MedCrave
Step into the World of Research

Journal of Microbiology & Experimentation

Research Article

Open Access

CrossMark

Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from a healthy student population

Abstract

Phenotypic tests, PCR analysis, and DNA sequencing were used to determine the frequency of *S. aureus* in a healthy student population. Sterile swabs from the nostrils were streaked on mannitol salt and blood agar. All isolates exhibiting beta hemolysis and mannitol fermentation were analyzed by using the coagulase test and Gram stain. PCR amplification of the isolates DNA detected a 273 bp DNA fragment encoding for a 16S rRNA gene. DNA sequencing and BLAST analysis of the amplified ribosomal genes demonstrated a 98% homology with *S. aureus* 16S rRNA genes. Six percent of the subjects were confirmed to carry *S. aureus* in the nares. Antimicrobial testing showed that the *S. aureus* isolated from a healthy population were susceptible to several antibiotics, peppermint oil, and lavender oil.

Keywords: *Staphylococcus aureus,* antibiotics, natural oils, nasal carriers, PCR, 16S rRNA gene

Volume 1 Issue 5 - 2014

Luis Jimenez, Elizabeth Kulko, Elyssa Barron
Biology and Horticulture Department, Bergen Community College, USA

Correspondence: Luis Jimenez, Bergen Community College, Biology and Horticulture Department, Paramus, New Jersey 07652, USA, Tel 201-446-7143, Email papojbmicro@gmail.com

Received: September 05, 2014 | **Published:** November 26, 2014

Introduction

Infections by *Staphylococcus aureus* are the number one cause for nosocomial outbreaks in the United States.[1] *S. aureus* is responsible for causing a variety of diseases such as skin eruptions, bacteremia, endocarditis, toxic shock syndrome, and pneumonia.[2] However, *S. aureus* is part of the commensal micro flora of the anterior nares in some humans. Most carriers are not infected by the bacteria but they are reservoirs assisting the spreading of *S. aureus* through the community.[3–7] Nasal carriage is influenced by host and bacterial factors.[2] The distinction between colonization and infection is critical. Colonization is the presence of *S. aureus* without signs or illness or infection. Infection presents clinical signs of illness and inflammation. Antimicrobial resistance is a common problem in both community and health-care associated infections.[4] Antibiotic susceptibility studies provide important information to determine the appropriate treatment to control or eliminate the infections. Several studies have shown that natural oils such as peppermint oil inhibited or killed *S. aureus*.[8,9] The objective of this study was to determine the presence of *S. aureus* in a healthy student population and ascertain the sensitivity of the isolates to different antibiotics and natural oils such as peppermint, lavender, and eucalyptus oil.

Materials and methods

Sampling

The students were ranging from ages of 18 to 44 years old. The gender distribution of the sampled population was 23% males and 77% females.

Phenotypic analysis

Different swab samples from the nostrils of 354 subjects were streaked on mannitol salt agar (MSA) and blood agar (BA) plates. The plates were incubated at 35°C for 48 hours. After incubation, all colonies showing beta-hemolysis on BA and mannitol fermentation on MSA were analyzed by using the Gram staining reaction, catalase test, and the tube coagulase test. *S. aureus* obtained from Ward Scientific (www.wardsci.com) was used as a quality control strain for all tests.

DNA extractions

Bacterial isolates were transferred to tryptic soy broth (TSB) and incubated overnight at 35°C. After overnight incubation, DNA extractions were performed as described in the ZR Soil Microbe DNA Mini Prep protocol (Zymo Research, Irvine, CA). Different aliquots of extracts were used in the PCR reactions.

PCR reactions

PCR conditions and DNA primers for *S. aureus* 16S rRNA genes were previously described.[10] Ready-To-Go (RTG) PCR beads (GE Healthcare, Buckinghamshire, UK) were used for each PCR reaction as previously described.[11] The beads contain BSA, dATP, dCTP, dTP, dGTP, and 2.5 units of PuRe Taq Polymerase. When reconstituted to a final volume of 25ul, concentrations for each nucleotide were 200 uM in 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl, and 1.5 mM $MgCl_2$.

DNA sequencing

Sequencing of the amplified PCR fragments from bacterial isolates were performed by Gene wiz, Inc. (South Plainfield, New Jersey). Sequencing reactions were carried out using primer16SSAIII.[10] Homology searches were performed using the Gen Bank server of the National Center for Biotechnology Information (NCBI) http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the BLAST algorithm.[12]

Antimicrobial testing

Bacterial isolates were screened using the disk diffusion disk method to determine their sensitivity to ampicillin (10ug), penicillin (10ug), novobiocin (22ug), chloramphenicol (30ug), and doxycycline (30ug) (BD BBL Sensi-Disc; Becton Dickinson and Company, Sparks, MD). The diameters of the zones of inhibition were measured and breakpoints calculated for each antibiotic in accordance with

the standards recommended by the Clinical Laboratory Safety Institute.[13] Peppermint oil (PO) (100%), Lavender oil (LO)(100%), and Eucalyptus oil (EO) (100%) were purchased from Whole Foods Inc. (Paramus, New Jersey). Disks were soaked into each solution and placed on the growth media previously inoculated with bacteria. Muller Hinton Agar (MHA) was used as the growth media in accordance with the standards recommended by the Clinical Laboratory Safety Institute.[13] *S. aureus* obtained from Ward Scientific was used as a quality control strain. The control strain identity was confirmed by 16S rRNA sequencing.

Results and discussion

The nostrils of 354 subjects were swabbed and swabs were streaked on MSA and BA. Twenty-two bacterial isolates showing mannitol fermentation and beta-hemolysis were gram stained (Table 1). All isolates were identified as Gram-positive cocci with positive catalase test and tube coagulase test. Chromosomal DNA extracted from all isolates was analyzed by PCR using specific primers targeting the 16S rRNA gene of *S. aureus*.[10] All isolates showed the 273 bp fragment specific for *S. aureus* (Table 1). DNA sequencing and BLAST analysis of the amplified fragments showed a minimum of 98% homology with the 16S rRNA gene of *S. aureus* (Accession number NC 007795.1). *S. aureus* was confirmed to be present in the nostrils of 6% of the students analyzed. Previous studies reported nasal carriage percentages from 15% to 32%.[1–6] Lower carriage, e.g., 6.3%, was reported in children under the age of 5.[7] Because the nostrils of the students were samples once, we could not distinguish between transient and persistent *S. aureus*. In a previous study, carriers were shown to be more likely to have *S. aureus* in one nostril than in both.[1] In other studies, the human throat appeared to be colonized more frequently than the nose.[3] No throat samples were analyzed in this study. Therefore, there is a possibility that the numbers of carriers in the studied population were underestimated by not sampling more than one nostril and the throat.

Table 1 Identification of nasal isolates

Isolate	Mannitol Fermentation	Beta-Hemolysis	Coagulase	*Staphylococcus aureus* 16S rRNA gene (273 bp)
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
11	+	+	+	+
12	+	+	+	+
13	+	+	+	+
14	+	+	+	+
15	+	+	+	+
16	+	+	+	+
17	+	+	+	+
18	+	+	+	+
19	+	+	+	+
20	+	+	+	+
21	+	+	+	+
22	+	+	+	+
S. aureus control	+	+	+	+

Disk diffusion studies were performed to determine the sensitivity of the isolates to different antibiotics and natural oils (Figure 1). All *S. aureus* isolates were found to be resistant to penicillin (Table 2). Penicillin resistance was not unexpected because of the long term and widespread use of this antibiotic to treat common infections.[2,4] The antimicrobial activity of ampicillin was higher than penicillin with 29% of the isolates showing high susceptibility. However, 71% of the isolates were resistant to ampicillin. Doxycycline showed a better antimicrobial activity than penicillin and ampicillin. Eighty six percent of the isolates showed to be susceptible to the agent. The percentage of *S. aureus* resistant to doxycycline was found to be 14%. Chloramphenicol was found to have intermediate activity against 67% of *S. aureus* isolates. Only 19% of the isolates were susceptible and 14% resistant. The percentage of *S. aureus* isolates susceptible to novobiocin was 86% while 14% were resistant.

Citation: Jimenez L, Kulko E, Barron E. Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from a healthy student population. *J Microbiol Exp.* 2014;1(5):168–171. DOI: 10.15406/jmen.2014.01.00030

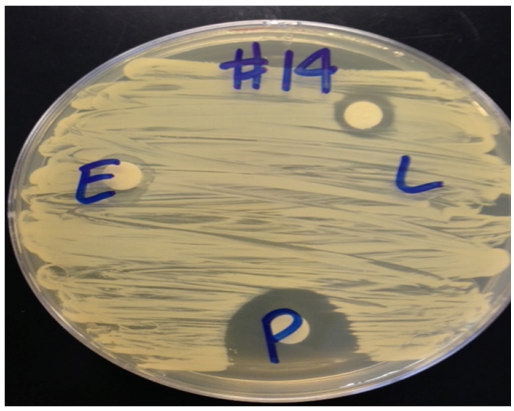

Figure 1 Disk diffusion assay.

Table 2 Antimicrobial activity of antibiotics against *S. aureus* isolates

Bacteria	C	P	A	N	Do
S. aureus Control	R	R	R	S	S
1	I	R	R	S	S
2	I	R	S	S	S
3	I	R	R	S	R
4	I	R	S	S	S
5	I	R	R	R	S
6	I	R	S	S	S
7	S	R	S	S	S
8	I	R	R	S	S
9	I	R	R	S	S
10	I	R	S	S	S
11	I	R	R	S	S
12	I	R	R	S	S
13	R	R	R	S	S
14	I	R	R	R	S
15	R	R	R	S	S
16	I	R	R	S	S
17	S	R	R	S	S
18	I	R	S	S	S
19	R	R	R	S	R
20	I	R	R	R	S
21	S	R	R	S	R
22	S	R	R	S	S

Sensitivity reaction; R, Resistant; I, Intermediate; S=Susceptible; C, Chloramphenicol= ≤12=R; 13-17, I ≥18=S; P, Penicillin= ≤28=R, ≥29=S; A, Ampicilin= ≤28=R, ≥29=S; N, Novobiocin= ≤16=R, ≥17=S; Do, Doxycycline= ≤12=R, 13-15=I, ≥16= S.

The control strain was resistant to penicillin, ampicillin, and chloramphenicol. However, it was susceptible to novobiocin and doxycycline. Only one *S. aureus* isolate, e.g., number 15, showed a similar antibiotic sensitivity profile to the control strain. Of all the isolates 24% percent showed a profile with chloramphenicol intermediate, penicillin and ampicillin resistant and novobiocin and doxycycline sensitive. Only one isolate, e.g., number 19, showed resistant to all the tested antibiotics commonly used to treat human infections. However, the isolate was susceptible to novobiocin. Novobiocin is not currently used in the USA for the treatment of human infections but veterinary applications are still available. Seventy six percent of the isolates showed an antibiotic profile with intermediate or susceptible activity against both chloramphenicol and doxycycline. Isolates with an antibiotic profile showing intermediate or susceptible activities against chloramphenicol, ampicillin, and doxycycline accounted for 29% of the isolates.

When the *S. aureus* isolates were exposed to natural oils using the disk diffusion method, PO was found to have the strongest antimicrobial activity (Table 3). The average zone of inhibition was 21.62 mm. The largest zone of inhibition was shown by the control strain with 36 mm. Zone diameters for PO ranged from 13 to 36 mm. The percentage of isolates with diameters greater or equal than 20 mm was 67%. PO is a common treatment for abdominal pain, coughs, and nausea.[8] PO was previously reported to decrease the production of virulence factors such as hemolytic activity and toxin production by *S. aureus*.[8] In that study minimum inhibitory concentration (MIC) values ranged from 64-256 ug/ml. Eradication of biofilm formation by *S. aureus* after treatment with natural oils was also reported.[9] When the red thyme, clove, and cassia oils were tested, all biofilms samples were killed.[9]

Table 3 Antimicrobial activity of natural oils against *S. aureus* isolates. Zone diameters in millimeters (mm)

	Zone of inhibition	(mm)	
Bacteria	**EO**	**LO**	**PO**
S. aureus Control	R	29	36
1	R	10	20
3	R	10	20
4	R	15	18
5	R	15	20
6	R	12	19
7	R	14	15
8	R	12	15
9	R	21	24
10	R	21	24
11	R	16	26
12	R	25	28
13	R	24	29
14	R	11	18
15	4	11	14
16	6	11	13
17	R	20	20
18	R	28	31
19	R	30	31
20	R	16	26
21	15	20	25
22	R	11	18
AVERAGE	1.19	16.81	21.62

R, Resistant=No Zone; EO, Eucalyptus oil; PO, Peppermint oil; LO, Lavender oil.

Citation: Jimenez L, Kulko E, Barron E. Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from a healthy student population. *J Microbiol Exp.* 2014;1(5):168–171. DOI: 10.15406/jmen.2014.01.00030

LO was the second most effective natural oil with an average zone of inhibition of 16.81mm (Table 3). The control strain and isolates 18 and 19 exhibited the strongest sensitivity to LO. Thirty eight percent of isolates showed zones of inhibition greater or equal than 20mm. Zone diameters for LO ranged from 10 to 30mm. LO was previously found to inhibit the growth of *S. aureus* with zones of inhibition ranging from 8 to 30mm.[14] EO showed the lowest antimicrobial activity against the isolates with 86% of bacteria showing no measurable zone of inhibition including the control strain. The average zone of inhibition for EO was 1.19mm (Table 3). Zone diameters ranged from 0 to 15mm.

The antimicrobial activity of natural oils seems to be driven by damaging the cell wall and cell membrane leading to cell lysis, leakage, and inhibition of the proton motive force. Due to the rapid emergence of resistant to antibiotics, new therapies for the prevention and treatment of *S. aureus* are needed. Fortunately, most isolates detected in the nasal carriers were found to have intermediate or susceptible profiles to doxycycline and chloramphenicol. Both antibiotics are commonly used for the treatment of human infections. Furthermore, based upon the zone of inhibition results, PO and LO appear to be reasonable alternatives to antibiotics for the treatment of common infections. Future studies will determine the kinetics of the antimicrobial action of PO and LO and the use of more diluted samples to ascertain their antimicrobial effect on the *S. aureus* isolates.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Kildow BJ, Conradie JP, Robson RL. Nostrils of healthy volunteers are independent with regard to *S. aureus* carriage. *J Clin Microbiol*. 2012;50(11):3744–3746.
2. Sivaraman K, Venkataraman N, Cole AM. *Staphylococcus aureus* nasal carriage and its contributing factors. *Future Microbiol*. 2009;4(8):999–1008.
3. Hamdan-Partida A, Sainz-Espunes T, Bustos-Martinez J. Characterization and Persistence of *Staphylococcus aureus* Strains Isolated from the Anterior Nares and Throats of Healthy Carrier sin a Mexican Community. *J Clin Microbiol*. 2010;48(5):1701–1705.
4. Du J, Chen C, Ding B, et al. Molecular Characterization and Antimicrobial Susceptibility of Nasal *Staphylococcus aureus* isolated from a Chinese Medical College Campus. *PLoS One*. 2011;6(11):e27328.
5. Jimei Du, Chun Chen, Baixing Ding, et al. Molecular Characterization and Antimicrobial Susceptibility of Nasal *Staphylococcus aureus* Isolates from a Chinese Medical College Campus. *PLoS One*. 2011;6(11):e27328.
6. Bischoff WE, Wallis ML, Tucker KB, et al. *Staphylococcus aureus* nasal carriage in a student community: prevalence, clonal relationships, and risk factors. *Infect Control Hosp Epidemiol*. 2014;25(6):485–491.
7. Pathak A, Marothi Y, Iyer RV, et al. Nasal carriage and antimicrobial susceptibility of *Staphylococcus aureus* in healthy preschool children in Ujjain, India. *BMC Pediatr*. 2010;10:100.
8. Li J, Dong J, Qiu JZ, et al. Peppermint Oil Decreases the Production of Virulence- Associated Exoproteins by *Staphylococcus aureus. Molecules*. 2011;16(2):1642–1654.
9. Kavanaugh NL, Ribbeck K. Selected Antimicrobial Essential Oils Eradicate *Pseudomonas* spp. and *Staphylococcus aureus* Biofilms. *Appl Environ Microbiol*. 2012;78(11):4057–4061.
10. Saruta K, Hoshina S, Machida K. Genetic identification of *Staphylococcus aureus* by polymerase chain reaction using single- base-pair mismatch in 16S ribosomal RNA gene. *Microbiol Immunol*. 1995;39(11):839–844.
11. Jimenez L, Smalls S, Grech P, et al. Molecular detection of bacterial indicators in cosmetic/pharmaceuticals and raw materials. *Journal of Industrial Microbiology & Biotechnology*. 1999;22:93–95.
12. Altschul SF, Madden TL, Schaffer AA, et al. Gaped BLAST and PSI--BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. 1997;25(17):3389–3402.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 18th informational supplement (M100-S18). Clinical and Laboratory Standards Institute: Wayne, PA, USA; 2008.
14. Roller S, Ernest N, Buckle J. The antimicrobial activity of high-necrodane and other lavender oils on methicillin-sensitive and -resistant *Staphylococcus aureus* (MSSA and MRSA). *J Altern Complement Med*. 2009;15(3):275–279.