REVIEW OF LITERATURE

- In the beginning of 1940s, the major treatment available to combat this organism was penicillin and all the isolates were sensitive to penicillin.
- During the next 2 decades, this pathogen developed resistance to penicillin and now a days virtually all strains of *S. aureus* are resistant to natural penicillins, aminopenicillins, and antipseudomonal penicillin.
- The reason for resistance to these drugs occurs because of the acquisition of genes that encode for the drug-inactivating enzymes, initially known as penicillinas and now called β-lactamases.
- The prevalence of penicillin resistant strains have increased in the 1960s. It was during this period that a new class of antibiotics was developed to target this pathogenic organism specifically.
- One year after this class of antibiotics was developed, soon a resistant strain known as methicillin-resistant *S. aureus* (MRSA) was reported.
- *Methicillin* was considered to resist β-lactamases degradation, but MRSA strains that were resistant to all β-lactam Antibiotics were identified soon after the introduction of methicillin into the clinical practice.
- The first case of MRSA was identified as early as 1961-1962, soon after the introduction of Methicillin in 1960.
- By 1980 they had spread throughout the world. From the late 1970s to the early 1990s, MRSA was usually a hospital-acquired pathogen, rendering the entire β- lactam class of antibiotics ineffective.
- In the last 20 years, methicillin-resistant *S. aureus* has spread world widely in the healthcare settings, leading to an increased reliance on vancomycin for the empiric treatment.
- Vancomycin had been considered to be the “gold standard antibiotic” of the last resort and has been the option for the treatment of MRSA infections over the last 3 decades.
- Until recently vancomycin was believed to have retained activity against all strains of *Staphylococcus aureus*: therefore the spread of MRSA has led to increased vancomycin usage and hence increased in selective pressure for the development of resistance.
- The first clinical strain of *S. aureus* resistant To vancomycin was reported in 1996 from Japan and 1997 from United States[^10].
Vancomycin resistance in S. aureus is a major problem from a clinical and public health standpoint.

Limited treatment options for infections caused by such multi-drug resistant microorganisms prompted the search for the novel compounds with a wide spectrum of activity and new therapeutic strategies.

In the news media, hundreds of reports of MRSA outbreaks in prisons appeared between 2000 and 2008. For example, in February 2008, one case was reported in the Tulsa County jail in Oklahoma started treating an average of about 12 \(^{[41]}\) S. aureus cases per month. A report on skin and soft tissue infections in the Cook country jail was reported in Chicago in 2004–05 demonstrated that MRSA was the most common cause of these infections among the cultured lesions, and also some of the risk factors were more strongly associated with MRSA infections than infections caused by methicillin-susceptible S. Aureus.

Tiwari HK et al.,(2006)\(^{[14]}\) had done a study for the period of three years from August 2002 to July 2005 in Banaras Hindu University and studied that Out of 783 S. aureus two S. aureus strains were found to be vancomycin and as well as teicoplanin resistant (one strain with MIC 32 µg/ml and the other strain with MIC 64 µg/ml); six strains of S. aureus have shown to be vancomycin intermediate (two strains with MIC 16 µg/ml and four strains with MIC 8 µg/ml); and two strains with teicoplanin intermediate (MIC 16 µg/ml). One CoNS strain was resistant to vancomycin and also to teicoplanin (MIC 32 µg/ml), and two CoNS strains were the intermediate to vancomycin and teicoplanin (MIC 16 µg/ml). All the VRSA, VISA and as well as the vancomycin resistant CoNS had shown growth on BHI vancomycin screen agar (vancomycin µg/ml) and were meca PCR positive.

Tsering et al., (2011)\(^{[42]}\) had done a cross sectional study in Sikkim Manipal Institute of Medical Sciences India during January to December 2006. In their cross sectional study they found the magnitude and antibiotic susceptibility pattern of the MRSA infection in the referral tertiary hospital of Sikkim. 827 clinical specimens were collected from the different departments of Central Referral Hospital and 196 carrier screening nasal swabs were obtained from the health care workers of the hospital. The antimicrobial susceptibility test was performed for the confirmed of the MRSA isolates as per the Clinical and Laboratory Standards Institute (CLSI). Methicillin resistance was seen in 152 isolates of S. aureus out of which 111 were from clinical specimens and 41 from carrier screening samples. MRSA
positivity among the males was significantly higher than females. The strains tested exhibited decreased in the susceptibility to vancomycin and imipenem. Most vulnerable of the carrier were the cleaners, that were a significant observation. Incidentally, it was observed that there was no resistance in the carriers to both vancomycin and imipenem.

In a 2011 study it reported 47% of the meat and poultry sold in a surveyed U.S. grocery stores was contaminated with the S. aureus, and out of those, 52% — or 24.4% of the total were resistant to atleast three classes of the antibiotics. "Now we need to determine that what does this means in terms of the risk to the consumer," said Dr.Keim, a co-author of the paper. Some samples of commercially sold meat products in Japan were also found to be harboring MRSA strains. Locker rooms, gyms, and related athletic facilities offer a potential sites for MRSA contamination and infection.

Joshi et al.,(2013) reported that Methicillin resistant Staphylococcus aureus (MRSA) is endemic in India and is a dangerous pathogen for the hospital acquired infections. This was a retrospective study which was conducted in 15 Indian tertiary care centres during the two year period from January 2008 to December 2009 to determine the prevalence of MRSA and also the susceptibility pattern of S. aureus isolates in India. Of the 13975 isolates of S. aureus in 2008, 5864 (42%) were the MRSA. In 2009, of the 12335 isolates, 5133 (40%) were MRSA. The majority of the isolates were obtained from inpatients - 3664 in 2008 and 4487 in 2009. This study demonstrates that MRSA is a problem in India. More number of MRSA isolates were supposed to be multidrug resistant as compared to the MSSA isolates. Glycopeptides and linezolid continue to remain the main stay for treatment for MRSA infections.

Dr.S.Kulkarni et al.,(2014) have done a study in MIMER Medical College, Talegaon (Dabhade), Pune from 1st April 2007 to 31st Dec. 2012 where they planned to determine prevalence of Methicillin Resistant S. aureus (MRSA) from clinical specimens (urine, pus, blood, sputum and the throat swab, miscellaneous(cervical and vaginal swabs, ear swabs, corneal scrapings/swab, rectal swab etc). S. aureus strains were subsequently tested for the methicillin resistance based on the Kirby-Bauer Disc Diffusion method by using the oxacillin disc [1mcg] obtained from Hi-media Laboratories Pvt. Ltd. The Isolates were considered to be methicillin-resistant if the zone of inhibition was observed to be 10mm or less. A total of 1217 S.aureus strains were isolated from clinical specimens like urine, pus, blood, sputum etc. Out of 1217 S.aureus, 856 (70.33%) isolates were MRSA. The highest percentage of the MRSA strains was obtained from the urine sample (82.38%). More than
70% of MRSA strains were found to be resistant to Cefuroxime, Augmentin, and Ofloxacin & Ciprofloxacin (74%). The lowest rate of resistance was noted to Linezolid (21.22%) & Ampicillin-Sulbactum combination (24.12%). They concluded that S. aureus is the most important nosocomial pathogen which is seen in tertiary care hospitals. The worst feature of MRSA is the simultaneous drug resistance to many antibiotics.

S. Mantri Rupali et al., (2014) [47] studied the antibiotic sensitivity pattern of Staphylococcal isolates with special reference to MRSA. A total of 285 strains of Staphylococci isolated from persons having different staphylococcal diseases were included in this study. Methicillin resistance was shown by 112 (58.33%) of the coagulase positive and 12 (12.9%) coagulase negative strains. In the present study all the MRSA strains as well as non-MRSA strains were found sensitive to vancomycin and Linezolid.

Liaqat et al., (2015) [48] stated that Vancomycin resistant Staphylococcus aureus (VRSA) have been reported from many parts of the world which are also including Asian countries. Hence, main objective of this study was to evaluate the possible occurrence of the VRSA in hospitals of Lahore city and to ensure the effectiveness of the various substitute therapeutic options. A total of 150 samples including that of pus/wounds were collected from three hospitals of the city and VRSA were isolated and was confirmed through recommended method as per Clinical and Laboratory Standards Institute. Out of the 51 (49.04%) methicillin resistant S. aureus (MRSA) isolates, 5 (9.8%) were found to be resistant to vancomycin. Ethanolic extracts of the Turmeric, Mint, Coriander, Garlic, Kalonji, Cinnamon and Cloves illustrate average MIC values of 140.84 µg/ml, 563.2 µg/ml, 486.4 µg/ml, 614.4 µg/ml, 409.6 µg/ml, 281.6 µg/ml and 64 µg/ml, respectively against 5 VRSA strains. The Concentration dependent increase in the growth inhibition zones of ethanolic plant extract was recorded by the agar well diffusion test. This study was helpful to find out the effectiveness of the plant extracts against VRSA.

Hasan et al., (2016) [49] investigated the vancomycin resistance traits of MRSA isolates collected from burn patients from Stamford University Bangladesh, Dhaka, from April 23, 2012 to January 15, 2013. A total of 40 wound samples from the patients with tertiary burns of partial or also full thickness (deep reticular dermis) were collected aseptically with a sterile cotton swab by a clinician who is properly wearing gloves (US Safety & Supply Co.) and a mask in the burn unit. Twenty-nine of 40 isolates of Staphylococcus spp. were identified as S.
aureus out of which twenty-one of the 29 strains of S. aureus were the MRSA, of which 11 were resistant to vancomycin when employing by the disc diffusion method. However, when the broth microdilution method was used for measuring the minimum inhibitory concentration (MIC) of vancomycin, the eight isolates were resistant to vancomycin, six with an MIC of 32 mg/mL and two with an MIC of 64 mg/mL.

They concluded that a significant fraction of VRSA was found among MRSA strains in this study, revealing that there is a necessity for new and effective drugs against MRSA.

The in vitro antimicrobial activity of garlic against the Mycobacterium tuberculosis has been reported way back in 1946 (Rao RR et al., 1946) [50]

Garlic (Allium sativum) is a natural plant, which is being used over years as a food as well as folk medicine all over the globe since centuries. Garlic has been reported as a plant with a widespread of biological properties including antimicrobial activity, anticancer, antioxidant, immunomodulatory, antiinflammatory, hypoglycemic, and cardiovascular effects (Reuter HD et al., 1996) [51]

According to a study by (Chehregani A et al., 2007) [52], in most of the plants bulbous extracts are more effective as antibacterial agents as they contains Allicine. Allium plants have the higher concentration of Allicinein their bulbs than other organs.

Numerous modern studies have confirmed that garlic has definite antibiotic properties and is effective against a wide spectrum of bacteria, fungi and viruses (Shuford JA, Steckelberg JM et al., 2005), (Low CF et al., 2008) [53,54]. In addition, the antimicrobial activity of garlic are linked to the presence of some bioactive compounds (Tsao SM et al., 2001) [55]. Garlic has been demonstrated to be one of the powerful remedy to protect against infections of many bacteria, fungi and viruses (Tsao SM et al., 2007), (Cutler RR et al., 2004) [56,57]. Garlic has been scientifically proven to be one of the powerful natural antibiotic against MRSA infections (Cutler RR et al., 2004) (Tsao SM et al., 2007) [57,56]. Ingredients in fresh garlic, other than illicit, have been found to have strong natural antibiotic effects (Tsao SM et al., 2007) [56].

(Ron et al., 2008) [58] reported that Hospital-patient Properly prepared antimicrobial garlic products in the form of powder and liquid form have been scientifically proven to kill MRSA.
in human clinical studies. In 2008, Dr. Ron Cutler and the University of East London (UEL) released the results from a human clinical study performed on 52 patients with the hospital acquired MRSA (or HA-MRSA). All 52 patients were treated with a form of stabilized allicin and were recovered fully from their MRSA infections. Many of the wounds healed in just 4 to 12 weeks and many patients had previously been treated with the multiple antibiotics with no improvement.

Garlic is one of the established remedies for the treatment of tuberculosis, according to Ayurvedic and Greek systems of medicine (Hannan A et al., 2011) [59].

Lekshmi et al., (2011) [60] have done the study on Garlic (Allium sativum) and stated that garlic has an important dietary and as well as medicinal role for centuries. It is a large annual plant of the Liliaceae family, garlic is used in traditional medicine for the infectious disease and also for some other cases. Five Staphylococcus aureus were isolated from the collected pus samples and three was identified as methicillin resistant Staphylococcus aureus (MRSA). This study aimed at assessing the antibacterial activity of garlic against three MRSA. Various solvent extracts of garlic inhibited the growth of the MRSA at a concentrations of 200, 300, 400 and 500 μg. Chloroform extract and ethanol extract of the Allium sativum was found to be highly effective against the MRSA1 and MRSA2 respectively. All the three organic solvent extracts have shown high activity against MRSA3 (26 mm). The extract showed concentration dependent antibacterial activity against Methicillin resistant Staphylococcus aureus (MRSA). All bacterial strains showed promising sensitivity to the solvent extracts of garlic. The chloroform extract, ethanol extract of garlic was found to be potent against the MRSA1 and also MRSA2 respectively. The antibacterial activity of all the organic solvents was found to be effective against MRSA3. It was observed that a very little effect was found with water extract of garlic against all the three MRSA used in this study.

MRSA1 was highly sensitive to chloroform extract of garlic at 500 μg concentration (27 mm). Petroleum ether extract of garlic was ineffective to MRSA1. MRSA2 was found to be sensitive to ethanol extract of garlic at 400 μg concentration (27 mm) MRSA3 was also found to be equally sensitive to all the extracts of garlic. The water extract
showed less inhibition when compared to other three extracts of garlic (26mm).
From this study, we conclude that the organic solvent extracts were very effective against all the three MRSA strains which were isolated than the water extracts of garlic.
Unfortunately, there is a lack of interest of pharmaceutical companies for investing and developing garlic into a drug and for performing clinical trials. The traditional use of garlic for infectious diseases and also for controlling MRSA infection appears to be justified.

Anuswedha et al.,(2015) [61] reported that Garlic (Allium sativum L.) and ginger (Zingiber officinale R.) have been used for thousands of years for medicinal purposes. Multi-drug resistant bacteria and their incidence had risen dramatically in the recent years. Allium vegetables, particularly a garlic (Allium sativum L.) exhibit a broad antibiotic spectrum against both the grampositive and as well as gram-negative bacteria. The in vitro antibacterial activity of the crude extracts and fresh juice of Allium sativum L. (garlic), Allium ampeloprasum L. (elephant garlic) and also Zingiber officinale R. (ginger) was determined and was compared against clinical strains of the methicillin resistant Staphylococcus aureus. Allium ampeloprasum fresh juice showed the maximum inhibition against all the strains of MRSA at a concentration of 100μl/ml followed by the fresh juice of the Allium sativum. The crude extracts and the fresh juice of Zingiber officinale showed no activity at the concentration tested. This study confirms that the higher therapeutic potential of Allium ampeloprasum and Allium sativum against MRSA.

Li et al.,(2015) [62] reported that Infections caused by strains with multi-drug resistance are difficult to treat with the standard antibiotics. Garlic is a powerful remedy to protect against infections of many bacteria, fungi and viruses. In this study, they have used the disk diffusion method to investigate the antimicrobial activities of fresh garlic extract (FGE) and the combination of antibiotics with the FGE, on methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and the Candida albicans, to evaluate the interactions between the antibiotics and FGE (fresh garlic extract). Clinical isolates were isolated from clinical specimens which were obtained from inpatients at First Affiliated Hospital of the Xi’an Jiaotong University Health Science Center. The isolates consisted of MRSA, (n = 30), C. albicans (n = 30) and the P. aeruginosa (n = 30). Quality control for the CLSI (Clinical and Laboratory Standards Institute) disk diffusion was performed using the S. aureus ATCC®25923, C. albicans ATCC®90028 and P. aeruginosa ATCC®27853. The 93 microorganisms were then divided into four groups in a factorial design as control (deionized
water), FGE, antibiotics without the FGE, and antibiotics with the FGE. Next, the antibacterial activity was evaluated by measuring the diameter of inhibition zones according to the performance standards for the antimicrobial susceptibility testing as Clinical and Laboratory Standards Institute (CLSI) which was formerly NCCLS). They observed that Fresh garlic extract have displayed evident inhibition properties against C. albicans and also MRSA, yet weak inhibition properties against P. aeruginosa. Additionally, FGE have shown the potential to improve the effect of the antibiotics on antibiotic resistant pathogens. The synergism of the fluconazole and itraconazole along with FGE on C. albicans yielded larger zones of inhibition as compared with fluconazole and itraconazole. They Conclusions that the FGE can improve the antibiotic sensitivity of these pathogens to some antibiotics.

*Rosa chinensis* (Rose) Flower of this plant has also been reported for its medicinal properties like antioxidant (Cai et al., 2005) [63], antifungal (Tripathi et al., 1977) [64], antibacterial (Abu-Shanab et al., 2006) [65]

Many bioactive natural products of polyalthia genus have been found (Chen et al.,2000) [66].

Studies on the antimicrobial activity of *Notonia grandiflora* in vitro activity against *Staphylococcus aureus*, *Shigellashigae*, *Salmonella typhi*, *Escherichia coli*. Vashant et al., (2001) [67]

Different biological activities of polyalthia (Ashoka) have been studied of extracts/isolated compounds of *P.longifolia* (Ashoka) like antibacterial, cytotoxicity, antifungal,antiinflammatory,hepatoprotective,antiulcer and leishmanial, and antimalarial etc.(Marthanda Murthy et al.,2005) [68];sterigny et al., 2005 [69];Ichino etal 2006 [70];Nair and Chanda [71] 2008;Mishra etal 2010 [72].

Different parts of polyalthia (ashoka) plant have been reported by many authors for their medicinal uses(Nair et al.,2007) [71].

Lampe JW et al.,(1990) [73] reported that traditionally onion has been used for the treatment of intestinal infections. It has been reported to be an antibacterial, anti-viral , anti-parasitic, antifungal and has anti-hypertensive,hypoglycemic, anti-thrombotic, anti-hyperlipidemic, anti inflammatory and also antioxidant activity.
In a study conducted by (N. Benkeblia et al., 2004) \(^{74}\) in Algeria, red and the purple onion exhibited a better antibacterial activity as compared to yellow onion against S. aureus and Salmonella enteritis. The zone of inhibition of extracts increased with an increasing concentration of extracts.

In a study by (RehanIrkin et al., 2007)\(^{75}\), in Turkey, onion extract with ethyl alcohol has inhibited Aspergillus niger.

Melvin JM, et al. (2009) \(^{76}\) the onion bulbs contain numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, Spropylcyteine sulfoxides and cycloallicin, flavonoids, phenolic acids, sterols also including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and also a trace of volatile oil compounds mainly that of sulphur compounds. The presence of these compounds may explain antimicrobial activity of this plant.

Masaudi et al. (2012) \(^{77}\) have studied the antimicrobial activity of the onion juice alone which extracted from the red Egyptian onion, honey alone (Langaneza honey, Black Forest) and honey-onion mixture the (v/v: 1/1, 1/4, 4/1) with different in the concentrations 100, 50, 20 and 10% respectively, against the 8 microbial species, Streptococcus pyogenes ATCC 19615, Staphylococcus aureus; (Methicillin- Sensitive Staphylococcus aureus -MSSA) ATCC 25923, (Methicillin Resistant Staphylococcus aureus -MRSA) ATCC 10442, Enterococcus faecalis; (Vancomycin -Sensitive Enterococci- VSE) ATCC 29212, the (Vancomycin -Resistant Enterococci-VRE) ATCC 51299, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Candida albicans ATCC 10291 were then investigated by broth dilution method. The results showed that the onion juice at the different percentage 100%, 50%, 20% and 10% concentration have a very strong effect on the growth of all the tested species of microbes comparing with that of the control and Staphylococcus aureus was the most sensitive microbe. Moreover, the Honey at 100, 50, 20 and 10% concentration possess the very strong effect on the growth of all species of microbes but was found to be significantly less than the effect of onion juice. Results also showed that the honey-onion mixture was found to be more significant effect comparing with onion or honey alone.
Hamza et al., (2014) \cite{78} reported that Medicinal plants Garlic and Onion (Allium sativum and Allium cepa), can be described as those plants in which one or even more of its organs contain substances that can be used as a chemotherapeutic purposes or precursors for the synthesis of a useful drug. The study aims in determining the antibacterial activity of the aquatic garlic, onion and garlic-onion combination extract, then the garlic oil, onion oil and garlic-onion oil combination. The antibacterial effect of aquatic garlic, onion and garlic onion combination extract then garlic oil, onion oil and garlic-onion oil Combination against the five Gram-positive organisms and eight Gram-negative bacterial & one yeast isolates including the Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus viridans, Streptococcus pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi, Proteus mirabilis, Serratia marcescens, Acinetobacter baumannii, and also the Candida albicans (fungi) all of them were studied. Antibacterial activity of these aqueous & as well as the oil extracts by the well diffusion method were characterized by inhibition zones of five Gram-positive, eight Gram-negative and one yeast pathogenic microbes. All the organisms which were tested were highly sensitive to the garlic-onion combination (aqueous and the oil extract), then garlic (aqueous and oil extract), whereas all the organisms which were tested were slightly sensitive to onion (aqueous and oil extract).

Adwan et al., (2008) \cite{79} has demonstrated the synergetic effect of various plant extracts such as Psidium guajava, Rosmarinus officinalis, Salvia fruitcosa, with known antimicrobial agents of different mechanisms of action eg Penicillin G (cell wall inhibitor), Oxytetracycline and Gentamycin (protein synthesis inhibitors) to have promising results.

Esimone CO et al. (2012) \cite{80} studied the antimicrobial activities of the water and methanolic extracts of Psidium guajava Linn. stem bark which was evaluated against eight methicillin-resistant Staphylococcus aureus (MRSA) isolates. The plant material was then extracted and phytochemical analyses were also performed by standard procedures. The phytochemical studies of P. guajava revealed that there is a presence of carbohydrates, glycosides, tannins, and proteins as its major constituents. Results shows that the methanolic and water extracts of P. guajava stem bark exhibited antibacterial activity against the methicillin resistant S. aureus bacteria.
Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) was been studied by (Biswas et al.,) (2013) [81]. The guava leaves were extracted in four different solvents of increasing polarities of the hexane, methanol, ethanol, and water. According to the findings of the antibacterial assay, the methanol and the ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had the antibacterial activity with the mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean inhibition zone of 6.11 and 11.0 mm against B. cereus and S. aureus, respectively. On the basis of this study it concluded that, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent.

Garode AM et al.,(2014) [82] reported the antimicrobial activity of Psidium guajava Linn (Guava) commonly known for its food and nutritional values. Five grams of powder were used for crude solvent extraction in Chloroform, Ethanol, Petroleum ether and Water. The solvents were then evaporated to dryness and extracted compound was used for its antibacterial assay by process of disc diffusion method. The bacterial pathogens were used as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi. Thus, the result obtained was found that, the extracts of Guava leaves were inhibiting the growth of Salmonella typhi to maximum extent followed by other pathogens. Finally, it was concluded that the leaves extract of Psidium guajava Linn plant showed effective and efficient result against the bacterial pathogen used. Psidium guajava leaves could serve as a good source of the antibacterial agents.

Piper betel is a member of family piperaceae. The leaves of this plant have been used traditionally in India for ages since ancient times as a prevention medicine for the dental caries. It has been found to contain certain active compounds in its ethanolic extract. These compounds are as 4chromanol while phenol-2-methoxy-4(2-propenyl) acetate was found in ethanol extract (Deshpande et al.,2013) [83]

Valle Jr DL et al.,(2015) [84] reported that the leaf extracts of Psidium guajava, Phyllanthus niruri, Ehretia microphylla and Piper betle (P. betle) showed the antibacterial activity against the Gram-positive methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus. P. betle showed the highest antibacterial activity for these bacteria by the disk
diffusion method (16–33 mm inhibition diameter), minimum inhibitory concentration (19–156 mg/mL) and minimum bactericidal concentration (312 mg/mL) assays. P. betle leaf extracts only showed remarkable antibacterial activity for all of the Gram-negative multidrug-resistant bacteria (extended spectrum b-lactamase-producing, carbapenem-resistant Enterobacteriaceae and metallo-b-lactamase-producing) in the disk diffusion (17–21 mm inhibition diameter), minimum inhibitory concentration (312–625 mg/mL) and minimum bactericidal concentration (312–625 mg/mL) assays. They concluded that P. betle had the greatest potential value against both for the Gram-negative and Gram-positive multidrug-resistant bacteria.

Valle Jr DL et al., (2016) reported Antimicrobial Activities of Methanol, Ethanol and Supercritical CO2 Extracts of Philippine study revealed that the bactericidal activities of all the P. betle leaf crude extracts on methicillin-resistant Staphylococcus aureus (MRSA), the vancomycin-resistant Enterococcus (VRE), extended spectrum β-lactamase-producing Enterobacteriaceae Piperbetle L. on the Clinical Isolates of Gram Positive and as well as the Gram Negative Bacteria with Transferable Multiple Drug Resistance, carbapenem-resistant Enterobacteriaceae, and metallo-β-lactamase-producing Pseudomonas aeruginosa and Acinetobacter baumannii., with minimum bactericidal concentrations ranging from 19 μg/ml to 1250 μg/ml. The extracts proved to be more potent against the Gram positive MRSA and also to VRE other than that for the Gram negative test bacteria negative. Data from the study firmly established P. betle as an alternative source for the anti-infectives against multiple drug resistant bacteria.

Duhan et al., (2013) Phytochemical Analysis And Antimicrobial Potential Of The Leaf Extract OfThuja orientalis was detected where Different leaf extract posses the antimicrobial activity almost against many the tested microbes among all the prepared extract of leaf the acetone extract was found to be most potent against B. subtilis maximum zone of inhibition 15.55 mm and than against A. furcalis 15.50 mm S. aureus were found sensitive against acetone extract 12.66 mm and 12.50 mm inhibition zone respectively.

Jasuja ND et al., (2013) studied the antibacterial, antioxidant and phytochemical investigation of Thuja orientalis leaves. The leaves of Thuja orientalis were powdered and extracted by soxhlet extractor in two solvent systems that is, the (E1) ethyl acetate:
chloroform: ethanol (40:30:30) and (E2) methanol: distilled water (70:30). The study conferred the screening up of the phytochemical constituents, antioxidant activity and antibacterial activity of crude extract E1 and E2 and its fractions. Antioxidant activity was carried out by 2,2-diphenyl-1 picrylhydrazyl (DPPH) assay. The results indicate that the E2 extract (70% methanolic extract) had the highest antioxidant effect (85.25% inhibition) at 100 µg/ml concentration and the crude extracts (E1 and E2 extract) showed significant inhibitory activity against both the gram positive and gram negative organisms. It was active against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and also against Agrobacterium tumefaciens. The minimum inhibitory concentrations (MICs) of E1 extract ranged from 0.40 to 0.85 mg/ml and E2 extract 0.55 to 1.15 mg/ml. The highest antibacterial potentiality was exhibited by E2 extract. The fractions was also found to exhibited antimicrobial activity against all the selected microorganisms. The study revealed that the T. orientalis is a promising phytomedicine for antioxidant and antibacterial activity.

Wasim et al.,(1995) have done the study on Antimicrobial activity of the leaf of Cannabis Sativa l on Gram positive Baccilus subtilus, Baccilus pumilus, Staphlococcus aureus and Micrococcus flavus. Gram negative Proteus vulgaris and Bordetella bronchoseptica. Fungi Candida albicans and Aspergillus niger. The In vitro antimicrobial studies were conducted with the aqueous, ethanolic and Petroleum ether extracts of the leaves of the Cannabis sativa L. The acidic fraction was obtained from the ethanolic extract and 2% of the Sodium Hydroxide extract. Ethanolic extract, petroleum ether extract and the acidic fraction exhibited antimicrobial activity both against Gram-positive and Gram-negative bacteria and also against the fungi. The aqueous extract however, did not show any antimicrobial activity.

The oil of the seeds, petroleum ether and the methanol extracts of the whole plant of Cannabis Sativa were screened for their antimicrobial activity against the two Gram positive organisms Bacillus Subtilis, Staphylococcus aureus, two Gram negative organisms (Escherichia coli, Pseudomonas aeruginosa) and also two fungi namely Aspergillus niger and Candida albicans using the cup plate agar diffusion method. The oil seeds of Cannabis sativa have exerted pronounced antibacterial activity (21-28 mm) against Bacillus subtilis and Staphylococcus aureus, the moderate activity (15 mm) against Escherichia coli and high activity (16 mm) against Pseudomonas aeruginosa and was inactive against the two fungi tested. The petroleum ether extract of the whole plant have exhibited pronounced antibacterial activity of zone of inhibition (23-28 mm) against both Bacillus subtilis and the
Staphylococcus aureus, high activity (16 mm) against Escherichia coli and was inactive against Pseudomonas aeruginosa and also against both fungi. The methanol extract of the whole plant showed also pronounced antibacterial activity (29 mm) against Bacillus subtilis, the low activity (12 mm) against Staphylococcus aureus and high activity (16 - 18 mm) against both Gram negative organisms, inactive against Aspergillus niger and low activity (13 mm) against Candida albicans. Thus, the oil seeds of Cannabis sativa have shown to exert pronounced antibacterial activities but inactive against two fungi tested (Esra M M Ali et al., 2011) [89].

The antimicrobial activity of Cannabis sativa is well known for a long time. The methanolic extracts of cannabis leaves have shown to possess the antimicrobial activity against several pathogenic organisms like E. coli, S. aureus, S. pneumoniae and S. typhi using the agar well diffusion method (Navneet et al., 2014) [90].

Maksumradji et al., 2013 [91] reported the Antimicrobial activity of the green tea extract against methicillin-resistant Staphylococcus aureus isolates and multi-drug resistant Pseudomonas aeruginosa where the results showed that the zone of inhibition diameter of green tea extract for S. aureus ATCC 25923 and MRSA were (18.970 ± 0.287) mm, and (19.130 ± 0.250) mm respectively. While the inhibition zone diameter for Pseudomonas. Aeruginosa ATCC 27853 and MRD-P. aeruginosa were (17.550 ± 0.393) mm and (17.670 ± 0.398) mm respectively. The MIC for MRSA were 400μg/Ml and for P. aeruginosa were 800μg/ml and thus concluded that Camellia sinensis leaves extract could be useful in combating the emerging drug-resistance caused by MRSA and P. aeruginosa.

AK S. Khan et al., (2012) [92] described a rapid and accurate PCR for the detection of clinically relevant antibiotic resistance gene of Staphylococcus aureus. In the present study, the PCR method was used for detection of mecA genes among the MRSA strains.

A total of 586 Staphylococcus positive clinical samples were collected under the aseptic precautions from the patients attending various departments in a tertiary care hospital in south India. The Staphylococcus aureus isolates were identified on the bases of their cultural characteristics, biochemical reactions and positive tube coagulase test. Methicillin resistance was determined by the Kirby-Bauer’s disc diffusion method. The PCR was used for the mecA gene detection from MRSA strains. Of the total 586 Staphylococcal aureus isolated, 236 (40.2%) strains were MRSA. Thirty-five MRSA strains were randomly selected for the PCR
assay. Thirty three MRSA strains (94%) were mecA gene positive and the two MRSA strains were mecA negative visualised on a 2% agarose gel electrophoresis.

Ali et al.,(2014)\textsuperscript{[93]} aimed to investigate S. aureus in some clinical samples by PCR and study the bacterial resistance to some antibiotics DNA fragments were amplified from isolated DNA. PCR was used to amplify the sequences of 16S rRNA, gap gene and nuc gene depending on the six specific primers. The PCR products were detected by the agarose gel electrophoresis. The antibiotics susceptibility tests was conducted on all the isolates by using the method of Kirby-Bauer disk diffusion method on the Mueller Hinton agar and Luria Bertani (LB) Agar. Out of the Eighty one isolates of S. aureus which were collected from the blood samples, urine samples and bronchial secretions. The results showed that DNA fragments of 16S rRNA, gap gene and the nuc gene were approximately equal to 479 bp, 933 bp and 270 bp, respectively and the result of antibiotics resistance for the 10 tested antibiotics was that the Chloramphenicol which was (97.5%), Tetracycline (50.6%) , Cefuroxime (37.0%), Oxacillin (33.3%), Levofloxacinc (37.0), Erythromycin (35.8%), Ciprofloxacin (32.1%), Rifampicin (7.4%), Vancomycin (3.7%), Imipenem (0%).