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A COMPARISON OF PHENOTYPIC METHODS WITH MECA GENE DETECTION FOR MRSA (Methicillin-Resistant S. Aureus)

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ABSTRACT

An independent risk factor for MRSA nasal colonization has been identified as HIV. Patients who are HIV positive have been shown to have a significant MRSA colonization rate. Uncertain factors such as frequent use of antibiotics, close contact with the medical community, and weakened immune systems may all contribute to the greater colonization rates among HIV-positive individuals, making them more vulnerable to the spread of resistant strains of bacteria. We are conducting a new study on the Nasal Carriage Position of MRSA in this research project. Their capacity to create bio-films will be of utmost importance in the successful management of immune-compromised patients like HIV positive patients, as HIV groups of people are susceptible to persistent infection, typically from their own flora. There is only a small amount of research about nasal MRSA carriage, particularly among HIV patients, in North India. Additionally, this subset of individuals' ability to build bio-film is completely absent, according to studies.

KEY WORDS: Phenotypic Methods, Gene Detection, MRSA (Methicillin-Resistant S. Aureus)

INTRODUCTION

Staphylococcus aureus (SA) is a gram-positive, spherical or coccus-shaped bacterium that is grouped in clusters resembling grapes, creates a distinctive yellow-gold pigmentation on nutrient agar, and is a member of the staphylococcaceae family. Due of the high rates of human morbidity and mortality linked to these infections, the abnormal rise in SA strains resistant to Methicillin and other antibiotics from 2% to 64% over the last three decades has garnered a lot of attention. It is widely known that Staphylococcus aureus can form biofilm on various human

body surfaces as well as on ingestible devices. Due to the pathogen's ability to form biofilm, persistent infections brought on by SA spread and increase disease and mortality in the community. S. aureus-produced biofilm is the primary cause of many nosocomial infections. Over 65% of hospital-based illnesses are detected by infecting organisms that have a propensity to form biofilms, according to published research. A biofilm is an expanding population of bacteria that colonizes biotic and abiotic surfaces and embeds itself in an extracellular matrix made of proteins, lipids, exopolysaccharides, and a few small molecules like DNA and RNA. There are numerous definitions of biofilm, but they all list the same three main components (A): bacteria (B), slime exopolysaccharide (C), and surface. Disabling any one of these components can prevent the development of biofilm. Because biofilm prevents medications from getting to the bacteria it is protecting, microorganisms that are protected by it are less susceptible to antibiotics. Additionally, biofilm causes bacteria to regularly escape from the host's defense mechanisms, leading to persistent infections that are frequently difficult to treat. Methicillin-resistant S. aureus (MRSA), which is prone to biofilm formation, can become multidrug resistant to the majority of antimicrobials now on the market, worsening an infection's prognosis. One of the most important steps in preventing and treating such infections may be the early detection of such an organism and the adoption of an effective biofilm control regimen. The essential pathogen Staphylococcus aureus is responsible for a wide range of infections in people, from minor localized and invasive infections like carbuncles, cellulitis, lymph node abscesses, and wound infections to severe ones like necrotizing pneumonia and necrotizing fasciitis. Close encounters, cross contamination, and fomites are the main ways in which it is spread from one patient to another. It is a pervasive organism that colonizes between 30 and 50 percent of adults and more than half of kids with serious skin conditions. Both community-acquired and hospital-acquired SA infections have become more common over the past few years. The extent of drug-resistant strains of SA, which are resistant to several drugs (MDR), including methicillin and, more recently, vancomycin, is making it harder to treat these infections with antibiotics. Due to its remarkable susceptibility to all beta-lactam antibiotics and propensity to develop biofilm, MRSA has drawn extraordinary attention. Since the population contains a large number of SA carriers, the infection may spread within the community. It has been established that the nose (anterior nasal mucosa) is the main ecological

reservoir of SA in humans, and that nasal carriage is a significant risk factor for SA infections in a variety of clinical contexts. It has been reported that screening recently admitted patients is effective in reducing the occurrence of MRSA at the various clinical settings. Additionally, while there are a ton of data from other nations, such Taiwan, Turkey, and others, that show an increase in the rate of nasal colonization over the past several years, there are not enough studies on the occurrence of MRSA nasal colonization in India. As the community isolates were distinct from nosocomial isolates, the appearance of community associated MRSA (CA-MRSA) strains has been noted in the last decade of the 20th century when four people died from invasive MRSA infection despite none of them had recognized MRSA risk factors. The majority of CA-MRSA infections affect the skin and soft tissues, and while they are typically successfully treated, they are more challenging to handle than HA-MRSA infections that are linked to hospitals and are capable of leading to serious illnesses like toxic shock syndrome and necrotizing pneumonia, among others. This might be because exotoxins such Panton Valentine leucocidine (PVL), a bi-component exotoxin generated by CA-MRSA, are present. SA librating PVL-toxin promotes the development of a disease pattern. Clinical and epidemiologic data further demonstrated that CA-MRSA with pvl genes has high virulence and that the typical risk factors for MRSA infections are different from those for HA-MRSA. Several studies used a retrospective analysis of the patient's medical records to describe various risk variables in MRSA-infected patients.

Additionally, few studies have highlighted the risk factors for MRSA infections; however, there is little knowledge of the risk factors for MRSA nasal carriage among HIV-positive individuals.

Age, sex, fasting blood sugar level, and smoking were recently confirmed to be independent predictors of SA nasal carriage. Other factors that have been linked to colonization risk include having a history of recurrent needle use, having a critical skin condition, or being on dialysis or diabetes medication.

Their family size, parental education, and career are additional risk factors for colonization. Epidemiological statistics have traditionally been effectively used to track and mobilize illness patterns among different patient populations as well as for program planning. Unfortunately, the

majority of the MRSA-related material that has been published relies on information gathered from specific groups or from medical records kept in specialized locations, which does not really change the prevalence of nasal carriage. Early in 1961, just after the development of methicillin to treat SA that was resistant to penicillin, MRSA was initially recognized. It has since become a significant global cause of hospital acquired infections. MRSA infections are difficult to treat because there aren't many effective antibiotics available, which increases mortality and morbidity as a result. Since MRSA has a propensity to evolve into MDR strains, glycopeptide antibiotics like Vancomycin are the preferred treatment. Lengthy hospital stays, inappropriate antibiotic use, a lack of hygiene knowledge, self-medication, etc. are some potential risk factors for MRSA infections. Because MRSA is easily transmitted in the community by infected or colonized individuals, and because healthcare professionals encourage further transmission in healthcare settings, infections brought on by MRSA are contagious all over the world. Therefore, knowledge of the prevalence of carrier state MRSA and their current antimicrobial pattern has become essential for both the establishment of institutional antibiotic policy and the suitable & empirical treatment of such type of infections. The incidence of MRSA nasal isolates, their antimicrobial resistance pattern, ability to build bio-film, and their impact on antimicrobial susceptibility among newly diagnosed HIV seropositive Case group and Healthy normal control group were examined in the current research effort.

Recently, among clinical isolates of MRSA, VISA and VRSA outbreaks have been recorded from North India and other countries. Unfortunately, healthcare personnel in Delhi, North India, have also reported having nasal carriage VRSA, which is a worrying ailment. To identify the staphylococcus aureus's vancomycin resistance, we have included included the vancomycin MIC in this study. The ability of a disease to survive in the presence of an antimicrobial agent or another treatment, such as an antiseptic, is known as antimicrobial resistance.

Resistance develops in bacteria by "natural selection" or "genetic mutation." The varied forms of drug resistance that exist today may result from mutations occurring in different segments of the genome. For instance, a mutation in a chromosomal gene that transports a specific antibiotic

into the bacterial cell decreases the pathogen's capacity to transport that antibiotic, while a mutation in a different gene changes the intracellular target protein for a different antibiotic, reducing the antibiotics' inhibitory effects. The antibiotic-resistant gene is passed down from altered parental cells to its offspring by natural selection. Every each generation has the potential to strengthen any existing resistance characteristics. However, bacterial genetics alone cannot explain the rise in resistance; human behavior, such as the careless use of antibiotics in the cattle industry, also plays a crucial role. Avoiding these behaviors could help the community's resistance from spreading.

SA uses biofilm as a significant and highly effective method of immune evasion. Biofilms are "a microbial derived sessile community and are typified by cells that are attached to a substratum, interface, or each other, are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression, and protein production,". A biofilm is just a collection of organisms that are forming a coating on a surface. Because the biofilm produces a different sort of antigen than the typical planktonic form, it is an efficient method for bacteria to evade the immune system. As a result, the host cells that were sensitive to MRSA in its planktonic state will not react to biofilm right away. Additionally, there's a possibility that a bacterial strain that produces biofilms will detach and enter the bloodstream, causing a serious infection. Staphylococcus aureus' ability to build biofilms also rely on the "agr and arlRS gene controlled transduction system," which can be affected by certain environmental factors that make MRSA resistant to the complement system.

This shows that the MRSA genome is closely linked to increased morbidity and antibiotic resistance. Patients with HIV have a higher chance of contracting SA. As MRSA colonization is associated with high risk groups for SA infection, individuals at high risk for MRSA colonization or infection serve as sources of both HA-MRSA and CA-MRSA. MRSA colonization typically occurs in people who regularly expose themselves to hospital environments and in those who repeatedly take antibiotics because their immune systems are compromised. Age, length of hospital stay, location of hospitalization, primary illness, use of persistent tools and methods, prior hospitalization, quality of care, and proximity to a patient

with MRSA colonization are a few other factors that have been mentioned in the literature as traits for the health environment coupled MRSA infection. Male-to-male sexual contact and the atmosphere in the home may both be significant risk factors for CA-MRSA. Due to the limited antibiotic treatment options and potential MRSA drug resistance to other antimicrobial agents, treating both forms of MRSA infections is a challenge for clinicians. Due to the high expense of hospital treatments, treating MRSA is extremely challenging for people. Due to other concerns like cancer, tuberculosis, HIV, malaria, and other dangerous diseases that are already present in that region, it is difficult to treat MRSA at an affordable cost. Specialized MRSA infection control facilities are available to try to stop the spread of these features. There are very few studies of MRSA infection among HIV-positive individuals in this area, and those that do are primarily based on risk factor evaluation and laboratory experimental value. There has never been a report on the prevalence of MRSA colonization and the antimicrobial susceptibility characteristics of these isolates among HIV-positive individuals in northern India.

Additionally, the researchers have proposed that a higher colonization rate can result in more infections. Additionally, MRSA has been shown to be more virulent than MSSA isolates. MRSA first surfaced in the 1960s and has continued to establish itself as a significant nosocomial disease among hospitals all over the world. While SA was predominantly linked to infections obtained in healthcare settings, MRSA began to emerge as a substantial source of infections acquired in the community in the late 1990s. Clinical studies range from systemic to external illness settings, which is responsible for a high death rate. SA typically concentrates in the delicate skin and mucous membranes of normal human noses, and about 30% of the healthy population has SA colonization. The majority of the time, infections are associated with more than SA traits that are coagulase negative, but it has been noted that some other Staphylococcus species, including Staphylococcus capitis, Staphylococcus cohnii, and Staphylococcus lugdenensis, have also been linked to nasal infections. The many mechanisms of antibiotic resistance and the abundance of virulence factors responsible for the spread of staphylococcal infections make SA isolates unique. MRSA has caused public health problems in countries due to its capacity to resist all known antimicrobial drugs and its rapid spread in hospitals. S.aureus is a serious pathogen that affects people who have HIV. Although there are many reports on

how severe and persistent S. aureus infections are in HIV patients, they have not been thoroughly researched up to this point.

The main cause of nosocomial infections is SA, and numerous MRSA isolates are constantly being found in hospitals and the community, infecting both children and adults. Community-Associated MRSA (CA-MRSA) and Hospital-Associated MRSA are the first and second, respectively (HA-MRSA). Regarding the clinical side, CA-MRSA is a significant pathogen linked to skin and soft tissue infections; it typically affects younger patients who are predisposed to more non-lactam antibiotics. As a result, MRSA is a heterogeneous group of organisms with various pandemic potentials, which causes its epidemiology to constantly change. Different virulence potentials and intricate interactions with the vulnerable host may be the cause of this heterogeneity. In HIV-positive individuals, the prevalence of MRSA isolates varies with different types of healthcare facilities, the population, geographic regions, and patient economic circumstances. The prevalence of SA is approximately 80% on the Asian continent, 20-35% in Europe, 40-60% in the US, and up to 70% in the USA. While it is rare in Sudan and Somalia, the prevalence of SA is very high in nations like Zimbabwe, Egypt, and Algeria. By breaching the epithelial barrier, nasal carriage serves as a platform for SA to spread to other body sites including the circulatory system. These SA strains either become a biofilm or are destroyed by the host's defense system. During many infectious processes, SA creates biofilm, which contains the primary virulent component and host defense mechanism. Treatment for diseases brought on by biofilm is typically challenging. According to estimates, biofilm development is linked to around 65% of hospital acquired illnesses. It is exceedingly challenging to eradicate these infections because biofilm-producing Methicillin-resistant isolates from healthy members of the community exhibited a greater incidence of multi-resistance than biofilm-non-producing isolates from the same population. Several antimicrobials, including vancomycin, cefotaxime, and oxacillin, exhibited less penetration into SA biofilms, according to a recent study. In nasal isolates of discovered a high rate of biofilm generation and substantial resistance was seen among biofilm-producers than Non-biofilm producers.

SCOPE OF THE STUDY

The epidemiological characteristics of MRSA infections in India were mostly discussed in the literature with respect to the MRSA colonization rates. An essential first step in the evaluation of any infectious disease's eruption is the identification of the source of infection and the mode of transmission. This will make it easier to map behavior and break the chain of infection. The most significant source of MRSA infections appears to be nasal colonization, which may also play a significant part in disseminating infections throughout communities. Decolonization of carriers is therefore assumed to be very important in managing these diseases.

The circulating Staphylococcus aureus in the community or in healthcare settings, as well as the population of high risk groups, affect the prevalence of nasal carriage staphylococcus aureus. HIV seropositivity is high in this area, and individuals in these patient groups may have a higher likelihood of nasal staphylococcus carriage due to their immunocompromised state. In HIV positive patients, positive nasal carriage of Staphylococcus, particularly MRSA, may have a role in subsequent opportunistic infections. These isolates can form biofilm, become resistant to many drugs, and are challenging to get rid of. Therefore, for efficient care, it will be helpful to know whether Staphylococcus aureus is nasally carried in this group of patients. However, this part of north India hasn't seen this kind of research done there. In order to prevent and treat MRSA infections among HIV patients in this region, the public health department and other medical professionals will be able to use the information from this study.

RESEARCH METHODOLOGY

The purpose of the study was to determine the prevalence of S. aureus and MRSA nasal carriage among HIV-positive individuals as the case group and healthy individuals as the control group in Agra and the surrounding areas, as well as to determine the likely risk factors and the impact of biofilm on antibiotic resistance.

SCREENING AND SELECTION OF STUDY POPULATION:

Case Group: Individuals who were HIV-positive after being tested at the ICTC, microbiology department, S. N. Medical College, Agra, and who wanted to contribute their samples and take part in the study. All patients' acquaintances, family members, and other participants who volunteered to participate in the study and who were tested as HIV-seronegative served.

PATIENT S DEMOGRAPHY:

Patients' demographic data, including age, sex, marital status, level of education, occupation, socioeconomic standing, close contact with someone who has been diagnosed with staphylococcal infection, prior staphylococcal infection, use of antistaphylococcal antibiotics, history of hospitalization, recent surgery, fever, skin lesions/SSTI, smoking, alcohol use, HIV status, antiretroviral therapy, and CD4 count, are collected during the sample collection process.

SAMPLES PROCESSING:

Nasal swabs from every patient and the control group were taken, and they were subsequently seeded into the blood agar (BA) and mannitol-salt agar (MSA) media. Overnight, seeded Petri plates were incubated aerobically at 370°C in the incubator. While colonies from MSA-medium were yellow/pink/colorless, those from BA-medium were white/yellow/creamy in color. The acquired colonies were tested for DNase, coagulase, catalase, and Gram's staining. The isolates that tested positive for the enzymes catalase, coagulase, DNase, and mannitol fermentation were identified as SA. 62,63 Colonies of SA were then put into MHA medium with 6 g/ml of Oxacillin and 4% sodium chloride, and any growth identified in this medium was determined to be MRSA. The 30-gram dose of cefoxitin was also evaluated for susceptibility during the antibiotic susceptibility testing. MRSA is defined as the antibiotic inhibition zone with a diameter of 21 mm.

RESULTS AND DISCUSSION

As a well-known carrier of both CA-MRSA and HA-MRSA, S. aureus poses an increasing hazard to the immunocompromised population as well as to healthy individuals in the general community.

Therefore, it is crucial to have an accurate, quick, and reliable approach for detecting methicillin resistance in S. aureus in order to select the proper course of treatment, avoid overusing glycopeptide antibiotics, guarantee the affected patient receives optimal care, and stop future transmission.

The penicillin binding protein a is encoded by the mecA gene, which is found on the staphylococcal chromosomal cassette mec and causes methicillin resistance in S. aureus (PBP2a). In the bacterial cell wall, -lactam antibiotics have a low affinity for PBP2a. Therefore, MRSA or MSSA is indicated by the presence or absence of the mec-A gene. Methicillin resistance in S. aureus is currently detected using polymerase chain reaction (PCR) for the mec-A gene. Despite the growing support for this method in the literature, it is not currently available in all clinical laboratories due to cost considerations and the necessity for technical expertization; for this reason, phenotypic methods continue to be the method of choice in environments with limited resources. There have been numerous studies in recent years on the use of cefoxitin as a substitute marker for finding mecA gene-mediated methicillin resistance. The mecA gene is effectively induced by cefoxitin. The CLSI recommendation recommends utilizing disk diffusion testing to detect methicillin resistance in Staphylococcus aureus (MRSA). Our study's objective was to assess the effectiveness of phenotypic MRSA detection techniques and compare them to the gold standard of PCR-based mecA gene detection. In the current investigation, S. aureus were isolated from cases and controls; of these were found to be MRSA-positive by mecA-gene PCR, while were shown to be MRSA-positive by oxacillin disc diffusion, oxacillin screen agar, and cefoxitin disc diffusion methods (Table-1). For measurements of diagnostic accuracy, a statistical comparison of phenotypic approaches using the mecA gene as the "Gold Standard" for MRSA detection is presented in tabular form (Table-2).

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Table- 1: Detection of MRSA by different methods & compare with mecA gene PCR; taken as Gold standard

mecA PCR		**Oxacillin Disc Diffusion Method**		**Oxacillin Agar Screening Method**		**Cefoxitin Disc Diffusion Method**	
n=140		Positive	Negative	Positive	Negative	Positive	Negative
Positive	21	16	8	20	5	21	0
Negative	123	2	121	0	123	0	123
Sensitivity		76.19%		95.24%		100.00%	
Specificity		98.37%		100.00%		100.00%	

The sensitivity of the Oxacillin Disc Diffusion Method was 76.19% (95% CI = 52.83% to 91.78%), the Oxacillin Agar Screening Method was 95.24% (95% CI = 76.18% to 99.88%), and the Cefoxitin Disc Diffusion Method was 100.00% (95% CI = 83.89% to 100.00%).

Similar to the sensitivity, the specificity for the Oxacillin Disc Diffusion Method was 98.37% (95% CI = 94.25% to 99.80%); for the Oxacillin Agar Screening Method, it was 100.00% (95% CI =97.05% to 100.00%); and for the Cefoxitin Disc Diffusion Method, it was 100.00% (95% CI =97.05% to 100.00%). The accuracy for the Oxacillin Disc Diffusion Method was 95.14% (95% CI =90.24% to 98.02%); for the Oxacillin Agar Screening Method, it was 99.31% (95% CI =96.19% to 99.98%); and for the Cefoxitin Disc Diffusion Method, it was 100.00% (95% CI =97.47% to 100.00%). As a result, cefoxitin makes a good stand-in marker for methicillin resistance. Cefoxitin disc diffusion should be used with another highly sensitive and specific test, such as the E-test (98.3%, 100%), to confirm MRSA. Oxacillin screen agar method for Methicillin resistance was described as the best screening instrument, with sensitivity 92.15% & specificity 90.90%. Similar to this, claimed that the latex agglutination test was the most effective and trustworthy method for detecting methicillin, with 100% sensitivity and specificity.

Table- 2: Comparison of different phenotypic methods for detection of MRSA (taking mecA gene PCR as Gold standard)

	Oxacillin Disc Diffusion Method		Oxacillin Agar Screening Method		Cefoxitin Disc Diffusion Method	
Statistic	**Value**	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**
Sensitivity	76.18 %	52.82% to 91.77%	95.23%	76.17% to 99.87%	100.00%	83.78% to 100.00%
Specificity	98.26 %	94.14% to 99.79%	100.00%	97.04% to 100.00%	100.00%	97.04% to 100.00%
Positive Likelihood Ratio	46.85	11.60 to 189.11	--	--	--	--
Negative Likelihood Ratio	0.23	0.10 to 0.50	0.05	0.01 to 0.31	0	--
Disease prevalence	14.58 %	9.26% to 21.41%	14.56%	9.26% to 21.42%	14.58%	9.26% to 21.42%
Positive Predictive Value	88.88 %	66.47% to 97.00%	100.00%	--	100.00%	--
Negative Predictive Value	96.03 %	91.83% to 98.10%	99.19 %	94.77% to 99.88%	100.00 %	--
Accuracy	95.13%	90.23% to 98.02%	99.30%	96.18% to 99.98%	100.00%	97.46% to 100.00%

CONCLUSION

The cefoxitin disc diffusion approach was discovered to be the most effective in this investigation for identifying methicillin resistance in S. aureus among the three different phenotypic methods. The cefoxitin disc diffusion approach had a 100% sensitivity and specificity rate for identifying mecA-mediated resistance in S. aureus. Because oxacillin, a poor inducer of PBP2a synthesis, is less effective than cefoxitin at inducing the expression of the mecA gene, heterogeneous MRSA populations that express the mecA gene differently are better detected by disk diffusion with cefoxitin.

An independent risk factor for MRSA nasal colonization has been identified as HIV. Patients who are HIV positive have been shown to have a significant MRSA colonization rate. Uncertain factors such as frequent use of antibiotics, close contact with the medical community, and weakened immune systems may all contribute to the greater colonization rates among HIV-positive individuals, making them more vulnerable to the spread of resistant strains of bacteria. MRSA prevalence, which was determined to be as low as 16.5% in 2009 but rose to 37.5% in 2012, is a major issue in this area, which is a center of world heritage. As HIV and MRSA epidemiology continue to change over time, SA interactions and disease transmission with HIV patients may be complex and diverse. In order to illustrate the epidemiology, antibiotic sensitivity pattern, biofilm development & their impact on resistance, and associated risk factors of nasal colonization of MRSA in the HIV-infected population, this study will also highlight how these factors relate to resistance. Vancomycin is an antibacterial drug that is frequently used to treat MRSA infections. Among India, MRSA infection in HIV patients is a significant public health issue. As is common knowledge, HIV patients' compromised immune systems make them vulnerable to a wide range of opportunistic diseases. Vancomycin has been utilized for the past ten years to treat MRSA infections in HIV patients to lower infection rates. As a result, the study also emphasizes the Vancomycin Minimum Inhibitory Concentration (MIC) of MRSA and the Vancomycin Susceptibility Testing demonstration.

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