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DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM VARIOUS CLINICAL SPECIMENS FROM A TERTIARY CARE HOSPITAL BY PHENOTYPIC METHOD AND ANTIMICROBIAL EFFECT OF CINNAMOMUM VERUM BARK EXTRACT

Anuradha N. Choudhary and Mili J. Thakkar
Post Graduate Department of Microbiology, Sevadal Mahila Mahavidyalaya,
Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, INDIA
Corresponding Email: - anuradhachoudhary291@gmail.com

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ABSTRACT:
Methicillin-resistant Staphylococcus aureus (MRSA) and coagulase-negative staphylococci (CoNS) are key agents of healthcare-associated infections. MRSA is a strain of Staphylococcus aureus resistant to β-lactam antibiotics, including penicillins and cephalosporins, and often displays resistance to other antimicrobial agents such as aminoglycosides, chloramphenicol, and ciprofloxacin. The first observations of staphylococci in human lesions were made by Von Recklinghausen in 1871, followed by Louis Pasteur demonstrating their pathogenicity in 1880. Sir Alexander Ogston, also in 1880, linked staphylococci to abscesses and named them based on their grape-like clusters. Invasive MRSA infections are categorized into healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and unknown. HA-MRSA infections are more common in hospitalized patients due to factors like improper antibiotic use, medical procedures, and ineffective cleaning.

Keywords: - Staphylococcus aureus, Aminoglycosides, Pathogenicity, Antimicrobial.

INTRODUCTION:

Methicillin-resistant Staphylococcus aureus (MRSA) is a significant human pathogen that causes both nosocomial (hospital-acquired) and community-acquired infections. It has become a serious public health concern. Resistance to penicillin in Staphylococcus aureus strains emerged in the 1940s, primarily among hospital strains, with resistance rates ranging from 75% to 95%. MRSA is associated with a variety of infections, including skin and soft tissue infections, endovascular infections, urinary tract infections, pneumonia, endocarditis, and septic shock. Methicillin, introduced in the 1960s, was effective against Staphylococcus aureus strains that produce the penicillinase enzyme, which rendered penicillin ineffective. MRSA strains, which emerged in 1961, are characterized by an alteration in their penicillin-binding protein (PBP), allowing them to spread worldwide. This resistance is primarily due to the modified PBP2a, which has a low affinity for β-lactam antibiotics. These strains carry the mecA gene, which can be detected using Polymerase Chain Reaction (PCR). Several molecular markers have been identified that are useful for epidemiological and diagnostic purposes; among these is the Staphylococcal Cassette Chromosome (SCCmec). Staphylococcus aureus possesses a variety of virulence factors, including peptidoglycan, teichoic acid, various enzymes and toxins, adhesion molecules, and four types of cytotoxins. These factors can lead to different types of infections, many of which are acquired in hospital settings. Approximately 20% to 30% of the human population is colonized by this bacterium, with the nasal mucosa serving as its primary ecological niche.[5] There are significant genetic and microbiological differences between healthcare-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) and community-associated methicillin-resistant

Staphylococcus aureus (CA-MRSA) infections. These infections exhibit distinct virulence factors and vary in their sensitivity to antibiotics.

Cinnamon, known scientifically as Cinnamomum verum and also referred to as sweet wood or Ceylon cinnamon (Darchini or Dorchini), has been highly valued for centuries in the Orient. It is one of the earliest known tree spices in India. The bark of cinnamon has a sweet and pleasant taste and contains the following components: moisture (9.9%), protein (4.6%), fat (2.2%), fiber (20.3%), carbohydrates (59.5%), total ash (2.5%), calcium (1.6%), phosphorus (0.05%), iron (0.004%), sodium (0.01%), potassium (0.4%), and various vitamins per 100 grams, which include B1 (0.14 mg), B2 (0.21 mg), C (39.8 mg), A (175 IU), and niacin (1.9 mg). The calorific value is 355 calories per 100 grams.

Cinnamon is native to Sri Lanka and the Malabar coast of India. In addition to these regions, the Seychelles Islands are also significant producers of cinnamon, with the spice spreading to places like Java and other parts of the world.

The antibacterial activity of cinnamon essential oil was evaluated using the Kirby-Bauer well plate method.[7]

MATERIALS AND METHOD:

The study was carried out in the microbiological laboratory of Sevadal Mahila Mahavidyalaya and Research Academy, Nagpur. All the laboratory work was performed according to the standard methodology. **Sources of isolated pathogen:**

Collection of samples was done from Navratra Hospital, Lakadganj, Nagpur. The specimens included blood and urine samples from patients admitted in various wards like ICU, General wards, General surgery, and gynecology department. Methodology: The total plate count was determined by the pour plate method by performing the serial dilution method. Isolation of pathogens and their characterization is done by morphological characteristics, cultural characteristics, biochemical characteristics, enzyme detection test, sugar fermentation test, antimicrobial susceptibility test and antimicrobial effect of cinnamon extract against MRSA.

Cefoxitin disc diffusion test:

Methicillin resistance can be detected phenotypically through disk diffusion testing using β-lactam molecules such as oxacillin or cefoxitin. However, the heterogeneous expression of PBP2a resistance can particularly impact the results obtained with oxacillin. In contrast, cefoxitin is a highly sensitive and specific marker for mecA-mediated methicillin resistance, making it the preferred agent for disk diffusion tests.

Recent studies have highlighted the antimicrobial effects of Cinnamomum verum extract against methicillin-resistant Staphylococcus aureus (MRSA). Cinnamomum and its derivatives, particularly cinnamaldehyde, possess potent antibacterial properties. These compounds have been utilized to inhibit the growth of prevalent bacterial and fungal biofilms. By inhibiting flagella protein synthesis and suppressing swarming motility, Cinnamomum can hinder bacterial attachment, colonization, and biofilm formation at an early stage. Furthermore, by downregulation of cyclic- di-guanosine monophosphate (c-di-GMP), biofilm-related genes, and quorum sensing, this compound suppresses intracellular adherence and accumulation of bacterial cells in biofilm and inhibits important bacterial virulence factors.[10]

Antimicrobial susceptibility test:

The test was conducted using the Kirby-Bauer disc diffusion method. Susceptibility refers to the inability of microbes, such as bacteria and fungi, to grow in the presence of one or more antimicrobial drugs. Susceptibility testing is performed on the bacteria or fungi responsible for an individual's infection after they have been isolated in a culture from the specimen. This testing helps determine the potential effectiveness of specific antibiotics against the

bacteria and assesses whether the bacteria have developed resistance to certain antibiotics. The results of this test can be used to select the drug(s) that are most likely to be effective in treating the infection.

RESULT & DISCUSSION:

MRSA isolates were obtained from the samples taken from the hospital.

Morphological Characteristics: Members of the genus Staphylococcus aureus are Gram positive cocci measuring 0.5 – 1.5 µm in diameter and divides to form the clusters characteristic of the genus, they also occur singly, appears in pairs, in tetrads and short chains mainly in liquid media.

Antimicrobial effect of cinnamon extract against MRSA: All the samples were sensitive to Cinnamon with zone of inhibition 17-20 mm.

(Results of tests mentioned in the table below)

DISCUSSION:

Despite the introduction of newer, effective antimicrobial agents and improvements in infection control measures, particularly hand hygiene techniques, Staphylococcus aureus remains a significant pathogen in both hospital and community settings. This organism is responsible for a range of infections, from superficial skin and soft tissue infections to serious systemic infections that can lead to illness or even death. The situation is further complicated by the development of methicillin resistance.

Methicillin resistance indicates resistance to all β-lactam antibiotics, including cephalosporins and monobactams—the most important group of antibiotics for treating Staphylococcal infections. Our prospective study examined the overall prevalence of methicillin-resistant Staphylococcus aureus (MRSA) and analyzed the resistance patterns of MRSA isolates against a predetermined panel of antimicrobials.

We found a high degree of resistance among MRSA isolates for several antibiotics: penicillin (91.4%), cotrimoxazole (91.4%), ciprofloxacin (84%), and erythromycin. The statistical resistance patterns for MRSA against various antimicrobials were observed as follows: Erythromycin (15 mcg, p-value: 0.001), Ciprofloxacin (5 mcg, p-value: 0.0001), Penicillin (10 units, p-value: 0.08), and Gentamicin (10 mcg, p-value: 0.4), with a reference chart showing a resistance rate of 52.4%.

The cefoxitin disc diffusion test was found to correlate well with the presence of the mecA gene. This method has proven to be a reliable and cost-effective way to detect MRSA, with results consistent with those obtained through PCR mecA gene detection. Cefoxitin is also considered superior to oxacillin as an indicator for detecting methicillin resistance. In laboratories where routine molecular methods are not feasible, the cefoxitin disc diffusion test serves as a good surrogate marker for identifying methicillin resistance.

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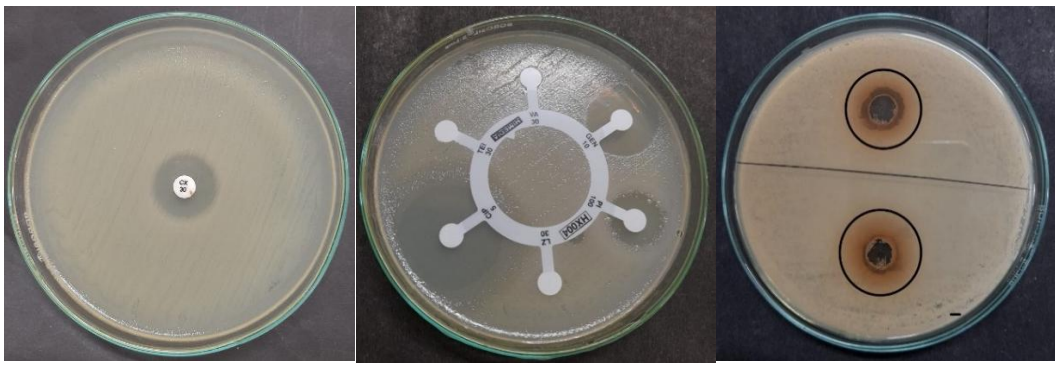
Table No.1 Results: Cultural, Biochemical, Enzyme detection test, Sugar Fermentation test, Cefoxitin disc diffusion test, Antimicrobial Susceptibility test

Cultural Characteristics				
Sr. No.	**Medium**		**Colonies**	
1	Mannitol Salt Agar		Yellow coloured colonies	
2	Baird Parker Agar		Black coloured colonies	
3	Blood Agar		Whitish grey colonies with beta haemolysis	
Biochemical Characteristics				
Indole	**MR**	**VR**	**Citrate**	**TSI**
Negative	Positive	Positive	Positive	A/A
Enzyme Detection Test:				
Sr. No.	**Tests**		**Results**	
1	Urease		Positive	
2	Catalase		Positive	
3	Coagulase		Positive	
Sugar Fermentation Test				
Sr. No.	**Sugars**		**Results**	
			Acid	**Gas**
1	Glucose		+	+
2	Lactose		+	+
3	Mannitol		+	+
Cefoxitin disc diffusion test :				
All samples were resistant to cefoxitin				
Antimicrobial susceptibility test:				
Sr. No.	**Antibiotics**	**Conc (in mcg)**	**Zone of inhibition (in mm)**	
1	Piperacillin(Pi)	100	18-25	
2	Linezolid(LZ)	30	25-27	
3	Ciprofloxacin(CIP)	5	22-25	
4	Teicoplanin(TEI)	30	15-18	
5	Vancomycin(VA)	30	17-21	
6	Gentamicin(GEN)	10	15-20	



1 **2** **3**

1. Cefoxitin disc diffusion test
2. Zone of inhibition in antibiotic susceptibility test
3. Zone of inhibition in antimicrobial action of *Cinnamomum verum* against MRSA