The present research work was a detailed study on MRSA. This study investigates the effect of turmeric on MRSA and gram negative bacteria which includes *E.coli, Klebsiella* sps, and *Enterobacter* sps.

Since first being identified, *Staphylococcus aureus* infections have been associated with significant morbidity and mortality. The mortality number is higher than the rates of death produced by HIV, virus hepatitis, tuberculosis, and influenza combined together (Hoyert et al. 2012).

Among gram positive bacteria Methicillin Resistance *Staphylococcus aureus* (MRSA) has emerged as a serious public health problem. Burden has been strengthening by the ability of Staphylococci to acquire antimicrobial Resistance over time.

Hospital-acquired MRSA usually causes infections in the elderly, paediatric and immune compromised patients. Whereas community acquired MRSA infections occur as Skin and Soft tissue infection in healthy individuals (Farzana et al. 2006).

It is estimated that 20% of the human population are long-term carriers of *S. aureus* (Kluytmans F et al. 1997).

There is a growing concern about the rapid rise in resistance of *S. aureus* to antimicrobial agents. (Mulla S et al. 2007).

The increasing trend of antimicrobial resistance is most worrisome for Gram-negative bacteria (GNB) because there has been little successful development of new antibiotic agents targeting this class of pathogens (Talbot GH et al. 2006). Furthermore, we are now in the presence of GNB that have “extreme drug resistance,” indicating complete resistance of strains to first-line antibiotics used for the treatment of GNB infections (Amikacin, Tobramycin, Cefepime, Ceftazidime, Imipenem,
Meropenem, Piperacillin-Tazobactam, Ciprofloxacin, and Levofloxacin) plus second-line drugs such as tigecycline and polymyxins (Paterson DL et al. 2007).

Even more worrisome is the continued emergence of new mechanisms of multidrug resistance among GNB. Two recent examples of this include aminoglycoside 16S ribosomal RNA methylation and production of the New Delhi metallo-β-lactamase (Doi Y et al. 2007).

Among gram positive bacteria *S. aureus* continues to cause skin, soft tissues and invasive infections in both communities 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 *Staphylococcus 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 survey conducted in Europe, the most common organisms in skin soft tissue infections were *S. aureus* with 22.5% being MRSA (Sader HS. et al. 2010). The proportion of MRSA varied among countries ranging from 0.4 percent in Sweden to 48.4 percent in Belgium (Sader HS et al. 2010).

In the present study, 31 (25.2%) MRSA strains were isolated from orthopaedic patients, followed by paediatric 15.44% and surgical wards (13.82%). Srinivasan et al. found that surgical units accounted for 80% of the MRSA isolates and post-operative infections in orthopaedic surgery accounted for 28% (Srinivasan et al. 2006).

In the present study, both male and female had a high rate of MRSA infections although males had a slightly higher rate of infection than females. Study conducted by Jacques et al. found that females had a higher rate of MRSA infections than males (Jacques et al 2004, Gemmell et al. 2006, Graham et al. 2006). Out of 123 MRSA isolates 36 were HA-MRSA and 87 were CA-MRSA.
MRSA infection can occur at any age group, however elderly people are more prone to contract the infection as their immune system is not strong enough to tackle the MRSA infections. In our study, around 44% of the MRSA infections occurred in individuals who were 61 years and older. Study conducted by Anjali et al. also showed that elderly people are more prone to MRSA infections (Anjali et al. 2008). Whereas 0 to 15 year age group are least infected with MRSA infections.

In India, recent study showed the prevalence of MRSA was 42% in 2008 and 40% in 2009 (Sangeeta Joshi et al. 2013). The recent study conducted by Arora et al. shows that prevalence of MRSA in Punjab is up to 46%. Other studies have also shown such a high prevalence of MRSA in various parts of the country ranging from 40.6% to 59.3% (Muldharan et al. 2009, Anurapraba et al. 2003, Tiwari HK et al. 2006). However, 31% and 23.6% MRSA prevalence has also been reported (Majumder D et al. 2001, Rajaduraipandi K et al. 2006). A study conducted by Ansari et al in Nepal found that prevalence of MRSA was 43.1 % which is higher as compared to other studies conducted in Nepal (Ansari et al. 2014, Rai CKS 2001, Subedis et al. 2005).

These variations 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).

There was a marked difference between antibiogram of MRSA and MSSA isolates. The association of multi-drug resistance with MRSA adds to the problem. The MRSA strains were found to be more resistant to other antibiotics than MSSA strains. Significant difference (p value <0.05) was observed in case of Doxycycline, Erythromycin, Ciprofloxacin, Gentamicin, Clindamycin and Cotrimoxazole. Data analysed using the statistical package for social sciences (SPSS 16.0). Vidhani S. et al. found that there was a marked difference between sensitivity pattern of MRSA and MSSA isolates (Vidhani S et al. 2001). Mojumdar D et al. also reported that coexisting resistance to different antibiotics (except Penicillin) with Methicillin was significantly higher in comparison to Methicillin sensitive strains (Mojumdar et al. 2001).

In our study, MRSA isolates were resistant to Ciprofloxacin (84.54%), Erythromycin (79.67%), Gentamicin (77.23%) and Cotrimoxazole (86.17%). The percentage of resistance is quite high. In the various reports from other parts of the country, the burden of such MDR strains has ranged from 23.2 % to 63.6% (Rajadurapandi K.et al. 2006, Anuprba S et al. 2003, Majumdar D et al. 2001).

In recent times Clindamycin has become an excellent drug for some Staphylococcal infections particularly skin and soft tissue infections and as an alternative in Penicillin allergic patients (Drinkovix D et al. 2001). The increasing prevalence of Methicillin resistance among the Staphylococci is an increasing problem (Yilmaz G et al. 2007). This has led to renewed interest in the usage of Macrolide, Lincosamide, Streptogramin B (MLS\textsubscript{B}) antibiotics to treat Staphylococcus
*aureus* infections with Clindamycin being the preferred agent due to excellent pharmacokinetic properties (Delialioglu N et al. 2005).

Widespread use of MLSB antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLSb antibiotics (Deotale V et al. 2010, Ajantha GS et al. 2008). Clindamycin has good oral bioavailability making it a good option for outpatient therapy and for change after intravenous antibiotics (Laclercq R et al. 2002). However, Clindamycin resistance can develop in Staphylococcal isolates with inducible phenotype and from such isolates spontaneous constitutively resistant mutants have arisen both in vitro and in vivo during Clindamycin therapy (Yilmaz G et al. 2007). Reporting *Staphylococcus aureus* as susceptible to Clindamycin without checking for inducible resistance may result in institution of inappropriate Clindamycin therapy. On the other hand, negative result for inducible Clindamycin resistance confirms Clindamycin susceptibility and provides a very good therapeutic option (Deotale et al. 2010). Since iMLSb resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographical location, D-test becomes an imperative part of routine antimicrobial susceptibility for all clinical isolates of *S.aureus* (Gupta V et al. 2009).

In our study, we found that 21.95% of isolates tested positive for inducible Clindamycin resistance by D-test. It was also observed that percentage of inducible Clindamycin resistance was higher amongst MRSA as compared to MSSA (6.90%). This was in concordance with few of the studies reported before (Yilmaz et al. 2007, Kavita P et al. 2011). On the contrary few studies have shown higher percentage of inducible resistance in MSSA as compared to MRSA (Schreckenberger et al. 2004,
Levin TP et al. 2005). Study conducted by Ansari et al. in Nepal had found that 12.4% were D-test positive amongst MRSA isolates (Ansari et al. 2014).

In a study done in Nepal, MDR MRSA was reported to be 40.1% (Tiwari HK et al. 2009). Reporting of high rates of MDR MRSA isolates leads to the possibility of exploitation of vancomycin by clinicians. The 100% sensitivity of MRSA to vancomycin suggests its prudent use and continuous monitoring of MIC levels so that we may not fall back into a pre-antibiotic era.

The heterogeneous nature of Methicillin-Resistance is an inherent limitation of the accuracy of susceptibility testing. Thus, detection of the mecA gene by PCR has been found to be a potentially sensitive method to identify heterogeneous strains of Methicillin-Resistant Staphylococcus (Prasad et al. 2000). Many Polymerase chain Reaction Techniques were used with different primers to detect the mecA gene for Oxacillin-Resistance. The mecA gene is highly conserved among Staphylococcal Species (Arches et al. 1994). Selection of Primers for the amplification of mecA gene can have significant impact on the accuracy of Test Results.

In the present study we have used 3 parameters to determine MRSA that includes – phenotypic methods like Cefoxitin disc diffusion method, automated system like VITEK 2 COMPACT and Molecular method that is Polymerase Chain Reaction. In our study, we could detect 123 MRSA by PCR, on the other hand Cefoxitin disc diffusion and VITEK 2 COMPACT could detect 117 and 121 respectively. The difficulty in the detection of Methicillin resistance is because of its heterogeneous nature. Cefoxitin disc was found to be superior to Oxacillin disc for the detection of MRSA in many studies (Gupta M et al. 2009, Anand KB et al. 2009).
Although Oxacillin Screen Agar could also detect equivalent number of resistant strains as those detected by Cefoxitin disc, it is however cumbersome to perform (Shilpa Arora et al. 2010).

In the present study, DNA extraction was done by kit method. mecA gene was amplified with two set of primers set1 (5’-AAATCGATGGTAAGGTTGGC-3’ and 5’-AGTTCTGCAGTACCAGTITTTGC-3’ (533bp)) and set2 (5’-TAGAAATGACTGAACGTCCG-3’ and 5’-TTGCGATCAAATGTTACCGTAG-3’ (154bp)). No specific oligonucleotides have been suggested for the detection of mecA gene in MRSA by PCR (Freboug et al. 1998, Louie et al. 2000). Primer set 1 could detect total of 123 MRSA, whereas set 2 detected 121. The appearance of bands on agarose gel electrophoresis was more prominent with primer set 1 than set 2. All the 123 MRSA, were also positive by Real Time PCR. This suggests that set 1 primer was more appropriate for the detection of mecA gene. This was in concordance with few of the studies reported before (Taweeporn et al. 2002, Murakami et al. 1991).

Herbs and spices have been used for generations by humans as food and to treat ailments. Many herbs and spices have medicinal properties that prevent disease. Some commonly used herbs and spices such as garlic, black cumin, cloves, cinnamon, thyme, all spices, bay leaves, mustard and rosemary possess antimicrobial properties and can be used as Therapeutics (Lai P.K. et al. 2004). In this study, antibacterial activity of different varieties of *Curcuma longa* was monitored using agar well diffusion and disc diffusion methods. *Curcuma longa* is a well-known indigenous herbal medicine having many biological activities (Ammon H.P. et al. 1991).

*Curcuma longa* is well known universally for its culinary and medicinal properties (Revaty S et al. 2011). Plant originated antimicrobial drugs are of great
Interest because many human and animal pathogens show multidrug resistance and the antibiotics used have undesirable side effects (Ahmad et al. 2003).

Yong Ouk et al. showed that *C. longa* extract inhibited the MRSA addition and invasion to human mucosal fibroblasts (Yong Ouk et al. 2003). Recent studies have also reported that *C. longa* showed promising results in elimination of *Enterococcus faecalis*, one of the common organism responsible for root canal failure and it is a good cost effective to all the historical intra canal medicaments with fewer side effects and least resistance is developed by this species (Himanshi Kumar et al. 2013). The concentration of Staphylococcus protein as well as DNA significantly decreased with the increase in concentration of turmeric extract (Nadia Gul et al. 2004). In the present study, different varieties of *C. longa* had been used. The varieties used are- *C. longa* TCP 129, *C. longa* NYST-II, *C. longa* RH7/90, *C. longa* NYST-24 and *C. longa* Sughandham. All the varieties were extracted by ethanol and aqueous extraction methods. Ethanol extract showed more inhibitory activity than other extracts which include heated and 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 the main ingredient of *C. longa* extract is considered very safe and is well tolerated at very high doses (Sarker S.D. et al. 2007).

The presence of Curcumin in both ethanol and aqueous extract (unheated) was confirmed by UV- spectrometry as well as high performance liquid chromatography (HPLC). Study conducted by Yuling Long et al. also reported the detection of Curcumin by HPLC (Yuling Long et al. 2014).
All the *C. longa* varieties extracts did not show any antibacterial activity against gram negative bacilli (*E. coli*, *Klebsiella* sps, and *Enterobacter* sps). Study conducted by Nadia gul et al also found that gram negative isolates were resistant to *Curcuma longa* extracts (Nadia gul et al. 2004)

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 of 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 in reducing the incidence of MRSA infection. Routine testing of Methicillin Resistance should be done using Cefoxitin disc which is the most sensitive and simple method and which helps in early diagnosis, proper treatment and management of MRSA infections.

Present study shows a high number of MRSA isolates in tertiary care hospitals in western Uttar Pradesh region and only few drugs like Vancomycin, Linezolid remains the drug of choice in many MDR MRSA infections. Clearly new therapies are needed to tackle the on-going problem. Present study reveals the potential medicinal use of turmeric as antimicrobial agent. *Curcuma longa* may provide a valuable tool for the development of a therapeutic agent against methicillin resistant *Staphylococcus aureus*. In addition, prevention and rapid identification are essential. Determining the optimal methods of treating this evolving organism will require that
both clinicians and researchers understand the organism, the patients and the antibacterial being employed more clearly.