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

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Research note

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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 37°C for 24 hours then inoculated by using sterile loop into Mannitol Salt Agar (MSA) and incubated aerobically at 37°C 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 37°C, 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 94°C for 10 minutes, followed by 10 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 75 seconds. Moreover, another 25 cycles of 94°C for 45 seconds, 50°C for 45 seconds, 72°C for 45 seconds, and a final extension step at 72°C 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 Cigarette 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.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>DNA sequence (5to 3)</th>
<th>Amplicon size (bp)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MecA1 – F</td>
<td>5- AACTCTGTTATTAGGAAGAACA-3</td>
<td>310 bp</td>
<td>MecA</td>
</tr>
<tr>
<td>MecA1 – R</td>
<td>5- CCACCTTCCTCCGGTTCACC-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph 756- F</td>
<td>5- AACTCTGTTATTAGGAAGAACA-3</td>
<td>756 bp</td>
<td>16S rRNA</td>
</tr>
<tr>
<td>Staph 750- R</td>
<td>5- CCACCTTCCTCCGGTTCACC-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Smoker (%)</th>
<th>Non smoker (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cigarette</td>
<td>Hookahs</td>
<td>P.value</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
<td>41 (100)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptibility test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>5 (12.2)</td>
<td>16 (39)</td>
<td>0.004</td>
</tr>
<tr>
<td>1- MSSA</td>
<td>0 (0)</td>
<td>1 (2.4)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>2- Intermediate</td>
<td>2 (4.8)</td>
<td>1 (2.4)</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>3- MRSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mec A gene</td>
<td>4 (10.7%)</td>
<td>12 (31.6)</td>
<td>0.647</td>
</tr>
<tr>
<td>Positive</td>
<td>3 (7.9)</td>
<td>5 (13)</td>
<td>8 (21)</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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](https://doi.org/10.6084/m9.figshare.12401675.v1)

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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.
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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.