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Research Article

MULTI DRUG RESISTANCE PROFILE AND ANTIBIOFILM ACTIVITY OF GARLIC OIL ON *STAPHYLOCOCCUS AUREUS* ISOLATED FROM MILK OF CATTLE SUFFERING WITH MASTITIS

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ABSTRACT: The present study was undertaken on 164 bovine milk samples (cow = 113 and Buffalo = 51) collected from various dairy farms in and around Rewa (Madhya Pradesh, India) and only 24 samples (6.83%) were found to be positive for mastitis when screened through California Mastitis Test (CMT). The prevalence of subclinical mastitis in cattle was 21%. All 17 isolates were phenotypically characterized by mannitol fermentation, biochemical tests (acetoin production by Voges-Proskauer test), beta-galactosidase test susceptibility to novobiocin (5 µg disk), resistance to polymyxin B (300 µg disk). We also observed the biofilm formation ability of all the *Staphylococcus aureus* strains (n=17) by Congo Red Agar (CRA), Microtiter plate (using crystal violet), and light microscopy method, 90% sensitivity was seen through microtiter plate method. Antibiofilm activity of garlic oil was undertaken on positive isolates (3%, 2%, and 1% concentration) along with positive and negative control in every microtiter plate assay. Maximum inhibition was observed at 3% concentration with O.D values ranging from 0.021±0.007 to 0.291 ± 0.005 in all the samples with percent inhibition (50 to 60%). Multi-drug resistance profiles of biofilm-producing isolates were also undertaken against polymyxin, novobiocin, tetracyclines, cotrimoxazole, clindamycin, cefoxitin, and cefoperazone. Eighty-two (82) percent of isolates showed resistance against polymyxin, 52% against novobiocin and erythromycin, and all the isolates showed 100% sensitivity against tetracyclines and cefoxitin.

Keywords: Biofilm, Bovines, Garlic oil, Mastitis, Milk, Microtiter plate assay.

INTRODUCTION

Bovine mastitis is one of the most dreadful infections inside the dairy industry and harms the economy and well-being of the animals. Some of the mastitis cases are not resolved which may be due to several reasons one being, increased antibiotic use in dairy farms resulting in treatment failures despite standard antibiotic protocols [1]. The rise of multidrug-resistant (MDR) bacteria is associated with selective pressure instigated by the misuse of antibiotics, which lessens therapeutic possibilities available resulting in serious public health issues recurrently allied with an increase in healthcare costs and high morbidity and mortality rates.

Staphylococcus aureus is called one of the leading pathogens responsible for mastitis in animals and has proven a notable capacity of resistance to several antibiotics, specifically in mastitis situations, moreover, *S. aureus* has a highlighted capacity to build surface-related bacterial clusters (group), called biofilm, one

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of the most determinant factors for the development of persistent infections. It also serves as the root cause of therapeutic failure. The formation of this biofilm represents more than 80% of microbial infections worldwide and provides a shield to the bacteria from a hostile environment in the extracellular matrix that is created during the multi-step process of biofilm development [2]. By decreasing their growth, metabolism, and the amount of antimicrobial that penetrates their biofilm, bacteria residing in biofilms can fight the host immune response and antimicrobial effect, they shield the microorganism using allowing them to continue to exist in unreceptive environments, hinder antibiotic uptake [3]. Bacterial biofilms are well acknowledged for their corporeal and biotic possessions that confer resistance against antibiotics, and thus it is one of the key challenges in the existing antibiotic therapy. All organisms whether pathogenic or not tend to inhibit these biofilms for their existence and confer themselves to the substrate or surface present in such matrix. The part of biofilm in the pathogenesis is still uncertain, *in vivo* research could be an option for quick and easy biofilm diagnostics in human or veterinary medicine, no such biofilm marker, particular biofilm product, or distinct biofilm immune response has been found as of yet [1].

Fresh garlic and its essential oil play an important role in the food industry as an antioxidant, antimicrobial, and flavoring agent, especially in processed chicken and meat products. Being a rich source of important nutrients and health-promoting phytochemicals which not only help to improve gut health and encourage growth in poultry, cattle, and other livestock but are highly useful against disease conditions as well. Biological effects of garlic associated with sulfur-containing compounds such as allicin and other functionally active components too were reported [4, 5]. Antibacterial effect of garlic and basil essential oil screening the highest antimicrobial activity ($p \leq 0.05$) against *Bacillus cereus* and *S. aureus* and total inhibition of biofilm-producing pathogens with the use of garlic extract was reported [6, 7]. Commercially available essential oils are used in the form of topical applications and oral hygiene and have a long-standing benefit by daily practice when associated with products with chlorhexidine. Looking into the above scenario study on the *Staphylococcus aureus* with biofilm-producing ability, isolated from the milk of cattle suffering from mastitis was undertaken and the effect of garlic essential oil was also observed on these isolates.

MATERIALS AND METHODS

Isolation of *Staphylococcus aureus* from milk of cattle suffering from mastitis

Collected samples of milk from different dairy farms were subjected to CMT for screening of subclinical mastitis following standard protocol. Milk was (300 µl) inoculated into a tube containing 30 ml of Mueller-Hinton broth (Hi-media, India make) supplemented with 6.5 % NaCl. The incubation of tubes was done at 35 °C for 16-20 hours. One loopful of this first pre-enrichment culture was then inoculated by streak method into Mannitol Salt agar plates, positive samples were then streaked onto Baird-Parker agar plate (prepared in the lab with chemicals of Himedia, India make) with egg yolk-tellurite supplement, incubated at 35 °C for 18-24 hour later streaked onto a Tryptone soy agar plate (prepared in the lab from ingredients of Hi-media, India make) at 35 °C for 16-20 h. The colony morphology (size and coloration) obtained on each plate was observed. The characterization of presumptive isolates was undertaken by standard biochemical tests, Novobiocin, and Polymyxin B susceptibility [8]. Multidrug resistance profile- an antibiotic sensitivity test was performed on the antibiotics- polymyxin, novobiocin, tetracyclines, cotrimoxazole, clindamycin, cefoxitin, and cefoperazone antibiotics. (as per the CLSI recommendation and antibiotics used in the area).

Biofilm forming ability of *S. aureus* isolates

Positive isolates were further subjected to test for their biofilm-forming ability by Congo red agar, light microscopy, and Microtiter plate assay [9]. The Congo red agar was prepared as Brain Heart Infusion agar with Congo red and sucrose 0.08 % and 5% respectively, 0.5 McFarland adjusted overnight culture was inoculated in the plate at 35 °C for 18- 24 hours of incubation, presence of dry crystalline black or pink colonies indicated positive result. In the light microscopy method, overnight culture in tryptone soy broth (TSB) (Hi-media make) added with 0.25% glucose was further diluted 1:40, 2 ml of this suspension used in petri plate with a coverslip using all the standard protocol with slight modifications. Cover slip kept on a clean oil-free glass slide was observed under a 40-x light microscope [10].

Biofilm forming ability through Microtiter plate assay was observed in bacterial culture kept overnight in tryptone soy broth (TSB) added with 0.25% glucose (TSB-glucose), 1:40 dilution in TSB-glucose. This cell suspension/inoculum (200 µl) was transferred in sterile,

96-well polystyrene microtiter plates (Tarson) [11,12]. The plate was kept at 37°C for 18 h. After incubation gentle washing of wells with 200 µl of sterile phosphate-buffered saline (PBS) was done thrice, air dried in an upturned position, and marked with 0.1% crystal violet for 10 minutes. The amount of released stain was calculated by measuring the absorbance at 492 nanometers (A 492) in a microplate reader (LISA plus, Rapid Diagnostic, China).

Antibiofilm activity of garlic oil on *S. aureus* isolates

Garlic oil was obtained as commercial preparation (Ranbaxy Laboratories) (100% pure) and diluted in DMSO in a range of 3%, 2%, and 1% by using the drop dilution method. Micro titer assay was executed as per the CLSI standards with slight modifications. The essential oil in broth was set in microtubes of 1 mL with a concentration range of 1%, 2%, and 3% (v/v). Then, 180 µl of different dilution was put into a 96-well microplate in 2 × 11 columns. Fresh overnight bacterial culture (adjusted to 0.5 McFarland standards) (20 µl) was inoculated in each well with a last volume of 200 µl well. Both positive control (broth with bacterial inoculum) and negative control (only broth) were placed into the first column of the microplate, and cefoperazone 5 µg/mL was added as standard control. Finally, the microplate was kept in the incubator with a sterile film cover at 37°C for 18 h without any shaking. After incubation contents of each well was carefully were washed with PBS/distilled water 3-4 times and dried for 45 min. To accomplish the crystal violet staining assay, the plate was stained with 1% crystal violet, incubated at room temperature for 15 min, and washed thrice with sterile distilled water further de-stained with ethanol and acetic acid. The absorbance was noted in three replicates at OD492 nanometer with the help of a microplate ELISA reader (Bio Tek Instruments, USA). The percentage inhibition was calculated with the following formula [13].

OD of negative control – OD of experimental data/ OD of negative control x 100

After the biofilm formation for 4 hr. at 37°C, essential oil was added to yield a final concentration similar to that mentioned earlier, the mixture was incubated for 24 hr. Crystal violet staining assay was conducted as described in the previous section to determine the inhibition of biofilm growth and development.

Phytochemical screening of garlic oil

Phytochemical analysis on the oil of garlic was done using standard qualitative procedures with slight modifications for saponins, steroids, alkaloids, phenols, and cardiac glycosides [14,15,16].

Quantitative analysis of essential oil by GCMS

Phytochemical analysis was performed through GCMS-QP2010 Plus equipment. Experimental Conditions were Ion Source Temp: 220°C Interface Temp: 270°C ; Solvent Cut Time: 2.50 min ; Detector Gain Mode : Relative ; Detector Gain: 0.00 kV; Threshold : 100; Column Oven Temp 50°C; Injection Temp: 260°C; Injection Mode-Split Flow; Control Mode-Linear; Velocity Pressure - 69.0 kPa Total Flow - 137.3 mL/min; Column Flow - 1.21 mL/min; Linear Velocity 39.9 cm/sec; Purge Flow - 3.0 mL/min; Split Ratio - 110.0 Sample Inlet Unit: GC; MS Program: off.

RESULTS AND DISCUSSION

Subclinical mastitis is an alarming situation in veterinary clinics because infectious mastitis pathogens might be mistakenly spread to susceptible animals and reach back again to their area of origin. Out of 164 samples collected from different farms, only 24 samples were found to be mastitis positive when screened through CMT (California Mastitis test) and 140 samples were negative in CMT screening (Table 1). Overall 17 dairies were included in the present study (Table 2). All the buffalo milk was negative and among the 113 samples of cows, 24 samples were mastitis-positive (Table 2). All 17 isolates were phenotypically characterized by mannitol fermentation, biochemical tests (acetoin production (Voges-Proskauer test), beta-galactosidase test), susceptibility to novobiocin (5 µg disk), resistance to polymyxin B (300 µg disk) for characterization of *Staphylococcus aureus*. Out of the total 17 *S. aureus* strains, 70% of samples (n = 12) were positive for biofilm formation on Congo red agar, the results in some of the plates showed pink colonies giving less accuracy, whereas 52% of samples (n = 8) were positive for biofilm on light microscopy. All 17 samples showed biofilm production through the microtiter plate method with almost 90% sensitivity contributing to the earlier report of microtiter plate assay in finding biofilm-producing isolates than Congo red agar [17].

The formation of biofilm and quick development of resistance against the antimicrobials are two main factors behind the problems in the treatment of mastitis [18,19]. Antibiofilm activity of garlic essential oil was

Table 1. List of Samples collected from different areas of Rewa.

Name of the area	No. of samples	Positive samples	Negative samples
Kuthuliya	11	3	8
Silpara	62	4	58
Nipaniya	7	0	7
Rani talab	31	1	30
Padokhar	16	9	7
Hardi	37	7	30
Total	164	24	140

observed by microtiter plate assay method using the dye crystal violet (CV) at 492 nm wavelength. O.D. values were taken in triplicates (Mean±S.E.) Percent inhibition was calculated based on O.D. values of negative control and sample. Antibiofilm activity and percent inhibition in 3% concentration were 0.021±0.007 to 0.291 ± 0.005 in all the samples with percent inhibition (50 to 60%). In 2% concentration per cent inhibition was from 16 to 20 % and very less inhibition effect was observed at 1% concentration. (Table 3.) Essential oil of garlic in the concentration range of 50 to 500µl/ml has also been reported to show an inhibitory effect on numerous bacteria [20].

Quantitative analysis through GCMS (Fig. 1) revealed the presence of Diallyl sulfide, Disulfide, methyl 2-propenyl, Diallyl disulfide, (E)-1-Allyl-2-(prop-1-en-1-yl)disulfane, (E)-1-Allyl-2-(prop-1-en-1-yl)disulfane, Allyl methyl trisulfide, 4-Methyl-1,2,3-trithiolane, Allitridin, 1-Allyl-3-propyltrisulfane, 5-Methyl-1,2,3,4-tetrathiane, Copaene, Cyperene, Caryophyllene, Rotundene, Gurjunene, Methylparaben, 7-isopropenyl-4a-methyl-1-methylene deca hydro naphthalene, Naphthalene,8a-octahydro-1, 8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.-selinene, Tetrasulfide, di-2-propenyl, Caryophyllene oxide, Propyl 4-hydroxybenzoate, Cyperotundone,1-Allyl-3-[2-(allylthio)propyl] trisulfane, 3-Heptene, 4-methoxy (Table 6). Some workers also reported the antibacterial activity of oil-soluble organosulfur compounds like allicin, ajoenes, allyl sulfides, and other principal phytochemicals [21]. The organosulfur compounds of garlic show an array of antibacterial activities like bactericidal, antibiofilm, antitoxin, and anti-quorum sensing activity against a widespread choice of microbes and multi-drug resistant (MDR) strains [14].

MIC of essential oils was also undertaken by Resazurin-based 96-well plate microdilution method in the concentration range of 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.2%, 0.1%. For garlic oil MIC of 1% was observed in most of the samples except 2 samples in which MIC was 2%. Correlating with our findings Rohani *et al.* (2011) [22] also reported 100 µg/ml MICs for garlic oil. MIC and MBC of garlic oil and garlic powder were also reported in the range of 8 to 32 µg/ml and 16 to 32 µg /ml respectively, higher for garlic powder than those of undiluted garlic oil [23].

Table 2. List of dairy farms included in sample collection areas of Rewa.

Dairy farm area	No. of samples	Cow	Buffalo
Kuthuliya	7	6	1
Kuthuliya	4	4	0
Silpara	17	12	5
Silpara	9	7	2
Silpara	13	10	3
Silpara	23	14	9
Nipania	7	6	1
Rani talab	17	11	6
Rani talab	9	7	2
Rani talab	5	2	3
Padokhar	3	2	1
Padokhar	7	5	2
Padokhar	2	2	0
Padokhar	3	3	0
Padokhar	1	1	0
Hardi	20	13	7
Hardi	17	8	9
Total	164	113	51

Multidrug resistance profile of biofilm-producing isolates against polymyxin, novobiocin, tetracyclines, cotrimoxazole, clindamycin, cefoxitin and cefoperazone (antibiotics selected as per the CLSI recommendation and used in this area) revealed 82% isolates resistant against polymyxin, 52% against novobiocin and erythromycin and all the isolates showed 100% sensitivity against tetracyclines and cefoxitin (Table 4, 5). In our study isolates showed 17% resistance to cotrimoxazole, the highest resistance against penicillin (84.2%), and 78.9% resistance against erythromycin [24]. In the same way, the moderate biofilm producers were completely resistant to penicillin followed by cefoxitin (85.7%) and erythromycin (71.4%). All the antibiotics observed resistance against strong biofilm-producing isolates. Simulating with our finding, maximum resistance to *S. aureus* was reported against ampicillin, penicillin (96.7%), rifampicin and clindamycin (80%), oxacillin (73.3%) and erythromycin

Table 3. Antibiofilm activity and percent inhibition of garlic oil.

Sl. No.	Sample	O.D. Values (Mean ± SE)	Per cent Inhibition	Sl. No.	Sample	O.D. Values (Mean ± SE)	Per cent Inhibition
		Control 0.688±0.016 (sample 1-8) Control 0.644 ±0.017 (sample 9-17)				**Control 0.688±0.016 (sample 1-8) Control 0.644 ±0.017 (sample 9-17)**	
1	K3C 1%	0.376a±0.016	14.149	10	U2C 1%	0.531a±0.009	17.598
	K3C 2%	0.368a±0.005	15.263		U2C 2%	0.387b±0.029	28.830
	K3C 3%	0.047b±0.010	62.017		U2C 3%	0.236c±0.024	63.406
2	I1B 1%	0.387a±0.006	12.598	11	ST1C1%	0.562a ± 0.010	12.785
	I1B 2%	0.345a±0.029	18.703		ST1C 2%	0.395b ± 0.005	17.754
	I1B3%	0.055b±0.025	60.854		ST1C 3%	0.294c ± 0.009	54.400
3	R1C 1%	0.383a±0.018	13.180	12	U3C 1%	0.564a ± 0.017	12.371
	R1C 2%	0.344a±0.005	18.800		U3C 2%	0.498b ± 0.004	22.619
	R1C3%	0.055b±0.004	60.854		U3C 3%	0.255c ± 0.004	60.455
4	RV1C 1%	0.343a±0.019	18.897	13	SK1C1%	0.550a ±0.009	14.596
	RV1C 2%	0.336a±0.018	20.011		SK1C 2%	0.514b ±0.006	20.186
	RV1C3%	0.045b±0.021	62.259		SK1C3%	0.216c ±0.050	66.408
5	R2B1%	0.384a±0.019	12.986	14	V1C 1%	0.609a ±0.013	11.128
	R2B2%	0.339b±0.006	19.575		V1C 2%	0.386b ±0.024	13.509
	R2B3%	0.036c±0.003	63.519		V1C 3%	0.263c ±0.021	59.110
6	SIC1%	0.402a±0.055	10.321	15	KTIC 1%	0.518a ± 0.008	19.565
	SIC2%	0.301b±0.009	25.002		KTIC 2%	0.500b ± 0.009	22.412
	SIC3%	0.028c±0.007	64.730		KTIC 3%	0.291c ± 0.005	54.865
7	A1C 1%	0.389a±0.005	12.308	16	RL2C1%	0.580a ± 0.014	12.164
	A1C 2%	0.349b±0.049	18.122		RL2C2%	0.534b ± 0.013	17.029
	A1C3%	0.031c±0.006	64.246		RL2C 3%	0.228c ± 0.002	64.596
8	JB1C1%	0.392a±0.008	11.775	17	BP1C 1%	0.574a ±0.026	10.870
	JB1C 2%	0.342b±0.025	19.091		BP1C 2%	0.528b ±0.007	17.961
	JB1C 3%	0.021c±0.007	65.796		BP1C 3%	0.239c ± 0.013	62.836
9	U1C 1%	0.547a±0.005	15.062				
	U1C 2%	0.518a±0.004	19.617				
	U1C 3%	0.289b±0.004	55.124				

Table 4. Multi drug resistance profile of positive isolates.

Sample	PB	NB	TE	CX	E	CD	CIP	COT
K3C	R	S	S	S	S	S	S	S
R1C	R	S	S	S	S	S	S	S
R2B	S	R	S	S	S	I	S	S
A1C	R	S	S	S	S	I	S	S
I1B	R	S	S	S	R	I	S	S
RV1C	R	R	S	S	R	R	I	R
S1C	S	R	S	S	S	I	S	S
JB1C	R	R	S	S	S	S	S	S
U1C	S	R	S	S	S	I	S	S
U2C	R	R	S	S	R	R	R	S
U3C	R	R	S	S	S	R	I	S
V1C	R	R	S	S	R	R	I	I
RL2C	R	S	S	S	R	I	R	R
ST1C	R	S	S	S	R	I	R	I
SK1C	R	R	S	S	R	R	R	S
KT1C	R	S	S	S	R	I	S	S
BP1C	R	S	S	S	R	S	R	R
% S	17.65	47.06	100.0	100.0	47.06	23.53	52.94	70.59
% R	82.35	52.94	0.0	0.0	52.94	29.41	29.41	17.65
% I	0.00	0.00	0.0	0.0	0.00	47.06	17.65	11.76

Table 5. Zone of Inhibition of positive isolates against different antibiotics.

Sample	Zone of Inhibition (mm)							
	PB	**NB**	**TE**	**CX**	**E**	**CD**	**CIP**	**COT**
K3C	7	28	28	32	23	25	26	25
R1C	8	29	29	31	25	22	29	26
R2B	10	21	30	33	25	16	25	23
A1C	6	32	32	35	23	17	28	30
I1B	6	29	31	33	20	16	27	28
RV1C	5	22	21	17	15	10	18	11
S1C	10	22	29	30	26	17	27	26
JB1C	7	22	27	30	24	22	26	27
U1C	10	22	30	33	25	16	25	20
U2C	6	21	15	22	12	11	14	17
U3C	7	16	17	21	27	10	17	34
V1C	5	21	21	17	15	10	18	11
RL2C	9	33	31	32	5	20	13	10
ST1C	9	33	31	32	5	20	13	14
SK1C	6	15	15	22	12	11	14	17
KT1C	6	29	30	32	5	20	30	20
BP1C	6	29	30	32	21	22	11	10

[PB- Polymyxin-B, NB- Novobiocin; TE- Tetracycline; CX- Cefoxitin; E- Erythromycin; CD- Clindamycin; CIP- Ciprofloxacin; COT- Co-trimoxazole].

Table 6. Compounds identified in garlic oil with its peak report.

Peak	R. Time	Area	Area%	Name
1	4.724	2615151	6.70	Diallyl sulfide
2	6.375	1325915	3.40	Disulfide, methyl 2-propenyl
3	12.784	11626671	29.79	Diallyl disulphide
4	13.410	1190860	3.05	(E)-1-Allyl-2-(prop-1-en-1-yl)disulfane
5	13.707	1447262	3.71	(E)-1-Allyl-2-(prop-1-en-1-yl)disulfane
6	15.342	2452452	6.28	Allyl methyl trisulfide
7	16.128	167628	0.43	4-Methyl-1,2,3-trithiolane
8	22.682	7687432	19.69	Allitridin
9	23.254	310217	0.79	1-Allyl-3-propyltrisulfane
10	25.528	150519	0.39	5-Methyl-1,2,3,4-tetrathiane
11	25.894	506420	1.30	Copaene alpha
12	26.976	2697091	6.91	Cyperene
13	27.690	149316	0.38	Caryophyllene <(E)->
14	29.406	763645	1.96	Rotundene
15	29.891	190314	0.49	Gurjunene<gamma->
16	30.225	1683753	4.31	Methylparaben
17	30.543	19015	40.49	7-Isopropenyl-4a-Methyl-1-Methylenedecahydronaphthalene
18	30.709	411699	1.05	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7].
19	30.841	247608.	0.63	Alpha.-Selinene
20	32.633	316528	0.81	Tetrasulfide, di-2-propeny
21	34.183	45835	11.17	Caryophyllene oxide
22	36.603	1598183	4.09	Propyl 4-hydroxybenzoate
23	38.410	334556	0.86	Cyperotundone
24	42.595	190315	0.49	1-Allyl-3-(2-(allylthio)propyl)trisulfane
25	47.609	322955	0.83	3-Heptene, 4-methoxy

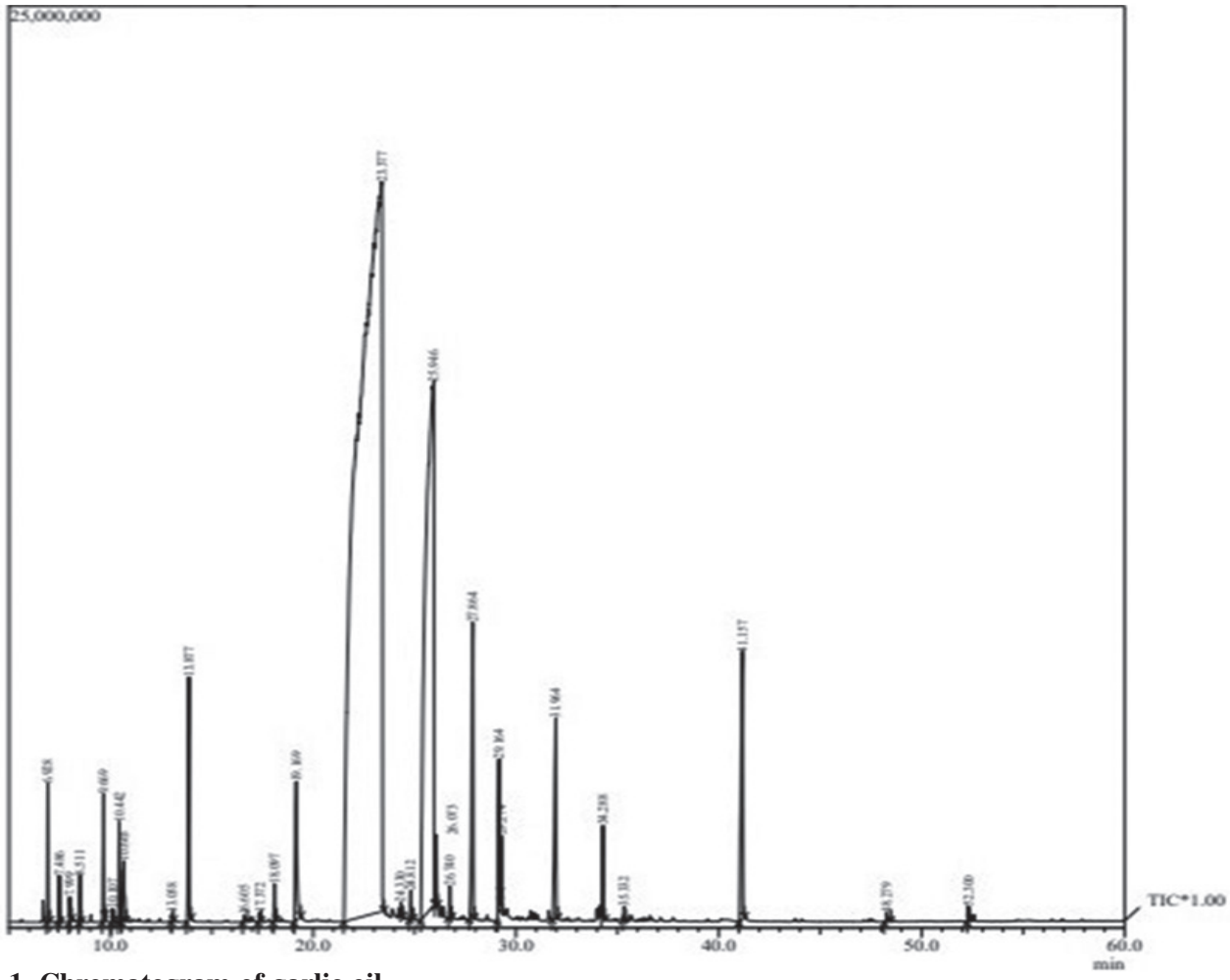

Fig. 1. Chromatogram of garlic oil.

(70%) [25]. In another finding, variable susceptibility to imipenem (96.7%), levofloxacin (86.7%), chloramphenicol (83.3%), cefoxitin (76.7%), ciprofloxacin (66.7%), gentamycin (63.3%), tetracycline sulfamethoxazole-trimethoprim (56.7%), vancomycin and doxycycline (50%) was seen against *S. aureus* isolates, our study also exhibited several antibiotic-resistant patterns including three or more antibiotics against 100% of the *S. aureus* isolates. In other findings *S. aureus* obtained from wound showed 92.4%, 63.0%, 44.2%, 35.8%, 52.4%, 61.9%, 15.5%, 31.2%, 7.1%, 78.9%, 76.6%, 100%, 71.4%, 30.7% and 100% sensitivity pattern against gentamicin, amoxycillin/ clavulanate, streptomycin, cloxacillin, erythromycin, chloramphenicol, cotrimoxazole, tetracycline, penicillin, ciprofloxacin, ofloxacin, levofloxacin, ceftriaxone, amoxycillin and vancomycin respectively. Methicillin-resistant isolates were sensitive to levofloxacin 93.7% and ofloxacin 68.7% [26]. In other findings, it was reported that biofilm-producing isolates showed resistance to trimethoprim + sulphamethoxazole and amoxicillin (93%), gentamycin (87%) and 0.5% with imipenem [27, 28].

CONCLUSION

The presence of biofilm-producing organisms is very detrimental to the proper treatment of mastitis. Our study observed biofilm-producing Staphylococcus aureus isolated from mastitis milk samples of bovines in Rewa. Biofilm forming ability was best observed by the microtiter plate method as the Congo red agar method and light microscopy method showed less sensitivity than the microtiter plate assay. Garlic oil showed antibiofilm-producing ability at 3% concentration. Organosulfur compounds present in garlic may be responsible for its antimicrobial activity. GCMS analysis observed the highest peak of diallyldisulphide present as the major component in the essential oil along with other organosulphur compounds. Multi-drug resistance profile showed

maximum resistance with erythromycin, clindamycin, novobiocin, and polymyxin-B giving an alarming sign of antimicrobial resistance in bovines.

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