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# The Emergence of Antibiotic Resistant *Staphylococcus aureus* in Hospital Sewage: A Threat to Public Health

Pratibha Goyal, Nupur Mathur, Anuradha Singh, Jaya Dayal
Environmental Molecular Microbiology Lab,
Department of Zoology, University of Rajasthan, Jaipur

**Abstract:- Antibiotic resistance is adversely affecting public health. The excess and overuse of antibiotics leads to the growth of antibiotic resistant *Staphylococcus aureus* (MRSA) in the hospital environment. Raw tank of sewage treatment plant receives pathogen from all over the units of hospital. In this study MRSA was isolated from raw tank and identified by using mannitol salt agar and Kirby Bauer disc diffusion test. Yellow colonies on mannitol salt agar with resistance zone on cefoxitin, oxacillin and vancomycin confirmed the resistance of MRSA according to the guidelines of clinical laboratory standards institute.**

***Keywords***:- *Antibiotic Resistance, Staphylococcus aureus, Mannitol Salt Agar, Kirby Bauer Disc Diffusion Test.*

## I. INTRODUCTION

A significant concern to human health is the emergence of bacteria that are multidrug resistant (MDR). Multidrug resistance is the resistance established by microorganisms (bacteria, viruses, fungi, and parasites) against their medications that were once useful for treating infections, which makes it difficult to treat infections and raising the possibility of disease transmission, severe sickness, and mortality. The World Health Organization (WHO) has recognized the rapid evolution of antibiotic-resistant bacteria as one of the ten greatest dangers to public health.

About 700,000 deaths are recorded each year worldwide from drug-resistant diseases (1). In the United States alone, approximately 2 million people infected with multidrug resistant to antibiotic every year and at least twenty-three thousand individuals died as a direct result of those illnesses, according to the Centre for Disease Control (2). However above 100,000 deaths were caused per year by methicillin-resistant *S. aureus* alone due to antibiotic resistance in 2019.

MRSA is the abbreviation for methicillin-resistant *Staphylococcus aureus* (*S. aureus*), a strain of *Staphylococcus* bacterium which is resistant to the antimicrobial medicines typically used to treat its infections. There are two categories of MRSA infections: healthcare-associated and community-associated *S. aureus* is commonly found in people's nasal passages and on their epidermis, where it is regarded as part of the normal flora.

Methicillin-resistant *Staphylococcus aureus* (MRSA) first appeared in England during 1961 (3), as soon as methicillin antibiotic was introduced for clinical use (4). Resistance in MRSA developed due to excess and unnecessary use of antibiotics in human and animal treatment. The mecA gene, which codes for a mutated penicillin-binding protein known as penicillin- binding protein 2a (PBP2a) (5), confers methicillin resistance. Unlike the original penicillin- binding proteins, this protein has a weak affinity for beta-lactams such as methicillin. Although CA-MRSA strains are typically found in community settings and HA-MRSAstrains have historically been connected to hospital settings, the two populations of MRSA are starting to mix. Due to these factors, minimising human interaction with any MRSA strains is crucial if the spread of MRSA is to be managed.

Since many different types of bacteria and antibiotics are released into the wastewater at sewage treatment plants, this environment is ideal for the emergence of antibiotic-resistant bacteria (6). This study's objective was to check samples from a hospital's sewage treatment system for Methicillin-Resistant *Staphylococcus aureus* (MRSA) pathogens.

This aim of this study was to carried out the isolation and identification of Methicillin- Resistant *Staphylococcus aureus* (MRSA) bacteria in waste water samples taken from the sewage treatment facility of a hospital located in Jaipur. Isolation was performed by using Mannitol Salt agar. On the other hand, Kirby Bauer disc diffusion test was done to analyse the antibiotic resistance in MRSA against oxacillin, cefoxitin and Vancomycin.

## II. MATERIALS AND METHODS

- *Sample collection*

Samples were taken from a waste water treatment plant of a private hospital located in Bapu nagar, Jaipur. It was collected from Waste water was collected from raw tank of treatment plant in 50 ml autoclavable transparent plastic bottle, immediately transported for storage at 4°C for further experiment in laboratory. The water appeared to be black and was hazy, included microscopic black particles floating in the water.

- *Isolation on Mannitol salt agar*

Mannitol salt agar (MSA) media was used to isolate *Staphylococcus aureus* from the raw sewage because it is selective and differential for detecting the existence of *S.*

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*aureus* and because *S. aureus* colonies grow in MSA as a bright yellow colour. To identify bacterial isolates that can thrive on mannitol salt agar (MSA), samples were taken by cotton swab from sewage sample and directly inoculated over this medium and incubated aerobically at 37 °C for 24 hours. The presence or lack of mannitol fermentation on MSA, results in yellow colour of colonies was used to first quickly and conventionally identify colonies.

➢ *Antibiotic susceptibility testing*

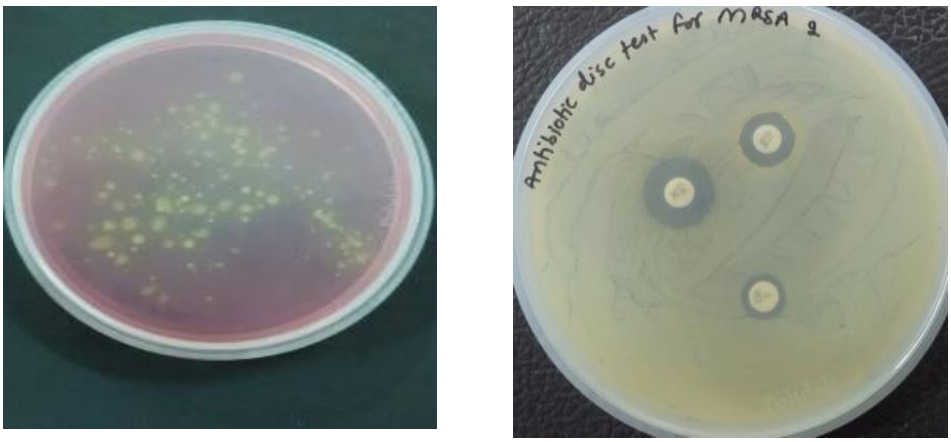
The Kirby-Baur Disk Diffusion Assay was used on Mueller Hinton agar (MHA) plates to assess the antibiotic resistance characteristics of the recovered isolates. Pipetting 2 ml of the pure culture into a sterile test tube, we diluted it with sterile saline (0.9% dilution) until it reached the same turbidity as the McFarland standard (0.5 mL equal to 1.0 $10^8$ cfu/mL). The bacterial lawn was grown using a sterile cotton swab and placed on Mueller Hinton agar plates, where it was allowed to rest undisturbed for 5 minutes before the antibiotics were added. A disc of each antibiotic Cefoxitin 30mcg, Vancomycin 10mcg, and Oxacillin 5mcg was placed on to the MHA plate's surface. These antibiotics are frequently used to treat *S. aureus* infection belongs to the methicillin class. For 24 hours, the plate was incubated at 37°C. A manual calliper was used to measure the zone size in millimetres following the incubation period and zone of lysis were interpreted according to the clinical laboratory standards institute guidelines (7).

➢ *Storage of MRSA in TSA agar*

Yellow colonies isolated were further added to tryptic soy broth and incubated at 37°C for 24 hours. Later serial dilution of tryptic soy broth culture was pour plated on tryptic soy agar plate. Colonies obtained were streaked on TSA agar plate and after 24 hrs, plates were stored at 4°C.

## III. RESULTS

Yellow colonies were observed on Mannitol salt agar plate (Fig. 1A). and the small zone formed around antibiotic disc (Oxacillin :10mm, cefoxitin: 16mm and vancomycin: 12mm diameter of the zone) (Fig. 1B).

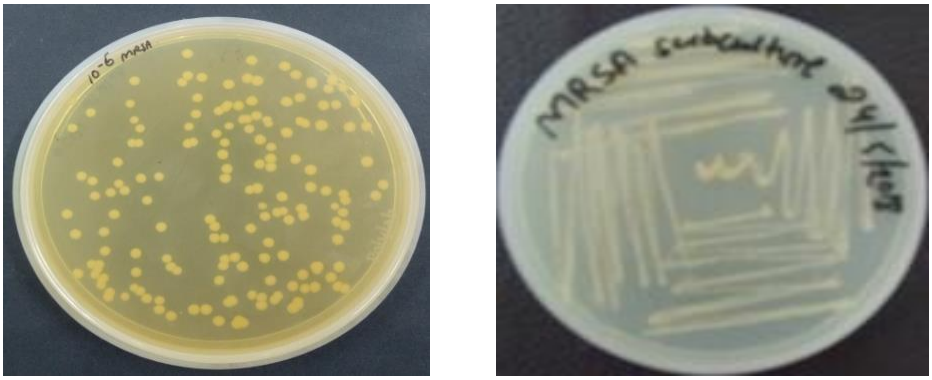



A B

Fig. 1: Showing results of MRSA identification and confirmation A) Mannitol salt agar and B) Antibiotic disc test for the same str ain. Inhibitory zone for antibiotics (vancomycin10mcg =12mm, cefoxitin30mcg=16mm, oxacillin5mcg=10mm) were observed.

➢ *Storage of isolated strain*

The pure TSB cultures' isolated bacteria (Fig. 2A) were streaked onto TSA agar plates for storage, and the TSA plates (Fig. 2B) was then incubated at 37°C for 24 hours before being stored at 4°C.




A B

Fig.2: Plates showing MRSA morphology on tryptic soy agar plate (A and B)

## IV. DISCUSSION

In this study MRSA was successfully isolated from waste water treatment plant located in hospital, which indicates the presence of antibiotic resistance MRSA strains in hospital environments. The source of these strains are mainly clinical patients having any kind of skin or burn infections. Since 1945, the selective medium mannitol salt agar (MSA) has been utilised to isolate pathogenic staphylococci (8,9). In 1985 (10), MSA go through its initial evaluation as a susceptibility testing medium. Several studies (11, 12) have examined its utility as an MRSA screening medium.

Using the selective and differential MSA medium, *S. aureus* in the sample was positively detected. Due to its 7.5% salt content, this kills the majority of bacteria, and the fact that only *S. aureus* can metabolise the substrate mannitol in the medium to create fatty acids. The presence of fatty acids makes the medium acidic, which causes the pH indicator to change colour from blue to yellow (13). Colonies with a yellow hue are a sure sign that *S. aureus* is present in the sample. The assessment of antibiotic resistance was conducted through the utilization of the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar, in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (7).These techniques shorten the time needed to identify *S. aureus* and evaluate its susceptibility to antibiotics, and they save money by eliminating the need for any extratesting (14).

Mannitol salt agar-positive *S. aureus* isolates were tested for oxacillin (5mcg), cefoxitin (30mcg), and vancomycin (10mcg) sensitivity using disc diffusion to evaluate clinical resistance to methicillin. 24 hours were used for incubating inoculated plates at 37°C. All oxacillin disc-resistant isolates were assumed to be MRSA. Inhibitory zone for antibiotics were observed as van10mcg =12mm, cefox30mcg=16mm and oxa5mcg=10mm. According to Clinical Laboratory Standards Institute 2014, standard zone for resistance for antibiotic disc on Muller Hinton Agar were (vancomycin =less than 17mm, cefoxitin=less than 22 and oxacillin=less than 27). Thus, results confirmed the multidrug resistance in *Staphylococcus aureus* isolated. Previous studies also confirmed the presence of MRSA in raw sewage of treatment plant (15).

## V. CONCLUSION

Bacterial load of antibiotic resistant strains is high in hospital environment. The results of this study reported the presence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in raw tank of waste water treatment plant. . The utilization of Mannitol Salt Agar when combined with the Kirby Bauer disc diffusion test represents a practical and effective method for the isolation and identification of Methicillin-Resistant Staphylococcus aureus, which requires less time and effort.

## REFERENCES

[1]. WHO 2019: Ten threats
[2]. Center for Disease Control (CDC). Antibiotic resistance threats in the United States. http//: CDC.gov. 4, (2015).
[3]. Jevons, M. “Celbenin”-resistant staphylococci. BMJ 1, 124–125 (1961).
[4]. Gordon, R.J.& Lowy F.D. Presence of methicillin resistant *Staphylococcus aureus* in hospitals. Journal of Clinical Infectious Disease. 46, 350-359 (2008).
[5]. Morell, E.A. & Balkin, D.M. Methicillin resistant *Staphylococcus aureus*: A pervasive pathogen highlights the need for antimicrobial development. Yale Journal of Biology and Medicine. 83, 223-233 (2010).
[6]. Everage, T.J., Boopathy, R., Nathaniel, R., LaFleur, G. & Doucet, J. A survey of antibiotic resistant bacteria in a sewage treatment plant in Thibodaux, Louisiana, USA, Internat Biodet Biodeg. 95, 2-10 (2014).
[7]. Clinical Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement.* Clinical Laboratory Standards Institute; Wayne, PA, USA: CLSI Document M100-S18 (2014).
[8]. Blair, E.B., Emerson, J.S. & Tull, A.H. A new medium, salt mannitol plasma agar, forthe isolation of *Staphylococcus aureus*. Am J Clin Pathol. 47(1), 30-9 (1967).
[9]. Chapman, G.H. The significance of sodium chloride in studies of staphylococci. Journal of bacteriology.50(2), 201-3 (1945).
[10]. Lally, R.T., Ederer, M.N. & Woolfrey B.F. Evaluation of mannitol salt agar with oxacillin as a screening medium for methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol. 22(4), 501-4 (1985).
[11]. La Zonby, J.G. & Starzyk, M.J. Screening method for recovery of methicillin- resistant *Staphylococcus aureus* from primary plates. J Clin Microbiol. 4(2), 186-8 (1986).
[12]. Marchese, A., Saverino, D., Debbia, E.A., Pesce, A., Schito, G.C. Antistaphylococcal activity of cefdinir, a new oral third-generation cephalosporin, alone and in combination with other antibiotics, at supra- and sub-MIC levels. J Antimicrob Chemother. 35(1), 53-66 (1995).
[13]. Duguid, J.P. *Staphylococcus*: cluster forming gram positive cocci. In: Practical Medical Microbiology, 13th edition. (Eds) Colle J.G., Duguid J.P., Fraser A.G., Marmion B.P. Churchill Livingstone, NY. 13, 303-316 (1989).
[14]. Sharp, S.E. & Searcy, C. Comparison of mannitol salt agar and blood agar plates for identification and susceptibility testing of *Staphylococcus* aureus in specimens from cystic fibrosis patients. J Clin Microbiol. 44(12), 4545-6 (2006).
[15]. Boopathy, R. Presence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in sewage treatment plant. Bioresour Technol. 240, 144-148 (2017).