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Antibacterial effect of some types of nanoparticles against methicillin- resistant Staphylococcus aureus isolated from milk.

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ABSTRACT

***Background**: Staphylococcus aureus is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. This bacterium is an opportunistic pathogen; it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of S. aureus such as methicillin-resistant S. aureus (MRSA) is a worldwide problem in clinical medicine. **Objectives**: To study the prevalence of Methicillin-Resistant S. aureus (MRSA) in raw milk, through isolation, identification by molecular methods and determined the effect of green synthesis silver nanoparticles on recovered isolates. **Results**: Fourteen bacterial strains (F01-F14) were isolated from raw milk as follows: 9 camel, 3 goat. 1 cow and sheep, then characterized by morphology and biochemical reactions. Further the strains were identified by molecular methods. Fourteen isolates were identified by 93-97% S. aureus, except strain F02 and F03 were identified by 91-92%. Antibiotic resistance profiles of isolated were determined. All isolates were MRSA, seven isolates displayed multidrug resistance against 7, 8 types of antibiotics. From statistical analysis the Cardamom silver nanoparticles, the concentration of AgNo3 1mM, showed good inhibitory effect towards MRSA. **Conclusion**: It is confirmed that green silver nanoparticles are capable of rendering high antimicrobial efficacy. Conclusion, it has a great potential in the field of sanitation, disinfection, cleaning utensils or packages and nanomedicine.*

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium found in maxillae, nose, groin, perineal area (males), mucous membranes, mouth, mammary glands, hair, and intestinal, genitourinary and upper respiratory tracts of human and sometimes leads to production of pus and abscesses, sepsis and even fatal septicemia [8, 27]. *S. aureus* is amongst major human pathogens both in hospitals and the community [50]. The bacterium is an opportunistic pathogen that can bring about a multiplicity of self-limiting and even life-threatening diseases in humans [8].

Antibiotics such as cephalexin and cloxacillin are usually used to treat staphylococci infections [38]. Many strains of *S. aureus* have increased resistance to multiple different classes of antibiotics [16]. Methicillin Resistant *S. aureus* (MRSA) strains are common causes of nosocomial infections [12]. Increasing resistance to vancomycin, which is administered intravenously and used to treat MRSA, has been documented in many hospitals [16, 20]. The universal emerge of MRSA is considered as a major problem for public health [19, 52]. The ability of *S. aureus* strains to

cause disease depends on a wide range of virulence factors that contribute to colonization and disease in the host [52].

Nanotechnology has paved way to combat the challenges of multi-drug resistance through innate properties of AgNPs, which are in limelight since a long time due to their broad spectrum antimicrobial activities against bacteria [40]. Nowadays, plant extract has been used as reducing and capping agent for the synthesis of nanoparticles and may be safe or ecofriendly [14]. Many natural biomolecules in plants (inactivated plant tissue, plant extracts and living plant) such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins could be involved in bioreduction, formation and stabilization of AgNPs [30]. The use herbs in phytotherapy are mostly due to their various natural activities, such as spasmolytic, carminative, hepatoprotective, anti-carcenogenic properties [7]. Cardamom (*Elettaria cardamomum*) is a perennial shrub of the family Zingiberaceae, and commonly used as spices in households worldwide. It is called as the queen of spices and has several phyto-active compounds, and has maximum antibacterial activity against *S. aureus* [6]. *Rosmarinus officinalis* commonly referred as Rosemary belongs to Lamiaceae family. It is one of the most valued sources for natural bioactive compounds which are special interest in the functional food industry. In vitro study, the extracts from Rosemary have good inhibitory activity against all bacteria, especially against *S. aureus* [32]. *Laurus nobilis* (Laura) is belongs to the family Lauraceae, which comprises numerous aromatic and medicinal plants [9]. Laura oil reported very effective against several bacteria [36]. Therefore, the objectives of the present study are to: Isolate and identify MRSA existing in raw milk samples from dairy farms. Determine the antibacterial activity of green synthesis silver nanoparticles by use each plant (Rosemary, Laura and Cardamom) against bacterial isolates.

2. MATERIAL AND METHODS

2.1. Sampling:

A number of 51 samples of raw milk were collected from livestock (camels, cows, goats and sheep) from different farms around Taif city. The samples were collected in clean sterile screw capped bottles (100 ml capacity). Each sample was labeled to show the animal species, date of samples and serial number. Collected samples were transferred to the laboratory in 24 hrs. Each sample represented one case and was incubated at 37 °C for 24 hrs. before cultivation onto solid media.

2.2. Isolation and identification of *S. aureus:*

2.2.1. Milk samples.

Raw milk samples 1ml was added in 9 ml of normal saline solution (8.5g NaCl / L) homogenized for 2min, suspensions were serially diluted up to 106. One ml each dilution was transferred to sterile petri dishes and then about 15 ml of Nutrient and Baird parker agar were poured. All plates were converted and incubated aerobically at 37° C for 24 h. After incubation, counted the total numbers of bacteria colonies for each collected sample. Individual, colonies on Baird Parker agar plates were selected according to their morphological differences such as color, shape and size then transferred into 10 ml sterile Nutrient broth. The inoculated tubes were incubated aerobically at 37° C for 24-48 hrs. A lactose fermenting colonies were spread on nutrient agar plates. According to the standard method of the microbiological examination, streak plate technique was applied in the present study to isolate and purify culture bacterial strains [22] in nutrient agar plates. For short- term preservation at 4° C, pure single colonies were streaked on nutrient agar tubes (slant). All isolates were periodically sub-cultured every two months on specific medium. Standard biochemical tests were performed following standard procedures [12].

2.3. Preliminary identification of isolates:

All isolates were confirmed to the genus level by colony and cell morphology, Gram stain - oxidase - catalase - coagulase - VP - detection of heamolysin - mannitol fermentation - urease - fermentation of sugar and TSI.

2.4. Partial sequencing of 16s rRNA gene:

2.4.1. DNA extraction from culturable bacteria.

The genomic DNA of culturable bacterial isolates was extracted using QIAamp DNMini kit (Qiagene) according to manufacturer's protocol.

2.4.2. DNA sequencing.

The PCR –amplified 16S rDNA fragments were amplified using two universal primers; fD1 (5'AGAGTTTGATCCTGGCTCAG3') and rP2 (5'ACGGCTACCTTGTTACGACTT3') [53]. The reaction mix was composed of × µL Template DNA, 2µL BigDye-Mix, 1µL universal primer (10 µmol), 1µL Taq DNA polymerase, and HPLC water to a final volume of 10µL. The amount of template DNA applied was dependent on the concentration of target sequences to obtain about 10 ng DNA in the final mix. The PCR program was as follows; initial denaturation at 96°C for 2min (1 cycle), denaturation at 96°C for 10s (30 cycle), annealing at 45°C for 5s (30 cycle),extension at 60°C for 4min (30 cycle), and then cooling at 4°C. The PCR product was purified using a DNA-purification kits as recommended by the manufacturer and then sequenced. PCR fragments were analyzed by cycle sequencing, using the Big Dye terminator cycle sequencing kit (Applied Biosystems, U.K.). This sequence step was commercially carried out by Macrogen Inc.,Seoul, South Korea, through 16S rDNA sequencing using universal primer, 518F (5' CCAGCAGCCGCGGTAATACG 3') [25]. 16S rRNA gene, were aligned using Clustal W from MEGA 4.0 software [45] and compared with the homologous sequences of the type strains, available in the GenBank database.

2.5. Antibiotic Susceptibility Test:

Antimicrobial susceptibility testing was done on Mueller-Hinton agar (MHA) (BD Difco) by the standard disc diffusion method recommended by [11]. Antimicrobial agents tested were AK (Amikacin), AMC (Augmentin [Amoxicillin + Clavulanic acid]), CIP (Ciprofloxacin), E (Erythromycin), CN (Gentamicin), OX (Oxacillin), N (Neomycin), TE (Tetracycline) and V (Vancomycin) (all disks charges were chosen as per the CLSI recommendations) [35].

2.5.1. Antibacterial assay.

The antibacterial activities of different concentrations of silver nanoparticles (AgNPs) synthesis by each plant (Rosemary, Laura, and Cardamom) were tested by using agar disc diffusion assay by (Clinical and Laboratory Standards Institute., 2011). Silver nanoparticles (AgNPs) and their concentration were synthesis by each plant (Rosemary, Laura, and Cardamom) according to Abu-Zaid [1].

2.6. Statistical analysis:

Results are presented as mean±SD. Statistical difference between the means of the various *S. aureus* species and diameter of inhibition zone were analyzed using one way analysis of variance (ANOVA). Data were considered statistically significant if $p < 0.05$.

3. RESULT AND DISCUSSION

3.1. Isolation of bacteria from raw milk:

The presence of MRSA in animals and humans is of public health concern. Where the multi-drug resistant MRSA strains were disseminate from animal to human and vice versa in Community [36]. Several reports shows the presence of MRSA in a variety of domestic species including dogs [48], cats [10] horses [21], sheep [18] pigs [51] and chickens [26] leading to an upsurge of reports and interest in MRSA colonization and infection in animals. Screening the prevalence of MRSA will be of much use in early prevention and control of community acquired infections. Hence, this study was carried out to address the prevalence of MRSA among domestic animals and to describe the antibiotics susceptibility pattern of these isolates.

Four types of raw milk were used in the present study as source for isolation some strains of *S. aureus* belong to family Staphylococcaceae. All samples (51) were collected from local farms, these samples were divided into; 37 camel, 7 goat, 4 cow and 3 sheep. The highest counts from *S. aureus* were found in camel samples, these results

revealed the possibility of spreading the diseases to the consumers. Similar results were also reported by other authors [4, 43]. Milk is one of the widely consumed products and highly susceptible to contamination by microorganisms and it is also a suitable medium for the rapid growth and multiplication of bacteria at favorable temperatures. It is necessary to use very great care in the collection and handling of milk samples to prevent any extraneous contamination and to control the growth of organisms during transportation and storage of the milk.

the average count value of *S. aureus* results from raw milk samples **table (1)** were nearly similar with the average count value of *S. aureus* results obtained by Umoh *et al*, [47] and Jørgensen *et al*, [24] who recorded that the average count value of *S. aureus* from milk samples was $2{\cdot}6 \times 10^3$ CFU g−1 and from 45 to $6{\cdot}8 \times 10^6$ CFU g−1, respectively.

Table (1): Prevalence and count of *S. aureus* among different types of raw milk samples (No. = 51)

Samples	Positive *S. aureus* isolates		*S. aureus* CFU/ml		
	No.	%	Min.	Max.	Median
Camel	37	72.5%	7×10	188×10^3	55×10^2
Cow	3	5.9%	116.5×10^2	103.5×10^3	6.5×10^3
Goat	7	13.7%	10	31×10^2	2.5×10^2
Sheep	4	7.8%	7×10	38.5×10^3	36.9×10^2

%: was calculated to the total numbers of *S. aureus* were divided on the total numbers of samples (51).

3.2. Preliminary identification:

Fourteen isolates that showed similarity in their morphological, biochemical were selected for further work. These isolates were named from F01 to F14 and subjected for further molecular identification. The results indicated clearly that all isolates exhibited coagulase positive, while 7 isolates were triple sugar iron (TSI) positive. Results of these biochemical tests were summarized and presented in **table (2)**, all selected isolates were Gram positive cocci, none spore forming, β blood hydrolysis, oxidase negative, catalase positive, 12 isolates fermented mannitol, variable fermented maltose and sucrose, 7 strains VP positive, and only four isolates did not hydrolysis urea. The same steps of study and identify isolates were carried out by authors [46, 26]. The biochemical tests results indicated that the bacteria isolates belonging to *S. aureus* were identified morphological examination revealed that their colonies on Baird Parker agar medium were black, convex, shining surrounding by two zones white zone and hallow zone then produces clear zones around the colonies by proteolysis action.

Table (2): Source of isolation, isolate number, some morphological and biochemical

Source of isolation	*Isolates No.*	*Cell shape*	*Gram stain*	*Spore-forming*	*Coagulase*	*Oxidase*	*Catalase*	*Urease*	*VP*	*TSI slant*	*Mannitol fermentation*	*Maltose fermentation*	*Sucrose fermentation*	*Blood haemolysis*
goat	**F01**	cocci	+	-	+	-	+	+	-	+	+	+	+	β
cow	**F02**	cocci	+	-	+	-	+	+	+	-	+	+	+	β
camel	**F03**	cocci	+	-	+	-	+	+	+	+	-	+	-	β
goat	**F04**	cocci	+	-	+	-	+	-	-	+	+	+	+	β
goat	**F05**	cocci	+	-	+	-	+	+	+	-	+	+	+	β
sheep	**F06**	cocci	+	-	+	-	+	+	+	-	+	+	-	β
camel	**F07**	cocci	+	-	+	-	+	+	-	-	+	+	+	β
camel	**F08**	cocci	+	-	+	-	+	-	+	-	+	+	+	β
camel	**F09**	cocci	+	-	+	-	+	-	-	+	-	+	+	β
camel	**F10**	cocci	+	-	+	-	+	+	+	+	+	-	-	β
camel	**F11**	cocci	+	-	+	-	+	+	-	-	+	-	+	β
camel	**F12**	cocci	+	-	+	-	+	-	-	+	+	+	+	β
camel	**F13**	cocci	+	-	+	-	+	+	-	-	+	-	+	β
camel	**F14**	cocci	+	-	+	-	+	+	+	+	+	-	-	β

3.3. Molecular Identification:

The analysis of 16S rRNA genes, aided by using PCR to amplify target sequences in environmental samples, had enabled molecular ecologists to provide better estimates of bacterial diversity [5]. PCR primers for amplification of 16S rRNA genes were widely available [3]. These isolates were identified by determine and analysis of the partial sequence of 16S rRNA gene. The species were initially determined by the BLAST program on NCBI (http://www.ncbi.nim.nih.gov\) based on the 16S rRNA sequences of type strains. The similarity and coverage percentage were presented in **table (3).** Fourteen isolates were identified by 93-97% *S. aureus*, except strain F2 and F3 were identified by 91-92%.

Table (3). Similarity and coverage percentage according to the obtained 16 s rRNA sequence

Isolate No.	Name and accession No. of the most related strain in NCBI Gene Bank	% Identity	% Coverage	Suggested Name of the isolates obtained in this work
F01	*Staphylococcus aureus* subsp. strain N315 NR_075000.1	**97**	**83**	*Staphylococcus aureus* F01
F02	*Staphylococcus aureus* strain NBRC 100910 NR_113956.1	**95**	**85**	*Staphylococcus aureus* F02
F03	*Staphylococcus aureus* strain S33 R NR_037007.1	**91**	**81**	*Staphylococcus aureus* F03
F04	*Staphylococcus aureus* subsp. anaerobius strain MVF-7 NR_036828.1	**92**	**82**	*Staphylococcus aureus* F04
F05	*Staphylococcus aureus* subsp. aureus N315 strain N315 NR_075000.1	**93**	**80**	*Staphylococcus aureus* F05
F06	*Staphylococcus aureus* subsp. aureus N315 strain N315 NR_075000.1	**93**	**91**	*Staphylococcus aureus* F06
F07	*Staphylococcus aureus* strain NBRC 100910 NR_113956.1	**93**	**97**	*Staphylococcus aureus* F07
F08	*Staphylococcus aureus* strain S33 R NR_037007.1|	**93**	**89**	*Staphylococcus aureus* F08
F09	*Staphylococcus aureus* strain S33 R NR_037007.1	**93**	**89**	*Staphylococcus aureus* F09
F10	*Staphylococcus aureus* strain NBRC 100910 NR_113956.1	**96**	**97**	*Staphylococcus aureus* F10
F11	*Staphylococcus aureus* strainNBRC100910 NR_113956.1	**98**	**97**	*Staphylococcus aureus* F11
F12	*Staphylococcus aureus* strain ATCC 12600 NR_118997.1	**94**	**80**	*Staphylococcus aureus* F12
F13	*Staphylococcus aureus* strain ATCC 12600 NR_118997.1	**94**	**84**	*Staphylococcus aureus* F13
F14	*Staphylococcus aureus* strain ATCC 12600 NR_118997.1	**95**	**94**	*Staphylococcus aureus* F14

3.4. Antibiotic resistance profile:

The present study showed the effect of nine commonly antibiotics against all isolated strains **table (4)**. All isolates were resistance to amikacin, oxacillin 100 %. The highest number of antibiotics resistance were recorded by *S. aureus* F01, F02 and F08 which displayed multidrug resistance against 8 types of antibiotics. While, the strains F03, F05, F06 and F07 showed multidrug resistance against 7 types of antibiotics. In agreement with these results, Alzohairy, [4] reported that the most isolates from *S. aureus* were multidrug resistant.

Most of *S. aureus* strains (13 out 14) showed resistant to gentamicin (92.9 %). In contrary, Aghazadeh *et al*, [2] stated that *S. aureus* had high susceptibility to gentamicin (91.7 %).

Statistical data and evidences from researches prove that multi drug resistant bacteria are emerging worldwide which causes many public health problems and challenges to healthcare. Moreover, uses of broad spectrum antibiotics,

insufficient aseptic condition and technique with inadequate control of infections spread had aggravated this problem concluded by Sikarwar and Batra, [40].Therefore, there is need for concerted efforts between Clinicians and Public health workers in educating the people from this state on the menace of indiscriminate use of antibiotics, especially when not prescribed by Physicians [34]. Statistical analysis demonstrated highly significant differences (P less than 0.05) between *S. aureus* species and diameter of inhibition zone *S. aureus.* Only F1 isolate was observed the most sensitive strains than others at 9:1 Cardamom silver nanoparticles with inhibition of zone 17 mm, which contain 1 Mm from AgNO3 and highest amount from plant extract, **table (5)**. Shirley *et al,* [39] concluded the same results; Cardamom silver nanoparticles inhibited *S. aureus* about 36 mm. The inhibitory factor natural products like spices for the antimicrobial activity can be identified and used as an alternative to currently used drugs against the pathogenic microbes. Cardamom extract assisted silver nanoparticles and it found to exhibit significant activity against the *Bacillus subtilis* [33].

No inhibition zones of *S. aureus*, when exposure to Rosemary, while all 14 strains were resistant to Rosemary AgNPs and did not show any antibacterial effect for any concentration. These results were disagreed with Ghaedi *et al*, [17] who used *R. officinalis* Ag-NPs as an effective antimicrobial agent against *S. aureus* strains. Moreno *et al*, [31] analyzed Rosemary water extract; it containing 30% of carnosic acid, 16% of carnosol and 5% of rosmarinic acid, these components were more effective against Gram-positive than Gram-negative bacteria or yeasts [15]. The rapid biological synthesis of silver nanoparticles using *R. officinalis* extract provides a stable, environmentally friendly, simple and efficient route for synthesis of nanoparticles. These obtained silver nanoarticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications, as well as large scale commercial production [44].

Table (4): Effect of Antibiotics resistance profile index of the bacterial strains from milk

strains	AK30	AMC 20/10	CIP 5	E1 5	CN1 0	N 30	OX 5	TE 30	VA 30	NO. of antibiotic resistance
Staphylococcus aureus **F01**	R	R	I	R	R	R	R	R	R	**8**
Staphylococcus aureus **F02**	R	R	I	R	R	R	R	R	R	**8**
Staphylococcus aureus **F03**	R	R	I	R	R	I	R	R	R	**7**
Staphylococcus aureus **F04**	R	R	I	R	-	I	R	R	R	**6**
Staphylococcus aureus **F05**	R	R	I	R	R	R	R	-	R	**7**
Staphylococcus aureus **F06**	R	R	I	R	R	R	R	-	R	**7**
Staphylococcus aureus **F07**	R	R	I	R	R	R	R	-	R	**7**
Staphylococcus aureus **F08**	R	R	I	R	R	R	R	R	R	**8**
Staphylococcus aureus **F09**	R	-	I	I	R	R	R	I	R	**5**
Staphylococcus aureus **F10**	R	-	I	I	R	R	R	I	R	**5**
Staphylococcus aureus **F11**	R	-	I	I	R	R	R	I	R	**5**
Staphylococcus aureus **F12**	R	-	I	I	R	R	R	I	R	**5**

Staphylococcus aureus **F13**	R	-	I	R	R	R	R	-	-	**5**
Staphylococcus aureus **F14**	R	-	-	R	R	R	R	I	-	**5**
Antibiotic resistance rate %	**100**	**57.1**	**0**	**71.4**	**92.9**	**85.7**	**100**	**35.7**	**85.7**	

*Inhibition zone diameter were measured inclusive of the diameter of the discs (-) sensitive; (I) Intermediate; (R) Resistant according to the table 2 **(Oxoid Manual, 1982)**. AK (Amikacin), AMC (Augmentin), CIP (Ciprofloxacin), (Erythromycin), CN (Gentamicin), N (Neomycin), OX (Oxacillin), TE (Tetracycline) and V (Vancomycin). (Antibiotic resistance rate = (number of strains resists certain antibiotic\ total number of tested strains) x 100.

3.5. Antibacterial activity of concentrations of Laura, Cardamom and Rosmary silver nanoparticles against *S. aureus* isolates:

The components of Laura oil are 1, 8 cineole terpenes (linalool), lactones and monoterpenes (camphene, alpha-pinene). In present study, the zone of inhibition was 10 mm in strain F08 when using concentration 1:9 Laura silver nanoparticles. Other concentration did not show significant antibacterial activity against all tested strains. In this study, the application of green silver nanoparticles as an antimicrobial agent was investigated and exhibited better antimicrobial activity against pathogens. Additionally, the

Cardamom silver nanoparticles showed good inhibition activity towards MRSA. Metal nanoparticles preparation using plant extracts is an important branch of biosynthesis processes. Plants have a potential to reduce metal ions both on their surface and in various organs and tissues remote from the ion penetration site [28]. Biomolecules existing in plant extracts including, enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrates are potentially able to reduce metal ions. The extract of various parts of plants such as leaves, flowers, seeds, barks and roots have been applied for synthesis of AgNPs [49]. Among noble metal nanoparticles, AgNPs have gained wide applications in different fields due to their strong multi drug resistant bacteria [23], and avoiding the occurrence of hazardous and toxic solvents [42].

The surface of AgNPs can easily form a layer of water and thus many silver ions can be released from AgNPs into the water. On the other hand, the main composition of bacteria cell membrane is phospholipid bilayers and protein molecules having negative electricity which make the whole cell membrane negatively charged. Therefore, the silver ions with positive electricity have the ability to attach to bacteria cell membrane quickly, which alters or damages the structures of bacteria. Moreover, Ag+ ions can be attracted to the sulfhydryl group (SH) of bacterial enzymes (respiratory enzymes), making the enzymes inactivated and even died out [54]. The AgNPs antimicrobial activity depends strongly on several factors including type of microorganisms, temperature, pH and AgNO3 concentration [29].

In conclusion, the rapid biological synthesis of silver nanoparticles using Cardamom, Rosemary, and Laura extract provides a stable, environmental friendly, simple and efficient route for synthesis of nanoparticles. Natural sources have the potential to reduce silver ions into AgNPs. It is understood that the variety of natural compounds that are present in plant extracts can act as both reducing and stabilizing agents for synthesis of AgNPs. Plants mediated AgNPs are stable due to the presence of natural capping agents such as proteins which prevent the particles from aggregation. Green synthesis of AgNPs using plant extracts has several advantages such as eco-friendliness cost effectiveness, compatibility for medical and pharmaceutical applications. It is confirmed that green silver nanoparticles are capable of rendering high antimicrobial efficacy and hence has a great potential in the field of nanomedicine.

Table (5): Antibacterial effect of various concentrations of bio-synthesized Cardamom (C), Rosemary (R) and Laura (L) nanoparticles against *S. aureus* strains from raw milk (14)

Zone of Inhibition (in mm) at different AgNPs conc.															
strains	**C-1**	**C-2**	**C-3**	**C-4**	**C-5**	**R-1**	**R-2**	**R-3**	**R-4**	**R-5**	**L-1**	**L-2**	**L-3**	**L-4**	**L-5**	**N**
F01	9	8	8	7	17	0	0	0	0	0	6	7	0	0	0	9
F02	8	8	8	0	0	0	0	0	0	0	7	6	0	0	0	10
F03	8	8	9	0	0	0	0	0	0	0	7	7	0	0	0	9
F04	7	8	8	0	0	0	0	0	0	0	0	0	0	0	0	9
F05	7	7	8	0	0	0	0	0	0	0	0	0	0	0	0	8
F06	9	9	8	8	7	0	0	0	0	0	0	0	0	0	0	8
F07	7	8	8	7	8	0	0	0	0	0	0	0	0	0	0	9
F08	8	9	9	7	7	0	0	0	0	0	10	8	0	0	0	9
F09	8	8	8	7	7	0	0	0	0	0	7	0	0	0	0	9
F10	8	8	9	7	0	0	0	0	0	0	7	0	0	0	7	8
F11	8	9	8	7	0	0	0	0	0	0	6	0	0	0	0	7
F12	9	8	8	7	0	0	0	0	0	0	7	7	0	0	0	8
F13	7	7	7	0	0	0	0	0	0	0	0	0	0	0	0	8
F14	9	8	10	8	7	0	0	0	0	0	7	0	0	0	0	9

* C-1 (1: 9), C-2 (3: 7), C-3 (5: 5), C-4 (7: 3), C-5 (9: 1), R-1 (1: 9), R-2 (3: 7), R-3 (5: 5), R-4 (7: 3), R-5 (9: 1), L-1 (1: 9), L-2 (3: 7), L-3 (5: 5), L-4 (7: 3), L-5 (9: 1) and N (Chemical silver nanoparticles).

REFERENCES

1.Abu Zaid,A., EL-Sehrawy,M.,Mohamed . H . and Nemari,A. Biosynthesis of Silver Nanoparticles by Plants Extract and Their Antimicrobial Activities.(2016). **Advances in Environmental Biology,** 10(9) September 2016, Pages: 114-127**.**

2. Aghazadeh, M., S. Sari, M. Nahaie, S.S.R. Hashemi and S. Mehri, 2015. Prevalence and Antibiotic Susceptibility Pattern of *E. coli* Isolated from Urinary Tract Infection in Patients with RenalFailure Disease and Renal Transplant Recipients. Tropical Journal of Pharmaceutical Research April, 14(4): 649-653.

3. Alm, E.W., D.B. Oerther, N. Larsen, D.A. Stahl and L. Raskin, 1996. The oligonucleotide probe database. Applied and Environmental Microbiology, 62(10), 3557.

4. Alzohairy, M., 2011. Colonization and antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia. J Bacterio Res, 3(4), 63–68.

5. Amann, R.I., W. Ludwig and K.H. Schleifer, 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews, 59(1), 143–169.

6. Aneja, K.R. and R. Joshi, 2009. Antimicrobial activity of *Amomum subulatum* and *Elettaria cardamomum* against dental caries causing microorganisms. Ethnobotanical Leaflets, 2009(7), p.3

7. Bajalan, I. and G.A. Pirbalouti, 2014. Variation in antibacterial activity and chemical compositions of essential oil from different populations of myrtle. Industrial Crops and Products, 61: 303-307.

8. Bannerman, T.L., S.J. Peacock, P.R. Murray, E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller, 2006. Staphylococcus, Micrococcus, and other catalase-positive cocci. Manual of clinical microbiology: Volume 1, (Ed. 9), pp.390-411.

9. Barla, A., G. Topçu, S. Öksüz, G. Tümen and D.G. Kingston, 2007. Identification of cytotoxic sesquiterpenes from *Laurus nobilis* L. Food chemistry, 104(4), pp.1478-1484.

10. Bender, J.B., S.M.F. Torres, S.M. Gilbert, K.E. Olsen, K.H. LeDell, 2005. Isolation of methicillin-resistant *Staphylococcus aureus* from a nonhealing abscess in a cat. Vet. Record, 157: 388–38.

11. Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards). 2011. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement, M100-S15; CLSI: Wayne, PA, USA.

12. Collins, C.H., 1988. Laboratory-acquired infections: history, incidence, causes and prevention. Butterworth & Co (Publishers) Ltd.

13. Collins, C., P. Lyne, J. Grange, P. Lyne and J. Falkinham III, 2004. Collins and Lyne's Microbiological Methods, 8Ed 8th Edition, pp: 67-120.

14. Dhiman, J., V. Kundu, S. Kumar, R. Kumar, and S.K. Chakarvarti, 2014. Eco-friendly Synthesis and Characterization of Silver Nanoparticles and Evaluation of Their Antibacterial Activity. American Journal of Materials Science and Technology, 3 (1):13-21.

15. Djeddi, S., N. Bouchenah, I. Settar and H.D. Skaltsa, 2007. Composition and antimicrobial activity of the essential oil of *Rosmarinus officinalis* L. from Algeria .Chemistry of Natural Compounds, 43(4):487- 490.

16. Eisenstein, B.I., 2008. Treatment challenges in the management of complicated skin and soft-tissue infections. Clinical Microbiology and Infection, 14(s2), pp.17-25.

17. Ghaedi, M., M. Yousefinejad, M. Safarpoor, H.Z. Khafri and M.K. Purkait, 2015. *Rosmarinus officinalis* leaf extract mediated green synthesis of silver nanoparticles and investigation of its antimicrobial properties. Journal of Industrial and Engineering Chemistry, 31, 167–172.

18. Goni, P., Y. Vergara, J. Ruiz, I. Albizu, J. Vila and R. Gómez-Lus, 2004. Antibiotic resistance and epidemiological typing of *Staphylococcus aureus* strains from ovine and rabbit mastitis. Int J Antimicrob Agents., 23(3): 268-72.

19. Grundmann, H., M. Aires-de-Sousa, J. Boyce and E. Tiemersma, 2006. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. The Lancet, 368(9538), pp.874-885.

20. Hansra, N.K. and K. Shinkai, 2011. Cutaneous community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus*. Dermatologic therapy, 24(2), pp.263-272.

21. Hartmann, F., S.S. Trostle and A.A.O. Klohnen, 1997. Isolation of a methicillin-resistant *Staphylococcus aureus* from a postoperative wound infection in a horse. J. American Vet. Med. Assoc., 211: 590– 592.

22. ICMSF International Commission on Microbiological Specification for Foods. 1996. Microorganisms in food. Characteristics of microbial pathogens. Blackie Academic & Professional. 5: 513-515.

23. Jeeva, K., M. Thiyagarajan, V. Elangovan, N. Geetha and P. Venkatachalam, 2014. Caesalpinia coriaria leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens. Industrial Crops and Products 52: 714-720.

24. Jørgensen, H.J., T. Mørk, H.R. Høgåsen and L.M. Rørvik, 2005. Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. Journal of Applied Microbiology, 99(1), 158–166.

25. Khan, M.S., A. Zaidi, M. Ahemad, M. Oves, and P. A. Wani, 2010. Plant growth promotion by phosphate solubilizing fungi--current perspective. Archives of Agronomy and Soil Science, 56(1), 73–98.

26. Lee, J.H., 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Applied and environmental microbiology, 69(11), pp.6489-6494.

27. Le Loir, Y., F. Baron and M. Gautier, 2003. *Staphylococcus aureus* and food poisoning. Genet Mol Res, 2(1), pp.63-76.

28. Makarov, V., A. Love, O. Sinitsyna, S.M.I. Yaminsky, M. Taliansky and N. Kalinina, 2014. Green nanotechnologies: Synthesis of metal nanoparticles using plants. Acta Naturae 6(1): 35-44.

29. Marambio-Jones, C. and E. M. Hoek, 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. Journal of Nanoparticle Research 12(5): 1531-1551.

30. Mohammadlou, M., H. Maghsoudi and H.*Jafarizadeh-Malmiri, 2016. A review on green silver nanoparticles based on plants: Synthesis, potential applications and eco-friendly approach. International Food Research Journal 23(2): 446-46

31. Moreno, S., T. Scheyer, C.S. Romano and A.A. Vojnov, 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research, 40(2): 223-231.

32. Oluwatuyi, M., G. W. Kaatz, and S. Gibbons, 2004. Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. Phytochemistry, 65(24), 3249–3254.

33. Omprakash and Sharada, 2015. Green Synthesis and Characterization of Silver Nanoparticles and Evaluation of their Antibacterial Activity using *Elettaria Cardamom* Seeds J Nanomed Nanotechnol 2015, 6:2.

34. Onifade, A.K., M.A. Oladoja and D.O. Fadipe, 2015. Antibiotics Sensitivity Pattern of *Escherichia coli* isolated from Children of School Age in Ondo State, Nigeria. Researcher Journal, 7(2).

35. Oxiod, 1982. Oxoid Manual: culture Media ingredients and other Laboratory service 5th Oxiod Limited, Basingstoke.

36. Persoons, D., S. Van Hoorebeke, K. Hermans, P. Butaye, A. De Kruif, F. Haesebrouck, J. Dewulf, 2009. Methicillin-resistant *Staphylococcus aureus* in poultry. Emerg. Infect. Dis., 15(3):452-3.

37. Santos, F.A. and V.S.N. Rao, 2000. Antiinflammatory and antinociceptive effects of 1, 8-cineole a terpenoid oxide present in many plant essential oils. Phytotherapy research, 14(4), pp.240-244.

38. Sharma, S. and K.K. Verma, 2001. Skin and soft tissue infection. Indian journal of pediatrics, 68, pp.S46-50.

39. Shirley, A., B. Dayanand, Sreedhar and S.G. Dastager, 2010. Antimicrobial activity of silver nanoparticles synthesized from novel Streptomyces species. Journal of Nanomaterials and Biostructures, 5(2): 447- 451.

40. Sikarwar, A.S. and H.V. Batra, 2011. Prevalence of Antimicrobial Drug Resistance of *Klebsiella pneumoniae* in India. International Journal of Bioscience, Biochemistry and Bioinformatics, 1(3):211-215.

41. Singh, D., V. Rathod, N. Shivaraj, J. Hiremath, A.K. Singh and J. Methew, 2014. Optimization and characterization of silver nanoparticles by endophytic fungi Penicillium sp. isolated from Curcuma longa (turmeric) and application studies against MDR *E.coli* and *S. aureus*. Bioinorganic Chemistry and Applications, 8.

42. Sinha, S.N. and D. Paul, 2014. Eco-friendly Green Synthesis and Spectrophotometric Characterization of Silver Nanoparticles Synthesized using Some Common Indian Spices. An International Peer Review E-3 Journal of Sciences, 3 (2): 401-408.

43. Stastkova, Z., S. Karpiskova and R. Karpiskova, 2009. Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at a goat breeding farm. Veterinarni Medicina. 54, 2009 (9): 419–426.

44. Sulaiman, G.M., E. H. Ali, I. Jabbar and A.H. Saleem, 2014. Synthesis characterization, and cytotoxic effects silver nanoparticles. Digest Journal of Nanomaterials and Biostructures, 9(2):787-796.

45. Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24(8), 1596–1599.

46. Tenover, F.C., L.M. Weigel, P.C. Appelbaum, L.K. McDougal, J. Chaitram, S. McAllister and others, 2004. Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. Antimicrobial Agents and Chemotherapy, 48(1), 275–280.

47. Umoh, V.J., K.S. Obawede and J.U. Umoh, 1985. Contamination of infant powdered milk in use with enterotoxigenic *Staphylococcus aureus*. Food microbiology, 2(4), 255-261.

48. Van Duijkeren, E., A. Box, M. Heck, W. Wannet and A. Fluit, 2004. Methicillin resistant staphylococci isolated from animals. Vet. Microbial. 103:91–7. DOI: 10.1016/j.vetmic.2004.07.014.

49. Velayutham, K., A.A. Rahuman, G. Rajakumar, S. M. Roopan, G. Elango, C. Kamaraj, S. Marimuthu, T. Santhoshkumar, M. Iyappan and C. Siva, 2013. Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of Ficus racemosa against *Culex quinquefasciatus* and *Culex gelidus*. Asian Pacific journal of Tropical Medicine 6(2): 95-101.

50. Verkaik, N.J., O. Dauwalder, K. Antri, I. Boubekri, C.P. de Vogel, C. Badiou, M. Bes, F. Vandenesch, M. Tazir, H. Hooijkaas and H.A. Verbrugh, 2010. Immunogenicity of toxins during *Staphylococcus aureus* infection. Clinical Infectious Diseases, 50(1), pp.61-68.

51. Voss, A., F. Loeffen, J. Bakker, C. Klaassen and M. Wulf, 2005. Methicillinresistant *Staphylococcus aureus* in pig farming. Emerg. Infect. Dis., 11: 1965–1966.

52. Voyich, J.M., M. Otto, B. Mathema, K.R. Braughton, A.R. Whitney, D. Welty, R.D. Long, D.W. Dorward, D.J. Gardner, G. Lina and B.N. Kreiswirth, 2006. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? Journal of Infectious Diseases, 194(12), pp.1761-1770.

53. Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173(2), 697–703.

54. Zhang, Y., X. Cheng, Y. Zhang, X. Xue and Y. Fu, 2013. Biosynthesis of silver nanoparticles at room temperature using aqueous aloe leaf extract and antibacterial properties. Colloids and Surfaces A: Physicochemical and Engineering Aspects 423: 63- 68.