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Activities of some important herbal drugs and common antibiotics against human pathogens predominantly *S. aureus* with special reference to determination of resistant genotype by multiplex PCR assay and its correlation with herbal drugs susceptibility pattern against *S. aureus*

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Abstract
Medicinal plant represents a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts are used for extract as raw drugs and it's obtaining varied medicinal properties. Several screening studies have been carried out in different parts of the world. On the antimicrobial activity of different herbal extracts. Considering the vast potentiality of plants as a source for antimicrobial drugs with reference to antibacterial and antifungal efficacy, this study was undertaken to screen the local flora for antibacterial activity. These local flora includes *Acacia nilotica, Tinospora cordifolia,* and *Withania somnifera*. The study was conducted in the Postgraduate Department of Microbiology, King George Medical University, Lucknow; from January 2011 to December 2011.The plants and pathogenic bacterium used in the research were *Acacia nilotica, Tinospora cordifolia*, *Withania somnifera* and *Staphylococcus aureus* respectively. The bacterium was cultured on nutrient agar and incubated at 37 °C for 24hours. The bacterium was repeatedly cultured to obtain pure isolates. Morphological and biochemical reactions were carried out to ascertain proper identification. the study shows the percentage of *Staphylococcus aureus*, and MRSA strains in the total specimens received. Mupirocin resistance is shown as determined by disc diffusion method, whereas methicillin resistance was determined by cefoxitin disc diffusion method. In the present study Gram-negative bacilli are the predominating pathogen (12.9%) while Gram positive cocci constituted only (4.37%). *S. aureus* isolates constitute only 31.4% among Gram-positive cocci while just 2.06% in overall samples received. The study shows Ashwagandha (12 µl/ml) and Babool (10mg/ml) has antimicrobial activity, but Guduchi did not show any type of antibacterial activity.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA) *Acacia nilotica, Tinospora cordifolia,* and *Withania somnifera*

Introduction
Historically, plants have supplied a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. In our country we are using crude plants as medicine since Vedic period. Globally too, the use of medicinal plants as a source illness can be traced back over five millennia documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts are used for extract as raw drugs and it's obtaining varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities through the local communities and folk healers for local used, many other raw drugs are collected in bigger quantities and traded in the market as the raw in larger quantities and traded in the market as the raw material for many herbal industries [1].

Even though many of plant species have been tested for antimicrobial properties, the vast majority of has not been adequately evaluated (Balandrin *et al.*, 1985) [2]. Several screening studies have been carried out in different parts of the world. Several reports on the antimicrobial activity of different herbal extracts in different regions of the world [3]. Although a wide range of medicinal plants are used for extract as raw drugs and they possess varied medicinal properties yet it is not possible to evaluate all of them in a single study. Considering the vast potentiality of plants as a source for antimicrobial drugs with reference to antibacterial and antifungal efficacy, this systematic investigation was undertaken to screen the local flora for antibacterial activity. These local flora *Acacia nilotica, Tinospora cordifolia,* and *Withania somnifera*.

All these herbs are traditionally being used as medicinal drugs and time and again have shown their efficacy as a therapeutic agent. The present study is targeted to evaluate the antibacterial efficacy of these drugs and to compare their relative efficacy too.

Staphylococcus aureus has been a major cause of infections in humans for as long as we have historical records. Pathological changes consistent with staphylococcal osteomyelitis are known from Egyptian mummies and other remains of similar antiquity. Along with several other organisms (e.g., group A *β-hemolytic streptococci* and *Mycobacterium tuberculosis*), this organism is uniquely equipped with virulence factors and defence mechanisms that enable it to cause rapidly progressive fatal infections in normal individuals.

Method and Materials

Materials/Chemicals

The study was conducted in the Postgraduate Department of Microbiology, King George Medical University, Lucknow, from January 2011 to December 2011.

The materials/chemicals used in this study were of analytical grade, include 96 % ethanol, chloroform, diethyl ether, methanol, dimethy - lsulphoxide (D. M. S. 0), Sabouraud dextrose and Mueller-Hinton agars, Petri dishes, Whatmann number 1. Filter papers, etc. The plants and pathogenic bacterium used in the research were Acacia nilotica, Tinospora cordifolia, Withania somnifera and *Staphylococcus aureus* respectively.

Methods

Extraction procedure

The pulverized plant materials; Acacia nilotica (200g), Tinospora cordifolia (400g) and Withania somnifera (400g), were soaked in 1,000m1, 1,200 ml and 1,200 ml of 96 % ethanol respectively for two weeks with intermittent shaking. The percolates were separately evaporated to dryness at room temperature (25 °C) to give crude extracts (ethanol extracts).

Fractionation of crude extracts

The crude extracts were fractionated using diethyl ether, chloroform and methanol solvents in the order of their increasing polarity indices. For l0g of ethanol extract of Acacia nilotica 30ml, 20ml and 15ml of diethyl ether, chloroform and methanol solvents were respectively used. For 40g of ethanol extract of Tinospora cordifolia 80ml, 60ml and 40ml of diethyl ether, chloroform and methanol solvents were respectively used. For 20g of ethanol extract of Withania somnifera 60ml, 40ml and 30ml of diethyl ether, chloroform and methanol solvents were respectively used. Solvent solubles of ethanol extracts were filtered and evaporated to dryness at room temperature 25 °C to give diethyl ether fractions, chloroform fractions methanol soluble fractions and methanol insoluble fractions.

Preparation of concentrations of solvents soluble fractions and extracts

Stock solutions of 10mg/m1 concentrations of solvent soluble fractions and extracts of the plants were prepared by dissolving 5mg of each fraction or extract in 0.5m1 of dimethyl sulphoxide (DMSO). From the stock solution, concentrations of 0.5mg/ml, 1.0mg/ml, and 0.5mg/m1 were prepared by serial dilution method. Concentrations of ciprofloxacin standard antibiotic were similarly prepared as those of the plant extracts. They were stored in sterilized vials and kept at the lower compartment of a refrigerator until required for use.

1. Initial isolation of Staphylococcus aureus

1. Specimens received in the bacteriology laboratory of the department were processed under routine laboratory protocol.
2. Staphylococcus aureus isolated from the specimens were sub-cultured and stock in 1% agar tube by stab culture for further processing.
3. These tubes were incubated at 37ºC for 24 hours. Next day after observing the growth, the caps of the tubes were paraffinised and stored at 4ºC.

2. Re-identification and processing of the Staphylococcus aureus isolates

1. Subcultures of Staphylococcus aureus isolates.
2. Re-identification of isolates were done under following protocol:
   a) Identification on the basis of colony characteristics
   b) Microscopic examination
   c) Catalase test
   d) Coagulase test
   i. Slide coagulase test
   ii. Tube coagulase test
3. Disc diffusion method for determination of low-level and high-level resistance
4. Broth microdilution method for determination of MIC (minimum inhibitory concentration) of mupirocin.
5. Agar dilution method for determination of MIC of mupirocin.
6. In vitro antibiotic susceptibility pattern of *Staphylococcus aureus* by Kirby-Bauer disc diffusion method.
7. Determination of methicillin resistance in *Staphylococcus aureus* by using cefoxitin disc.

Antibacterial sensitivity test: The bacterium was cultured on nutrient agar and incubated at 37 °C for 24hours. The bacterium was repeatedly cultured to obtain pure isolates. Morphological and biochemical reactions were carried out to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4.C. The agar diffusion method was used. Mueller-Hinton agar plates were inoculated with standard test inocula by direct streaking, and plates were properly labeled. A sterile cork borer (5mm in diameter) was used to make wells in the plates for the

extracts. 0.1m1 of the fractions was dispensed in to each well. The plates were left to allow diffusion of extracts before being placed in the incubator at 37 °C for 24hours. The relative susceptibility of the organism to the extracts was indicated by zones of inhibition produced after incubation which was measured and recorded in millimeters. But the resistant strains grew up to the edges of the wells. Experiment was conducted in triplicates and values for diameter of growth inhibition were used to calculate the sample standard deviation.

Result and Discussion

A Total of 19841 samples were received for bacterial culture in the bacteriology laboratory of Postgraduate Department of Microbiology, K.G.M.U., Lucknow during August' 2011 to July'2012. All the samples were grouped in 5 groups

a) Pus
b) Blood
c) Genitourinary specimens
d) Respiratory specimens
e) Miscellaneous which includes pleural fluid, ascetic fluid, other body fluids, ophthalmic specimens and biopsy tissues etc.

Percentage distribution of samples

Table 1: Distribution of samples

Sample	Percentage
Pus	13.2
Blood	24.5
Genitourinary specimens	43.1
Respiratory specimens	7.0
Miscellaneous	12.2

Table 1 shows the distribution of various samples. Genitourinary specimens contain maximum number of samples and constitute 43.1% of total samples received with urine comprising 38.8% of total samples followed by blood samples. Respiratory samples are least among them.

Table 2: Sample wise distribution of bacterial isolates obtained during August 2011- July 2012

Sample type	No. Of specimens	Total isolates	Gram negative bacilli							Gram positive cocci				Gpb	Cand.
			E. Coli	Kalb. Sapp	Pseudo spp.	Aconite sop	Proteus sop	Citro spp.	Enter sop	*S. Aureus*	Cons	Entero sop	Bhs	Dpitheroid	
Pus	2620	1287	404	96	388	145	6	32	-	142	14	24	-	24	12
Blood	4862	476	42	8	16	48	-	14	-	48	176	88	8	3	25
Genitourinary specimens	8551	1208	720	41	66	43	-	14	15	11	4	225	-	28	21
• Urine	7708	1074	657	36	65	38	-	12	12	20	4	208	-	10	12
• Vaginal Swabs	810	124	58	5	1	1	-	2	3	10	-	17	-	18	9
• Semen	31	8	4	-	-	3	-	-	-	1	-	-	-	-	-
• Urethral discharge	2	2	1	-	-	1	-	-	-	-	-	-	-	-	-
Respiratory specimens	1383	412	70	21	39	153	-	44	4	39	19	12	-	4	7
• Sputum	206	51	7	9	8	12	-	5	-	1	1	-	-	3	5
• Throat swab	310	115	9	7	4	39	-	26	-	19	7	3	-	-	1
• Tracheal aspirate	792	214	44	3	25	100	-	11	-	15	11	5	-	-	-
• Nasal swab	36	16	8	2	-	2	-	-	-	4	-	-	-	-	-
• Ear swab	10	5	2	-	2	-	-	-	-	-	-	-	-	-	1
• Oral swab	29	11	-	-	-	-	-	2	4	-	-	4	-	1	-
Miscellaneous	2425	186	38	14	16	69	-	1	0	10	15	12	-	1	10
• Pleural fluid	196	19	2	2	2	3	-	0	0	4	0	4	-	-	2
• Ascitic fluid	284	18	5	0	4	0	0	0	0	2	1	0	-	-	6
• Other body fluids	1920	145	31	12	9	66	-	-	-	4	12	8	-	1	2
• Ophthalmic specimens	11	0	-	-	-	-	-	-	-	-	-	-	-	-	-
• Disc. Material and Biopsy tissue	14	4	-	-	1	-	-	1	-	-	2	-	-	-	-
Total	19841	3569	1274	180	525	458	6	105	19	250	228	361	8	60	75
GPB= Gram positive bacilli, CAND.= Candida															

Distribution of isolates in different group of samples

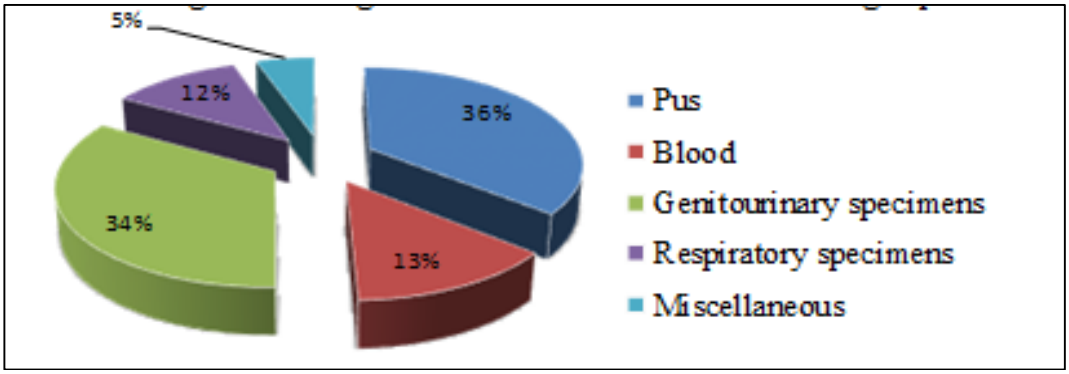

Fig 1: Percentage distribution of isolates in different groups

Figure 1 shows the percentage distribution of isolates in different group of samples. Pus samples had maximum isolation with 36% of total isolates obtained from various group of samples, 34% isolates from genitourinary specimens, 13% from blood and respiratory specimens and 5% from miscellaneous samples.

Distribution of isolates among total isolate

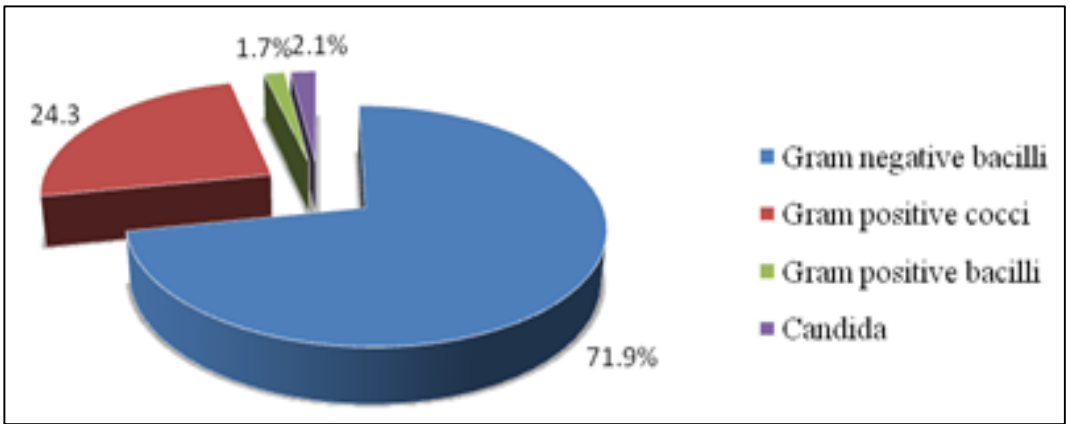

Fig 2: Percentage distribution of various isolates

Figure 2 shows the percentage distribution of isolates among the total isolates obtained from different group of samples. Out of 19841 specimens 3569 (17.98% of total sample received) samples were having pathogenic isolates. Overall Gram negative bacilli were predominating (12.9% of total samples), followed by Gram positive cocci (4.37%). *Candida* and Gram positive bacilli (esp. *diphtheroids*) were present in 0.4% in 0.3% of samples, respectively. Among pathogenic isolates 71.9 % were Gram negative bacilli, 24.3% Gram positive cocci, 1.7% Gram positive bacilli and 2.1% *Candida*.

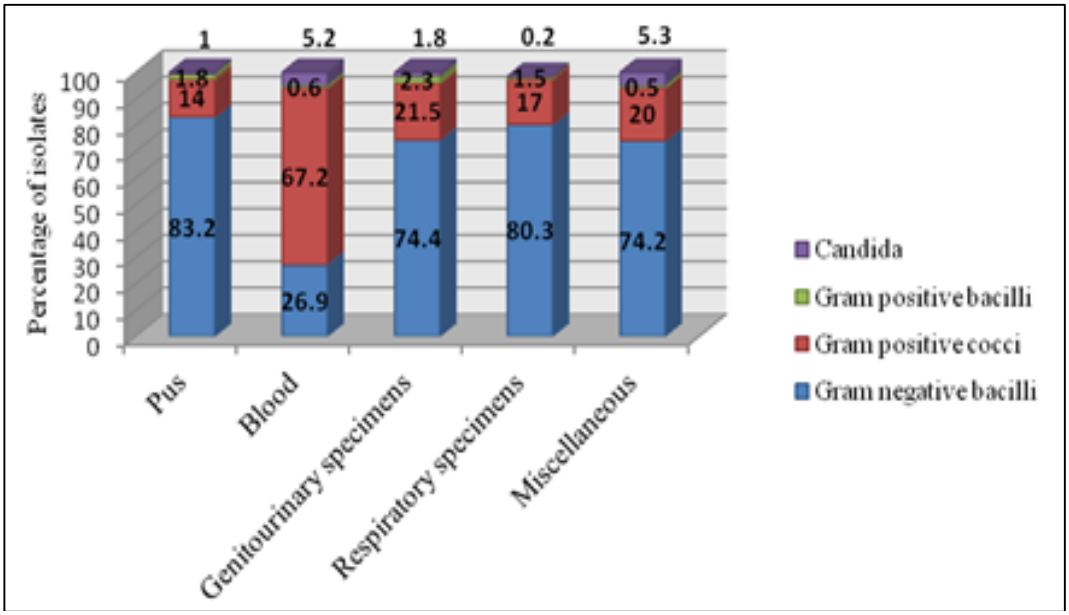

Fig 3: Percent distribution of various isolates in different samples

Figure 3 is showing distribution of various isolates in different specimens. Sixty seven percent of total pathogenic isolates in blood were Gram positive cocci, whereas Gram negative bacilli predominates in pus, genitourinary specimens, respiratory specimens and miscellaneous samples with 83.2%, 74.4%, 80.3% and 74.2% isolates, respectively. *Staphylococcus aureus* was isolated in only 0.2% urine samples. As urine shares a major percentage of samples, urine samples were excluded from the study. From now onwards total sample excludes urine samples.

Distribution of *Staphylococcus aureus*, MRSA in different samples

Table 3: Distribution of *Staphylococcus aureus*, methicillin resistant *S. aureus* (MRSA) isolates in different samples

Sample types	Total no. of samples	*S. aureus* (%)	MRSA (%)
Pus	2620	142 (5.42)	78 (2.97)
Blood	4862	48 (0.98)	27 (0.55)
Genitourinary specimens	843	11 (1.3)	1 (0.11)
• Vaginal Swabs	810	10 (1.23)	0 (0)
• Semen	31	1 (3.22)	1 (3.22)
• Urethral discharge	2	-	-
Respiratory specimens	1383	39 (2.09)	23 (1.66)
• Sputum	206	11 (5.34)	6 (2.91)
• Throat swab	310	21 (6.77)	12 (3.87)
• Tracheal aspirate	792	3 (0.38)	2 (0.25)
• Nasal swab	36	4 (11.11)	3 (8.33)
• Ear swab	10	-	-
• Oral swab	29	-	-
Miscellaneous	2425	10 (0.41)	4 (0.16)
• Pleural fluid	196	4 (2.04)	1 (0.51)
• Ascitic fluid	284	2 (0.70)	1 (0.35)
• Other body fluids	1920	4 (0.21)	2 (0.11)
• Ophthalmic specimens	11	-	-
• Disc. Material and Biopsy tissue	14	-	-
Total	12133	250 (2.06)	133 (1.09)

Table 3 shows the percentage of *Staphylococcus aureus*, and MRSA strains in the total specimens received. Mupirocin resistance is shown as determined by disc diffusion method, whereas methicillin resistance was determined by cefoxitin disc diffusion method as described. Two hundred fifty *Staphylococcus aureus* isolates were obtained during the study period of them 133 were detected as MRSA strains and 5 as mupirocin resistant *Staphylococcus aureus* (Mup RSA). *Staphylococcus aureus* isolates comprises 2.06% of the total samples received during the study period whereas Mup RSA and MRSA were 0.04% and 1.09% of total samples received.

Sample-wise distribution of MRSA among *Staphylococcus aureus* isolates

Table 4: Percentage of MRSA strains out of total *Staphylococcus aureus* isolates in different samples

Samples	*S. aureus*	MRSA (%)
Pus	142	78 (54.93)
Blood	48	27 (56.25)
Genitourinary specimens	11	1 (9.09)
Respiratory specimens	39	23 (58.97)
Miscellaneous	10	4 (40.0)
Total	250	133 (53.2)

Table 4 shows sample-wise distribution of MRSA among total *Staphylococcus aureus*. Methicillin resistance (56.25%) was found maximally in the *Staphylococcus aureus* isolated from the blood samples. In all 53.2% were resistant to methicillin.

Table 5: Sex-wise distribution of MRSA in tertiary care hospital

SEX	*S. aureus*	MRSA (%)
Male	194	112 (57.73)
Female	56	21(37.5)
	250	133

According to table 5 *Staphylococcus aureus* were predominantly isolated from male patients with 1.03% and 57.73% isolates were mupirocin resistant and methicillin resistant respectively. In case of female 37.5% were MRSA isolates.

Table 6: Plant extract with activity on bacteria

S.N.	Plant	Part	Concentration with inhibition	
1.	Guduchi	Leaves	-	No inhibition on *S. aureus*
2.	Ashwagandha	Leaves	12µg/ml	Inhibition to *S. aureus*
3.	Babool	Leaves	10mg/ml	Inhibition to *S. aureus*

Table 6 displays the antibiotic susceptibility pattern of MRSA strains for different group of antibiotics effective primarily against Gram positive organism. MRSA isolates shows 89.5% resistance to ampicillin and >60% resistance to ciprofloxacin and erythromycin. 60% of mupirocin resistant strains were susceptible to broad spectrum group of antibiotics tetracycline and septran. All MRSA isolates were susceptible to vancomycin and linezolid.

According to table 6 its shows that Ashwagandha (12µ/ml), Babool (10mg/ml), inhibition the growth of *S. aureus*. But Guduchi was not shows any type of inhibition against in this study.

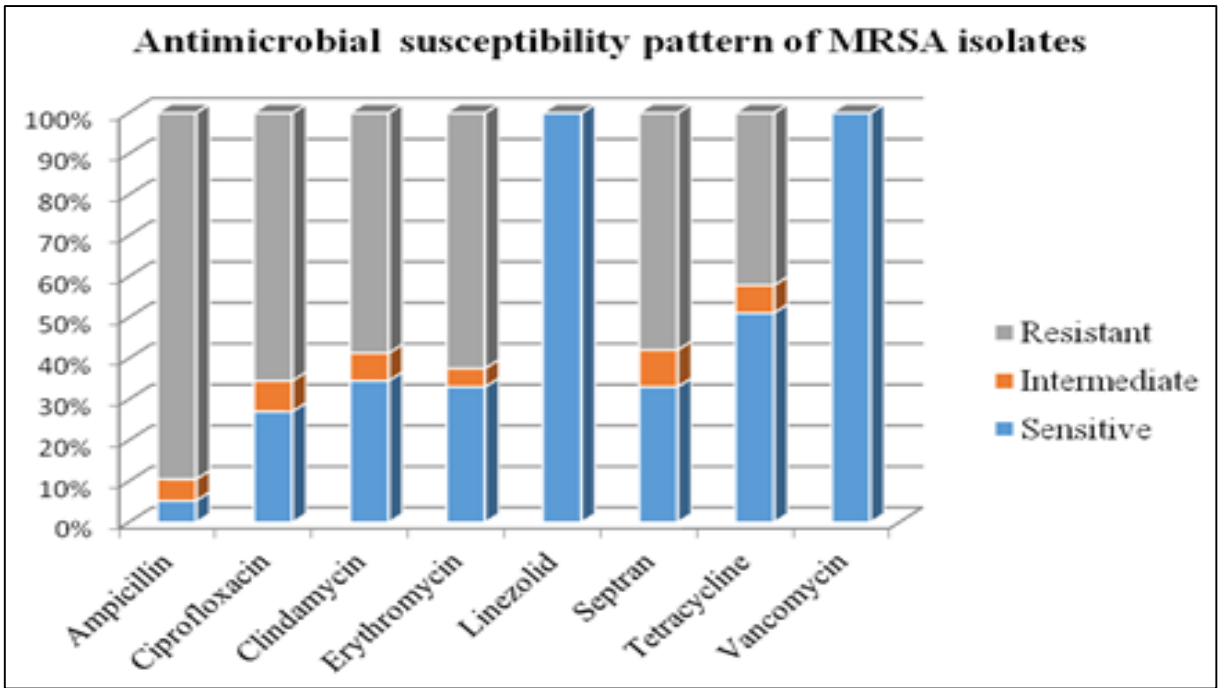

Fig 4: Antimocrobial susceptibility pattern of MSRA isolates

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay [10]. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials[11]. Continued further exploration of plant-derived antimicrobials is needed today. A total of 8 extracts from 4 different plant species were investigated. Extracts of the different parts of the tested medicinal plants used in this study were shown in Table 1. The Antibacterial susceptibility by means of disk diffusion method showed that the 3 plant extracts tested exhibited an antimicrobial effect against Bacillus circulans, Bacillus circulans, Bacillus circulans, Staphylococcus aureus and Serratia liquefaciens (Table 2). Barks extracts of the four tested medicinal plants possess a lower zone on inhibitory activity as compared to the leaf extracts of these plants. Noticeably no antimicrobial activity was found in methonolic bark extract of Acacia nilotica against the tested bacteria except Bacillu ciurlans (Table 2).

Though, the mechanism of the action of these chemical constituents is not yet fully known, it is clear that the effectiveness of the extracts largely depends on the type of solvent used. Perhaps it is one of the reasons behind the differences in the antibacterial activities of the plants. Moreover, the effectiveness of the extracts varies with its concentration and the kind of bacteria used in the study. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures[12, 13]. Thus, *S. aureus* was found to be resistant to all the extracts. Which is most probably due to its outer membrane? Nevertheless, it is the ability of the active principle of the extracts that disrupt the permeability barrier of cell membrane structures and thus inhibit the bacterial growth[12, 13]. The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [14]. It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally, ion leakage from the cells[15]. In our study B. subtillis was found to be highly susceptible to L. camara ethyl acetate extracts of pods and leaves. Similar to one of the early studies[16]. Z. mauritiana ethanol leaf extract was active against *S. aureus* supported by a previous study[17]. The ascending sequence of maximum antimicrobial activity against test microorganisms were as follows: Acacia nilotica, withania somnifera and tinospora cordifolia (Table 2). Medicinal plants can be poisonous if wrong plant parts or wrong concentrations are used. Herbal medicines are assumed to be harmless. Although herbal extracts need to be assured for quality control and efficacy for a particular dose, the results of present study clearly indicate that the antibacterial activity varies with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. MRSA is a major nosocomial pathogen causing significant morbidity and mortality [18, 19]. The important reservoir of MRSA in hospitals is infected or colonised patients. In India, the significance of MRSA had been recognised relatively late and it is emerged as a problem in the 1980s.

With increasing pressure to prevent methicillin-resistant *Staphylococcus aureus* (MRSA) infection, there is increased use of mupirocin (*Pseudomonas fluorescens*), a topical antibiotic for eradication of nasal carriage of MRSA. The indiscriminate use of mupirocin leads to development of resistance **[20]**. The resistance was reported soon after the introduction of the drug leading to a major concern to treat the carriage of MRSA. In the past few years, mupirocin resistance has been increasing among staphylococci in many parts of the world **[21, 22]**.

Very scanty data is available regarding the prevalence of mupirocin resistance in India. KGMU being a major referral centre of Uttar Pradesh, this study is conducted to observe

the mupirocin resistance in *Staphylococcus aureus*, to determine the prevalence in this region. The material for the present study was collected from the patient attending the outpatient department and admitted to inpatient department. Three different methods of mupirocin resistance detection have been evaluated in this study to find out the most suitable for routine purpose because mupirocin resistance is not detected routinely as no such guidelines is recommended for the resistance detection.

In this study, out of total 250 *Staphylococcus aureus* isolates 5 i.e. 2% showed mupirocin resistance by disc diffusion method. All the mupirocin resistant *Staphylococcus aureus* isolate are high-level resistant strains. MIC as determined by using two methods; broth microdilution method as recommended by CLSI 2012 guidelines and agar dilution method as described in various studies [19, 20, 21] showed that all the 5 mupirocin resistant *Staphylococcus aureus* isolates have MIC > 512 µg/ml which is a high-level resistant phenotype. This result is consistent with the finding of the disc diffusion method. Low-level resistant phenotype was found in this study.

Antimicrobial susceptibility pattern of MRSA

In this study among MRSA isolates 100 % resistance was seen to ampicillin, oxacillin and ciprofloxacin whereas in MRSA isolates it is 89.5% and 65.6%, respectively. None of isolates including MRSA found resistant to vancomycin and linezolid similar to as seen in the study conducted in Trinidad in 2008 by Orrett *et al*., [22] which has also reported 96.8%, 95.2%, 94.1%, 93.6% and 93.1% for gentamicin, ciprofloxacin, amikacin and tobramycin, cotrimoxazole and tetracycline, respectively.

MDR-MRSA

The epidemiology of MRSA strains is gradually changing since its emergence was reported. Initially there were occasional reports but now it has become one of the established hospital acquired pathogen. Moreover the association of multidrug resistance with MRSA has added to the problem. In the present study, the MRSA isolates resistant to $\leq$ 2 non β-lactam groups of antibiotics were considered as non-multi-drug resistant MRSA strains and the isolates resistant to $\geq$ 3 non β-lactam groups of antibiotics were considered as multi-drug resistant MRSA strains. This was the criteria given by Merlino J *et al*., (2002) [23].

We obtained high percentage of multidrug resistant MRSA from clinical specimens i.e. 67.7%. Majumder *et al*., [24] from Assam had reported 23.2% of the MRSA strains isolated from clinical specimens to be multidrug resistant. Anupurba from U.P. had reported a higher percentage of multidrug resistant MRSA strains (32%).Vidhani *et al*., [25] from Delhi reported even a higher percentage of multidrug resistant MRSA strains from high-risk patients admitted in burns and orthopaedic units. Rajaduraipandi K *et al*., [26] who observed 63.6% of multidrug resistant MRSA strains in clinical samples have somewhat similar results to the present study.

Conclusion

In the present study 250 S. aureus isolates were prospectively processed and observed for oxacilline resistance and studied in detail; following conclusions were drawn from the above work. In all clinical samples received for bacterial culture, Gram-negative bacilli are the predominating pathogen (12.9%) while Gram positive cocci constituted only (4.37%). *S. aureus* isolates constitute only 31.4% among Gram-positive cocci while just 2.06% in overall samples received. Overall hospital load of MRSA strains are also less i.e. 1.09% of all samples received during one-year study period.53.2 % of total *S. aureus* isolates are resistant to methicillin (MRSA strains), this prevalence is quite high. All the oxacilin resistant *S. aureus* showed to have high level resistant. Maximum percentage of MRSA strains is found among *S. aureus*, isolated in blood (56.25% and 6.25% respectively). MRSA isolates was obtained maximally from OPD (62.1%> of *S. aureus* from OPD samples) patients as compared to Indoor patients. Among the indoor patients MRSA isolated maximally (60%) of *S. aureus*) from surgical wards. MRSA isolated from surgical wards was isolated maximally from orthopaedics wards (72.4%>) followed by neurosurgical wards (62.5%). Urine in spite of being maximal in sample input, the isolation of *S. aureus* and MRSA strains is quiet low. No significant difference is seen in the distribution of MRSA isolates in different age group and sex. All MRSA isolates of clinical samples were susceptible to vancomycin and linezolid. MRSA isolates from clinical samples were showing high level resistance to all groups of antibiotics with 89.5% resistance to ampicillin and >60% resistance to ciprofloxacin and erythromycin. Most of MRSA isolates from clinical samples (67.7% and 80%) were found to be MDR strains i.e. resistant to more than or equal to 3 non β lactam group of antibiotics. Three different phenotypic methods were used to detect high-level and low- level oxacillin resistant i.e. disc diffusion assay, agar dilution and broth microdilution method. All of them found to be equally efficacious to detect the resistance. These MRSA strains showed high-level resistance to almost all group of antibiotics. 100% resistance is seen to ampicillin, ciprofloxacin, clindamycin and septran among isolates. Vancomycin and linezolid is the only drug to which all strains of MRSA isolates are susceptible. According our study Ashwagandha (12 µl/ml), Babool (10mg/ml) shows antimicrobial activity. But Guduchi was not shows any type antibacterial activity. From this study it is concluded that high-level oxacillin resistant is present among the Staphylococcus aureus isolates from both the patients admitted in the hospital and hospital staff taking care of patients which is a major concern as it may spread the disease among patients and other health care personnel. This indiscrimate and overuse of antibiotics with no antibiotic policy may be a reason for development of resistance in our hospital. This study also found that herbal drug is cheaper and safer than allopathic antibiotics. Thus, suggesting routine use of herbal drug against bacterial treatment in hospitals.

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