunosuppressive conditions; surgical variables, such as the type of procedure, duration of surgery, and the possibility of contamination of the surgical site; and healthcare variables, such as prior hospitalization, invasive interventions, and antimicrobial exposure. The introduction of risk factors with in the community, especially those related to contact sports, imprisonment, and illicit drug consumption has expanded the vulnerable range, far beyond the boundaries of the conventional medical care contexts. Identifying and

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mitigating these risk factors are essential for preventing MRSA SSIs and improving surgical outcomes [9].

The continual widespread occurrence of MRSA in the postoperative surgical site infections [SSIs] highlights the importance of the strict adherence to the evidence-based infection prevention and control measures in the healthcare organizations. Targeted approaches to preventing the dissemination of MRSA such as careful hand washing, broad-based environmental decontamination, prudent antimicrobial stewardship, active surveillance, and systematic decolonization interventions are considered central players in preventing SSIs and also curtailing the spread of multidrug-resistant organisms, as outlined by Green and colleagues [10]. The use of molecular epidemiology, in particular whole-genome sequencing has also changed the face of surveillance, making it now possible to accurately phylogenetically track the strains of MRSA. This genomic urgency allows target containment measures to be developed and hence improves outbreak management and forestalls nosocomial transmission. Furthermore, the formulation of cross-disciplinary teamwork, including surgeons, infection control practitioners, clinical microbiologists, and epidemiologists, has the utmost significance in the arrangement of holistic, integrative strategies aimed at mitigating the MRSA-related SSIs [11].

This research aims to investigate the prevalence of Methicillin-resistant Staphylococcus aureus [MRSA] in post-operative wound infections.

MATERIALS AND METHODS

The current study was conducted in the Microbiology department of Kalpana Chawla Government Medical College, Karnal. It included patients who had undergone operative processes in Departments of Surgery, Obstetrics and Gynaecology, and Orthopaedics. This group of operations was stratified into four groups, including clean, dirty, contaminated, and clean-contaminated and excluded those procedures that required compromised skin integrity, e.g., abscess drainage or burn wound management.

For this study a total of 130 samples were collected from clinically diagnosed SSIs following the guidelines of the CDC. S. aureus was recognized based on morphology of colonies, gram staining results, yellow pigmentation, and catalase tests. It was further confirmed by using tube and slide coagulase tests and bacterial growth on Mannitol salt agar, in accordance with the CLSI protocols [12, 13]. S. aureus ATCC 25923 strains was used as a positive control for comparison. The Antibiotic Susceptibility Test [AST] was performed with the use of the following antimicrobial agents: erythromycin [15µg], ciprofloxacin [5µg], levofloxacin [5µg], clindamycin [2µg], cefoxitin [30µg], linezolid [30µg], and doxycycline [30µg] disc concentrations.

In order to isolate genomic DNA, HiPurA® Bacterial Genomic DNA Purification Kit [SKU: MB505] was used and for PCR amplification, the Hi-PCR® MRSA [Multiplex] Probe PCR Kit [SKU: MBPCR133] was used [14, 15]. The statistical analysis was done with the help of the IBM SPSS software version 25 and other relevant statistical software used to analyze the data to establish meaningful insights and conclusions of the study findings.

RESULTS

Overall, 130 specimens were analyzed with each microorganism playing a contributing differently to the distribution. Staphylococci formed the major group comprising 52.3% of the total samples. This was followed by Escherichia coli [E. coli] at 20.0% and Klebsiella spp. at 16.1%. Other bacteria isolated were Pseudomonas aeruginosa [4.6%], Acinetobacter baumanii [3.1%], Enterococcus spp. [2.3%], Citrobacter [0.8%] and Proteus vulgaris [0.8%] [Table 1].

Table1: Distribution of various microorganisms identified in the study population

Microorganism	Total [%]
Staphylococci	68 [52.3]
E. coli	26 [20.0]
Klebsiella spp.	21 [16.1]
Pseudomonas aeruginosa	6 [4.6]
Acinetobacter baumanii	4 [3.1]
Enterococcus spp.	3 [2.3]
Citrobacter	1 [0.8]
Proteus vulgaris	1 [0.8]
Total	**130 [100.0]**

Out of 61 Staph aureus strains, 45 [73.7%] strains were identified as MRSA phenotypically

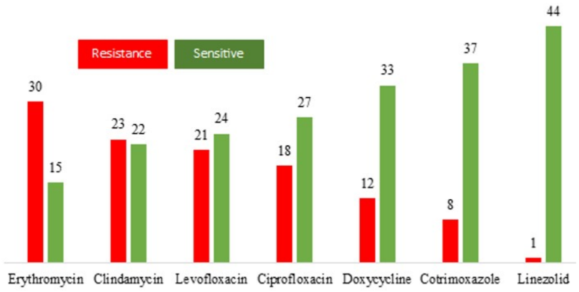

Figure 1: Sensitivity and resistance pattern to various antimicrobial agents

Out of 61 Staph aureus strains, 45 [73.7%] strains were identified as MRSA phenotypically on the basis of cefoxitin disc test and 16 [26.2%] strains were identified as MSSA. Among the participants infected with MRSA, there were a total of 10 females and 35 males, accounting for 22.2% and 77.8% of MRSA infections, respectively cases [15.60% of all MRSA cases]. All the 45 strains of MRSA were examined for inducible clindamycin resistance by D-test, where 17 [37.8%] showed positive result for inducible clindamycin resistance. 15 were sensitive to both, 23 were resistant to both, 7 were resistant to only erythromycin.

The isolates under investigation were found to have 12 [26.7%] doxycycline resistant of the total and the number of susceptible was 33 [73.3%]. In linezolid 44 [97.8%] of the isolates were susceptible with only one [2.2%] isolate being resistant. Cotrimoxazole had the resistance rate of 8 isolates [17.8%] and susceptibility rate of 37 isolates [82.2 %]. The results are relevant to the knowledge of the antimicrobial susceptibility trends among the methicillin-resistant isolates of the Staphylococcus aureus and can be used to refine the therapeutic regimen and infection-control measures.

Genotypic Analysis of Staphylococcus aureus: All 45 MRSA strains were tested for mec A and mec C gene by RT-PCR, mec A gene was found to be positive in all 45 strains.

DISCUSSION

Phenotypic Analysis

Distribution of Microorganisms Identified in SSI

In our study, 130 positive isolates from SSI samples were analysed. Staphylococcus aureus formed the major group comprising 61 [52.3%] of the total samples. This was followed by Escherichia coli [E. coli] at 20.0% and Klebsiella spp. at 16.1%. Other bacteria isolated were Pseudomonas aeruginosa [4.6%], Acinetobacter baumanii [3.1%], Enterococcus spp. [2.3%], Citrobacter [0.8%] and Proteus vulgaris [0.8%]. In a meta-analysis conducted by Ghia C and colleagues, a pooled prevalence of MRSA was 26.8% [8]. In the second meta-analysis involving Hasanpour A et al., 119 eligible studies [a total of 164,717 participants] across 29 nations revealed that the pooled global prevalence of MRSA was only 14.69% [16].

The detection of MRSA using the phenotypic tests such as cefoxitin disc diffusion test is completely congruous with the normal diagnostic protocols suggested by the healthcare bodies and professional associations in our study. In present study, out of 61 Staph aureus strains, 45 strains were identified as MRSA phenotypically on the basis of cefoxitin disc test. All the results were consistent with the PCR result with all strains giving positive results for mecA gene.

A study done by Abdelwahab et al. the cefoxitin disc diffusion test was found to have a better diagnostic capability of identifying the methicillin resistance in the S. aureus isolates than the mecA PCR. Specifically, the sensitivity of the cefoxitin disk diffusion test was determined as 100% i.e. the test can detect the presence of methicillin resistance in the tested isolates [17]. In a study conducted by Perazzi et al., the sensitivities of cefoxitin disks for all CoNS [Coagulase-Negative Staphylococci] species were reported at 80%, while the specificities were 100%. This result indicates that cefoxitin disk testing is both specific [recognizes CoNS species] and has good sensitivity; however, sensitivity can be inconsistent, which implies that the test is not sensitive enough to identify all CoNS isolates [18]. Comparatively, Bhattacharya et al. [19] reported 1049 cases of Staphylococcus aureus [34.93%] out of 3003 SSI cases, with Escherichia coli [611, 20.34%], Klebsiella spp. [543, 18.08%], Pseudomonas spp. [240, 7.99%], and Acinetobacter spp. [225, 7.49%] following.

Among Staphylococcus aureus, 267 strains were identified as MRSA [25.45%] which was much less than our study.

Gender-wise Distribution of SSI

In the study by Bhattacharya S et al, MRSA was isolated from 167 [62.54%] male patients and 100 [37.45%] female patients with surgical site infection [19]. Similar gender distribution was also observed by Pathak A et al in study of 720 patients where 76% being male and 24% female are found [20]. In our study we are also found the same result where 35 [77.8%] males and 10 [22.2%] female participants infected with MRSA. According to the meta-analysis by Ghia C et al [8], MRSA infection was more prevalent among male patients, accounting for 60.4% [95% CI: 53.9%-66.5%], compared to female patients, who represented 39.6% [95% CI: 33.5%-46.1%] of MRSA infections.

D-test specifically for MRSA

In our study, 37.8% of MRSA samples showed a positive outcome for inducible clindamycin resistance by D-test, constitutive resistance was seen as 51.1% and 15.5% were negative for D-test. While 62.2% yielded invalid results, indicating a negative outcome for the D-test. Che et al. [21] found that the most common MLSB phenotype among isolates was inducible clindamycin resistance [iMLSB or D-test positive], accounting for 52.3% of the total. Constitutive resistance [cMLSB or resistance to both erythromycin and clindamycin] and the MS phenotype [D-test negative] were observed much less frequently at 6.6% and 4.0%, respectively [21]. In the study by Grace F et al., all MRSA isolates processed exhibited positive D Test results [22]. Out of 209 S. aureus isolates investigated by the Manjhi et al., 22 % of the isolates showed an inducible phenotype of macrolide-lincosamide-streptogramin B [iMLSB], 19.6 % were methicillin-sensitive [MS], and 17.7% of the isolates had a constitutive iMLSB phenotype. The prevalence of the inducible, constitutive, and MS phenotypes was higher in MRSA [23].

Test conducted for MRSA

In the present research, the 45 analyzed samples all positive in test of fermentation of mannitol, test of slide coagulase, and tube coagulase were found to be 100 % positive of each test on MRSA samples. But contra, Kateete D et al [12] have noted cases of weak or negative responses of coagulase-negative Staphylococcus aureus isolates, which may be MRSA with the tube coagulase test. They also identified methicillin-resistant Staphylococcus aureus strains that were mannitol-negative [12]. In the study by Karmakar A et al., it was discovered that 92% of the isolates tested positive for coagulase, indicating the presence of Staphylococcus aureus. However, the remaining strains, although identified as Staphylococcus aureus through PCR analysis, were coagulase-negative [24].

Antibiotic resistance/susceptibility profile of MRSA

The antibiotic susceptibility testing conducted in our study showed different resistance patterns of the isolated strains. In case of linezolid, 97.8% of the isolates [44/45] were susceptible and only 2.2% of the isolates were resistant. Conversely, the rate of resistance to cotrimoxazole was higher [8 isolates 17.8% resistant and 37 isolates 82.2 susceptible]. The rate of resistance to levofloxacin, doxycycline, and ciprofloxacin was 46.7, 26.7, and 40.0 respectively. Such results highlight the need to conduct regular monitoring of the trends of antibiotic resistance to inform relevant empirical treatment and fight the process of developing multidrug-resistant pathogens. In the research by Bhattacharya et al., [19] it was found out that the MRSA strains were 100% sensitive to linezolid, which demonstrates that it is an effective treatment option.

This was followed by high sensitivity rates to levofloxacin [75.66%] and doxycycline [72.28%]. Clindamycin and cotrimoxazole also demonstrated moderate sensitivity, with rates of 56.55% and 28.09%, respectively. In Ranjan K et al.'s [25] study, Linezolid and Vancomycin demonstrated exceptional efficacy against both MRSA and MSSA strains. Amikacin was the most effective antibiotic used against MRSA [90.2%], and then Clindamycin [56%], Ciprofloxacin [36.2%], Erythromycin [32%] and Cotrimoxazole [30.3%] were found to be highly effective. In the case of MSSA, amikacin was still effective [92.6%] followed by Ciprofloxacin [81.6%], Clindamycin [72%], Erythromycin [63%] and Cotrimoxazole [60.2%]. In general, MRSA isolates were more drug-resistant than MSSA, and 24 % of MRSA isolates were resistant to more than one drug, which was 9 % of MSSA isolates [25]. In Rajaduraipandi K et al.'s study, a high degree of antibiotic resistance was observed among clinical MRSA strains. Nearly all isolates of MRSA [99.6%] exhibits resistance to penicillin about 93.6%. Ampicillin resistance was detected in a substantial percentage of strains whereas gentamicin, co -trimoxazole, cephalexin, erythromycin and cephotaxime resistance levels were more heterogeneous. It is notable that both clinical and carrier isolates had a high prevalence of multidrug resistance, making it crucial to take appropriate measures to mitigate antibiotic stewardship. However, all strains from both clinical and carrier subjects remained sensitive to vancomycin [26].

Genotyping of MRSA by RT-PCR

All the 45 cefoxitin resistant strains were subjected for PCR and showed presence of mecA gene thus proving consistency between the phenotypic and genotypic methods and mec A gene to be the prominent gene responsible for methicillin resistance and the fact is that staphylococcus is the predominant organism in SSIs within health care settings. Our results from the study on the detection of the mecA gene for MRSA using reverse transcription polymerase chain reaction [RT-PCR] indicate a high prevalence of the gene [100%] among MRSA. Nevertheless, none of the samples had the mecC gene showing no presence of the individual genetic source of methicillin resistance. Additionally, all the samples were positive in IC gene, which further confirmed the presence of Staphylococcus aureus.

These genotyping findings illuminated the molecular characteristics of strains of Staphylococcus aureus in question, especially in terms of methicillin resistance markers. PCR analysis in the study by Bhattacharya et al. [19] showed that 96.25 % of cefoxitin resistant Staphylococcus aureus contained the mecA gene, which is consistent with the results of methicillin resistance. In the research conducted by Kocagoz S et al., the most common form of resistance observed among the staphylococcal strain isolates was the presence of mecA with 94% of the cases being positive [27]. Banerjee et al. discovered that every tested isolate harbored the methicillin resistance gene mecA and some had mecC and SCCmec integration at attB or orfX. Other genotypic AMR assays of positive blood cultures found less pathogen, targeting either a wide range of Gram-positive bacteria or Staphylococcus aureus and methicillin resistance exclusively. The test has good performance in monomicrobial cultures, with a sensitivity and specificity of greater than 95 % of most targets [28].

CONCLUSIONS

In conclusion, the study reiterates the fact that staphylococcus is the predominant organism in SSIs within health care settings. Phenotypic and genotypic studies showed that a significant percentage of Staph aureus strains showed methicillin resistance, and thus, the issue of overcoming MRSA infections remains a challenge. Antibiotic resistance profiling also offers a definition on the effectiveness of antimicrobial agents against single isolated microorganisms and this is used in clinical decision-making on the approach to treatment. Interestingly, molecular testing reveals the presence of mecA gene, which proves the advantage of the modern diagnostic methods in the detection of the resistant strains and the implementation of the treatment process. All in all, the research is a contribution on the subject of microbial epidemiology and antimicrobial resistance patterns and highlights the need to further monitor and implement intervention measures to reduce the effects of resistant pathogens in healthcare facilities.

Declaration[s]

Competing interests: All authors report no conflicts of interest.

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Ethical Approval and Consent to Participate: The study adhered to ethical standards and received approval from the Institutional Ethical Committee [KCGMC/IEC/2022/49]. Additionally, written consent was obtained from all participants.

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