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Genotypic Profiling and Evaluation of Bio-assisted Silver Nanoparticles (*Nigella sativa*) against Multidrug-resistant Clinical Isolates of *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a severe healthcare issue because of its multidrug resistance feature, virulence, and biofilm formation. The present research study characterizes clinical MRSA isolates and evaluates the antibacterial potential of the *Nigella sativa* (NS) extract (seed). A total of 200 MRSA samples from bloodstream, skin and soft tissue (SST), and urinary tract infection (UTI) were subjected to antimicrobial-susceptibility testing and molecular characterization. We evaluated both antibacterial and antibiofilm effects of crude NS extract as well as NS-mediated silver nanoparticles (NS-AgNPs). The results reveal that SST infections accounted for 46.0% of isolates, followed by bloodstream infections (39.0%) and urinary infections (15.0%). All isolates were resistant to penicillin, and 156/200 (78.0%) were classified as multidrug resistant. All 200 isolates were reported susceptible to vancomycin. The nuc and mecA genes were detected in all isolates. lukS/F-PV was significantly more frequent in SST isolates (67.4%) than bloodstream (48.7%) and urinary isolates (40.0%; P = 0.003). At 100 µg/mL, crude extract and NS-AgNPs inhibited biofilm formation by 78% and 90%, respectively. NS-AgNPs demonstrated a lower MIC_{50}/MBC_{50} (0.5 mg/mL) than crude extract (1.0 mg/mL). MRSA isolates showed substantial multidrug resistance. NS-AgNPs demonstrated greater antibacterial and antibiofilm activity than crude NS extract. Further, multicentre studies involving larger sample sizes, complemented by in vitro and in vivo investigations, including cytotoxicity, pharmacokinetic, and animal model-based evaluations, are warranted to validate the therapeutic potential, safety, and biological activity of the identified compounds.

Keywords: MRSA, *Nigella sativa*, Antimicrobial Resistance, Biofilm, Silver Nanoparticles

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INTRODUCTION

The bacterium *Staphylococcus aureus* is Gram-positive and catalase-positive that commonly colonizes the anterior nares, skin, and mucous membranes.[1] Although usually present as a commensal, it can cause infections ranging from folliculitis, cellulitis, and abscesses to pneumonia, bloodstream infection, endocarditis, osteomyelitis, and sepsis. Its pathogenicity is related to adhesins, protein A, hemolysins, leukocidins, enterotoxins, and biofilm-associated factors, which assist in tissue invasion, immune dysregulation, and insistent infection.[2] It has been documented that treatment of *S. aureus* mediated infection is difficult, attributed to the resistance increment. The MRSA has spread internationally and targeted community settings and healthcare setups severely.[3] A recent clinical surveillance study reported that mortality rate at the hospitals was significantly higher with MRSA (38%) than methicillin-susceptible *S. aureus* (MSSA) (26%).[4] Furthermore, A pooled analysis of 62 studies comprising 17,563 patients demonstrated an increased mortality burden associated with MRSA compared with MSSA. The estimated ORs were 1.95 (95% CI: 1.73-2.21) for various cause mortality and 2.04 (95% CI: 1.63-2.55) for infection-related death.[5] It is demonstrated that methicillin resistance (MR) is primarily facilitated through *mecA* (gene name), carried on the staphylococcal cassette chromosome (SCC*mec*).[6] PBP2a is an altered penicillin binding protein (PBP) encoded by mecA, and its squat affinity for β-lactam antibiotics is a key molecular determinant of β-lactam resistance.[7,8] MRSA isolates may also possess resistance determinants against macrolides, lincosamides, aminoglycosides, tetracyclines, and fluoroquinolones, thereby considerably limiting therapeutic options.[9] MRSA is reported to be the main cause of SST and UTI among hospitalized patients.[10] Infection due to MRSA often associated with metastatic complications, extended hospitalization, higher treatment expenses, and substantial mortality.[11] For instance, a recent global epidemiological review reports that across European healthcare systems, MRSA infections lead to an average 1.26-fold rise in hospital length of stay (LOS) than to MSSA.[12] Furthermore, a major United States economic analysis evaluating multidrug-resistant infections among hospitalized patients found that MRSA carries an attributable medical cost of $22,293 per elderly patient, contributing to billions of dollars in aggregate healthcare expenditure.[13] It has been observed that SST infections may become severe, particularly when isolates harbour cytolytic toxins such as Panton–Valentine leucocidin.[14,15] MRSA isolated from urine may represent ascending infection, catheter-associated infection, colonization, or hematogenous dissemination.[16] Therefore, studying isolates obtained from different clinical specimens is essential for understanding their distribution, resistance profiles, and infection-specific virulence characteristics.

Accurate characterization of MRSA is imperative for managing patient's health, antimicrobial surveillance, and infection severity. Phenotypic identification includes culture, Gram staining, catalase and coagulase testing, followed by cefoxitin or oxacillin susceptibility testing.[17] Cefoxitin is generally preferred because it provides clearer and more reproducible detection of *mecA*-mediated resistance.[18] Genotypic recognition of *mecA* and other virulence or resistance genes provides additional confirmation and enables evaluation of the relationships among genotype, phenotypic antimicrobial resistance and clinical source.[19] The frequent rise of multidrug-resistant in MRSA has encouraged the investigation of medicinal plants as potential sources of antimicrobial compounds. The plant *Nigella sativa* (NS) L., usually called black seed or kalonji, possesses antibacterial, antioxidant, antiallergic, anxiolytic, anti-inflammatory, and immunomodulatory potential.[20,21] Its principal bioactive constituent is thymoquinone, while thymohydroquinone, dithymoquinone, thymol, p-cymene and carvacrol may also contribute to its antimicrobial activity. Proposed mechanisms include bacterial membrane disruption, leakage of intracellular components, enzyme inhibition and interference with biofilm formation.[22]

A study demonstrated that ethanolic NS seed extract inhibited all 99 tested clinical MRSA isolates, with 0.2-0.5 mg/mL of minimum inhibitory concentrations (MIC).[23] Ugur et al. reported MIC of <0.25-1.0 µg/mL against 45 clinical MRSA isolates, with no detectable cytotoxicity at the tested concentrations.[24] Elmowalid et al.

also demonstrated antibacterial and potential resistance altering activity of NS extract against MRSA isolates.[25] Nevertheless, its reported antibacterial activity varies according to seed origin, extraction technique, solvent, phytochemical composition, extract concentration, and susceptibility-testing procedure.[26] The objective of this study is to determine the distribution of MRSA causing bloodstream, SST and UTI; perform phenotypic and genotypic characterization; assess virulence and antimicrobial-resistance genes and correlate them with phenotypic resistance. Furthermore, this study evaluates the antibacterial and antibiofilm activities of crude NS seed extract and green-synthesized NS-mediated silver nanoparticles (NS-AgNPs) against these clinical MRSA isolates.

MATERIALS AND METHODS

A laboratory investigation and experimental study were carried out in the Department of Microbiology, JNMC (AMU), Aligarh, India. We performed isolation and characterization of MRSA, assessed antimicrobial resistance, and detected virulence genes. We also evaluated the antibacterial and antibiofilm effect of NS seed extract and NS-mediated silver nanoparticles (NS-AgNPs).

Ethics Approval and Informed Consent

The study was approved by the Institutional Ethics Committee of Jawaharlal Nehru Medical College and Hospital, Aligarh, Uttar Pradesh, India (Approval No. IECJNMC1648/B; dated 04 September 2026). Written informed consent was obtained from all participants before their enrolment in the study.

Study isolates

A total of 200 unique (non-duplicate) MRSA isolates were obtained from hospitalized patients with bloodstream infections, SST, and UTIs. Patient age, sex, specimen type, and infection source were recorded. Only one isolate from each patient was considered for each distinct infection episode.

Inclusion criteria

The study included clinical isolates identified as *S. aureus* and confirmed as MRSA. The isolates recovered from blood, SST specimens, or urine were considered eligible for inclusion. Only viable, non-duplicate isolates with sufficient material for microbiological and molecular characterization were considered in the study.

Exclusion criteria

Isolates were excluded if they were duplicates, mixed, contaminated, non-viable, or inadequately characterized. MSSA isolates were also excluded. In addition, colonizing or surveillance isolates obtained in the absence of suspected clinical infection were also omitted from the study.

Specimen collection and processing

The collected clinical specimens were obtained under aseptic conditions, subsequently transferred to laboratory for further processing. Blood specimens were subjected to culture using a [blood-culture system], whereas pus, wound swabs, aspirated materials, tissue samples, and urine specimens were cultured on suitable microbiological media. Following inoculation, the culture dishes were incubated at 35-37 °C for 18-24 hours aerobically.

Identification of *Staphylococcus aureus*

Bacterial isolates were characterized initially according to their colony characteristics and Gram-staining reactions, followed by biochemical testing, like catalase, tube coagulase, mannitol fermentation, and DNase assays, as appropriate. The species level validation of *S. aureus* was subsequently carried out using polymerase chain reaction (PCR) amplification targeting the species specific *nuc* gene.

Detection of methicillin resistance

MR among the confirmed *S. aureus* isolates was initially assessed through cefoxitin disc diffusion assay performed on Mueller-Hinton agar.[27] Bacterial inocula were prepared to a turbidity equivalent to 0.5 McFarland before inoculation of the agar plates. The resulting

inhibition zone diameter was evaluated in accordance with the relevant CLSI interpretive criteria.[28] Molecular confirmation of MR was subsequently achieved via PCR extension of the *mecA* resistance determinant.

Susceptibility assessment

Susceptibility testing of the bacterial isolates was done by using the Kirby-Bauer disc diffusion (KBDD) technique. Antibiotic test included penicillin, erythromycin, clindamycin, cotrimoxazole, doxycycline, gentamicin, ciprofloxacin, and linezolid. Vancomycin susceptibility was checked by utilizing the broth microdilution/E-test/automated system. Results were interpreted according to CLSI criteria.[28] *S. aureus* ATCC 25923 was used for disc-diffusion, while MRSA ATCC 43300 served as the methicillin-resistant reference strain.

Molecular characterization

Extraction of the DNA

The genomic DNA was obtained from pure overnight cultures using [boiling method/ commercial extraction kit]. The extracted DNA was assessed for concentration and purity and stored at -20 °C until PCR investigation.

Recognition of antimicrobial resistance and virulence genes

The PCR was performed in order to detect the following genes:

- *nuc* for confirmation of *S. aureus*
- *mecA* for methicillin resistance
- lukS/F-PV for Panton–Valentine leukocidin
- *spa* for staphylococcal protein A
- *coa* for coagulase
- *vanB* for vancomycin resistance

PCR fragments (amplified) were resolved by the gel technique called agarose gel electrophoresis (AGE) and subsequently examined using a gel documentation system. Appropriate positive, negative, and no-template controls were considered.

Cassette chromosome mec (SCC*mec*) typing

SCC*mec* typing was done through multiplex PCR. Isolates were categorized as SCC*mec* types I, II, III, IV, or V according to their amplification patterns.

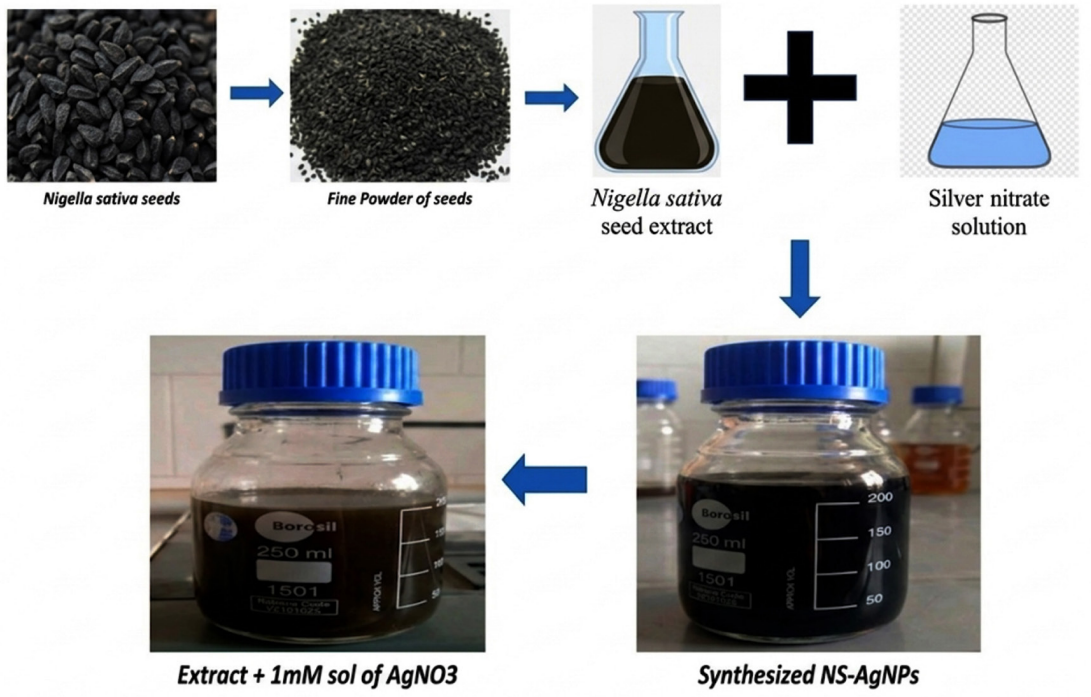


Figure 1. Overview of the synthesized silver nanoparticle solution.

Nigella sativa seed extract preparation

Authenticated NS seeds were cleaned, shade-dried, and crushed into a fine powder. Subsequently, powdered seeds were extracted using [solvent] by [maceration/Soxhlet extraction]. The extract was filtered, concentrated, dried, and stored at 4 °C. A stock solution was prepared in [solvent] and diluted to the required concentrations.

Green synthesis of NS-AgNPs

NS-AgNPs were prepared by mixing NS seed extract with aqueous silver nitrate solution under controlled conditions. The development of a brown colour indicated nanoparticle formation. The nanoparticles were recovered by centrifugation, washed with sterile distilled water, dried, and stored until testing (Figure 1).

Table 1. Demographic and Clinical Characteristics of Study Participants (N = 200)

Characteristic	Frequency (n)	Percentage (%)
Clinical source		
Skin and soft-tissue	92	46.0
infection		
Bloodstream infection	78	39.0
Urinary tract infection	30	15.0
Age group (years)		
<20	22	11.0
21-40	74	37.0
41-60	68	34.0
>60	36	18.0
Age, mean ± SD (years)	44.6 ± 16.8	
Age, median (IQR) (years)	43 (28-58)	
Sex		
Male	118	59.0
Female	82	41.0

Evaluation of antibacterial activity

Agar-well diffusion assay

The preliminary antibacterial activities of crude NS extract and NS-AgNPs were evaluated through the agar well diffusion method.[29] After incubation at 35-37 °C for 18-24 hrs, the diameter of each zone of inhibition was determined and recorded in millimeters. Appropriate positive, negative, and solvent controls were included.

Minimum Inhibitory Concentration (MIC)

The MICs were established by the broth microdilution technique. Furthermore, serial dilutions (twofold) of the crude extract and NS-AgNPs were set in cation assisted Mueller-Hinton broth (MHB). Each well was inoculated to obtain approximately 5×10^5 CFU/mL. The MIC stands for the lowest concentration required to prevent the growth of microorganisms. MIC values, such as MIC_{10}, MIC_{50}, and MIC_{90}, were calculated.

Minimum Bactericidal Concentration (MBC)

Samples inside the wells showed no visible bacterial growth and were transferred to

Table 2. Antimicrobial susceptibility profile of MRSA isolates (N = 200)

Antimicrobial agent	Resistant, n (%)	Susceptible, n (%)
Penicillin	200 (100.0)	0 (0.0)
Erythromycin	156 (78.0)	44 (22.0)
Ciprofloxacin	140 (70.0)	60 (30.0)
Clindamycin	124 (62.0)	76 (38.0)
Cotrimoxazole	98 (49.0)	102 (51.0)
Gentamicin	88 (44.0)	112 (56.0)
Doxycycline	72 (36.0)	128 (64.0)
Linezolid	8 (4.0)	192 (96.0)
Vancomycin	0 (0.0)	200 (100.0)

Table 3. Distribution of virulence and resistance genes according to clinical source

Gene	SSTI (n = 92), n (%)	BSI (n = 78), n (%)	UTI (n = 30), n (%)	Total (n = 200), n (%)	P-value
nuc	92 (100.0)	78 (100.0)	30 (100.0)	200 (100.0)	—
mecA	92 (100.0)	78 (100.0)	30 (100.0)	200 (100.0)	—
lukS/F-PV	62 (67.4)	38 (48.7)	12 (40.0)	112 (56.0)	0.003
spa	70 (76.1)	58 (74.3)	20 (66.7)	148 (74.0)	0.410
coa	78 (84.8)	60 (76.9)	22 (73.3)	160 (80.0)	0.320
vanB	8 (8.7)	6 (7.7)	4 (13.3)	18 (9.0)	—

antimicrobial-free agar plates for subculturing. MBC was considered the lowest antimicrobial concentration that resulted in the complete absence of detectable growth or achieved at least a 99.9% reduction in the original bacterial population. MBC ranges, and MBC_{10}, MBC_{50}, and MBC_{90} were calculated.

Evaluation of antibiofilm activity

Antibiofilm activity was assessed using the microtitre-plate crystal-violet assay. MRSA isolates were treated with crude NS extract and NS-AgNPs at concentrations of 25, 50, and 100 µg/mL. After the designated incubation period, the resulting adherent biofilms were stained with crystal violet. Biofilm biomass was subsequently quantified by measuring the optical density of the stained preparations at a wavelength of 570 nm.

Biofilm inhibition was calculated as:

$$\text{Biofilm inhibition (\%)} = \frac{OD_{control} - OD_{treated}}{OD_{control}} \times 100$$

Experiments were conducted in triplicate, with MRSA ATCC 43300 as the reference strain.

Table 4. Molecular types and multidrug-resistance patterns of MRSA isolates

A. Distribution of SCC*mec* types

SCC*mec* type	Number of isolates	Percentage (%)
Type I	38	19.0
Type II	52	26.0
Type III	44	22.0
Type IV	46	23.0
Type V	20	10.0
Total	200	100.0

B. Multidrug-resistance according to clinical source

Clinical source	MDR, n (%)	Non-MDR, n (%)	Total	P-value
SSTI	70 (76.1)	22 (23.9)	92	
BSI	68 (87.1)	10 (12.9)	78	0.010
UTI	18 (60.0)	12 (40.0)	30	
Total	156 (78.0)	44 (22.0)	200	

Outcome measures

The study outcomes included: (I) Clinical-source distribution of MRSA, (II) Antimicrobial-susceptibility and MDR patterns, (III) Distribution of resistance and virulence genes, (IV) Distribution of SCC*mec* types, (V) Association of detected genes with phenotypic resistance, (VI) Biofilm inhibition by crude extract and NS-AgNPs, (VII) MIC and MBC values of crude extract and NS-AgNPs.

Statistical analysis

Data processing and statistical evaluation were performed in SPSS version 26. Categorical variables were expressed as counts with their respective percentages, whereas continuous variables were reported either as mean ± standard deviation (SD) or as median with interquartile range (IQR), as appropriate. The relationships between clinical source and variables including multidrug resistance (MDR), resistance-associated genes, SCC*mec* types, and antimicrobial resistance patterns were assessed using either the Pearson chi-square test or Fisher's exact test, depending on the suitability of the data. Differences in biofilm inhibition among the different treatment groups and tested concentrations were evaluated using two-way analysis of variance (ANOVA) followed by an appropriate post hoc multiple-comparison procedure. Statistical hypotheses were evaluated using two-sided tests, and results with $P < 0.05$ were considered statistically significant.

RESULTS

A total of 200 non-duplicate MRSA isolates were collected from hospitalized patients. SST infections were the most common source, accounting for 92 (46.0%) isolates, followed by bloodstream infections with 78 (39.0%) and UTI with 30 (15.0%). Mean age of the patients was 44.6 ± 16.8 years, with median age of 43 years (IQR: 28-58 years). The 21-40 year age category remains high in proportion of participants (74/200, 37.0%), followed by 41-60 years (68/200, 34.0%), >60 years (36/200, 18.0%), and <20 years (22/200, 11.0%). Of the 200 patients, 118 (59.0%) were male, and 82 (41.0%) were female (Table 1).

All MRSA isolates were resistant to penicillin (200/200, 100%). High resistance rates were also observed against erythromycin

(156/200, 78.0%), ciprofloxacin (140/200, 70.0%) and clindamycin (124/200, 62.0%). Resistance to cotrimoxazole, gentamicin and doxycycline was detected in 98 (49.0%), 88 (44.0%) and 72 (36.0%) isolates, respectively. Eight isolates (4.0%) were resistant to linezolid, whereas all 200 isolates were susceptible to vancomycin (Table 2 and Figure 2).

PCR analysis demonstrated the occurrence of both the species explicit nuc gene and the methicillin-resistance-associated *mecA* gene (MAmecA) in every isolate, regardless of the clinical specimen from which it was obtained. The lukS/F-PV gene was detected more frequently among SST isolates (62/92, 67.4%) than among bloodstream (38/78, 48.7%) and urinary isolates (12/30, 40.0%). The observed difference reached statistical significance (P = 0.003).

The spa gene was detected in 70 (76.1%) SST, 58 (74.3%) bloodstream and 20 (66.6%) urinary isolates (p = 0.41). Similarly, the coa gene was detected in 78 (84.7%), 60 (76.9%) and 22 (73.3%) isolates, respectively (P = 0.32). The *vanB* gene was identified in 18 (9.0%) isolates, including eight SST, six bloodstreams and four urinary isolates (Table 3 and Figure 3).

SCC*mec* type II was the most frequently identified type, occurring in 52 (26.0%) isolates, followed by type IV in 46 (23.0%), type III in 44 (22.0%), type I in 38 (19.0%) and type V in 20 (10.0%) isolates. The distribution of SCCmec types differed significantly (P = 0.01) (Table 4A and Figure 4).

Overall, 156 of the 200 MRSA isolates (78.0%) were multidrug-resistant. Multidrug resistance was most frequent among bloodstream isolates (68/78, 87.1%), followed by skin and soft-tissue isolates (70/92, 76.1%) and urinary isolates (18/30, 60.0%). The association between multidrug resistance and clinical source was statistically significant (P = 0.01) (Table 4B).

Among the 112 lukS/F-PV-positive isolates, 82 (73.2%) were multidrug-resistant, while resistance to erythromycin, ciprofloxacin and clindamycin was observed in 88 (78.6%), 82 (73.2%) and 74 (66.1%) isolates, respectively. The association between lukS/F-PV carriage and antimicrobial resistance was statistically significant (P = 0.002).

Of the 148 spa-positive isolates, 108 (73.0%) were multidrug-resistant. Significant associations were also observed between spa carriage and resistance to erythromycin, ciprofloxacin and clindamycin (P = 0.001). Among the 160 coa-positive isolates, 118 (73.8%) were multidrug-resistant, with 128 (80.0%), 118 (73.7%) and 100 (62.5%) isolates resistant to erythromycin, ciprofloxacin and clindamycin, respectively (P = 0.001). In contrast, the association between *vanB* carriage and the evaluated resistance phenotypes was not statistically significant (P = 0.08) (Table 5 and Figure 5).

Both crude NS seed extract and NS-mediated silver nanoparticles (NS-AgNPs) produced concentration-dependent inhibition of MRSA biofilm formation. At 25, 50 and 100 µg/mL, the crude extract produced mean inhibitions of 32 ± 6.1%, 54 ± 7.3% and 78 ± 5.8%, respectively. The corresponding inhibition values for NS-AgNPs were 40 ± 5.2%, 62 ± 6.0% and 90 ± 4.1%. For the ATCC 43300 control strain, inhibition was 10 ± 4.5%, 30 ± 6.2% and 60 ± 4.3%, respectively. Differences among the tested preparations were statistically significant at 25 µg/mL (P = 0.002), 50 µg/mL (P = 0.012) and 100 µg/mL (P = 0.001) (Table 6A).

The MIC of crude NS extract ranged from 0.125 to 2.0 mg/mL, with MIC_{50} and MIC_{90} values of 1.0 and 2.0 mg/mL, respectively. NS-AgNPs demonstrated lower MIC values, ranging from 0.062 to 1.0 mg/mL, with MIC_{50} and MIC_{90} values of 0.5 and 1.0 mg/mL, respectively. For

Table 5. Association of selected genes with multidrug and antimicrobial resistance

Gene	Gene-positive isolates, n (%)	MDR, n (%)	Erythromycin resistant, n (%)	Ciprofloxacin resistant, n (%)	Clindamycin resistant, n (%)	P-value
lukS/F-PV	112 (56.0)	82 (73.2)	88 (78.6)	82 (73.2)	74 (66.1)	0.002
spa	148 (74.0)	108 (73.0)	118 (79.7)	110 (74.3)	94 (63.5)	0.001
coa	160 (80.0)	118 (73.8)	128 (80.0)	118 (73.8)	100 (62.5)	0.001
vanB	18 (9.0)	14 (77.8)	12 (66.7)	10 (55.6)	9 (50.0)	0.080

the ATCC 43300 control strain, the MIC range was 1-4 mg/mL. Differences in MIC_{50} and MIC_{90} were statistically significant (P = 0.003 and P = 0.001, respectively).

MBC of crude NS extract ranged from 0.125 to 2.0 mg/mL, with MBC_{50} and MBC_{90} values of 1.0 and 2.0 mg/mL, respectively. For NS-AgNPs, the MBC ranged from 0.062 to 1.0 mg/mL, with MBC_{50} and MBC_{90} values of 0.5 and 1.0 mg/mL. The corresponding MBC range for the ATCC 43300 control strain was 1-4 mg/mL. The differences in MBC_{50} and MBC_{90} were statistically significant (P = 0.008 and P = 0.004, respectively) (Table 6B).

DISCUSSION

All isolates exhibited resistance to penicillin, while resistance to erythromycin,

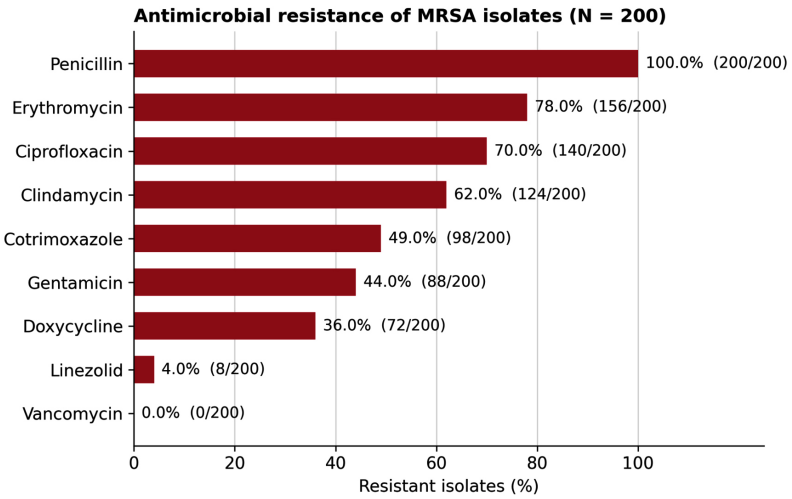


Figure 2. Antimicrobial susceptibility profile of MRSA isolates (N = 200)

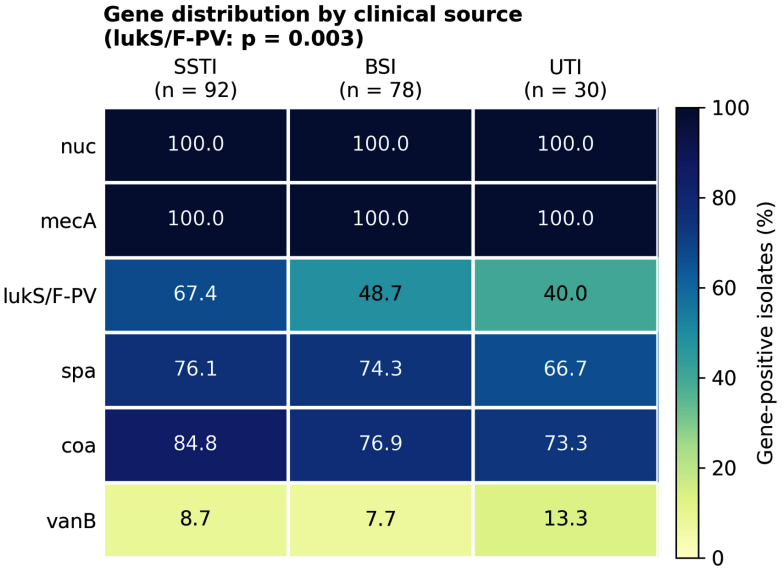


Figure 3. Distribution of virulence and resistance genes across clinical sources. Values are presented as percentages of gene-positive isolates. SSTI, skin and soft-tissue infection; BSI, bloodstream infection; UTI, urinary tract infection. The distribution of lukS/F-PV differed significantly across clinical sources (P = 0.003)

ciprofloxacin, and clindamycin was 78.0%, 70.0%, and 62.0%, respectively. Conversely, the susceptibilities to linezolid and vancomycin were 96.0% and 100%, respectively. Ammar et al.[30] recovered 40 MRSA isolates from 18.6% of the milk and meat samples examined, of which 85.0% were MDR. Among 20 isolates with discordant erythromycin–clindamycin resistance, eight demonstrated inducible clindamycin resistance. Two isolates had erythromycin MICs of 512 and 1,024 µg/mL and clindamycin MICs of 16 and 32 µg/mL. Similarly, Aljazzar et al.[31] recovered Campylobacter from 67.3% of poultry samples, with 100% resistance to erythromycin, ampicillin and trimethoprim-sulfamethoxazole. Although different organisms and sources were studied, the above findings support the widespread occurrence of antimicrobial resistance.

PCR analysis confirmed the presence of both *nuc* and *mecA* genes in all tested isolates. The lukS/F-PV gene was significantly more frequent in SST isolates (67.4%) than bloodstream (48.7%) and urinary isolates (40.0%; P = 0.003). The *spa*, *coa* and *vanB* genes were detected in 74.0%, 80.0% and 9.0% of isolates, respectively. Ammar et al.[30] reported good correspondence

Table 6. Antibacterial and antibiofilm activity of *Nigella sativa* preparations against MRSA
A. Biofilm inhibition at different concentrations

Preparation	25 µg/mL, mean ± SD (%)	50 µg/mL, mean ± SD (%)	100 µg/mL, mean ± SD (%)
Crude NS extract	32 ± 6.1	54 ± 7.3	78 ± 5.8
NS-AgNPs	40 ± 5.2	62 ± 6.0	90 ± 4.1
ATCC 43300 control	10 ± 4.5	30 ± 6.2	60 ± 4.3
P-value	0.002	0.012	0.001

B. Minimum inhibitory and bactericidal concentrations

Preparation	MIC range (mg/mL)	MIC_{50}	MIC_{90}	MBC range (mg/mL)	MIC_{50}	MIC_{90}
Crude NS extract	0.125-2.0	1.0	2.0	0.125-2.0	1.0	2.0
NS-AgNPs	0.062-1.0	0.5	1.0	0.062-1.0	0.5	1.0
ATCC 43300 control	1.0-4.0	3.0	4.0	1.0-4.0	3.0	4.0
P-value	-	0.003	0.001	-	0.008	0.004

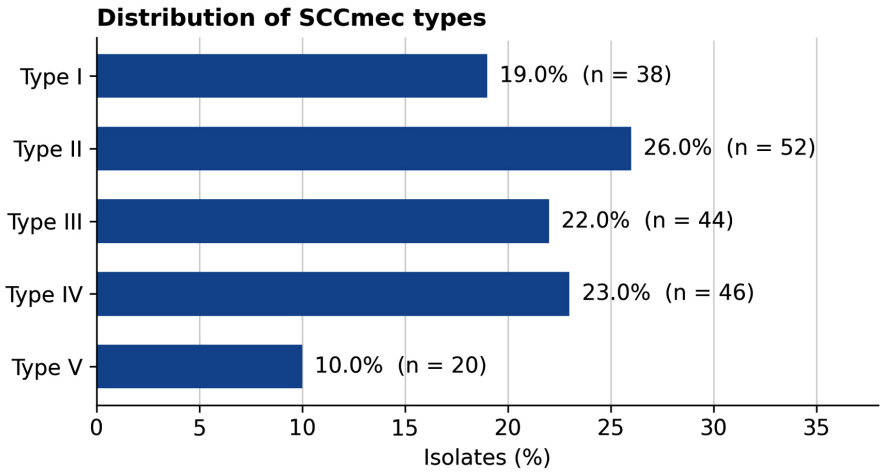


Figure 4. Distribution of SCC*mec* types

between methicillin, amoxicillin–clavulanate, and tetracycline resistance and the detection of *mecA*, *blaZ*, and *tet*(K), respectively. However, *norA* was absent from four ciprofloxacin-resistant isolates, while neither *qacA*/B nor smr was detected despite benzalkonium-chloride resistance in 55.0% of isolates. Similarly, the presence of vanB without phenotypic vancomycin resistance in our study demonstrates that gene carriage may not always correspond to phenotypic expression.

SCC*mec* type II was most frequent (26.0%), followed by type IV (23.0%), type III (22.0%), type I (19.0%) and type V (10.0%; P = 0.010). This distribution suggests the coexistence of healthcare- and community-associated MRSA lineages. Overall, 78.0% of isolates were MDR. MDR was significantly higher among bloodstream isolates (87.1%) than SST (76.1%) and urinary isolates (60.0%; P = 0.010). This was comparable to the 85.0% MDR prevalence reported by Ammar et al.[30] among food-associated MRSA isolates. Aljazzar et al.[31] similarly observed 100% resistance to erythromycin, ampicillin and trimethoprim–sulfamethoxazole among their Campylobacter isolates.

Among lukS/F-PV-positive isolates, 73.2% were MDR, while resistance to erythromycin, ciprofloxacin and clindamycin was 78.6%, 73.2% and 66.1%, respectively (P = 0.002). MDR was also present in 73.0% of spa-positive and 73.8% of coa-positive isolates, with significant associations between these genes and resistance (P = 0.001 each). In contrast, the association involving vanB was not significant (P = 0.080). Similarly, Ammar et al.[30] demonstrated correlations between resistance phenotypes and *mecA*, *blaZ* and *tet*(K) genes. The significant associations of lukS/F-PV, spa and coa with resistance in our study may indicate the circulation of MRSA lineages simultaneously carrying virulence and resistance determinants.

The crude NS extract produced 32 ± 6.1%, 54 ± 7.3% and 78 ± 5.8% biofilm inhibition at 25, 50

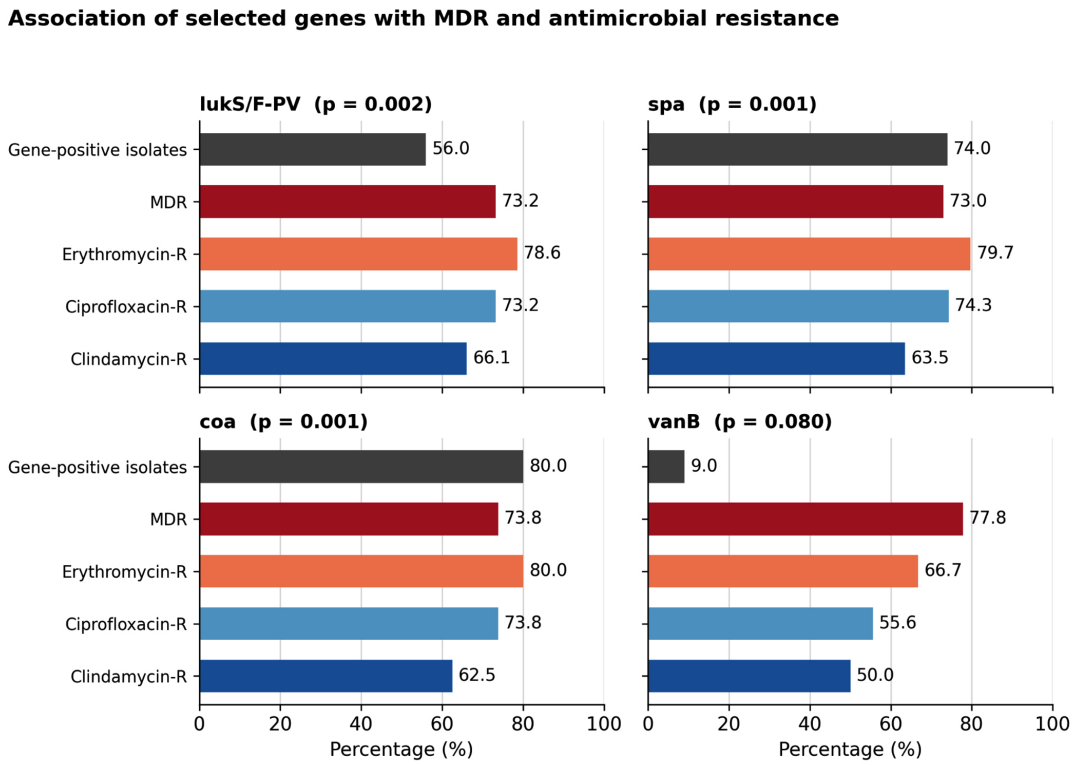


Figure 5. Association of lukS/F-PV, *spa*, *coa*, and *vanB* carriage with multidrug resistance (MDR) and resistance to erythromycin, ciprofloxacin, and clindamycin. Each panel represents one gene, with bars showing the percentage of isolates. P-values shown in the panels correspond to the statistical analyses presented in Table 5

and 100 µg/mL, respectively. NS-AgNPs produced greater inhibition of 40 ± 5.2%, 62 ± 6.0% and 90 ± 4.1%, respectively, with significant differences at all concentrations (P = 0.002, 0.012 and 0.001). Chaieb et al. reported thymoquinone MICs of 8–32 µg/mL against human pathogens and 50% biofilm inhibitory concentrations (BIC) of 22 µg/mL for *S. aureus* and 60 µg/mL for *S. epidermidis*.[32] These findings support the concentration-dependent antibiofilm activity observed in our study.

The crude extract demonstrated an MIC/MBC range of 0.125-2.0 mg/mL, with MIC_{50}/MBC_{50} of 1.0 mg/mL and MIC_{90}/MBC_{90} of 2.0 mg/mL. For NS-AgNPs, the MIC/MBC range was 0.062-1.0 mg/mL, with MIC_{50}/MBC_{50} of 0.5 mg/mL and MIC_{90}/MBC_{90} of 1.0 mg/mL. Toama et al. demonstrated that the volatile oil derived from NS, particularly thymohydroquinone, was active against Gram-positive bacteria and yeasts.[33] Suresh et al. found that supercritical carbon dioxide extract prepared at 120 bar and 40 °C produced greater bacterial inhibition than conventional extracts.[34] Aforementioned findings support the influence of extraction and formulation on antibacterial activity. Indirectly, a study by Aljazzar et al.[31] showed that eugenol, as well as trans-cinnamaldehyde, reduced *C. jejuni* loads and downregulated the virulence genes, including *flaA*, *virB11*, and *wlaN*. Ibrahim et al. found that a dose of about 200 mg/kg cornelian cherry extract can improve growth and antioxidant status in broilers. Together, these studies support the biological potential of plant-derived compounds. The lower MIC and MBC values and the higher biofilm inhibition observed with NS-AgNPs indicate greater activity than that of the crude NS extract.

CONCLUSION

MRSA isolates demonstrated high multidrug resistance, particularly among bloodstream isolates, and frequently carried important virulence genes. NS seed extract exhibited concentration-dependent antibacterial and antibiofilm activity against clinical MRSA isolates. NS-AgNPs produced greater biofilm inhibition and lower MIC and MBC values than the crude extract, supporting further preclinical investigation. Although plant-assisted antimicrobial nanomaterials may be relevant to broader One Health strategies for AMR mitigation, cross-sectoral efficacy, environmental safety, and sustainability were not evaluated in the present clinical-isolate study. This single-center study included a limited number of isolates, evaluated only selected resistance and virulence genes, and assessed biofilm formation only by the microtitre crystal-violet assay; Congo Red Agar confirmation and photographic documentation were not performed. The in vitro findings were not confirmed by cytotoxicity, pharmacokinetic, animal model, or clinical investigations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Jawaharlal Nehru Medical College and Hospital, Aligarh, U.P., India, approval number IECJNMC1648/B, dated 04/09/2026.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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