Bacteriological and Molecular Detection of Methicillin-resistant Staphylococcus Aureus Isolated From Cigarette and Hookah Smokers in Khartoum State

Jara Osman Ali Osman
Sudan University of Science and Technology
Esraa abdulraman Abdallah Bakri
Sudan University of Science and Technology
Ahmed Ibahim Hashim
Sudan University of Science and Technology
Ahmed Bakheet Abd Alla (ahmed.hassanab@gmail.com)
Sudan University of Science and Technology _ College of Medical Laboratory Science
https://orcid.org/0000-0002-5463-9892
Hisham N Altayb
King Abdulaziz University
Yousif Fadlallah Hamedenil
Sudan University of Science and Technology college of medical laboatory science

Research note

Keywords: Antibiotic, Staphylococcus aureus, Bacteriology

Posted Date: July 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38865/v1

Abstract

Objective

Staphylococcus aureus is an extra-ordinarily versatile pathogen that causes a wide variety of infections in humans and animals, capable of causing superficial lesions and systemic infections. The aim of this study was to detect mecA gene in smokers of cigarette and hookah.

Results

Forty-one isolates (41%) were identified as *S. aureus* by traditional culture methods. non-smokers, nasal carriers of *S. aureus* were 16 (39%) while 25 (60%) among smokers (P = 0.103). Of the overall 25 smoker nasal carriers, 18 were hookah smokers and 7 were cigarettes smokers (P = 0.004). MRSA was 1 (2.4%) in non-smokers and 3 (7.2%) in smokers, 2 in cigarettes and 1 in hookah (P = 0.009). Only 38 isolates were confirmed as *S. aureus* by PCR (mecA gene). In non-smokers group mecA were 6 (15.8%) positive and 8 (21%) negative, while in smokers were 16 (42.3%) positive and 8 (20.9%) negative isolates (P = 0.187). From hookah 12 mecA positive and 4 from cigarette (P = 0.647). The correlation of MRSA and using of antibiotic during last 6 months indicates significant (P = 0.031).

Introduction

Cigarette smoking (CS) is the leading preventable cause of death, disease, and disability worldwide. In 2010, nearly 6 million worldwide deaths were due to cigarette smoking and second-hand exposure. Forty percent of children worldwide regularly exposed to second- hand cigarette smoke [1–3].

Both direct and second-hand cigarette smoke exposures increase the risk and severity of developing respiratory tract and other invasive infections [4]. Invasive pneumococcal disease is 2–4 folds more common in cigarette smokers, in addition to influenza and tuberculosis infections [2, 5]. CS exposure can impair epithelial innate immune responses to microbial products, perhaps setting the stage for overgrowth and invasion [1, 4]. Adverse health effects linked to cigarette smoke, including carcinogenesis [4], and chronic pulmonary disease, are commonly believed to arise from the direct action of tobacco smoke components on human cells [2]. CS contains a variety of bioactive substances, including oxidizing, genotoxic, and immunomodulatory factors [7,8]. Exposure to cigarette smoke raises the risk of many infectious diseases [9,10]. Kulkarni and his colleagues demonstrated that CS can impair epithelial innate immune reactions to microbial products [11]. Perhaps setting the stage for overcrowding and invasion [9]. Invasive pneumococcal disease is 2–4 times more common among cigarette smokers, in addition to influenza and tuberculosis infections [12]. Moreover, direct and second-hand cigarette smoke exposures change the normal composition of the nasopharyngeal microflora, opening the door to opportunistic pathogens such as *S. aureus*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* [5,12].

Tobacco in hookahs is exposed to high heat from burning carbon dioxide, and smoke is at least as toxic as cigarette smoke, this could lead to absorption of more toxic substances than those absorbed by cigarette smokers [13].

The amount of smoke inhaled during a typical hookah session is about 90,000 millilitres (ml), compared with 500–600 ml inhaled when smoking a cigarette [14].

Hookah smokers can be at risk for some of the same problems as cigarette smokers [5]. These include oral cancer, prostate cancer, stomach cancer, oesophageal cancer, diminished lung function and decreased fertility [15].

S. aureus is Gram-positive cocci that causes a broad range of infections, including pneumonia, bacteraemia, and endocarditis [5,15]. Methicillin Resistant *S. aureus* (MRSA) is resistant to oxacillin, methicillin and all beta lactam agent [16]. Smokers have high rates of MRSA colonization than non-smokers, thus increasing risk of serious and difficult to treat infections [17]. The primary mechanism for this resistance is the production of an altered Penicillin Binding Protein 2a (PBP 2a). MRSA produces a PBP 2a mediated through the mecA gene, MRSA has a higher mortality rate for bacterial infections than for methicillin-sensitive *S. aureus* (MSSA) [17,18]. On the other hand, cigarette smoking promotes bacterial virulence, since the colonizing microbiota inhabits human mucosal spaces, microbes that share exposure to a variety of environmental stimuli, including CS [11,19]. Durmaz and his colleagues believed that exposure of *S. aureus* to smoking could lead to pathways for survival against antimicrobial activity [20]. Smokers have elevated levels of MRSA colonisation relative to non-smokers, raising their risk of severe and difficult infections to treat [11,20]. Smokers with acute and chronic sinusitis have a high prevalence of *S. aureus* and MRSA as pathogens relative to non-smokers [5,11,21].

Staphylococci grow well aerobically and in a carbon dioxide enriched atmosphere [22]. MRSA strains can be identified using a latex agglutination test kit [23].

MRSA was treated by vancomycin [24], but recently uses antibiotic such as linezolid, daptomycin and mupirocin [25, 26].

Methods

Methods

This was case-control study, conducted in Khartoum state from 15th January to the 30th of July 2018.

Participant of this study were males of age from 20 to 65 years old. Fifty adult males who smoke cigarette or hookah as case, another fifty non-smokers males as control were enrolled in this study. The case divided into two groups, twenty-five were cigarette smokers and the others were hookah smokers, participants under treatment or immunocompromised were excluded from study.

Samples were collected based on probability convenience technique, then data were collected by direct non-self –constricting questionnaire from participant.

Specimens Collection

The specimens were collected from anterior nares by using sterile cotton swab emulsified by peptone water, collection was done by inserting the swab and gently rotating for several times, collected specimens were labelled by number, packaged and processed within one hour of collection to Microbiology Laboratory in Sudan University of Science and Technology.

Each nasal swab inoculated into peptone water and incubated aerobically at 37Co for 24 hours then inoculated by using sterile loop into Mannitol Salt Agar (MSA) and incubated aerobically at 37Co for overnight. After incubation, isolates show significant growth were identified using standard microbiological methods, which included colonial morphology, Gram's stain, biochemical tests and molecular techniques (PCR).

Bacterial Identification

Colonies were examined in the next day. The organisms were identified according to the morphology of the colonies, Gram stain [27], biochemical tests and gene detection for the 16 s RNA of *S. aureus*.

In-vitro Antibiotic Sensitivity Testing

Kirby-Bauer method was used in the current study; the antibiotic discs used were from HI media (HI media Laboratories Pvt. Ltd, Mumbai 400086, India). The Oxacillin antibiotic (10 mg) and Vancomycin (30 mg) were used. The discs of the antibiotics placed in the diagnostic susceptibility test agar (Muller Hinton Agar). The distance between the two adjacent discs was at least 20 mm and from the edge of the plate was 15 mm. The media were incubated aerobically for 24 hours in 37 °C. After 24 hours of incubation, the diameter of the zone inhibition was measured and compared with the published tables of the control strains according to Clinical Labratorey Stander Institute guidelines (CLSI)[28]. The results were compared with *S. aureus* control ATCC 25923.

Genotyping of Staphylococcus aureus **resistant genes**

DNA was extracted by boiling method [29], by tacking small inoculums of bacteria cultured on nutrient agar, dissolving it in 0.5 ml of D.W in 1.5 ml Eppendorf tube and 10 ml of proteinase K was added for overnight at 37C^{o}, then boiled for 20 minutes, then incubated in refrigerator at -20 for 10 minutes. Repeated this process four times (heat shock), then centrifuged at 12000 rpm for 5 min. The supernatant used as template DNA for PCR.

The multiplex PCR was done by using a thermo-cycler (techne 312, England). The primer in table (1) [30], the following conditions: denaturation at 94Co for 10 minutes, followed by 10 cycles of denaturation at 94Co for 45 seconds, annealing at 55Co for 45 seconds, and extension at 72Co for 75 seconds. Moreover, another 25 cycles of 94Co for 45 seconds, 50Co for 45 seconds, 72Co for 45seconds, and a final extension step at 72Co for 10 minutes.

The Amplicon were separated at 120 Volt for 15 min in 1.5% (w/v) agarose gel containing ethidium bromide, bands were visualized under U.V trans-illuminator (UVitec – UK) to detect the specific amplified products by comparing with 50 base pairs standard ladders (INtRON biotechnology. Korea). All samples were confirmed as *S. aureus* by specific housekeeping gene primer (16 s RNA), negative sample were excluded.

Statistical analysis

Data were analysed using SPSS version-20. The Chi-squared test was performed to determine bivariate correlation and statistical significance and a *P*-value of less than 0.05 was considered statistically significant.

Results

Phenotypic analysis

S. aureus were found in the nasal of smokers 7 (17.1) and 18 (43.9) in Ciggrate and Hookahs respectively and non-smokers 16 (39) with insignificant association (P.value = 0.103). The distribution of *S. aureus* in cigarette and hookah smokers and it was very high in hookah smokers, 7 (28%) and 18 (72%) respectively. There was association between hookah and nasal carriers (P = 0.000).

S. aureus was susceptible to Vancomycin (41 were sensitive). In this study four participants from total number of *S. aureus* carriers were MRSA, while 35 carriers were MSSA and (2) isolates were showed intermediate result.

MRSA presence was higher in cigarette smokers than in hookah (2 folds), while only one case of MRSA was found in non-smokers as showed in Table 2.

Table 1
Nucleotide sequences of the primers used in the Multiplex PCR for the simultaneous detection of 16 s rRNA gene, and the mecA gene.

Primer name	DNA sequence (5to 3)	Ampilicon size (bp)	Specificity
MecA1 – F	5- AACTCTGTTATTAGGGAAGAACA-3	310 bp	MecA
MecA1 –R	5- CCACCTTCCTCCGGTTTGTCACC-3		
Staph 756- F	5- AACTCTGTTATTAGGGAAGAACA-3	756 bp	16S rRNA
Staph 750- R	5- CCACCTTCCTCCGGTTTGTCACC-3		

Table 2
Distribution of *Staphylococcus aureus*, susceptibility test and Mec A gene among study group.

Variable	Smoker (%)			Non smoker (%)	Total
	Ciggrate	Hookahs	P.value		
Frequency Staphylococcus aureus	7 (17.1)	18 (43.9)	0.004	16 (39)	41 (100)
Susceptibility test	5 (12.2)	16 (39)	0.009	14 (34.1)	41 (100)
Oxacillin	0 (0)	1 (2.4)		1 (2.4)	
1- **MSSA**	2 (4.8)	1 (2.4)		1 (2.4)	
2- **Intermediate**					
3- **MRSA**					
Vancomycin					
Mec A gene	4 (10.7%)	12 (31.6)	0.647	6 (15.8)	38 (100)
Positive	3 (7.9)	5 (13)		8 (21)	
Negative					

The relationship between nasal carriers of *S. aureus* and smokers group indicated significant relationship (P = 0.001), Moreover, the relationship between nasal carriers and number of cigarette or hookah per day indicated significant (P = 0.008), while, the relationship between nasal carriers and smoking years indicated insignificant (P = 0.479).

Genotypic Analysis

Forty one isolate of *S. aureus* were examined by the multiplex PCR to detect the presence of 16 s rRNA and mecA gene (Fig. 1), three samples were negative to 16 s rRNA, so were excluded from the study, and mecA was detected in the remainder 38 isolates.

From (38) *S. aureus* isolates (22) were MRSA positive, which was observed to be higher in smokers than non-smokers (16 and 6) respectively as showed in Table 2.

Discussion

In all nasal swabs, the phenotypic confirmation of *S. aureus* was performed using culture methods. The total number of confirmed *S. aureus* isolates was 41. Smoking carriers were 25 (61%), this finding differs from that reported by Durmaz *et al.* (2001) [20] Who was found (33%) and Abdullah *et al.* (2010) [31] who was found (22%). While there were 16 (39%) *S. aureus* isolates in non-smokers carriers, which was slightly near to the result obtained by Chattergee, (2009) [32] who was found (30%) and Lu *et al.* (2005) [33] who was found (32%).

These findings indicate a substantial rise in *S. aureus* in the smoker group rather than the non-smoker group. The distribution of *S. aureus* among smokers' group (n = 25) was: hookah group 18 (72%) while in cigarette 7 (28%), this result has shown an increase in the colonization of *S. aureus* in the hookah group more than a cigarette; this may be due to an increase in inhalation of more than a cigarette in the hookah, it may also be due to the sharing of hookah devices between different personnel, which may increase the colonization of *S. aureus* in this group.

In smokers 3 (7.3%) MRSA were found (2 (4.2%) from cigarette and 1 (2.4%) from hookah), this finding closed to Durmaz *et al.* (2001) [20] who was found (6%). In other hand MRSA in non-

smoker was only one isolate (2.4%), this finding was lower from reported by Abdallah *et al.* (2010) [31] who was found (4%). This phenotypic analysis shows that the cigarette increases the resistance of *S. aureus* more than the hookah.

There was significant association between nasal carriage and smoking (P. value = 0.001), and number of smoking per day (P. value = 0.008), this means that, smoking increases the colonization of *S. aureus* by smoking per day (P. value = 0.479).

This study revealed 6 (15.8%) isolates were mecA positive from non smoker, while there were 16 (42.3%) *S. aureus* in smokers. This genotypic analysis removes 3 phenotypically reported samples as *S. aureus*, which indicates that it is more accurate than phenotypic analysis. In addition, genotypic study reveals a sharp rise in Oxacillin resistant to more than phenotypic study, because of its ability to identify an unexpressed gene, it is an innovative diagnostic method and more efficient than a phenotypic study. In addition, the genotypic study revealed a higher concentration of mecA in the hookah than the cigarette according to the phenotypic study; This may increase the amount of nicotine and inhaled smoke in the hookah by more than a cigarette due to the presence of toxic substance in the hookah. Furthermore, the

way in which genes are transmitted (through plasmid) and the way in which hookah is used (different people share one device) helps to spread the mecA gene between different *S. aureus* carriers.

Conclusion

Smoking show significant association with nasal colonization of *S. aureus*. There was a high rate of MRSA nasal carriage in hookah group. Hookah smoking consider as strong risk factor for MRSA colonization.

Limitations

- Only male were included in this study.
- Low sample size due to financial issues

Abbreviations

MRSA
Methicillin Resistant *S. aureus*
PCR
polymerase chain reaction
CS
Cigarette smoking
ml
millilitres
PBP 2a
Penicillin Binding Protein 2a
MSSA
methicillin-sensitive *S. aureus*
MSA
Mannitol Salt Agar
rRNA
ribosomal ribonucleic acid
CLSI
Clinical Labratorey Stander Institute
DNA
deoxyribonuclease
D.W
distilled water
Rpm
rotate per minute
w/v

weight / volume
U.V
ultra violet
SPSS
Statistical Package for the Social Sciences
MLS – IEC
Committee of medical laboratory science -ethical approval number

Declarations

Ethics approval and consent to participate

Ethical clearance for this study was obtained from Committee of medical laboratory science, Sudan University of Science and Technology, ethical approval number (MLS – IEC – 08 – 17). Written informed consent for participation and publication of the data was obtained from the all participants included in this study.

Availability of data and materials

The data are available at https://doi.org/10.6084/m9.figshare.12401675.v1

Funding

None

Acknowledgments

Not applicable

Consent for publication

All participantS were informed for publication of data.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AIH and YFH designed the study, JOA, EAA, performed the experiments, HNA and ABA analyzed the data, ABA and AIH wrote the manuscript, all the authors approved the final version of the manuscript.

References

1. World Health Organization. Global status report on noncommunicable diseases 2014. World.
2. Health O. 2014.
3. Anonymous. (2014). The health consequences of smoking—50 years of progress: a report of.
4. th e surgeon general. U.S. Department of Health and Human Services Centres for Disease.
5. National Centre for Chronic Disease Prevention and Health Promotion
   Control, Prevention. National Centre for Chronic Disease Prevention and Health Promotion.
6. Office on Smoking. and Health, Atlanta, GA. Available at http:\\pubMed [accessed at 20, Jul.
7. 2018.
8. 3. Oberg, M., Jaakkola, MS., Woodward, A., Peruga, A. and Pruss-Ustun, A. (2011).Worldwide
9. burden of disease from exposure to second-hand smoke: a retrospective analysis of data from
10. 192. countries. Lancet. 377: 139–146.
11. 4. Stampfli, MR., Anderson, GP. (2009). How cigarette smoke skews immune responses to
12. promote infection, lung disease and cancer. Nat. Rev. Immunol. 9(5):377–384.
13. 5. Prasad, DS., Kabir, Z., Dash, AK., Das, BC. (2009). Smoking and cardiovascular health: a review of the epidemiology, pathogenesis, prevention and control of tobacco.Indian J. Med. Sci.
14. 63. (11):520–533.
15. 6. Yao, H., Rahman, I. (2011). Current concepts on oxidative/carbonyl stress, inflammation and
16. epigenetic in pathogenesis of chronic obstructive pulmonary disease. Toxicol. Appl.
17. Pharmacol. 254(2):72–85.
18. 7. Pryor, WA., Stone, K., Zang, LY., Bermudez, E. (1998). Fractionation of aqueous cigarette tar
19. extracts: fractions that contain the tar radical cause DNA damage. Chem. Res. Toxicol.11(5):
20. 441. –448.
21. 8. Feng, Y., Kang, Y., (2011). Exposure to cigarette smoke inhibits the pulmonary T-cell response
22. to influenza virus and Mycobacterium tuberculosis. Infect. Immun. 79:229–237.
23. 9. Huvenne, W., Ellen, A., Lanckacker, Olga Krysko, Ken R Bracke, Tine Demoor,Peter W Hellings, Guy G Brusselle, Guy F Joos, Claus Bachert, and Tania Maes, (2011). Exacerbation of cigarette smoke-induced pulmonary inflammation by Staphylococcus aureus enterotoxin B in
24. mice. Respir Res. 12(1): 69.
25. 10. Yageta, Y., Ishii, Y., Morishima, Y., Masuko, H., Ano, S., Tadahiro Yamadori,Ken Itoh, Kaoru Takeuchi, Masayuki Yamamoto, and Nobuyuki Hizawa. (2011). Role of Nrf2 in host defense against influenza virus in cigarette smoke-exposed mice. J. Virol.85(10):4679–4690.
26. 11. Kulkarni, R., Rampersaud, R., Aguilar, LJ., Randis, MT., Kreindler, LJ., and Ratner,JA.
27. 2010. Cigarette smoke inhibits airway epithelial cell innate immune responses to
28. bacteria. Infect. Immun. 78(5):2146–2152.

29. 12. Garmendia, J., Morey, P. and Bengoechea, JA. (2012). Impact of cigarette smoke exposure
30. on host-bacterial pathogen interactions. Eur Respir J. 39:467–477.
31. 13. American Lung Association. (2011). Hookah Smoking: A Growing Threat to Public
32. Health Issue Brief… Smoke free Communities Project, [accessed 2015 Sep 14].
33. 14. Yadav S, Rawal G. Waterpipe tobacco smoking: A mini-review. Journal of translational
34. internal medicine. 2018 Dec;6(4):173.
35. 15. Cobb, CO., Ward, KD., Maziak, W., Shihadeh, AL., Eissenberg, T.(2010). Waterpipe Tobacco
36. Smoking an Emerging Health Crisis in the United States. Americ J. Health. Behavior.
37. 34. (3):275–285.
38. 16. Durai, R., Ng, PC. and Hoque, H.(2010). Methicillin-resistant Staphylococcus aureus:an
39. update. AORN J 91(5):99–606.
40. 17. Kristiansen MM, Leandro C, Ordway D, Martins M, Viveiros M, Pacheco T, Kristiansen JE,
41. Amaral L. Phenothiazines alter resistance of methicillin-resistant strains of Staphylococcus
42. aureus (MRSA) to oxacillin in vitro. International journal of antimicrobial agents.2003 Sep
43. 1.;22(3):250-3.
44. 18. Porto, JP. Santos, RO., Gontijo, Filho, PP. and Ribas, RM. (2013). Active surveillance to
45. determine the impact of methicillin resistance on mortality in patients with bacteraemia and
46. influences of the use of antibiotics on the development of MRSA infection. Rev Soc Brasil Med
47. Trop 46(6):713–718.
48. 19. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell.2014 Mar
49. 27.;157(1):121 – 41.
50. 20. Durmaz, R., Tekerekoglu, MS., Kalcioglu, T. and Ozturan, O. (2001). Nasal carriage of
51. methicillin-resistant Staphylococcus aureus among smokers and cigarette factory workers.New Microbial24:143–147.
52. 21. Brook, I., and Hausfeld, JN.(2011). Microbiology of acute and chronic maxillary sinusitis in
53. smokers and non-smokers. Ann Otol Rhinol Laryngol. 120(11):707–712.
54. 22. Stepanović S, Djukić V, Djordjević V, Djukić S. Influence of the incubation atmosphere on the production of biofilm by staphylococci. Clinical microbiology and infection.2003 Sep;9(9):955-8.
55. 23. Carr FJ. Microbiology: A fundamental introduction. EC Microbiology. 2017;8(3):123 – 83.
56. 24. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, Stellrecht K.
57. Relationship between vancomycin MIC and failure among patients with MRSA bacteremia
58. treated with vancomycin. Antimicrobial Agents and Chemotherapy. 2008 Jun 30.
59. 25. Lee DK, Kim YN, Park KS, Yang JW, Kim KJ, Ha NJ. Antimicrobial activity of mupirocin,
60. daptomycin, linezolid, quinupristin/dalfopristin and tigecycline against vancomycin-resistant
61. enterococci (VRE) from clinical isolates in Korea (1998 and 2005). BMB Reports. 2007;40(6):881-7.

62. 26. Saravolatz LD, Pawlak J, Johnson L, Bonilla H, Fakih MG, Fugelli A, Olsen WM.In vitro
63. activities of LTX-109, a synthetic antimicrobial peptide, against methicillin-resistant,vancomycin-intermediate, vancomycin-resistant, daptomycin-nonsusceptible, and linezolid-
64. nonsusceptible Staphylococcus aureus. Antimicrobial agents and chemotherapy. 2012 Aug
65. 1.;56(8):4478-82.
66. 27. Cheasbrough, M., (2012). District Laboratory practice in Tropical Countries part 2. Second
67. Ed. New York. Cambridge University press.157–158, 238, 239, 400–402.
68. 28. Clinical Laboratory Standards Institute (2014). Interpretative charge zone size performs
69. standerds for untimicrobial susceptibility test USA, 27(1): 32–52.
70. 29. Compain, F., Babosan, A., Brisse, S., Genel, N., Audo, J., Ailloud, F., Kassis-Chikhani,N., Arlet, G. and Decre, D. (2014). Multiplex PCR for detection of seven virulence factors and K1/K2
71. capsular serotypes of Klebsiella pneumoniae. J. Clinical Microbiol. 52(12):4377–4380.
72. 30. Sambrook, J., Fritsch, E.F. and Maniatis, T.(1989). Gel electrophoresis of DNA.In: Molecular
73. Cloning: a Laboratory Manual. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,USA.
74. 31. Abdullah, HM., Zaki, WK. and Elnaggar, MK. (2010). Risk of nasal colonization of MRSA in
75. tropical medicine and microbiology. Egyptian J. Medical Microbiol. 19 (2): 95–102
76. 32. Chatterjee, SS., and Ray, P. (2009). Community based study on nasal carrier of
77. Staphylococcus aureus. India J Med Res. 130:742–748.
78. 33. Lu, P.L., Chin, C.F., Peng, Y.H., Chang, T.P. and Chen, L.A. (2005). Risk factors and molecular analysis community acquired methicillin-resistant S.aureus. J. Clin Microbiol.43:

Figures

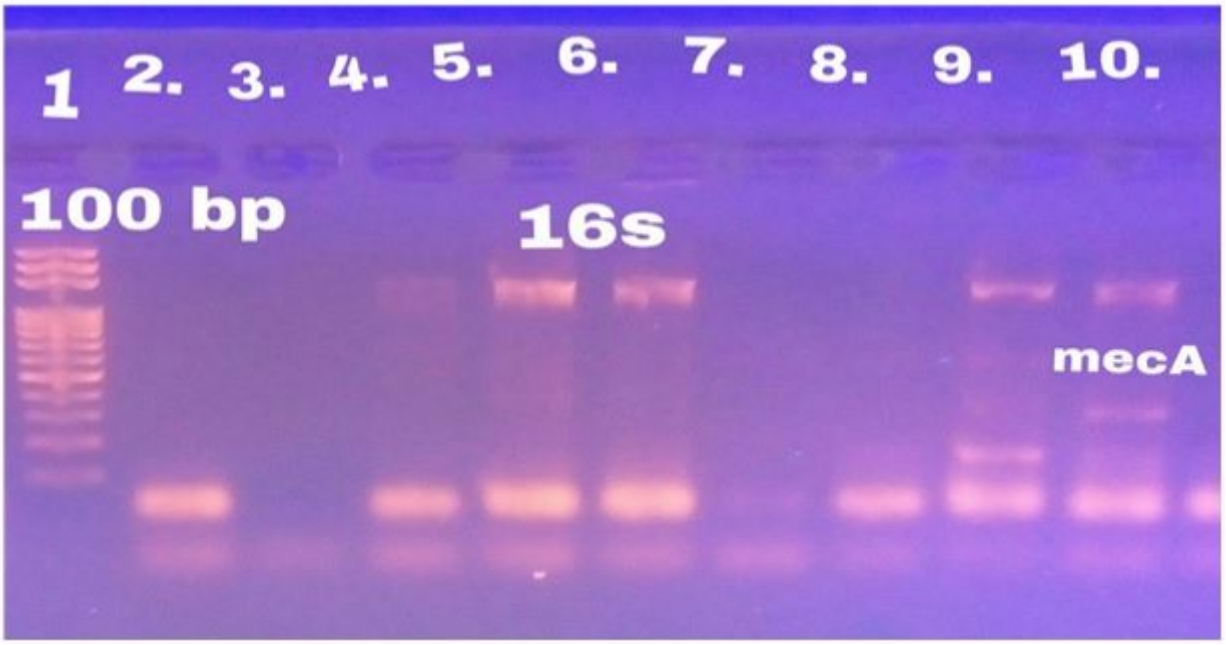

Figure 1

Detection of mecA gene by gel electrophorisis: Lane one is ladder samples 6,7,9 and 10 show 16s rRNA, only sample 10 show mecA positive.