*Pure Appl. Biol., 10(4):969-977, December, 2021*
*http://dx.doi.org/10.19045/bspab.2021.100101*

**Research Article**

# Screening of methicillin resistant *Staphylococcus aureus* from dogs and cats in Hyderabad Pakistan

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**Citation**

Imtiaz Ali Ujjan , Jam Kashif Zaman, Nazeer Hussain Kalhoro, Amjad Hussain Mirani, Abdul Latif Bhutto, Ahmed Nawaz Khosa, Banzir Sahito, Chandni Wajid, Asma Babar, Zain ul Aabdin, Muhammad Azhar Memon and Waseem Ali Vistro. Screening of methicillin resistant *Staphylococcus aureus* from dogs and cats in Hyderabad Pakistan. Pure and Applied Biology. Vol. 10, Issue 4, pp969-977.
http://dx.doi.org/10.19045/bspab.2021.100101

Received: 19/10/2020 Revised: 18/12/2020 Accepted: 28/12/2020 Online First: 02/01/2021

**Abstract**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a "superberg", an important cause of human nosocomial and as a universal community-acquired infection cause resistant to β--*lactam* antibiotics. Till to date no detailed and authentic report on MRSA in nasal swabs of diseased dogs and cats reported. This study was planned to check the presence of MRSA in nasal swabs of dogs and cats. Total 100 samples were collected, and 37 samples were positive with *S. aureus* in both diseased dogs and cats which were confirmed by their colony characteristic, morphology, gram stain reaction and biochemical properties. These isolates were subjected to minimum inhibitory concentration (MIC) through Micro broth dilution method by using methicillin in 96 well plates. Among them, 8/37 (21.62%) were found methicillin resistant. In diseased dog, 5/21 (23.80%) and cat 3/16 (18.75%) isolated were found moderately resistant to methicillin as they grew at 32µg/µl concentration of methicillin. *mec-A* gene in methicillin resistant samples were detected through polymerase chain reaction (PCR). This resistance was due to phenotypically and moderate level of the resistance against methicillin with absence of *mec-A* gene shows that the examined pets were not the reservoir of *mec-A* gene. However, more studies are recommended to further confirm that, pets are reservoir of mec-A gene or not or this may be due to involvement of sharing mechanism of resistance to penicillin group. So far, this is first study on MRSA in diseased dog and cats, which revealed that, there is no MRSA in this area of Pakistan.

**Keywords:** Cat; Dog; Methicillin resistant; Screening; *Staphylococcus aureus*

## Introduction

Antibiotics are generally used to treat for variety of bacterial infection in veterinary and human practice and these antibiotics produced their action by either stopping the bacterial growth or directly killing microbes. The broad spectrum antibiotics have killed all type of bacteria including gram positive and gram negative whereas narrow spectrum antibiotics are effected on

either gram positive or gram negative bacteria [1].

Antibiotic agent of β-*lactam* antibiotics contains a β-*lactam* ring in their molecular structures. The β-*lactam* resistant penicillin consist of methicillin, oxacillin and cloxacillin were used in first line of treatment as penicillin-resistant *S. aureus* and the β-*lactam* antibiotics are frequently used for *Staphylococcal* infections as a result antibiotic acquired resistance has developed [2]. The different derivates of Penicillin including Methicillin has been used for the infection occurred through *S. aureus* [3]. The most penicillin derivatives are resistant to 90% of *S. aureus* strains However, many species of *S. aureus* have developed resistance recognized as MRSA [4]. The first antibiotic was Methicillin which introduced in human medicine used as methicillin resistant *Staphylococcal* infection in 1950s and more than 80% of *S. aureus* have produced Penicillinase enzyme, caused resistant to *β-lactam* antibiotics which commonly used to treat *Staphylococcal* infections in dogs and cats [5].

Antimicrobial resistance is a major threat in veterinary and human medicine and the most important antimicrobial resistance has developed due to use and misuse of antibiotics in veterinary field and human medicine [6]. MRSA known as an important cause of human nosocomial and as a universal community-acquired infection cause resistant to β--*lactam* antibiotics was facilitated by the *mec-A* gene, present in bacterial cell wall which encodes to penicillin-binding protein (*PBP2a*) for reducing the affinity of β--*lactam* antibiotics [7]. MRSA kills more people than the acquired immunodeficiency syndrome (AIDS) because of it known as the "superbug" [8]. The mobile genetic element (*mec-A)* gene is fragment of a 21kb to 60-kb *Staphylococcal chromosome cassette mec* (SCC*mec*). The *mec-A* gene is characterized by the combination of *ccr* and *mec* which encode resistance to β-lactam antibiotics [9]. Pets contain emerging and prevalent pathogenic species of *Staphylococcus aureus* [10]. Dogs and cats can be a potential reservoir of MRSA infection for human and contact with large part of human population so there is the potential for transfer of MRSA or *mec-A* gene among human and cats and dogs. The risk factor for MRSA infections were intravenous catheter and drugs used in veterinary medicine in cats and dogs and MRSA infection is becoming a public health concern because dogs and cats are in close connection with their owners, risk them for transmission of highly pathogenic strain of MRSA [11].

Considering this screening present study was designed to check the presence of highly pathogenic and zoonotic organism in pets (cats and dogs) from in order to analyses the possible pathogenic thread of spread of MRSA from cats and dogs to human. Results of this study will provide information to the veterinary field as well as human clinicians about the prevalence of Methicillin resistant strains of *S. aureus* in pets.

## Materials and Methods

### Study area

All samples were collected from nasal swabs of diseased dogs and cats from the Hyderabad and its vicinity. The clinically ill dogs and cats were selected in this study and samples were collected from different Pets clinics of Hyderabad and its vicinity.

### Collection of sample

A total of 100 samples were collected, 50 samples were collected from diseased dogs and 50 samples were collected from the diseased cats and brought to the veterinary clinics for collection of nasal swabs. Samples were collected in Tran swab tube containing Amies medium.

All samples were brought to the bacteriology section of Central Veterinary Diagnostic Laboratory (CVDL) Tandojam in a portable cooler containing an ice pack and then swabs were refrigerated at 4 ˚C until being processed within 48 hr of collection for isolation and identification of *S. aureus* by chain of laboratory technique

including colony characteristics, gram staining, and growth on blood agar. The isolates of *S. aureus* were cultured on Tryptic Soy Broth (TSB) at 37 °C for further experimental use.

**Antimicrobial susceptibility test**

The *S. aureus* isolates were determined through Minimum inhibitory concentration test by using (Micro Broth 2-fold dilution method) on Muller Hinton (MH) agar (Oxide, UK) and 1:1000 dilution was prepared for MIC test for that 6µl of bacterial culture of *S. aureus* in Nutrient broth were added into 6ml of Muller Hinton broth.

96 well plates of micro titer tray were used for MIC test and a concentration 1280µl/ml of Methicillin (Sigma, USA) was used for minimum inhibitory concentration against *S. aureus.*

*S. aureus* isolates in nutrient broth culture were added in each well of micro titer tray. In first well 180µl of culture were added and in remaining all wells 100µl of bacterial culture were added than 20µl of Methicillin (Sigma, USA) were added in first well and mixed by push up of Eppendorf tube then 100µl of culture from first well were introduced into next well and repeat till last well. The final sub cultures of bacterial isolates were discarding. Then MIC plates were placed in incubator at 37 °C for 24 hours. Results of MIC test were obtained by performing MIC test three times.

**Molecular examination of resistant *S. aureus* Isolates**

Molecular examination of resistant *S. aureus* isolates was done in molecular laboratory of Sindh Poultry Vaccine Centre (SPVC) Karachi.

**DNA extraction**

The DNA was extracted by using commercial kit (Geneaid GEE 300, GEE O3K) as per manufacturers' instruction. Following Specific primers were used for detection of *mec-A* gene from *Staphylococcus aureus*.

F-ATGAAATGACTGAACGTCCGATT A,

R-CAAATTCCA CATTGTTTCGGTCT AA.

The PCR reaction mixture 50µl, which include following reagents.

1. PCR master mixture: 25µl
2. RNase free water: 17µl
3. Forward primer: 2µl
4. Reverse primer: 2µl
5. DNA/sample: 4µl

**PCR protocol**

After DNA extraction, PCR was performed in Applied Bio system 2720 thermal cycler (Eppendorf). Total of 50µlof PCR reaction mixture was prepared for each supernatant DNA for that 25µl PCR master mixture was added into Eppendorf tube then 17µl of RNase free water was added into same Eppendorf tube then added 2µl of forward primer followed by 2µl of reverse prime were mixed with 4µl of supernatant DNA Gently, mixed by vortex and then kept tubes in thermal cycler. A thermal cycler was used for amplification of DNA then cycling condition of PCR was set such as initial denaturation at 95 °C for 05 minutes, followed by 35 cycles of denaturation at 95 °C for 01minute annealing at 55 °C for 45 seconds, extension at 72 °C for 45 seconds and final cycling of amplification was done at 72 °C for 5minutes. The amplified product was analyzed through gel electrophoresis processes by using 3µl ethidium bromide and 2% agarose gel and DNA band was visualized by ultraviolet light.

**Results**

**Percentage prevalence of *S. aureus* in diseased dogs and cats**

To isolates the *S. aureus,* one hundred samples were collected from nasal swabs of diseased dogs and cats. All samples were cultured on different media to isolates pure colony of *S. aureus* then pure cultures of organism were confirmed through grams staining. Further pure cultures were confirmed by the biochemical properties. From all samples only 37 were found positive with *S. aureus* colony in both diseased dogs and cats (Table 1).

**Percentage prevalence of *S. aureus* isolated from nasal swabs of diseased dogs and cats.**

A total of 50/50 samples were collected from nasal swabs of diseased dogs and cats, only 21 and 16 samples were found positive with *S. aureus* isolates shown in (Table 2).

**Colony characteristic of *Staphylococcus aureus* isolated from diseased Dogs and Cats**

The colonies of *Staphylococcus aureus* produced yellow or white colony and typical characteristic of *β- hemolysis* of red blood cell was recorded on blood agar.

**Morphological characteristic of *S. aureus* isolated from diseased dogs and cats**

*S. aureus* was confirmed through grams staining. The positive samples of *S. aureus* were characterized as gram positive, spherical in shape, and arranged in pairs like cocci.

**Biochemical characteristic of *S. aureus* isolates**

The *S. aureus* isolates was recorded positive for coagulase, urease, methyl red and Voges- Proskauer and *S. aureus* was found negative for indole and oxidase test. *S. aureus* isolates on Triple Sugar Iron agar (TSI) medium were showed A/A properties that meant this *S. aureus* isolates showing the property of acidic slant and acidic butt that indicate that the sugar present in the media was fermented (Table 3).

**Minimum inhibitory concentration of *S. aureus* isolates**

All 21 and 16 positive isolates of *S. aureus* were selected for MIC test. Only 05 and 03 samples were shown resistance at different concentration of Methicillin in diseased dogs and cat shown in (Table 4).

**Table 1. Percentage prevalence of S. aureus isolated from nasal swabs of diseased dogs and cats**

| Total number of samples | No. of positive samples | Percentage |
|---|---|---|
| 100 | 37 | 37% |

**Table 2. Percentage prevalence of S. aureus in diseased dogs and cats**

| Animal | Total number of samples | No. of positive samples | Percentage |
|---|---|---|---|
| Dog | 50 | 21 | 42% |
| Cat | 50 | 16 | 32% |

**Table 3. Biochemical characteristic of *S. aureus* isolated from nasal swabs of diseased dogs and cats**

| *S.aureus* | Coag | Ure | M.R | V.P | Ind | Oxid | Cit | TSI | Cat |
|---|---|---|---|---|---|---|---|---|---|
| | +ve | + ve | +ve | +ve | -ve | -ve | +ve | A/A | +ve |

**Table 4. Number and percentage of Methicillin Resistant *S. aureus* isolates from diseased dogs and cats**

| Animal | Positive | Methicillin resistant | Percentage |
|---|---|---|---|
| Dog | 21 | 05 | 23.80% |
| Cat | 16 | 03 | 18.75% |

**Minimum inhibitory concentration of *S. aureus* isolates against Methicillin from nasal swabs of diseased dogs**

Total of five samples were shown their resistant at the concentration of 16µg/ml against methicillin in diseased dogs and two samples were shown their resistant at 64µg/ml concentration of methicillin while other samples no resistant at any concentration of methicillin. The positive sign indicates the bacterial resistant against methicillin and negative sign shows no

growth of *S. aureus* against methicillin (Table 5).

**Table 5. Minimum inhibitory concentration of *S. aureus* isolates against methicillin from nasal swabs of diseased dogs**

| Sample No. | 128 µg/ml | 64 µg/ml | 32 µg/ml | 16 µg/ml | 8 µg/ml | 4 µg/ml | 2 µg/ml | 1 µg/ml | 0.5 µg/ml |
|---|---|---|---|---|---|---|---|---|---|
| **1** | - | + | + | + | + | + | + | + | + |
| **2** | - | + | + | + | + | + | + | + | + |
| **3** | - | + | + | + | + | + | + | + | + |
| **4** | - | - | - | + | + | + | + | + | + |
| **5** | - | - | - | + | + | + | + | + | + |
| **6** | - | - | - | - | - | - | - | - | - |
| **7** | - | - | - | - | - | - | - | - | - |
| **8** | - | - | - | - | - | - | - | - | - |
| **9** | - | - | - | - | - | - | - | - | - |
| **10** | - | - | - | - | - | - | - | - | - |
| **11** | - | - | - | - | - | - | - | - | - |
| **12** | - | - | - | _ | _ | _ | _ | _ | _ |
| **13** | - | - | - | - | - | - | - | - | - |
| **14** | - | - | - | - | - | - | - | - | - |
| **15** | - | - | - | - | - | - | - | - | - |
| **16** | - | - | - | - | - | - | - | - | - |
| **17** | - | - | - | - | - | - | - | - | - |
| **18** | - | - | - | - | - | - | - | - | - |
| **19** | - | - | - | - | - | - | - | - | - |
| **20** | - | - | - | - | - | - | - | - | - |
| **21** | - | - | - | - | - | - | - | - | - |

**Minimum inhibitory concentration of *S. aureus* isolates against methicillin from nasal swabs of diseased cats**

Total of three samples were shown their resistant at the concentration of 16µg/ml in diseased cats while remaining shown no resistant at any concentration of methicillin. The positive sign indicates the bacterial resistant against methicillin and negative sign shows no growth of *S. aureus* against methicillin (Table 6).

**Molecular characterization of *S. aureus* isolated from diseased dogs and cats**

The result of PCR assay showed that all the five phenotypically methicillin resistant samples of dogs did not carry *mec-A* gene and all three phenotypically methicillin resistant samples of cats were not carry *mec-A* gene hence confirmed that there was no MRSA in nasal swabs of diseased dogs and cats. The electrophoresis processes showed absence of *mec-A* gene in any samples. The PCR amplified picture shows the marker of 2000bp.

**Discussion**

This study was designed to detect the Methicillin resistant *S. aureus* from nasal swabs of diseased dogs and cats and to confirm that *S. aureus* carrying genetic material responsible for their resistant to methicillin or not. During this study, 37 samples were found positive with *S. aureus* from100 nasal samples in dogs and cats (Table 1). Prevalence was observed comparatively higher in diseased dogs than diseased cats for *S. aureus* at Hyderabad. Similarly, Habibullah reported that among the 93 samples, 38 (40.86%) was found positive for *S. aureus* in pet dogs and cats at

Dhaka city. This prevalence of *S. aureus* result is in line with present study.

During present study, no positive strain of Methicillin resistant *S. aureus* from nasal swabs of diseased dogs and cats but, 8% of pet samples were infected with MRSA and in addition 5 (10%) samples of dog were positive with MRSA and 3 (6%) cat samples were positive with the MRSA which is in disagreement with our study as we found no positive samples for MRSA.

The bacterial isolates of this study were confirmed by their typical growth pattern and colony characteristics, which include beta hemolytic activity on blood agar (Fig. 1) [12] mentioned that *S. aureus* is found hemolytic on blood agar, whereas *S. epidermis* is no hemolytic, thus it confirms that bacterial colonies observed during this study were of *S. aureus*, which has ability to produce hemolysin enzyme that lyses the red blood cells.

Morphologically *S. aureus* was found gram positive *cocci* often arranged in single, double or grapes like (Fig. 2). Habib *et al.* [13] defined morphological properties as gram positive *cocci* arranged in single, pairs or in chain and *S.aureus* were produced golden yellow and shiny colonies on nutrient agar. The bacterial isolates of this study is confirmed by the findings of above studies.

**Table 6. Minimum inhibitory concentration of *S. aureus* isolates against methicillin from nasal swabs of diseased cats**

| Samples No. | 128 µg/ml | 64 µg/ml | 32 µg/ml | 16 µg/ml | 8 µg/ml | 4 µg/ml | 2 µg/ml | 1 µg/ml | 0.5 µg/ml |
|---|---|---|---|---|---|---|---|---|---|
| **1** | _ | - | + | + | + | + | + | + | + |
| **2** | _ | - | + | + | + | + | + | + | + |
| **3** | _ | - | + | + | + | + | + | + | + |
| **4** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **5** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **6** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **7** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **8** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **9** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **10** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **11** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **12** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **13** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **14** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **15** | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| **16** | _ | _ | _ | _ | _ | _ | _ | _ | _ |

The *Staphylococcus aureus* were confirmed by its biochemical properties, which included catalase, methyl red, urease, gelatin liquification, coagulase, oxidase positive, indole oxidase negative (Table 3). Rusenova & Rusenov [14] reported that the biochemical properties of *S. aureus* as catalase coagulase urease positive, indole and oxidase negative. The above biochemical result is in agreement with present study.

The bacterial susceptibility to methicillin was confirmed through minimum inhibitory concentration (MIC) through using micro broth 2-fold dilution method. A concentration of 1280µg/ml of methicillin was used. The 20µg/ml concentration of methicillin was break point for MIC were observed in this study. Out of 37 isolates of *S. aureus* only 08 isolates were shown resistant to methicillin phenotypically (Table 4) 5(23.80%) isolates were found

moderate level of resistance to methicillin in diseased dogs. Out of 16, 3(18.75%) isolates were found low level of resistant to methicillin in diseased cats (Table 5) [15]. stated that, Methicillin Resistant *S. aureus* was treated with different antibiotic, but Methicillin was the most effective antibiotic against all MRSA isolates they mentioned that some samples showed resistance to the drug even at low concentration, which indicated that bacteria are methicillin resistance.

The PCR results of the present study suggest that all methicillin resistant samples did not carry *mec-A* gene (Fig. 3). The DNA fragment of 533bp was amplified and confirmed as *mec-A* in 25% of human isolates by [16]. The findings of this study are in disagreement with our study, as we found that no *mec-A* gene, But in this study no band was seen at any stage of gel electrophoresis methicillin resistant isolates were only found phenotypically resistant to methicillin and the level of resistant was moderate, this phenotypical resistance may be due to the Penicillin binding protein (PBP), *Beta-lactmase* enzyme and methicillinase enzyme production and different strain of *Staphylococcus aureus*. Absence of the *mec*-A gene in this study can be attributed to many causes subsequently there are other mechanisms that are non-*mec* dependent mechanism that donate individually or in combination with different antibiotic resistance in *Staphylococci* isolates [17].

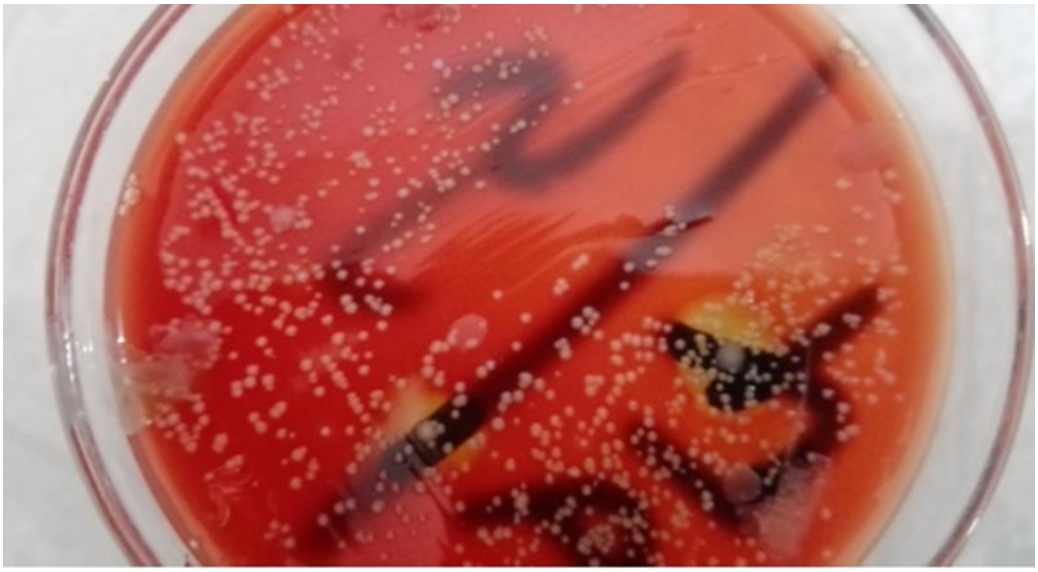

**Figure 1. Colony characteristic of *S. aureus* isolates from nasal swabs of diseased dogs and cats**

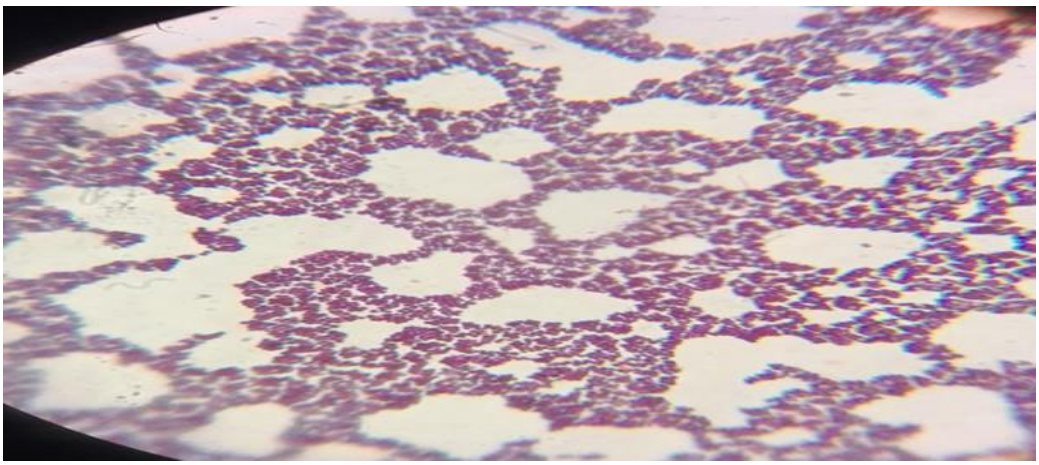

**Figure 2. Add Caption biochemical characteristic of *S. aureus* isolates**

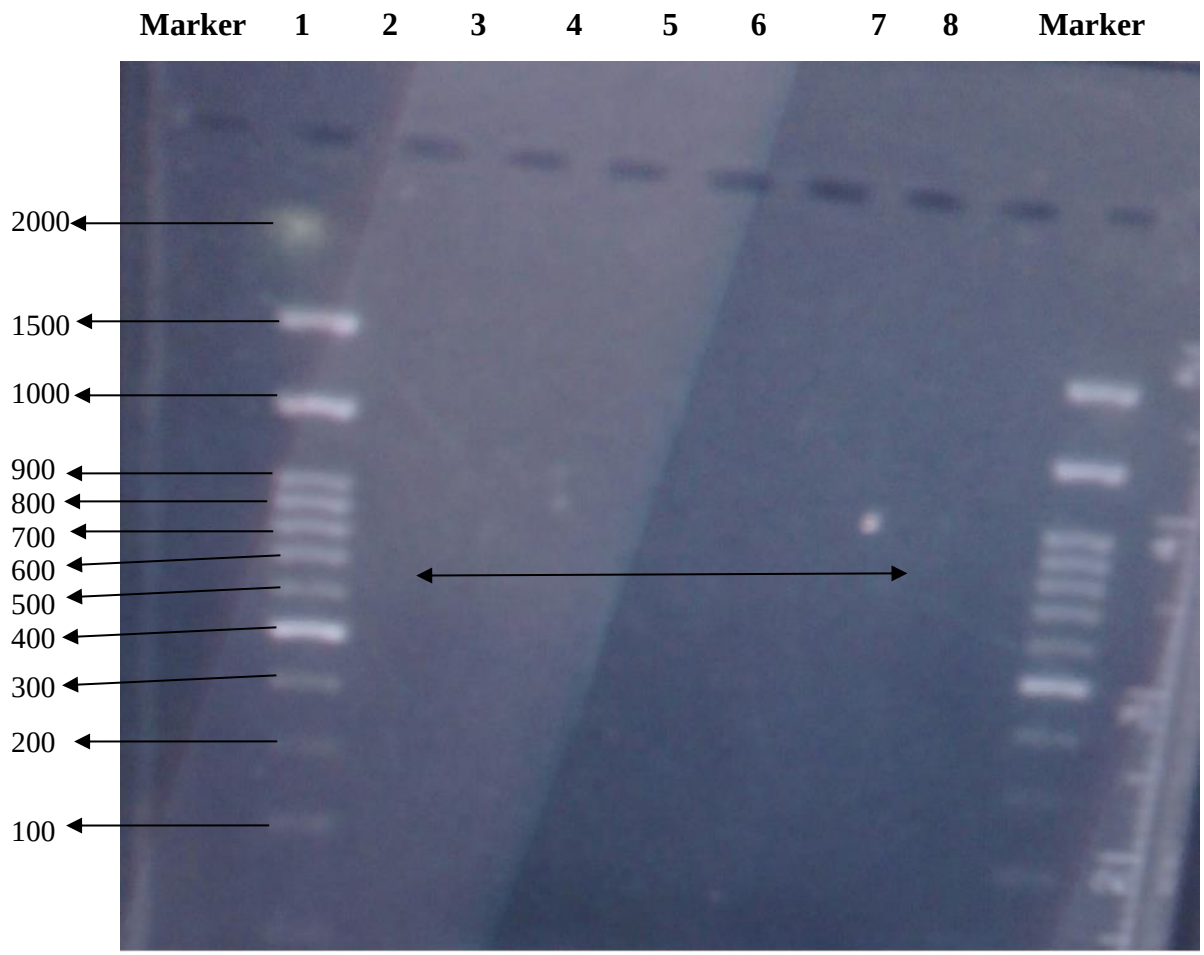


**Figure 3. This gel electrophoresis result shown that no any amplified product was found at 530bp)**

## Conclusion

On the basis of present finding it is concluded that *S. aureus* is prevalent in nasal swabs of diseased dogs and cats. The prevalence Percentage is higher in diseased dogs as compared to diseased cats and *S. aureus* showed moderate resistant to methicillin when MIC test done but this resistant were recorded more in dogs as compared to cats. This resistance was due to phenotypically and moderate level of the resistance against methicillin with absence of *mec-A* gene shows that the examined pets are not the reservoir of *mec-A* gene. However, more studies at the larger scale at (country level) are recommended to further confirm that the pets are reservoir for *mec-A* gene or not.

## Authors' contributions

Conceived and designed the experiments: IA Ujjan, JK Zaman & NH Kalhoro, Performed the experiments: IA Ujjan, AH Merani & AL Bhutto, Analyzed the data: IA Ujjan, AN Khosa, B Sahito & WA Vistro, Contributed reagents/ materials/ analysis tools: IA Ujjan, C Wajid & MA Memon, Wrote the paper: IA Ujjan.

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