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Efficacy of Some Medicinal Plants on MDR Staphylococcus saprophyticus Isolated from Urine sample

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ABSTRACT

Staphylococcus saprophyticus is Gram positive spherical shaped bacteria. It is opportunistic pathogen. It is a normal flora of skin and periurethral area. It can cause UTI particularly in sexually active young women.UTI is second most common type of infection in community practice. UTI refers to the presence of microorganism in the urinary tract including urinary bladder, prostate, collecting system or kidney. The syndrome ranges from asymptomatic bacteriuria to perinephrid abscess with sepsis. Due to indiscriminate use of antibiotics there is an increase in resistance among microorganisms. Bacteria become resistant to antibiotic by different ways. Patent suffering from antibiotic resistant strain fail to respond antibiotic treatment. So there is a continuous demand of new drug in the world. This problem of drug resistance among microorganism could overcome by drugs of plant origin. Due to this reason now a day the demand of herbal products as therapeutic agents is increasing all over the world. In the present study antibacterial activity of *Aloe vera, Azadirachta indica* & *Citrus limon* was studied against MDR *Staphylococcus saprophyticus* isolated from urine sample collected from civil hospital. Antibiotic susceptibility test of the clinical isolates of *Staphylococcus saprophyticus* was done by using modified Kirby-Bauer disc diffusion method in accordance with the guidelines of the clinical & laboratory standards institute. Interpretation of resistance was based on the NCCLS criteria. The most common pattern of multiple drug resistance of isolates of *Staphylococcus saprophyticus* observed was erythromycin-nalidixic acid-penicillin-sulfamethoxazole-chloramphenicol. The antibacterial activity of solvent extract of plant was tested by agar well diffusion method. *Citrus limon* and *Azadirachta indica* showed moderate antibacterial activity while *Aloe vera* showed least antibacterial activity.

Keywords : Antibacterial activity, Disc diffusion method, MDR, Medicinal plant extracts, *Staphylococcus saprophyticus.*

I. INTRODUCTION

Staphylococcus saprophyticus is Gram positive, facultative anaerobic spherical shaped bacteria arranged in grape like clusters belongs to family Micrococcaceae. It is opportunistic pathogen. Young women are very susceptible to colonize this organism in the urinary tract and it is spread through sexual intercourse [2, 15, and 21]. It is a normal flora of skin and periurethral area. It can cause UTI particularly in sexually active young women. UTI is second most common infection in community practice. UTI refers to the presence of microorganism in the urinary tract including urinary bladder, prostate, collecting system or kidney. The syndrome ranges from asymptomatic bacteriuria to perinephrid abscess with sepsis [13]. All over the world *Escherichia coli* account for 75 to 90% of UTI isolates and *Staphylococcus*

saprophyticus accounts for 5 to 15 % of cases of uncomplicated cystitis [23]. Several studies showed that rectal, vaginal and urethral colonization of *Staphylococcus saprophyticus* was associated with UTI caused by this bacterium [16]. Indiscriminate use of antibiotics has led to an increase in antibiotic resistance in the microorganisms [3, 17]. Bacteria become resistant to antibiotic by different ways. R-plasmid often contains genes for resistance to different antibiotics [7]. Plasmid can be transferred between closely related bacterial populations [17].

The application of plants is as old as 4000 to 5000 B.C. In India earliest references of curative properties of medicinal plants appear in Rig-Veda which is said to be written between 3500 to 1600 B.C. The rural population in different parts of the world is more disposed to traditional treatment [12, 14]. It is estimated that about 80 % of the rural population in developing Asian nation depend on home care and traditional medicine for major therapies [11]. Plants produce different types of bioactive compound, making them rich sources of different types of medicine [19]. These compounds are secondary metabolites such as alkaloids, flavonoids , resins , steroids, , fatty acids, tannins ,phenol compounds etc .The problem of drug resistance could overcome by drugs of plant origin. Due to this reason now a day the demand of herbal products as therapeutic agents is increasing throughout the world. Keeping this view in mind in the present study an attempt was made to isolate multi drug resistant *Staphylococcus saprophyticus* from human urine sample & to study the effect of medicinal plant extract on it.

II. METHODS AND MATERIAL

2.1. Isolation of MDR *Staphylococcus saprophyticus* from urine sample

Staphylococcus saprophyticus was isolated from urine sample collected from civil hospital in Latur. Midstream urine sample were collected in sterile small bottles. Urine samples were streaked specific media. Mannitol salt agar (MSA), *Staphylococcus* isolation agar & Blood agar were used for isolation. Isolates of *Staphylococcus saprophyticus* were identified by using different morphological, cultural characters & biochemical tests [2, 6 and 8]. Antibiotic susceptibility testing was carried out by Kirby-Bauer's disk diffusion method [4] for drug susceptibility according to National Committee for Clinical Laboratory standards [20]. The Muller Hinton agar plates were spread with isolates of *Staphylococcus saprophyticus.* Then antibiotic discs were kept by using sterile forceps & then gently pressed down onto the agar. Plates were kept at low temperature in the freeze for 30 minutes for proper diffusion and then incubated at 37 ºC for 16- 48 hrs. Antibiotics used in this study were Amoxicillin (20mcg), Chloramphenicol (30mcg), Ciprofloxacin (5mcg), Erythromycin (5mcg), Gentamycin (10mcg), Nalidixic acid (30mcg), Penicillin (10 Units), Rifampin (5mcg), Sulfamethoxazole (50mcg), Vancomycin (30mcg) supplied by Hi-Media Laboratories, Mumbai.

2.2. Preparation of Herbal extract

Fresh leaves of *Azadirachta indica*, *Aloe vera* and peels of *Citrus limon* were collected and cleaned to remove soil and dirt. Plant materials were completely dried, powdered and mixed with methanol. Methanol extract of above plant material (5% w/v) were prepared separately. The obtained liquid extract was subjected to rotary evaporator and subsequently concentrated and stored in refrigerator at 4 ºC & tested by using agar well diffusion method [18].

2.3. Antibacterial testing of herbal extract

A 0.2ml of suspension of isolates of *Staphylococcus saprophyticus* was thoroughly mixed with sterile molten nutrient agar and poured into sterile Petri plates under aseptic conditions. After solidification,

plates were used for making of well by using flamed, cooled cork borer. 0.5 ml of single plant extract was added in each well. Plates were kept at low temperature for diffusion then incubated at 37 ºC for 1 to 2 days. After incubation, zones of inhibition were measured & noted [8].

III. RESULTS AND DISCUSSION

Isolates of *Staphylococcus saprophyticus* were isolated from urine sample. 10 isolate *Staphylococcus saprophyticus* showed antibiotic resistance to one or more antibiotics. Isolates of *Staphylococcus saprophyticus* were Gram-positive, non motile, non spore forming cocci, arranged in grape-like clusters. It forms white, raised colonies about 1-2 mm in diameter. It is coagulase -ve, DNAse -ve ,golden pigmentation -ve, hemolysis on blood agar -ve, growth on mannitol salt agar +ve, catalase +ve, urease +ve, DNase –ve, nitrate reduction test +ve, MR +ve, VP +ve, Indole –ve, novobiocin resistance +ve.

Table 1 : Percent resistance of *Staphylococcus saprophyticus* isolates against antibiotic.

Sr No	Antibiotics	No of isolate showing resistance	Percent resistance
1.	Amoxicillin (20mcg)	00	00
2.	Chloramphenicol (30mcg)	01	10
3.	Ciprofloxacin (5mcg)	00	00
4.	Erythromycin (5mcg)	06	60
5.	Gentamycin (10mcg)	00	00
6.	Nalidixic acid (30mcg)	04	40
7.	Penicillin (10Units)	03	30
8.	Rifampin (5mcg)	00	00
9.	Sulfamethoxazole(50mcg)	03	30
10	Vancomycin (30mcg)	00	00

The most common pattern of multiple drug resistance patterns of isolates of *Staphylococcus saprophyticus* observed was erythromycin-nalidixic acid-penicillin-sulfamethoxazole-chloramphenicol.

The MAR index of each isolate was calculated by using Eq. (1). The MAR index of each isolate was calculated by using following formula:

$$\text{MAR Index} = \frac{\text{No. of Antibiotics to which the isolate was resistant}}{\text{Total no. of antibiotics tested}} \quad (1)$$

The antibacterial activity of plant extract on isolates of *Staphylococcus saprophyticus* was studied, zone of diameter were measured & noted in the Table 2.

Table 2 : Antibacterial activity of herbal extract on isolates of *Staphylococcus saprophyticus*

Isolate of *Staphylococcus saprophyticus*	Zone Diameter in mm		
	Aloe vera	*Azadirachta indica*	*Citrus limon*
ISS01	---	---	++
ISS02	---	++	----
ISS03	+	+	++
ISS04	---	++	---
ISS05	+	---	++
ISS06	---	++	++
ISS07	--	---	+
ISS08	---	+	---
ISS09	--	--	--
ISS10	+	--	+

(--: no antibacterial activity, +: antibacterial activity)

Citrus limon and Azadirachta indica showed moderate antibacterial activity while *Aloe vera* showed least antibacterial activity. *Aloe vera* has long been used in Ayurveda for its anti-burn effect and

also as a tonic, alternative, antiseptic, antibiotic, anti-diarrheal, anti-fungal, anti-viral and good hair conditioner [10]. *Aloe vera* gel that contains 97-99% water and 1-2% active compounds like aloesin, aloin, aloe-emodin, aloe-mannan, flavonoids, saponin, sterols, amino acids and vitamins. Free anthroquinones and their derivatives like barbaloin-IO-aloe emodin-9 anthrone, isobarbaloin and chromones in *Aloe vera* leaves exert a strong purgative effect and are potent anti-microbial agents [22]. Antimicrobial properties of *Azadirachta indica* can be attributed to several bioactive compounds found in it, which are categorized into two major classes, isoprenoids and non- isoprenoids . Azadirachtin is mainly responsible for antibacterial activity of neem [5]. More than 130 compounds have been isolated from different parts of neem, which have tremendous biological activity as antiarthritic, antipyretic, hypoglycemic, spermicidal, antifungal, antibacterial, diuretic, antimalarial, antitumour, antiinflammatory, immunomodulatory etc [9]. *Citrus limon* fruits have a broad spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities due to alkaloids [1].The indiscriminate use of antibiotics has led to an increase in antibiotic resistance among microorganisms. The problem of drug resistance could overcome by drugs of plant origin. Hence plants can be used for treatment of serious infections caused by multi drug resistant bacteria.

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