APPENDIX -4

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PREVALENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN TERTIARY CARE HOSPITALS OF WESTERN UTTAR PRADESH

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Abstract:

Introduction: Methicillin Resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial and community infections which may range from minor skin diseases to life threatening infections. The present study estimates the prevalence of MRSA in and around Western UP.

Methodology: The study included 353 Coagulase Positive Staphylococci isolated from different clinical samples of patients who visited to two large more than 500 bedded each teaching hospitals situated in the western U.P. Identification of MRSA and their antibiotic susceptibility pattern was done according to the standard guidelines. MRSA was identified by using Cefoxitin disc diffusion method and resistance to Methicillin was confirmed by Vitek 2 Compact automated system as well as PCR.

Result: Of the 353 isolates of Saureus, 230(65.15%) were MSSA and 123(34.84%) were MRSA mecA gene positive. However other two methods, Cefoxitin disc diffusion method and Vitek 2 compact could detect MRSA in 117(33.14%) and 121(34.27%) isolates respectively.

Conclusion: The reported prevalence rate of MRSA in Western UP is fairly high. Regular surveillance of Hospital-associated infections, monitoring of antibiotic sensitivity pattern and adherence to rational use of antibiotics may reduce the spread of Methicillin-Resistant Staphylococcus aureus in hospitals as well as community.

Keywords: Prevalence, MRSA, Cefoxitin

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**Introduction**

*Staphylococcus aureus* is responsible for causing a variety of human infections, which may range from minor skin diseases like furuncles, impetigo, carbuncles to life threatening infections like Pneumonia, Meningitis, Endocarditis, Osteomyelitis and Toxic Shock Syndrome (Tiwari HK et al., 2009). The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) has become a worldwide problem in clinical medicine. Irrespective of its resistance to Methicillin, *S. aureus* is a frequent cause of both community-acquired and hospital acquired infections, with substantial morbidity and mortality. *Staphylococcus aureus* infection used to respond to beta-lactam and related groups of antibiotics but the emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA) has posed a serious therapeutic challenge (Muralidharan S et al., 2009).

Methicillin resistant *Staphylococcus aureus* (MRSA) was first described in 1961 reported after one year of introduction of Methicillin and has emerged as one of the most important nosocomial pathogens especially in the last two decades (Maple PAC et al., 1989).

Infected and colonized patients in hospitals mediate the dissemination of MRSA strains, and hospital staff is the main source of transmission. This leads to serious endemic and epidemic MRSA infections (Rajaduraiapandi K et al., 2006). The predisposing factors for the emergence and spread of MRSA are prolonged and repeated hospitalization, indiscriminate use of antibiotics, intravenous drug abuse and presence of indwelling medical devices (Anupurba et al., 2003).

The incidence of MRSA varies according to the region, 25% in western part of India (Patel AK et al., 2010) to 50% in South India (Gopalakrishnan R et al., 2010).

MRSA is of serious concern not due its sole resistance to Methicillin, but also because of resistance to many other
antimicrobials that are used on a regular basis in hospitals. Current therapeutic options for MRSA are limited to few expensive drugs like Vancomycin, Linezolid, Teicoplanin, Daptomycin and Streptogramins. Another alarming sign is that emergence of resistance to Vancomycin, although at a low level has been reported (Assadullah S et al., 2003).

The knowledge of prevalence of MRSA and their antimicrobial-susceptibility pattern is a must for appropriate treatment of these infections. The present study was conducted to know the prevalence and antimicrobial susceptibility pattern of MRSA in two tertiary care hospitals of western UP.

Materials and Methods:
The study included 353 Coagulase Positive Staphylococci isolated from different clinical samples of patients who visited to two large more than 500 bedded each teaching hospitals situated in the western U.P. from Nov 2012-Nov 2013.

Culture and Identification of the organism:
For the isolation and identification of S. aureus, brain heart infusion broth, blood agar, chocolate agar, MacConkey agar were used according to type of the sample received.

The isolated colonies on culture media were identified by standard procedures which includes Gram’s staining, Catalase test, Mannitol fermentation, Slide coagulase and Tube coagulase test (Baird D et al., 1996).

Antibiotic susceptibility test
The strains were then subjected to antimicrobial-susceptibility testing by Kirby- Bauer disc diffusion method in compliance with clinical and laboratory standard institute (CLSI) guidelines using Mueller –Hinton agar media. Antibiotics tested were, Cefoxitin (30µg), Erythromycin (15µg), Cephalexin (30µg), Ciprofloxacin (5µg), Gentamycin (10µg), Amikacin (30µg), Linezolid (30 µg), Vancomycin (30 µg), Norfloxacin (10µg) and Nitrofurantoin (300µg) (Hi media Mumbai).
Norfloxacin and Nitrofurantoin were used only for urine samples. *Staphylococcus aureus* ATCC 25923 was used as the control strain.

**Identification of MRSA**

MRSA was identified by using Cefoxitin disc diffusion method recommended by the Clinical and Laboratory Standard Institute (Wayne, PA et al., 2010). The resistance to Methicillin was further confirmed by Vitek 2 compact automated system and amplification of mecA gene by polymerase chain reaction. The primer pair used 5′-AAATCGATGTAAGGTTGC-3′ and 5′-AGTTCTGCAGTACCGATTTG-3′ (fig 3).

**Result:**

Of the 353 isolates of *S. aureus*, 230(65.15%) were MSSA and 123 (34.84%) were MRSA mecA gene positive. However other two methods, Cefoxitin disc diffusion method and Vitek 2 compact could detect MRSA in 117(33.14%) and 121(34.27%) isolates respectively (table 1). Out of 123 (34.84%) MRSA, 87 (70.73%) strains were from inpatient department while 36 (29.26%) were from outpatient department (fig 1).

Antibiotics sensitivity pattern of all the isolates of MRSA was shown in fig 2. All the isolates were sensitive to Vancomycin and Linezolid.

**Table 1. Occurrence of MRSA versus MSSA among S. aureus (n= 353) and Detection of MRSA by Cefoxitin disc diffusion method, Vitek 2 compact and PCR**

<table>
<thead>
<tr>
<th>Total no. of <em>S. aureus</em> isolates</th>
<th>MSSA</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>353</td>
<td>230(65.15%)</td>
<td>By PCR</td>
</tr>
<tr>
<td></td>
<td>123 (34.84%)</td>
<td>BY Cefoxitin disc diffusion method</td>
</tr>
<tr>
<td></td>
<td>117 (33.14%)</td>
<td>By Vitek 2 Compact system</td>
</tr>
<tr>
<td></td>
<td>121 (34.27%)</td>
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</tbody>
</table>

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Distubustions of IPD and OPD in MRSA infections

Fig.1 Distributions of inpatients and outpatients in MRSA infections

Antibiotic Sensitivity pattern of MRSA

Fig.2 Antimicrobial susceptibility pattern of Methicillin Resistant strains of S.aureus (MRSA, n=123)

Fig.3 AGAROSE GEL ELECTROPHORESIS FOR meca (540BP) GENE. LANE 1: MOLECULAR WEIGHT LADER; LANE 2-4: CLINICAL ISOLATES OF MRSA; LANE 5: ATCC 43300 MRSA; LANE 6-7: CLINICAL ISOLATES OF MSSA; LANE 8: ATCC 29213 MSSA

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Discussion:
There is a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents (Mulla S et al., 2007). Among the gram positive pathogens, *S. aureus* continues to cause skin, soft tissue, and invasive infections in the both community as well as in the hospitalized patients. In a study at Aligarh, India (Sangeeta Joshi et al., 2013), it was shown that 35.1% of *S. aureus* isolates were resistant to Methicillin. In another study (Rajaduraipandi K et al., 2006) conducted in Tamil Nadu, out of 906 strains of *S. aureus* isolated from clinical samples, 250 (31.1%) were found to be Methicillin resistant. In a recent wide survey conducted in the Europe, the most common organisms in skin soft tissue infections were *S. aureus* with 22.5 per cent being MRSA (Sader HS et al., 2010). The proportion of MRSA varied among countries ranging from 0.4 per cent in Sweden to 48.4 per cent in Belgium (Sader HS et al., 2010). In India, recent study shown the prevalence of MRSA was 42% in 2008 and 40% in 2009 (Sangeeta Joshi et al., 2013). This variation in prevalence may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

The present study also reports a fairly high prevalence (34.84%) of MRSA infection. In our present study all the MRSA isolates were sensitive to Vancomycin and Linezolid. This is in accordance with other studies (Tiwari HK et al., 2009, Rajaduraipandi K et al., 2006, Anupurba S et al., 2003). However Vancomycin intermediate and Vancomycin resistant *S. aureus* (VISA and VRSA) strains have been reported recently from different parts of the country (Menezes GA et al., 2008, Tiwari HK et al., 2006).

Since first being identified, *S. aureus* infections have been associated with significant morbidity and mortality. MRSA is a highly versatile, virulent pathogen with the potential to evolve and adapt to its host as well as to the
treatments developed to control its invasive damage. Although MRSA initially was a nosocomial pathogen, it is now clear that individuals within the community as well as outside of healthcare institutions are also at risk for acquiring MRSA infections. Most common reason for multi-drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or patients poor economic status. Thus, consistent surveillance of hospital associated infections and formulation of definite antibiotic policy may be helpful for reducing the incidence of MRSA infection. Routine testing of methicillin resistance should be done using Cefoxitin disc which is the most sensitive, simple method and helps in the early diagnosis, proper treatment, and management of MRSA infections. In conclusions our study shows that fairly high number of MRSA isolates which is multidrug resistant and only few drugs like Vancomycin, Linezolid remains the drug of choice in many MDR MRSA infections.
References:


xvi. Tiwari HK, Sen MR. Emergence of Vancomycin resistant Staphylococcus aureus (VRSA) from a Tertiary care hospital from Northern part of India. BMC infect Dis 2006; 6:156.
ANTIMICROBIAL ACTIVITY OF CURCUMA LONGA EXTRACTS ON METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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ABSTRACT

Introduction: Methicillin Resistant Staphylococcus aureus (MRSA) can cause a wide spectrum of infections which may range from mild localized skin infection to severe life threatening infections. Only few drugs are available for the therapy of resistant Staphylococci. Curcuma longa belongs to the family Zingiberaceae commonly known as Turmeric, widely used as an aphrodisiac, colouring agent and is known for its medicinal properties. Aim: the present study was undertaken to check the antimicrobial activity of Curcuma longa against MRSA. Material and Methods: Heat and Room Temperature aqueous extracts and Ethanol extract were made from the fresh rhizomes of Curcuma longa. Total of 102 MRSA were isolated and identified from various clinical specimens according to standard protocol. Antibacterial activity of different types of extracts of Curcuma longa was checked against MRSA using agar well and disc diffusion methods. Result: Ethanol extract as well as unheated aqueous extract of Curcuma longa had shown antibacterial activity whereas heated aqueous extracts of C. longa did not show antibacterial activity against MRSA. Conclusion: Ethanol extracts and unheated aqueous extracts of Curcuma longa was found to be effective against MRSA, a major culprit of wound infections. Further standardisation of these extracts is needed to be done to use it as an effective economical antibiotic.

Key Words:

INTRODUCTION

Staphylococcal infections constitute one of the most important infections in the hospital as well as community settings. Despite the introduction of effective antimicrobial agents and improvements in hygiene, Staphylococcus has persisted as an important pathogen. The development of resistance to a wide range of antibiotics in S. aureus has diversified, such as resistance to methicillin that takes the account of S. aureus to most β-lactams, macrolides and Aminoglycosides. The infections caused by MRSA are serious and are difficult to treat. Only a few antimicrobial agents are available for treatment of such infections and the reports from India suggests increasing incidence of MRSA.

Alternative agents for decolonization and potentiation of existing anti-Staphylococcal therapy are the urgent requirement to combat these dreaded bugs in infection. Medicinal plants have a long history of existence and their use is widespread in both developing and developed countries. According to the report of the World Health Organisation, 80% of the world’s population relies mainly on traditional therapies which involve the use of plant extractors their active substances. Curcuma longa commonly known as Turmeric belongs to the family Zingiberaceae. It is cultivated primarily in India, China, Taiwan, Sri Lanka, Java, Peru, Australia, and West Indies. Turmeric is commonly known for its medicinal values in the Indian traditional systems of medicine. Its main active components include Curcuminoids, Monoterpenoids and Sesquiterpenoids. Turmeric is a well-known indigenous herbal medicine having many biological activities. It is an excellent anti-inflammatory herb and therefore is very useful in the treatment of arthritis, rheumatoid arthritis, injuries, and trauma. Curcuma longa also exhibit anti-tumour activities and helps prevent cancer. It directly helps a cell to retain its integrity if threatened by carcinogens. In the present study, however the Antimicrobial activity of Curcumalonga against MRSA was studied.

MATERIAL AND METHODS

Fresh rhizomes of Curcuma longa (INYST) were obtained from the National Agricultural University, Pantnagar, Uttarakhand.

Extractions of Curcuma Longa Rhizome: The different extractions of Curcuma longa were extracted by taking 200 grams of rhizome, which were cruched, dissolved in 1000 ml
of ethanol and water, and soaked for 7 days. It was then filtered, evaporated and concentrated and stored at 40°C until further applications. Heated extracts of Curcuma longa were prepared in the water by boiling in a water bath for 4-5 hours.

**ISOLATION AND IDENTIFICATION OF TEST ORGANISMS:**

Total of 102 MRSA were collected from various clinical specimens submitted to the Department of Microbiology, Subharti Medical College Meerut. Staphylococcus aureus were identified according to standard protocol including Gram stain, Catalase test, Coagulase test, Mannitol fermentation test. MRSA were detected by Kirby Baur disk diffusion method using Cefoxitin disk (30µg) according to Clinical and Laboratory Standard Institute (CLSI) guidelines using Mueller Hinton agar media. Further MRSA were confirmed by Vitek 2 compact automated system and amplifying mecA gene by Polymerase Chain Reaction.

The primer pair used was 5’-AAATCAGTGTAAGGTIGGTC-31 and 5’-AGTCCGGCAGTGATTTGG-31 (fig 3).

**ANTIBACTERIAL SCREENING:** Agar Well Diffusion and Disc Diffusion method was used to detect the antibacterial activity of different extractions of Curcuma longa. For agar well diffusion method, wells of 8mm diameter were punched in to the Mueller Hinton Agar having the lawn culture of the test organism, and filled with 150µl of Curcuma longa extract. For disc diffusion test, sterile paper discs were soaked with extracts of Curcuma longa, then air dried. The bacteria were aseptically lawn cultured on a Mueller Hinton Agar plate and the paper discs containing the extracts were placed at different areas on the surface of each plate (figure-1). The plates were incubated for 18h at 370°C. Antibacterial activity was evaluated by measuring the inhibition zone around the well and disc (table-1). Minimum inhibitory concentration (MIC) of Curcuma longa was determined by using Broth Dilution Method and it is then cultured on a solid media to see the minimum bactericidal concentration (figure-2).

**RESULT**

**Table-1**: antibacterial activity of Ethanol extract, aqueous extracts of Curcuma longa against MRSA

<table>
<thead>
<tr>
<th>Type of extraction</th>
<th>Average zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic</td>
<td>18</td>
</tr>
<tr>
<td>Aqueous (unheated)</td>
<td>15</td>
</tr>
<tr>
<td>Aqueous (heated)</td>
<td>No zone</td>
</tr>
</tbody>
</table>

**DISCUSSION AND CONCLUSION**

Herbs and spices have been used for generations by humans as food and to treat ailments. Many herbs and spices have the medicinal properties that prevent disease. Turmeric is well known universally for its culinary and medicinal properties. Plant originated antimicrobial drugs are of great interest because many human and animal pathogens show multi-drug resistance and the certain antibiotic used have undesirable side effects. Yong uk our et al. showed that C. longa extract inhibited the MRSA adhesion in vivo to Human mucosal fibroblasts. Recent Studies have also reported that C. longa showed promising results in elimination of E. aecalis, one of the common organism responsible for root canal failure and is a good cost effective to all the historical intracanal medicaments with fewer side effects and least resistance developed by the species. The concentration of Staphylococcus protein significantly decreased with the increase in concentration of turmeric extract.

In the present study ethanol extract exerted more inhibitory activity than unheated aqueous extract. On the other hand, heated aqueous extract did not show any antibacterial activity against MRSA. Even though many herbs and medicinal plants contain various biologically active compounds that may trigger side effects and interact with other herbs, supplements or medications, Curcumin in the main ingredient of Curcuma longa extract is considered very safe and is well tolerated at very high doses.

This study reveals the potential medicinal use of turmeric as an antibacterial agent. Curcuma longa may provide a valuable tool for the development of a therapeutic agent against Methicillin Resistant Staphylococcus aureus, however further standardization of these extracts are needed to be done to use it as an effective economical antibiotic.

**Figure-1**: Antibacterial activity screening by Disc Diffusion Method. 1- Ethanolic extract, 2- Aqueous extract (Room Temperature), 3-4- Aqueous extract (Heated).
Appendix

Figure-2: Minimum Bactericidal Concentrations (MBC) of Curcuma longa extract. MBC = 256µl

Figure-3: Agarose gel electrophoresis for mec A (540BP) gene. lane 1: molecular weight ladder; lane 2-4: clinical isolates of MRSA; lane 5: ATCC 43300 MRSA; lane 6.7: clinical isolates of MSSA; lane 8: ATCC 29213 MSSA

REFERENCE


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Appendix - 5

UP MICROCON 2013
Saturday 9th February 2013
IXth Annual Conference of
Indian Association of Medical Microbiologists - UP Chapter
Organized by: Department of Microbiology, Subharti Medical College, Meerut

CERTIFICATE

This is to certify that Dr./Mr./Ms. Lakshmikantha M has participated as Delegate/Resource person/presented a paper (Oral/Poster) in UP MICROCON 2013 and was awarded......

Uttar Pradesh Medical Council has granted 3 credit hours to the participants.

Reference NO. UPM/6796/2012 Date: 01-10-2012

Dr. T N Dhole
President, IAMM-UP Chapter

Dr. Molly Madan
Organizing Chairperson

Dr. Vivek Agarwal
Organizing Secretary

UP MICROCON 2014
Saturday 1st February, 2014
X Annual Conference of
Indian Association of Medical Microbiologists - U.P. Chapter

DEPARTMENT OF MICROBIOLOGY
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Sharda University, Greater Noida (U.P.) 201 306

Certificate

This is to certify that Dr./Mr./Ms. Lakshmikantha M has participated as Delegate/Resource Person/Presented a paper (Oral/Poster)/Chaired Session in UP MICROCON 2014 and was awarded......

Uttar Pradesh Medical Council has granted 4 credit hours to the participants.

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Dr. Anita Jais
President, IAMM-UP Chapter

Dr. Yogesh Chander
Organizing Chairperson

Dr. Vivek Rastogi
Organizing Secretary
This is to certify that, *Curcuma longa* verities including *C. longa* TCP 129, *C. longa* NYST-II, *C. longa* RH7/90, *C. longa* NYST-24 and *C. longa* sughandhum has been given to Mr. Lakshmikantha M. from G.B. Pant University of Agriculture & Technology, (G.B.P.U.A.T) Pantnagar for his research work on Methicillin Resistant *Staphylococcus aureus*.
Personal research scholar profile

I’m a research scholar in the Department of Microbiology, Subharti Medical College, Swami Vivekanand Subharti University. I have completed my post-graduation in medical microbiology from Kasturba Medical College, Manipal. I have also completed a post-graduation diploma in computer applications (PGDCA). I was cadet in the national cadet corps (NCC) and I have completed all the certificates (A, B and C). I have won the best poster presentation in bacteriology section, in IX Indian Association of Medical Microbiologists, UP chapter 2013 held at Meerut.

LAKSHMIKANTHA.M