Isolation and Characterization of *Staphylococcus aureus* From Food of Bovine Origin in Mekelle, Tigray, Ethiopia

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Abstract:

**Background:** Among food-borne diseases, *S. aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food.

**Objectives:** The study aimed to isolate and characterize *S. aureus* from raw milk, yogurt and meat and determine its antibiotic sensitivity pattern.

**Materials and Methods:** A cross-sectional study was conducted from December 2014 to June 2015 in Mekelle. A total of 284 samples were collected purposively. Enumeration of total viable bacteria count (TVBC), bacteriological isolation and identification, antimicrobial susceptibility testing, as well as PCR amplification of *fem A* and *mec A* genes were performed. Chi-square (χ²) and one way ANOVA tests were used for analysis.

**Results:** Overall TVBC mean was found to be 1.29x10⁸ cfu/ml/g. The highest TVBC (1.38x10⁸ cfu/ml) was from the yogurt sample and the lowest (1.26x10⁸ cfu/g) was from meat. The overall prevalence of coagulase positive *S. aureus* (CoPS) was 39.1% (111) and of the samples, 51(56.04%), 38(26.20%) and 22(45.83%) were isolated from meat, raw milk, and yogurt, respectively. There was a statistically significant difference (p<0.05) among the different sample types and sources in the prevalence of *S. aureus*. Almost half of the CoPS isolates were sensitive to Tetracycline, Gentamycin, and Kanamycin, but resistant to Amoxicillin (96.9%) and Penicillin G (93.8%). Moreover, 93.75% of the isolates developed multidrug resistance. All isolates carried the *fem A* gene and among these isolates, 12 (37.5%) carried *mec A* gene.

**Conclusion:** The present study revealed that foods of bovine origin of the study area are found to be having less bacteriological quality, high prevalence of CoPS and development of drug resistance.

**Keywords:** Antimicrobial, Bacteriological, Bovine, Food, Mekelle, PCR, *S. aureus*.

Article History

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1. INTRODUCTION

To date, around 250 different food-borne diseases have been described, and bacteria are the causative agents of two-thirds of food-borne disease outbreaks [1]. Factors that contribute to outbreaks of the bacterial food-borne diseases include, obtaining food from unsafe sources, contaminated raw food items, improper food storage, poor-personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time-lapse between preparing and consuming food items [2]. Among the bacteria predominantly involved in these diseases, *Staphylococcus aureus* (*S. aureus*) is a leading cause of gastroenteritis resulting from the...
consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins pre-formed in the food [3]. *S. aureus* is a ubiquitous organism frequently isolated from raw milk that is manually drawn from individual animals, bulk raw milk and naturally, from the milk of dairy cattle suffering from mastitis [4]. Similarly, meat and its products are important reservoirs for many of the food-borne pathogens including *S. aureus* [5].

Despite advances in food science and technology, food-borne diseases remain one of the major public health and economic problems all over the world [6]. Up to one-third of the population of developed countries may be affected by food-borne diseases each year. The problem is likely to be even more widespread in developing countries like Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers [7, 8]. In Ethiopia, both food shortage and lack of appropriate food safety assurance systems are problems that have become obstacles to the country’s economic development and public health safety [9, 10]. Data regarding food-borne diseases are extremely scarce at a national level and a few studies conducted in the different parts of the country showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like Campylobacter, Salmonella, *S. aureus*, *Bacillus cereus* and *Escherichia coli* [11 - 15]. Therefore, there is a pressing need to systematically investigate this recurring food-borne disease complex and to initiate appropriate intervention measures for the said problems. In the study area, only a few BIOLOG identification and biochemical characterizations of *S. aureus* from different food sources were conducted by some researchers [16, 17], and [15]. Moreover, in the study area, there is no published and/or accessible research work done on molecular characterization of *S. aureus*. Hence, the objectives of the current study were to isolate and characterize *S. aureus* from raw milk, yogurt and meat of different sources in the study area and to determine the antibiotic sensitivity pattern of the isolates.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted from December 2014 to June 2015 in Mekelle city. Mekelle is the capital city of Tigray Regional State, situated about 783 Kms North of Addis Ababa at 38.5° East longitude and 13.5° North latitude at an altitude of 2300 above sea level. The city has a total population of 215,546 [18], 308 cafeterias, 292 restaurants, 258 supermarkets and an active urban-rural exchange of goods which has 30000 micro- and small enterprises [19]. The weather condition is hot and dry. The mean annual rainfall of the area is 628.8 mm and an annual average temperature of 21°C [20].

2.2. Study Design and Study Population

A cross-sectional study on *S. aureus* was conducted from December 2014 to June 2015 on raw milk, yogurt and meat samples collected from different sources and parts of Mekelle, Tigray, Ethiopia. The study populations comprised of purposively selected slaughtered cattle and milked dairy cows found in Mekelle.

2.3. Sampling Technique and Sample Collection

A total of 284 samples of bovine raw milk (n=145), yogurt (n=48) and meat (n=91) were collected using a purposive sampling technique from Mekelle. These samples were collected based on the willingness of the owners. Raw milk samples were aseptically collected directly from the teats of lactating cows (n=100), whole seller (n=17), and cafeterias (n=28). Similarly the yogurt samples were collected from dairy farms (n=26) and cafeterias (n=22) using a sterile universal bottle. However, the raw meat samples were collected from abattoirs (n=55), butcher shops (n=16) and restaurants (n=20) during slaughtering and selling. Then afterward, the sections of meat samples were aseptically removed and placed in separate sterile plastic bags to prevent spilling and crosscontamination. Both samples were transported to the Veterinary Microbiology Laboratory of College of Veterinary Medicine, Mekelle University using icebox and stored at 4°C until the performance of laboratory experiments.

2.4. Enumeration of Total Viable Bacteria Count

One ml of milk and yogurt or 1 g of meat samples were homogenized into 9 ml of sterile peptone water using vortex mixer and from the 10 fold dilutions of the homogenates, 1 ml of 10^-6 dilution of the homogenates were plated on standard plate count agar (HiMedia, India) for the targeted organism using pour plate method. The plates were incubated at 37°C for 48h. Then after, plates containing 25-250 colonies were counted using the illuminated colony counter. The counts for each plate were expressed as colony-forming unit of the suspension (cfu/g/ml) [21].

2.5. Bacteriological Isolation and Identification

Isolation of *S. aureus* was attempted according to the method described [21] with slight modification. A part of each sample (10 ml or g) was enriched in sterile peptone water (HiMedia, India) (90 ml) and was incubated at 37°C for 24 h. A loopful of the enriched sample was aseptically streaked onto 5% Sheep Blood Agar (Oxoid Ltd., Basingstoke, Hampshire, England) and the plates were incubated aerobically at 37°C and examined after 24h of incubation for growth. The colonies were provisionally identified on the basis of staining reaction with Gram's stain, cellular morphology and hemolytic pattern on blood agar. The representative colonies were sub-cultured on Manitol Salt Agar (MSA) (Oxoid, UK) and incubated at 37°C. Then the colonies that grew on MSA were sub-cultured on nutrient broth and agar media and cultures were preserved and maintained for characterizing the isolates. Growth on MSA, catalase test, oxidase test, oxidation-fermentation test, detection of hemolysis, DNase test (Spot inoculation and Line streak inoculations) and tube coagulase test were performed [21]. Bacterial strain that was used as quality control organism in this study was the standard strain of *S. aureus* American Type Culture Collection (ATCC) 25923. The samples that were considered as coagulase positive *S. aureus* (CoPS) were further characterized.
2.6. Polymerase Chain reaction Amplification of femA and mecA genes of *S. aureus*

The *S. aureus* genomic DNA extraction and purification were performed as per the protocol provided by Thermo Scientific, GeneJET Genomic DNA Purification Kit for Gram positive organisms and the total genomic DNA was checked by running on 1.0% agarose gel. The total genomic DNA containing tubes were stored in the fridge for use as the template DNA. All antimicrobial susceptibility tested isolates were screened for the presence of the mec A and fem A genes by multiplex PCR according to the procedure given [22] using the following specific primers: F-5’GTA GAA ATG ACT GAA CGT CCG ATA A3’ and R-5’CCA ATT CCA CAT TGT TTC GGT CTA A3’- for mec A gene (having a band size of 310 bp) and F-5’-AAA AAA GCA CAT AAC AAG CG-3’ and R-5’GAT AAA GGA GAA ACG AGC AG-3’ for fem A gene (*S. aureus* species specific and encoding a factor responsible for methicillin resistance and having an ampiclon size of 132bp). Each PCR reaction mixture (50μl) was prepared from 5μl of 10X reaction buffer, 5μl of template DNA, 1μl of each primer, 3μl of 10mM dNTP mixture, and 1μl of Taq polymerase. The remaining volume was nuclease-free deionized water. Amplification was carried out in a Tianlong PCR Thermocycler with thermal cycling conditions of an initial denaturation at 94°C for 6 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and with final extension at 72°C for 6 min. Finally PCR products were separated by running on a 1.5% (w/v) agarose gel containing 0.5 g/ml ethidium bromide. Electrophoresis was conducted in a horizontal equipment system for 55 min at 110 V using 1X TAE buffer (40 mM Tris, 1 mM EDTA and 20 mM glacial acetic acid, pH 8.0). The amplicons were visualized under UV-light gel doc and their molecular weights were estimated by comparing with100 bp DNA molecular weight marker (Solis BioDyne, Tartu, Estonia) [23 - 25].

2.7. Antimicrobial Susceptibility Testing

The *S. aureus* isolates were screened for *in vitro* antimicrobial susceptibility using the agar disk diffusion method according to the procedure given [26] on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England). The following seven different antibiotic discs, with their concentrations given in parentheses, were used in the interpretation table of the Clinical and Laboratory Standard Institute [27].

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Site of Collection</th>
<th>Range of Viable Count CFU/g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>Meat</td>
<td>Abattoir</td>
<td>0.4x10^8</td>
</tr>
<tr>
<td></td>
<td>Butchery</td>
<td>0.4x10^8</td>
</tr>
<tr>
<td></td>
<td>Restaurant</td>
<td>0.7x10^8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.4x10^8</td>
</tr>
<tr>
<td>Raw milk</td>
<td>Farm</td>
<td>0.2x10^8</td>
</tr>
<tr>
<td></td>
<td>Whole seller</td>
<td>1x10^8</td>
</tr>
<tr>
<td></td>
<td>Cafeteria</td>
<td>0.4x10^8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.2x10^8</td>
</tr>
</tbody>
</table>

2.8. Data Management and Analysis

All collected data were entered into Microsoft Excel Sheet and analyzed through the Statistical Package for Social Sciences [28]. Accordingly, descriptive statistics such as percentages and frequency distribution were used to determine the prevalence and mean of the total viable count of microbial load in the food items and Chi-square (χ²) test was applied to assess the association. The mean of the total viable count of microbial load in the food items was compared with one way ANOVA.

3. RESULTS

3.1. Total Viable Bacterial Count (TVBC)

The overall total viable bacterial count mean was found to be 1.29x10^8 cfu/ml/g. The highest total viable bacterial count (1.38x10^8 cfu/ml) was found from the yogurt sample and the lowest (1.26x10^8 cfu/g) was from meat. The TVBC mean in meat samples collected from the butcher shop was higher followed by that from the restaurant. The highest viable bacterial count among the raw milk samples was found for the samples collected from whole sellers but in yogurt samples, the higher total viable bacterial count was from that of the cafeteria (Table 1).

3.2. Prevalence and Antimicrobial Susceptibility Profile of *S. aureus*

Out of the total 284 raw milk, meat, and yogurt food samples collected from different sources and examined, 111 (39.1%) were found CoPS and of these 111 isolates, 51 (56.04%), 38 (26.20%) and 22 (45.83%) were isolated from yogurt, meat and raw milk samples, respectively (Table 2).
The prevalence CoPS from different sources of meat samples was found 68.75%(17), 49.09%(29), and 65%(13) from butcher shop, abattoir, and restaurant, respectively; and 17%(17), 29.4%(5) and 57.14%(16) for raw milk samples collected from farm, whole seller, and cafeteria, respectively. Similarly, the prevalence of CoPS from different sources of yogurt samples was found to be 50%(13) and 40.9%(9) from farm and cafeteria, respectively. There was a significant difference (p < 0.05) among the different sample types and sample sources in the prevalence of S. aureus (Tables 2 and 3).

The antibiogram test results of the 32 CoPS isolates revealed that almost half of the CoPS isolates were sensitive to Tetracycline (53.1%), Gentamycin (50%), and Kanamycin (50%). But the high levels of resistance were recorded for Amoxicillin (96.9%), Penicillin G (93.8%), and Vancomycin (59.4%). Moreover, 93.75% of the isolates developed multidrug resistance (Table 4).

Besides the antibiogram test, the PCR amplification result indicated that all antimicrobial tested CoPS isolates carried the fem A gene, which has a band size of 132 bp and among these isolates, 12 (37.5%) carried mec A gene (MRSA), which has a band size of 310 bp.

### Table 2. Prevalence of S. aureus among the different sample types.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>No. of Examined Samples</th>
<th>No. of Positive Samples</th>
<th>( \chi^2 )</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>91</td>
<td>51 (56.04%)</td>
<td>22</td>
<td>0.000</td>
</tr>
<tr>
<td>Raw milk</td>
<td>145</td>
<td>38 (26.20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yogurt</td>
<td>48</td>
<td>22 (45.83%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>284</td>
<td>111 (39.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Prevalence of S. aureus among different collection sources and their sample types.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Site of Collection</th>
<th>No. of Examined Samples</th>
<th>No. of Positive Samples</th>
<th>( \chi^2 )</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>Abattoir</td>
<td>55</td>
<td>27 (49.09%)</td>
<td>40.1</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Butchery</td>
<td>16</td>
<td>11 (68.75%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Restaurant</td>
<td>20</td>
<td>13 (65%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>91</td>
<td>51 (56.04%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw milk</td>
<td>Farm</td>
<td>100</td>
<td>17 (17%)</td>
<td>40.1</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Whole seller</td>
<td>17</td>
<td>5 (29.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cafeteria</td>
<td>28</td>
<td>16 (57.14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>145</td>
<td>38 (26.20%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. In vitro antimicrobial sensitivity pattern of *S. aureus* isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>16(50%)</td>
<td>4(12.5%)</td>
<td>12(37.5%)</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>2(6.2%)</td>
<td>0(0%)</td>
<td>30(93.8%)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>1(3.1%)</td>
<td>0%</td>
<td>31(96.9%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>13(40.9%)</td>
<td>0(0%)</td>
<td>19(59.1%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>17(53.1%)</td>
<td>4(12.5%)</td>
<td>11(34.4%)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>16(50%)</td>
<td>5(15.6)</td>
<td>11(34.4%)</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>0(0%)</td>
<td>17(53.1%)</td>
<td>15(48.9)</td>
</tr>
</tbody>
</table>

The present study revealed that 39.1% (111/284) of the overall prevalence of CoPS and of these 111 isolates, 51(56.04%), 38(26.20%) and 22(45.83%) were isolated from meat, raw milk and yogurt, respectively. This overall prevalence of CoPS was nearly comparable with the findings of studies conducted [36] in Asella, South Eastern Ethiopia (35.71%) [17], in Northern Ethiopia (36%) [37], in and around Wolaita Sodo, Southern Ethiopia (37.14%) [38], in the region of Tirupathi, India (39.09%) [39], in Adama town, Ethiopia (42.14%), and [40] in Urban and peri-urban areas of Debere-Zeit, Ethiopia (44%). However, the present finding was higher than the reports [41] in Addis Ababa, Ethiopia (9%) [42], (9.1%) [43], in Addis Ababa, Ethiopia (10%) [16], in selected Districts of Tigray (9.8%) [44], in Mityana District, Uganda (10%) [45], in Zaria and Kaduna, Nigeria (12.6%) [46], in Nigeria (12.63%) [47], in South Italy (12.9%) [48], around Addis Ababa, Ethiopia (16.2%) [49], in Fars, Chahar Mahalva Bakhtiari and Ghom, provinces, Iran (17.9%) [50], in Fieri Region in Albania (18%) [51], in and around Gondar town, North Western Ethiopia (18.4%) [52], in and around Asella, Ethiopia (19.3%) [53], in South-West Uganda (20.3%) [54], in Bahir Dar town and its environs, Ethiopia (20.3%) [55], in Addis Ababa, Ethiopia (20.8%) [56], in Sibeta, Central Oromia, Ethiopia (23.4%) [57], in Bangladesh (25.53%) [58], in Adama, Ethiopia (28.1%) [59], in and around Addis Ababa, Ethiopia (28.8%) [60], in Pokhara, Nepal (29.7%) [61], in Plateau State, Nigeria (30.9%) [62], in Fazabad, India (31.78%) [63], in Wolayta Sodo, Ethiopia (32.14%) [64], in Mafikeng town, North-West Province of South Africa (32.5%), and [65] from Northern Plains of India (32.8%). But it was lower than the reports [66] in Southern Assam, India (47.86%) [67], in Hawassa area, Ethiopia (48.75%) [68], in the Selale/Fitche area, North Showa, Ethiopia (51.56%) [45], in Zaria and Kaduna, Nigeria (52.42%), and [69] in Hawassa town, Ethiopia (53.5%). The variation in the prevalence of *S. aureus* from the different studies might be due to differences in sample size and type, isolation techniques, husbandry practices, awareness and skill of the farm owners, animal health delivery systems, and geographic region of the sampled area.

The antibiogram test results of the 32 CoPS isolates revealed that almost half of the CoPS isolates were sensitive to Tetracycline (53.1%), Gentamycin (50%), and Kanamycin (50%). But the high levels of resistance were recorded for Amoxicillin (96.9%), Penicillin G (93.8%), and Vancomycin (59.4%). Moreover, 93.75% of the isolates developed multidrug resistance. Besides the antibiogram test, the PCR amplification result indicated that all antimicrobial tested CoPS isolates carried the *erm* A gene and among these isolates, 12 (37.5%) carried *mec* A gene (MRSA). This finding was in line with the findings of authors [70] who reported 100% resistance to Penicillin G and Amoxicillin and 42.7% to Cefoxitin [39], who reported 94.4% resistance to Penicillin G [48], who observed 92.2% resistance to Penicillin G and 33.3% to Oxacillin [71], in Solag Governorate, Egypt who reported 43.1% resistance to Oxacillin and 83.7% to one or more antimicrobial agents [57], who reported 100% resistance to Penicillin and Amoxicillin [63], who reported 93.3% resistance to Penicillin G [72], who observed 100% resistance to Penicillin G and Amoxicillin, and 42.7% to Cefoxitin [64], who illustrated a large proportion (60%-100%) of resistance to Penicillin G and Ampicillin [66], who reported 87.5% resistance to Penicillin G [65], who reported 96% resistance to Penicillin G and 93% to Ampicillin [37], who observed 100% resistance to Penicillin G and 71.8% multidrug resistance [61], who reported 35.6% resistance to Oxacillin [52], who reported 95.5% resistance to Penicillin G [68], who reported 36.6% MRSA [38], who reported 86.04% resistance to Penicillin G and 74.42% to Ampicillin [45], who reported 100% resistance to Penicillin G and 46.8% to Oxacillin, and 44.6% to vancomycin, detected *mec* A by polymerase chain reaction in 4 of the 18 MRSA isolates, and 88.9% multi-drug resistance [46], who reported 100% of resistance to Penicillin G, 65% to Amoxicillin, and 40% to Oxacillin [56], who reported 98.5% resistance to Penicillin G [55], who reported 95.3% resistance to Penicillin G and 46.8% to Oxacillin, and 44.6% to vancomycin, detected *mec* A by the PCR is considered a gold-standard technique for oxacillin resistance detection [74]. The resistance of *S. aureus*...
to Penicillin and closely related antibiotics might be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. Around 50% of mastitis-causing *S. aureus* strains produces β-lactamase [75]. Moreover, the development of antimicrobial resistance might be as a result of repeated therapeutic and/or indiscriminate use of them in the dairy and fattening farms, particularly penicillin and oxytetracycline for the treatment of mastitis cases as well as prophylaxis in the study area at large.

**CONCLUSION AND RECOMMENDATIONS**

The present study revealed that foods of bovine origin of the study area are found to be of less bacteriological quality. Moreover, there is a high prevalence of CoPS as well as MRSA in the different sample sources. This might be due to poor sanitary conditions and improper handling practices of foods. The antibiogram test results revealed that almost half of the CoPS isolates were sensitive to Tetracycline, Gentamycin, and Kanamycin. But almost all the isolates developed resistance to Amoxicillin and Penicillin G. Moreover, nearly all the isolates developed multidrug resistance. The development of antimicrobial resistance might be as a result of repeated therapeutic and/or indiscriminate use of them in the dairy and fattening farms. This higher percentage of multidrug resistance pattern indicates an alarming situation for designing prevention and control measures. However, the current study only targeted *mec A* and *fem A* genes for molecular characterization. In general, the low level of bacteriological quality, detection of CoPS and MRSA in different samples and development of drug resistance indicate that the products are not appropriate at all for human consumption. Hence, it poses a serious economic, animal welfare, food safety and public health problem. Therefore, strict hygiene should be implemented by creating awareness among different workers and managers of different food sources regarding transmission, zoonotic importance and control and prevention strategies of the disease; and dispensing of non-prescribed drugs and indiscriminate use of antibiotics should be avoided. Eventually, further studies on molecular characterization and sequencing of MRSA should be conducted by targeting other important genes in addition to the targeted genes in the study area and at large in the country.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**HUMAN AND ANIMAL RIGHTS**

Not applicable.

**CONSENT FOR PUBLICATION**

Not applicable.

**AVAILABILITY OF DATA AND MATERIALS**

The data supporting the findings of the article is available from the corresponding author [G.G.] on reasonable request.

**FUNDING**

None.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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[25] [http://dx.doi.org/10.1128/JCM.33.11.2864-2867.1995] [PMID: 8576325]


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[55] [http://dx.doi.org/10.1186/s12866-017-1048-9] [PMID: 28655298]
Isolation and Characterization of Staphylococcus aureus


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