

# Study of Antibiotics Sensitivity Pattern And Molecular Characterization of *Staphylococcus Aureus* Isolated From Human And Animal Pyogenic Cases

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## Research Article

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## Abstract

*Staphylococcus aureus* has been described as the most common cause of human and animal diseases and has emerged as superbug due to multidrug resistance. Considering these, a total of 175 samples were collected from pyogenic cases of humans (75) and animals (100), to establish the drug resistance pattern and also for molecular characterization of human and animal isolates. Thermonuclease (*nuc*) gene amplification was used to confirm all presumptive *S. aureus* isolates and then antibiotic sensitivity and slide coagulase tests were used for phenotypic characterization of isolates. Following that, all of the isolates were subjected to PCR amplification to detect the existence of the methicillin resistance (*mecA*) and coagulase (*coa*) genes. Lastly, typing was done by using the Randomly Amplified Polymorphic DNA-PCR. The overall prevalence of *S. aureus* in human and animal samples was found to be 39.4%. Drug sensitivity revealed the highest resistance against the β-lactam antibiotics such as ampicillin (94.8%) and penicillin (90.6%), followed by cephalosporin (cefixime-67.7%) and quinolone (ciprofloxacin-52.1%) group of drugs. The drug sensitivity was the highest against antibiotics like chloramphenicol (95%) followed by gentamicin (90%). Among the 69 *S. aureus* isolates, the overall presence of MRSA was 40.5% (27.5% and 50% in human and animal isolates respectively). Total 33 isolates exhibited *coa* genes amplification of more than one amplicons and variable in size of 250, 450, 800, and 1100 bp. The RAPD typing revealed amplification of 5 and 6 different band patterns in humans and animals, respectively, with two common patterns suggesting a common phylogenetic profile.

## Introduction

*Staphylococcus aureus* has become the greatest global concern. *S. aureus* has become an utmost problem due to its capacity to cause life-threatening infections, its inherent virulence, and its ability to adjust to various ecological conditions [1]. In recent times *S. aureus* has gained attention due to its ability to gain resistance against commonly used antibiotics. The *S. aureus* strain is considered as drug-resistant upon acquisition of the *mecA* gene, this gene encodes the penicillin-binding protein 2a (PBP2a), and its incorporation within a large chromosomal element, called staphylococcal cassette chromosome *mec* (SCCmec). Available information shows that the *mecA* gene is present in the resistant strains but absent in the susceptible ones [2]. “There is evidence that the methicillin-sensitive strains of *S. aureus* acquired the SCCmec element probably from coagulase-negative staphylococcal strains and became methicillin-resistant” [3]. Moreover, “the *nuc* gene amplification by PCR is considered a standard technique for the identification of *S. aureus*. The *nuc* gene in *S. aureus* encodes the thermonuclease enzymes and amplification of the *nuc* gene is potential for rapid diagnosis of *S. aureus* infection” [4].

*S. aureus* also produces extracellular proteins; one of them is coagulase, which can bind with prothrombin and fibrinogen. “The coagulase and prothrombin complex binds with fibrinogen and convert it to fibrin threads by a mechanism different from natural clotting” [5]. The coagulase is encoded by the *coa* gene which possesses a conserved and repeated polymorphic region [6].

To combat MRSA infections, quick and accurate typing of *S. aureus* strains is compulsory. Many typing methods have been described [7–9]; among these methods for genotyping of *S. aureus*, randomly amplified polymorphic DNA (RAPD) technique has been observed as a basic, effective, and quick method [10]. Based on these observations and increasing resistance to different antibiotics the present study was designed to evaluate the antibiotics sensitivity patterns, to determine the correlation of antimicrobial resistance *mecA* gene with antibiotics sensitivity patterns and with virulence *coa* gene in *S. aureus* isolated from pyogenic cases of humans and animals. Finally, the phylogenetic correlation between human and animal MRSA isolates was evaluated by RAPD-PCR.

## Materials And Methods

### Sample collection and identification of *S. aureus*

A total of 175 samples were collected, consisting of 75 pus samples from humans and 100 pus samples from cattle, dogs, and, buffalos. All the collected samples were immediately shifted to Tryptone soya broth (Hi-Media, India) containing 6.5% of NaCl (Merck, India) and then incubated at 37°C overnight. Then, obtained culture growths were subjected to isolation and identification of *S. aureus* based on cultural, morphological, and biochemical characteristics. These cultures were further subjected to Coagulase tests as per standard procedures [11].

The extraction of genomic DNA from all the isolates was done by the phenol-chloroform method [12] with slight modifications. All of the presumptive *S. aureus* isolates were subjected to PCR-based amplification of the 280 bp species-specific *nuc* gene using custom synthesized previously described primers [13]. All of the obtained amplicons were analyzed under a UV transilluminator (Spectroline, Westbury, NY) after electrophoresis in a 1.5% agarose gel containing ethidium bromide and standard molecular weight markers.

### Antibiotic sensitivity testing

The disc diffusion method was used to perform the antibiotic sensitivity test as recommended by the Clinical and Laboratory Standards Institute [14]. Different groups of antimicrobial drugs were used containing a designated amount of drug (HiMedia, India), including vancomycin (30µg), methicillin (10µg), amoxicillin (10µg), ticarcillin (75µg), penicillin G (10U), erythromycin (15µg), clindamycin (2µg), amikacin (10µg), streptomycin (10µg), gentamicin (10µg), cefazolin (30µg), chloramphenicol (30µg), cefoxitin (30µg), tetracycline (30µg), cefixime (5µg), and ciprofloxacin (5µg). Using an antibiotic zone scale (Himedia, India), the zone of inhibition was measured in millimeters after 24 hours of incubation.

### Determination of methicillin-resistant *mecA* and coagulase producing *coa* genes

All the confirmed *S. aureus* isolates were subjected to amplification of antimicrobial resistance *mecA* [15] and coagulase producing *coa* gene [16] on genomic DNA. The PCR reactions were carried out in a total volume of 25 µl made up of the 2 µl template DNA, 6.5 µl nuclease-free water, 2 µl each set of primers,

and 12.5 µl HotStart PCR Master Mix (Takara Bio Inc., Japan). The PCR amplification was performed by using previously reported procedures for *mecA* genes [15], and *coa* genes [16].

### RAPD Typing

RAPD Typing was done by using AP4 primer 5'-TCACGCTGCA-3'. PCR reactions were carried out in an absolute volume of 50 µl comprised of the 2 µl template genomic DNA, 4 µl AP4 primer, 25 µl 2X HotStart PCR Master Mix (Takara Bio Inc., Tokyo, Japan), and 19 µl nuclease-free water to make up the volume. All of the obtained amplicons were analyzed under a UV transilluminator (Spectroline, Westbury, NY) after electrophoresis in a 1.5% agarose gel containing ethidium bromide and standard molecular weight markers. For negative controls, AP4 PCR reactions without DNA were used [17].

## Results And Discussion

MRSA was first isolated from cows with mastitis in 1972 [18], followed by isolation of MRSA of human origin from dairy cows [19]. Since the first report of MRSA, the presence of MRSA has been reported almost from all the domestic species. In this study, a total of 175 samples were collected from human and animal pyogenic cases. The study revealed the 39.4% ( $n = 69$ ) overall prevalence of *S. aureus* in human and animal samples. These included 38.6% (J1-J29) and 40% (J30-J69) prevalence in human and animal pyogenic cases, respectively (Fig. 1).

All of the 69 *S. aureus* isolates were tested for antimicrobial susceptibility and the resistance was highest against the β-lactam group of antibiotics such as ampicillin (94.8%), penicillin (90.6%), methicillin (81.3%), and ticarcillin (70.8%) (Fig. 2, Table). In contrast to this study, the resistance rate recorded in another study was 95% to amoxicillin and 82.5% to penicillin [20]. Following the β-lactam, resistance was observed against cephalosporin drugs like cefixime (67.7%) and quinolone groups of drugs such as ciprofloxacin (52.1%) (Table). Earlier published report also recorded the same resistance to ciprofloxacin (54%) [21]. Irrespective of a group of drug resistance was higher in human isolates in comparison to animal isolates. The ciprofloxacin is commonly used in humans against *S. aureus* was found to be 78.6% resistant in humans and 15.0% in animals. A study has shown that after 3 months of ciprofloxacin use, the resistance rate was increased from none to 79% over 1 year [22]. However, other antibiotics were found to be sensitive. The highest sensitivity was observed against drugs like chloramphenicol (95%) followed by gentamicin (90%), cefoxitin (86.5%), streptomycin (86.5%), and tetracycline (83.9%). This study is in agreement with another report that reported the highest sensitivity to chloramphenicol (85.2%) [23]. In one study 83% and 81% sensitivity was obtained against gentamicin and tetracycline respectively [24]. The human isolates revealed the highest sensitivity to quinolones like cefoxitin (85.7%) followed by aminoglycosides like streptomycin (83.9%). The animal isolates revealed the highest sensitivity to aminoglycosides like streptomycin (90%) followed by cefoxitin (87.5%), chloramphenicol (85%), and clindamycin (74%) (Fig. 2, Table).

**Table.** Drug sensitivity pattern (in %) of *S. aureus* isolates against different groups of antibiotics

S. No.	Antibiotic Name	Sensitivity			Intermediate			Resistance			Total
		H	A	Total	H	A	Total	H	A		
1.	<b>Vancomycin (30 µg)</b>	1.8	57.5	25.0	14.3	42.5	26.0	83.9	0.0	49.0	
2.	<b>Methicillin (10 µg)</b>	0.0	10.0	4.2	0.0	35.0	14.6	100.0	55.0	81.3	
	<b>Ampicillin (10 µg)</b>	0.0	0.0	0.0	3.6	7.5	5.2	96.4	92.5	94.8	
	<b>Ticarcillin (75 µg)</b>	3.6	32.5	15.6	5.4	25.0	13.5	91.1	42.5	70.8	
	<b>Penicillin G (10 units)</b>	0.0	5.0	2.1	1.8	15.0	7.3	98.2	80.0	90.6	
3.	<b>Erythromycin (15 µg)</b>	1.8	12.5	6.3	51.8	70.0	59.4	46.4	17.5	34.4	
4.	<b>Clindamycin (2 µg)</b>	67.9	82.5	74.0	0.0	15.0	14.6	17.9	2.5	11.5	
5.	<b>Amikacin (10 µg)</b>	53.6	77.5	63.5	0.0	20	22.9	21.4	2.5	13.5	
	<b>Streptomycin (10 µg)</b>	83.9	90.0	86.5	10.7	5.0	8.3	5.4	5.0	5.2	
	<b>Gentamicin (30 µg)</b>	10.7	75.0	90.0	0.0	2.5	2.5	0.0	7.5	7.5	
6.	<b>Chloramphenicol (5 µg)</b>	3.5	85.0	95.0	0.0	0.0	0.0	0.0	5.0	5.0	
7.	<b>Cefoxitin (30 µg)</b>	85.7	87.5	86.5	5.4	5.0	5.2	8.9	7.5	8.3	
	<b>Cefixime (10 µg)</b>	3.6	10.0	6.3	1.8	60.0	26.0	94.6	30.0	67.7	
	<b>Cefazolin (30 µg)</b>	53.5	10.0	60.7	16.1	0.0	16.1	23.2	0.0	23.2	
8.	<b>Ciprofloxacin (30 µg)</b>	12.5	52.5	29.2	8.9	32.5	18.8	78.6	15.0	52.1	
9.	<b>Tetracycline (10 µg)</b>	71.4	17.5	83.9	3.6	0.0	3.6	12.5	0.0	12.5	

H - Human *S. aureus* isolates, A - Animal *S. aureus* isolates

In this study, out of 69 *S. aureus* isolates, 28 (40.5%) isolates showed amplification of the *mecA* gene (Fig. 3). These included 8 (27.5%) isolates from human pyogenic cases and 20 (50%) from different animal species. This study in concurrence with another study of Nepal reported 26.14% of the prevalence of MRSA was procured from various human samples of a tertiary-care hospital [25]. In the case of humans, out of 29 *S. aureus* isolates, only 8 showed antimicrobial resistance *mecA* gene amplification, however, out of 40 animal *S. aureus* isolates, 18 showed *mecA* gene amplification while all human and animal isolates were resistant to methicillin antibiotic. In another study out of 40 animal isolates, 23 isolates revealed amplification of the *mecA* gene [26].

The detection of coagulase can be made by slide/tube coagulase test, however, both human and sheep plasmas had very low specificity (11% and 7%) so may lead to misleading results [4]. Hence, it cannot be used as a single test to confirm *S. aureus*. Similarly in the present study, out of 69 isolates, 33 (47.8%) isolates revealed a positive slide coagulase test (Fig. 4). These were included 24 (34.7%) human and 9 (13%) animal isolates. These isolates were also confirmed for the presence of pathogenicity-related *coa* genes on genomic DNA. Total 33 (47.8%) isolates showed amplicons of variable size and number for *coa* genes amplicons irrespective of positive or negative slide coagulase test results. The *coa* genes positive isolates included 24 (34.7%) isolates from human pyogenic cases and 9 (13%) from animal isolates. Size of amplicons showed polymorphism with amplification of more than one amplicons product ranging from 250–1100 bp. Likewise in one study, two amplicons of size 680 bp and 750 bp were obtained from 21 (out of 30) clinical isolates of *S. aureus* [27]. In another study, “out of 58 MRSA isolates, 15 *coa* types were classified, and the amplification products showed multiple bands (1, 2, 3, 4, 5, and 8 bands)” [28]. The presence and absence of *coa* genes were not related to the presence of methicillin-resistant *mecA* genes or the drug resistance pattern of isolates. The *coa* gene-positive isolates comprised of both *mecA* positive and negative isolates. Consequently, the coagulase enzyme production was not related to the presence of methicillin resistance. The resistance is acquired through a different mechanism and there are reports that the methicillin-sensitive strains of *S. aureus* acquired the SCCmec element probably from coagulase-negative staphylococcal strains and became methicillin-resistant [3].

The RAPD-AP4 PCR revealed that 6 out of 8 human MRSA isolates had amplification of 2–5 bands with five different band patterns (Fig. 5). A previous study of Polyclinic hospital in Italy, also suggested the five distinct amplicons were found in human MRSA isolates [29]. In the case of animals, out of 20 MRSA, 18 isolates revealed amplification of 2–5 bands with six different band patterns (Fig. 5). Out of these six patterns 2 were similar to patterns of human isolates suggesting a common phylogenetic profile of MRSA isolates in humans and animals. In contrast to this study, one study reported RAPD patterns of six animal isolates were found to be similar to some human isolates [30]. The common patterns obtained in humans and animals also suggest that MRSA might have transferred from animal to human. Several studies have suggested that “the transfer of *S. aureus* between humans and cattle is possible” [31–33].

## Conclusion

In contrast to human samples, animal samples had a higher prevalence of *S. aureus*. Animal isolates were more susceptible to the antibiotics used in the study than human isolates. Resistance was higher in human isolates than in animal isolates, regardless of the drug type. Antibiotics often used to treat infections caused by *S. aureus* showed more resistance, whereas antibiotics that were no longer in use showed more sensitivity. As a result, other antibiotics must be used instead of widely prescribed antibiotics to treat *S. aureus* infections. It is also concluded that the presence and absence of *coa* genes were not related to the presence of methicillin-resistant *mecA* genes or the drug resistance pattern of isolates. In RAPD-PCR typing two-band patterns were similar in human and animal MRSA isolates, suggesting a common phylogenetic profile or transfer from animal to human or vice-versa.

## Declarations

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**Ethical Declaration** No ethical clearance was required as samples were collected from the clinical cases of both humans and animals.

**Conflicts of interest** The authors declare that they have no conflict of interest.

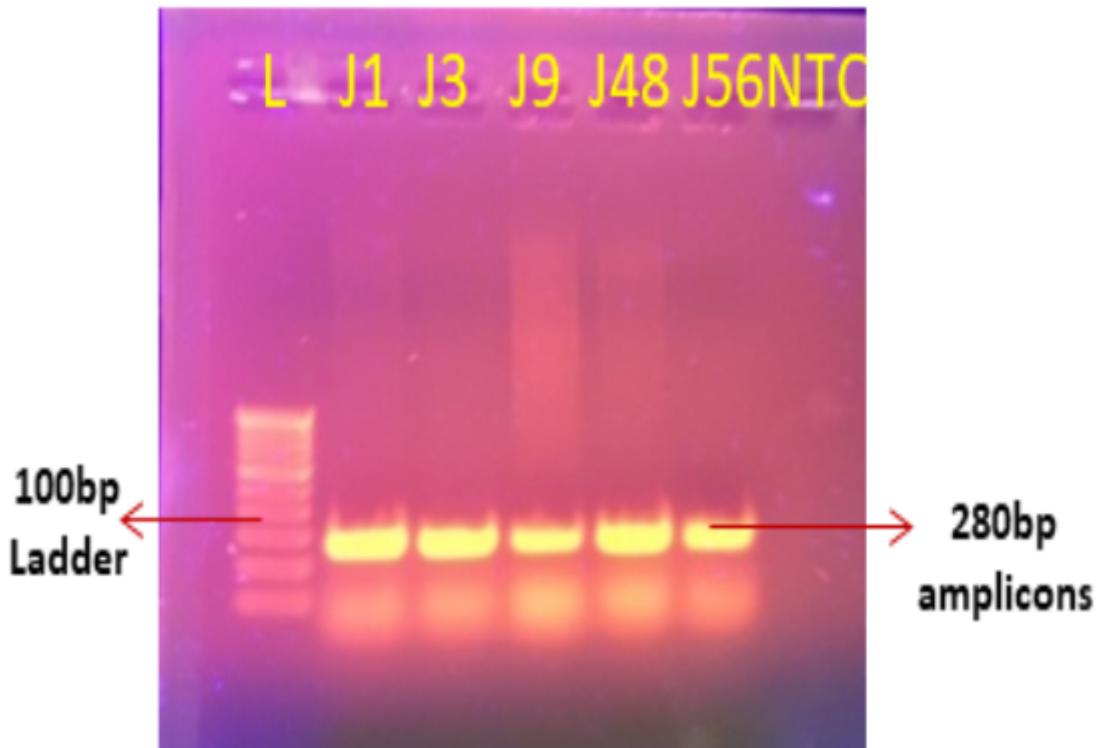
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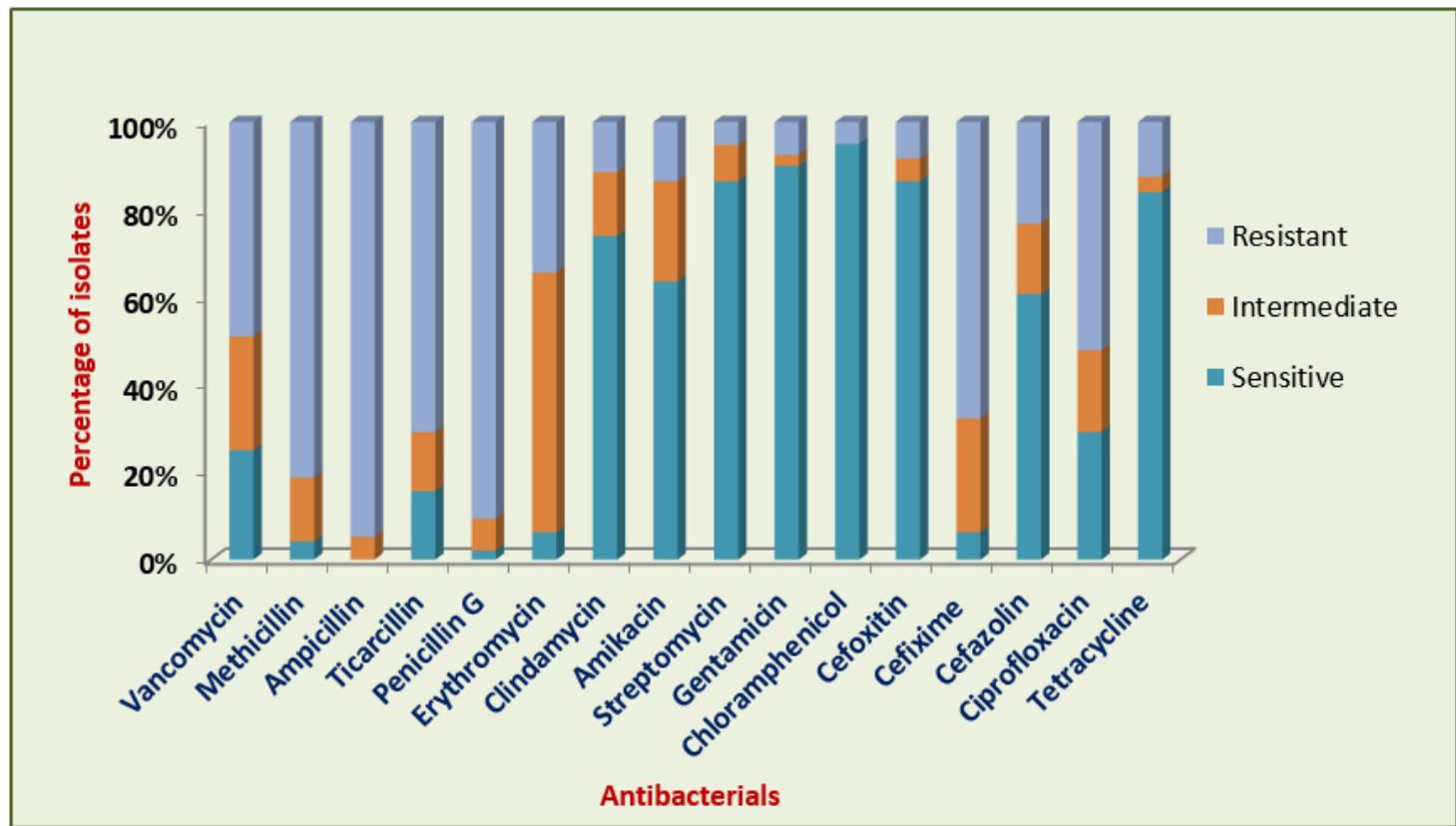
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## Figures



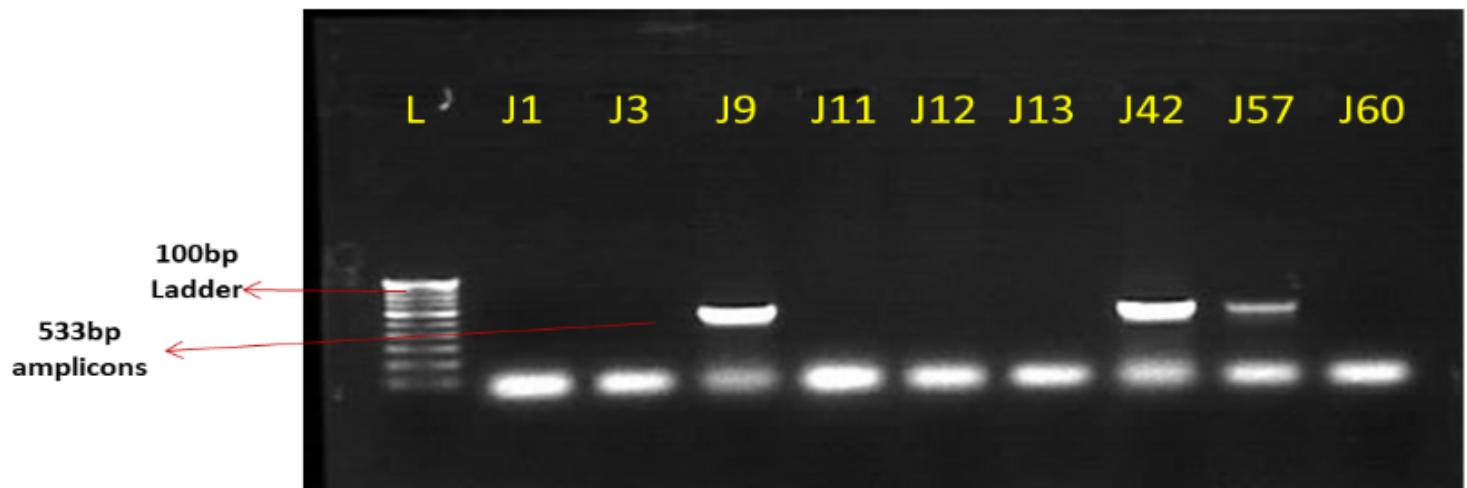
**Figure 1**

Amplification of the *S. aureus*-specific nuc gene yielded a 280-bp product



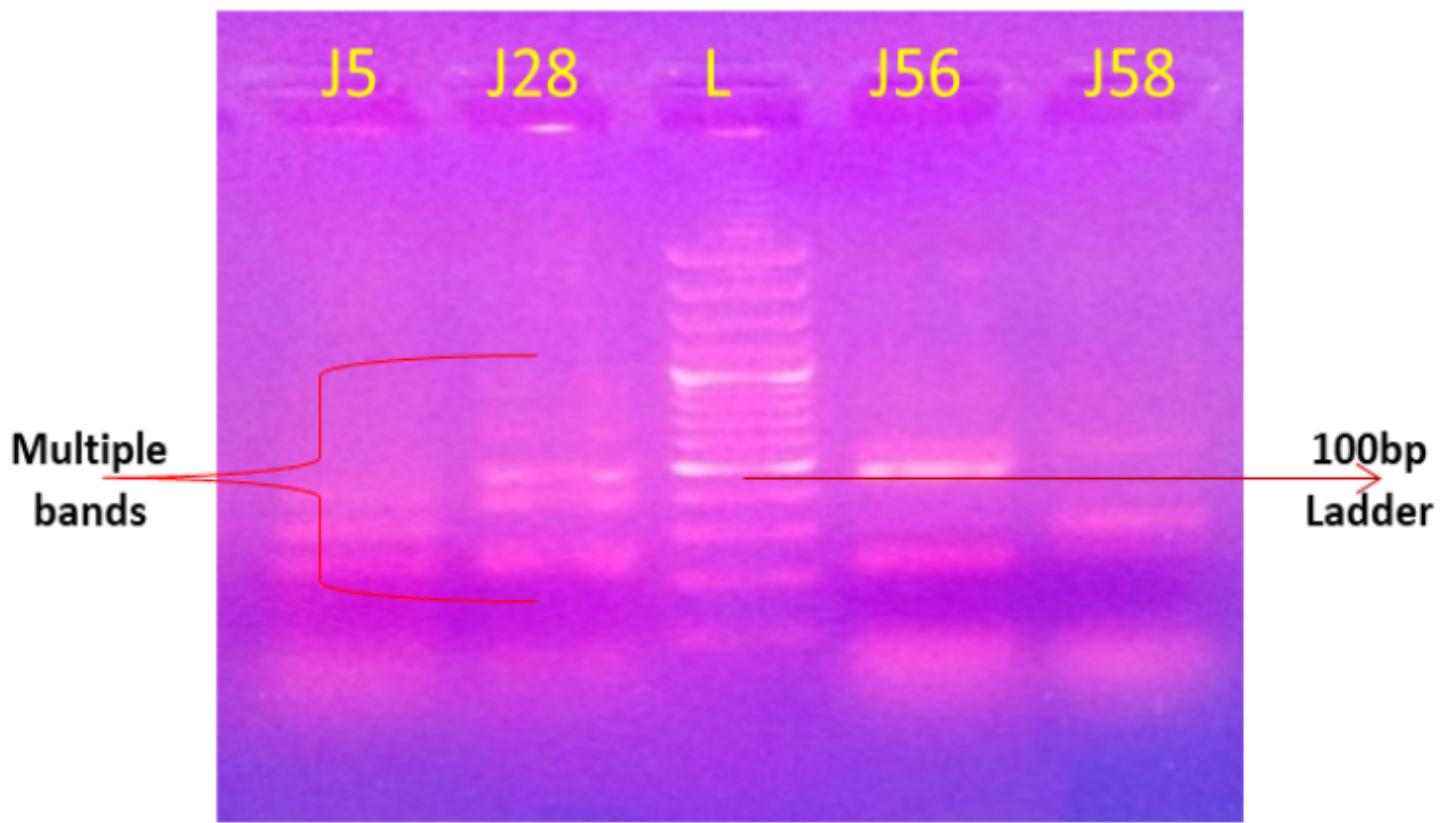
**Figure 2**

Overall drug sensitivity of *S.aureus* isolates. The sensitivity was highest against chloramphenicol (95%) and resistance was highest against ampicillin (94.8%)



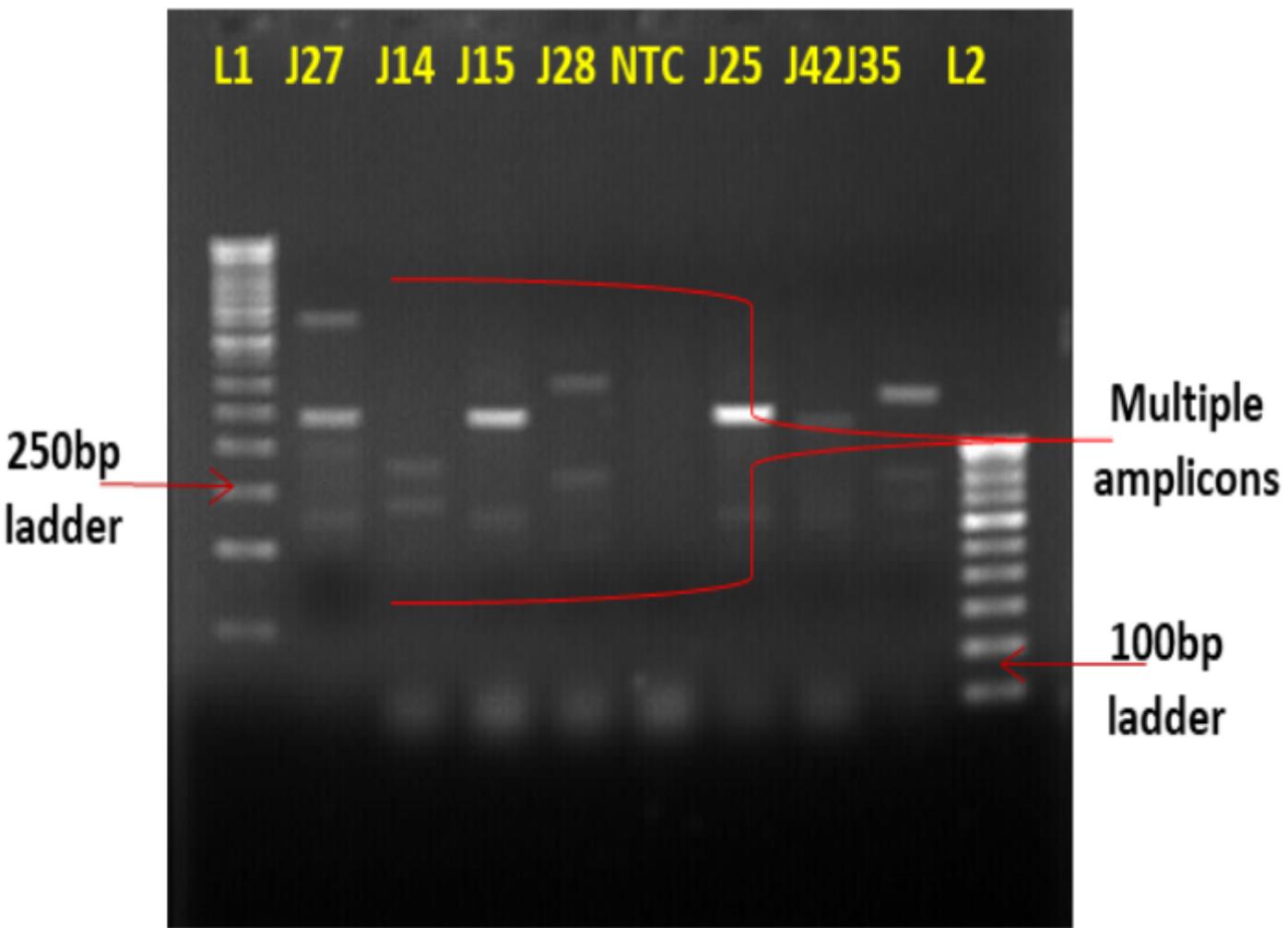
**Figure 3**

Amplification of methicillin-resistant *mecA* genes from *S. aureus* isolates resulted in a 533 bp product



**Figure 4**

Amplification of the *coa* gene yielded multiple amplicons with sizes ranging from 250 to 450, 800, and 1100 bp



**Figure 5**

Typing of *S. aureus* isolates using RAPD-AP4 PCR revealed 2-5 amplicons