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# Partial Sequencing of 16S rRNA Gene of Selected *Staphylococcus aureus* Isolates and its Antibiotic Resistance

**H. D. Kusumaningrum[a,b]*, L. Handayani[c], & R. Novrianti[c]**
[a]Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University
Campus IPB Darmaga, PO. BOX 220, Bogor 16002, Indonesia
[b]Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University
SEAFAST Center Building, Jl. Puspa No 1, Campus IPB Darmaga, Bogor 16680, Indonesia
[c]Food Science Study Program, Graduate School, Bogor Agricultural University, Indonesia
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## ABSTRACT

**The choice of primer used in 16S rRNA sequencing for identification of *Staphylococcus* species found in food is important. This study aimed to characterize *Staphylococcus aureus* isolates by partial sequencing based on 16S rRNA gene employing primers 16sF, 63F or 1387R. The isolates were isolated from milk, egg dishes and chicken dishes and selected based on the presence of *sea* gene that responsible for formation of enterotoxin-A. Antibiotic susceptibility of the isolates towards six antibiotics was also tested. The use of 16sF resulted generally in higher identity percentage and query coverage compared to the sequencing by 63F or 1387R. BLAST results of all isolates, sequenced by 16sF, showed 99% homology to complete genome of four *S. aureus* strains, with different characteristics on enterotoxin production and antibiotic resistance. Considering that all isolates were carrying *sea* gene, indicated by the occurence of 120 bp amplicon after PCR amplification using primer SEA1/SEA2, the isolates were most in agreeing to *S. aureus* subsp. *aureus* ST288. This study indicated that 4 out of 8 selected isolates were resistant towards streptomycin. The 16S rRNA gene sequencing using 16sF is useful for identification of *S. aureus*. However, additional analysis such as PCR employing specific gene target, should give a valuable supplementary information, when specific characteristic is expected.**

*Keywords: 16S rRNA gene sequencing, Staphylococcus aureus, enterotoxin-A, antibiotic*

## ABSTRAK

**Pemilihan primer yang digunakan dalam sekuensing gen 16S rRNA untuk identifikasi secara akurat terhadap spesies *Staphylococcus* pada pangan merupakan hal yang penting. Studi ini bertujuan untuk mengkarakterisasi isolat *Staphylococcus aureus* melalui sekuensing parsial terhadap gen 16S rRNA menggunakan primer 16sF, 63F atau 1387R. Isolat yang digunakan diisolasi dari susu, telur olahan dan ayam olahan yang dipilih berdasarkan keberadaan gen *sea* yang berperan dalam pembentukan enterotoksin-A. Kerentanan isolat terhadap enam antibiotik juga diuji. Penggunaan primer 16sF secara umum menghasilkan persentase homologi dan cakupan yang lebih tinggi dibandingkan dengan sekuensing menggunakan 63F atau 1387R. Hasil BLAST terhadap semua isolat yang disekuensing dengan 16sF menunjukkan 99% homologi terhadap 4 strain *S. aureus* dengan karakteristik yang berbeda dalam pembentukan enterotoksin maupun kerentanan terhadap antibiotik. Mengingat bahwa semua isolat mempunyai gen *sea*, yang ditandai dengan kehadiran amplikon 120 bp setelah amplifikasi menggunakan primer SEA1/SEA2, semua isolat homolog dengan *S. aureus* subsp. *aureus* ST288. Penelitian ini juga menunjukkan bahwa empat dari delapan isolat resisten terhadap streptomisin. Sekuensing terhadap gen 16S rRNA menggunakan primer 16sF sangat bermanfaat dalam mengidentifikasi *S. aureus*, tetapi tambahan analisis PCR terhadap gen spesifik, akan memberikan informasi yang berharga terutama jika diharapkan ditemukan karakteristik khusus pada bakteri.**

*Kata kunci: sekuensing gen 16S rRNA, Staphylococcus aureus, enterotoksin-A, antibiotik*

*Corresponding author:
E-mail: h_kusumaningrum@ipb.ac.id

## INTRODUCTION

Staphylococcal food poisoning is occurred when people consume food contaminated by *Staphylococcus aureus* that produce enterotoxins. Various staphylococcal enterotoxins (SE) incriminated in staphylococcal food poisoning have been reported. They were SEA, SEB, SEC, SED and SEE, and recently new serological types of SEs (SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER and SEU) were also identified (Argudin *et al*., 2010; Xie *et al*. 2011, Roussel *et al*., 2015). Staphylococcal enterotoxins-A (SEA), which is found on most food poisoning by *S. aureus*, is expressed in the mid-exponential phase, and its gene appears to be transferred by temperate bacteriophage (Argudin *et al*., 2010). The prevalence and genetic diversity of *S. aureus* has been investigated in raw and pasteurized milk (Rall *et al*., 2008), ready-to-eat foods (Huong *et al*., 2010), milk and food product (Salasia *et al*., 2011), and street-vend foods (Rohinishree & Negi, 2011). For epidemiological purposes, the accuracy of identification of *Staphylococcus* species isolated from various food products is critical. In this regard, molecular characterization is reported to be more accurate (Becker *et al*., 2004) than phenotypic identification (Rohinishree & Negi, 2011). The 16S rRNA gene is extensively used as taxonomic marker molecules during molecular characterization (Janda & Abott, 2007), particularly during the sequence analysis for differentiating species and sub species of bacteria (Rohinishree & Negi, 2011).

Generally, during sequencing analysis, the DNA target is firstly amplified using a pair primers followed by the sequencing using a single primer (SenGupta & Cookson, 2010). Identification based on highly conserved genes such as 16S rRNA usually uses long sequences primers (≥ 500 bp to about 1500 bp) (Janda & Abbott, 2007), although species specific shorter sequences can also be applied. For this purposes, universal primers for amplification of 16S rRNA genes are widely available, such as primers 63F and 1387R (1350bp) (Marchesi *et al*., 1998) and 27f and 1492r (Frank *et al*., 2008). Relatively shorter primer, i.e. 16sF and 16sR3 were also used (Lee *et al*., 2007). Since the choice of primers will affect the diversity of bacterial species that will be detected (Fredriksson *et al*., 2013), in this study three different primers targeting the 16S rRNA gene, i.e. 16sF, 63F and 1387R, was used separately to sequence DNA of selected *S. aureus* isolates from milk, egg dishes and chicken dishes. Milk, egg dishes and chicken dishes were reported contaminated by *S. aureus* in previous study (Handayani *et al*., 2014).

Furthermore, as information on the spreading of antibiotic resistance strains among *S. aureus* in food is also important, the antibiotic susceptibility testing of the isolates against antibiotics was also conducted in this study. The antibiotics tested were antibiotics that usually used to control human infections as well as to control and treat infections on farms. Many *S. aureus* strains that demonstrated resistance to different antibiotics were isolated from hospitals (Schmitz *et al*., 1999; Brown & Ngeno, 2007; Xie *et al*., 2011), hospital waste waters (Thompson *et al*., 2012), as well as from animal based food product, such as raw milk and dairy products (Jamali *et al*., 2015), poultry retail meat (Teramoto *et al*., 2016), and goat milk powder (Xing *et al*., 2016).

## MATERIALS AND METHODS

### Bacterial Strain and Isolates

The wild-type of *S. aureus* from raw milk (S1, S4 and S10), egg dishes (TB1), sautéed chicken cuts (UA2 and UA13) and chicken cuts satay (SJ1 and SJ4) were isolated in previous study (Handayani *et al*., 2014). *S. aureus* ATCC 25923 was used as a reference bacterium. All bacteria were grown in a tryptic soy broth or agar (Difco Laboratory, Detroit, MI, U.S.A.), and incubated at 37°C for 18h-24h. For confirmation, the isolates were spread onto Baird-Parker Agar (Oxoid Ltd., Hampshire, UK) supplemented with egg-yolk tellurite. Plates were incubated at 37°C for 18-24 h, thereafter the colonies were picked and streaked on Mannitol Salt Agar (Oxoid Ltd., Hampshire, UK). Typical colonies were then tested for production of catalase using Staphylase test kit (Oxoid Ltd., Hampshire, UK) and biochemical identification using API Staph (bioMérieux Inc., North Carolina, USA) according to manufacturer's instructions.

### DNA Extraction

Genomic DNA was isolated as described previously by Mason *et al.* (2001) with slight modification, as reported by Handayani *et al*. (2014), i.e. lysostaphin (10 mg/mL) was substituted by lysozyme (Bio Basic Canada Inc., Ontario, Canada) solution (10 mg/ml). The concentration of genomic DNA was determined by the Spectrophotometer UV -1800 (Shimadzu, Japan) at 260 nm while the quality was assessed based on the ration of the reading at 260/280 nm. The integrity of the DNA was checked by running in 1.5% agarose gel at 75V for 40 min electrophoresis (Bio-Rad Laboratories Pte. Ltd, Singapore).

### Detection of 16S rRNA and *Sea* Gene by PCR

The amplification of the gene encoding 16S rRNA and *sea* was performed using primers listed in Table 1 at Thermal Cycler 2720 (Applied Biosystems, California, USA). PCR master mix consisted of 12.5 μL of DreamTaq Green master mix (Thermo Fisher Scientific, Massachusetts, USA), 1 μL of each primer (10 μM), 2 μL of DNA template, and 8.5 μL nuclease free water (Thermo Fisher Scientific, Massachusetts, USA). Cycling parameters were one denaturation cycle for 5 min at 95°C and 30 amplification cycles for denaturation (1 min at 95°C), annealing (1 min at 55°), extension (1 min at 72°C) and termination for 5 min at 72°C, adopted from Lee *et al.* (2007). The amplification products were visualized on 2% agarose gel (Thermo Fisher Scientific, Massachusetts, USA) by electrophoresis (Bio-Rad, Bio-Rad Laboratories Pte. Ltd., Singapore) at 75 V for 40 min.

Table 1. Nucleotide sequences of primers used in PCR analysis

| Gene | Primer | Sequence (5′ → 3′) | PCR product of partial sequence | Temperature annealing (°C) |
|---|---|---|---|---|
| *sea*[a] | SEA1 | TTGGAAACGGTTAAAACGAA | 120 bp | 62.1 |
| | SEA2 | GAACCTTCCCATCAAAAACA | | 64.2 |
| 16S rRNA[a] | 16sF | CCGCCTGGGGAGTACG | 240 bp | 70.1 |
| | 16sR3 | AAGGGTTGCGCTCGTTGC | | 69.1 |
| 16S rRNA[b] | 63F | CAGGCCTAACACATGCAAGTC | 1350 bp | 70.8 |
| (universal) | 1387R | GGGCGGWGTGTACAAGGC | | 70.7 |

Note: [a] Lee *et al*. (2007); [b] Marchesi *et al*. (1998).

### Sequence Analysis of 16S rRNA Gene

The genotypic characterization of bacteria isolates was made through partial sequence analysis of 16S rRNA gene, using single primer 16sF, 63F, or 1387R. The PCR products that were amplified with primers 16sF/16sR3 were sequenced using primer 16sF. The PCR products amplified with primers 63F/1387R were sequenced using primer 63F and 1387R separately. The process of DNA sequencing was conducted by BigDye Applied Biosystem sequencer engine model 3730 at Macrogen inc., Singapore. Partial sequence data obtained, in FASTA format, was then submitted to the BLAST process at NCBI (National Center for Biotechnology Information) database for the identification of isolates (http://blast.ncbi.nlm.nih.gov/ Blast.cgi). The BLAST process was conducted using nucleotide collection searching setting (nr/nt) with *Staphylococcus aureus* subsp. *aureus* (taxid:46170) as organism of choice. The sequencing results were then compared to the reported 16S rRNA gene sequences of *Staphylococcus* species available in the GenBank database. The isolates were identified based on the highest homology percentages to the reported sequences.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was tested according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2012) using the disk diffusion technique with commercially available discs (Oxoid). The antimicrobials and concentrations in micrograms tested were gentamicin (10 mcg), streptomycin (10 mcg), kanamycin (30 mcg), chloramphenicol (30 mcg), tetracycline (30 mcg), and oxytetracycline (30 mcg). The inhibition zones, in mm, were measured in duplicate and scored as sensitive, intermediate susceptibility and resistant according to the CLSI recommendations, e.g. >19, 15-18 and <14 for tetracycline; >18, 13-17 and <12 for chloramphenicol; etcetera (CLSI, 2014). Gentamicin 10 μg S ≥15 13–14 ≤12; Kanamycin 30 μg ≥18 14–17 ≤13; Streptomycin 10 μg ≥15 12–14 ≤11; Chloramphenicol 30 μg ≥18 13–17 ≤12; Tetracycline 30 μg ≥19 15–18 ≤14 (CLSI, 2014).

## RESULTS

### Genotypic Characteristic of Isolates

Ten isolates of 78 presumptive *S. aureus* isolates were positive for *S. aureus* (Handayani *et al*., 2014). Eight of these isolates were reconfirmed in this study carrying *sea* gene that responsible for formation of staphylococcal enterotoxin-A (SEA). The presence of *sea* gene was indicated by the occurrence of 120 bp amplicon, after PCR amplification using primer SEA1/SEA2 (Table 2). The reference strain, *S. aureus* ATCC 25923, did not show this gene. In addition to the work of Handayani *et al*. (2014), all isolates also demonstrated 1350 bp amplicon after amplification by 63F/1387R primer (universal primer). The amplified PCR products of some isolates are shown in Figure 1.

The BLAST results are listed in Table 3. Sequencing by primer 16sF resulted in high identity percentages (almost all achieved 99%) towards the existing genome of *S. aureus* strains found in database of NCBI GenBank, in comparison to the sequencing by 63F and 1387R. Sequencing by 63F showed similarity to *S. aureus* strains in a range between 94% and 99%, while by 1387R in a range of 90% to 99%. In addition, using 16sF also resulted in low E-value. The lower the E-value, or the closer it

Table 2. Characteristics of *S. aureus* isolates

| Isolate | Sources | Coagulase | *sea* 120bp | 16S rRNA 240bp | 16S rRNA 1350bp |
|---|---|---|---|---|---|
| S1 | raw milk | + | + | + | + |
| S4 | raw milk | + | + | + | + |
| S10 | raw milk | + | + | + | + |
| TB1 | egg dishes | + | + | + | + |
| UA1 | sautéed chicken cuts | + | + | + | + |
| UA2 | sautéed chicken cuts | + | + | + | + |
| UA13 | sautéed chicken cuts | + | + | + | + |
| SJ1 | chicken cuts satay | + | + | + | + |
| SJ4 | chicken cuts satay | + | + | + | + |
| *S. aureus* | ATCC 25923 | + | - | + | + |

Note: + : positive results, - : negative results.

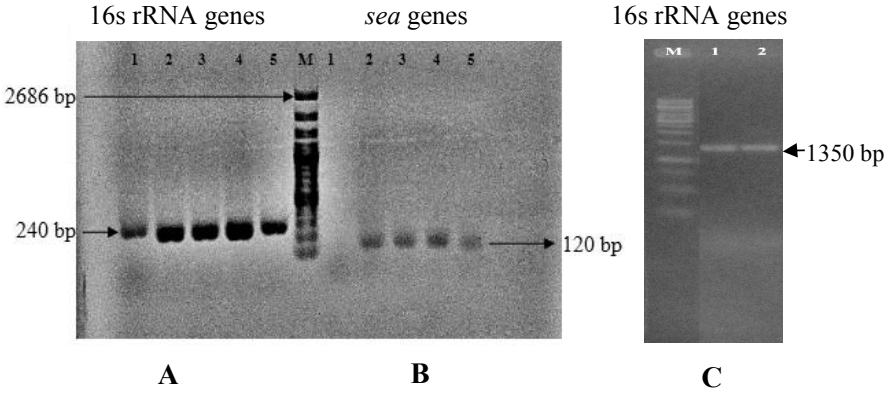


Figure 1. PCR products of partial sequence of 16S rRNA amplified by primer (A) 16sF/16sR3, (B) SEA1/SEA2, and (C) 63F/1387R. M= DNA ladder, 1= ATCC 25923, 2=S10, 3=UA13, 4=SJI, 5=TBI.

is to zero, the match is more significant (Pearson, *et al*., 2014). The expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size.

As shown in Table 3, by sequencing using primer 16sF, all isolates demonstrated 99% homology to the sequence of genome of 4 strains of *S. aureus.* They were *S. aureus* strain MSSA476, *S. aureus* subsp. *aureus* ST772-MRSA-V strain DAR4145, *S. aureus* subsp. *aureus* DSM 20231, and *S. aureus* subsp. *aureus* ST288 (isolate 10338, 10497, 15532, 16035, 18341, 18412 and 18583). Strain MSSA476, DAR4145 and DSM 20231 do not produce enterotoxin-A, as reported at accession BX571857.1, CP010526.1 and CP011526.1, respectively (NCBI, 2016). On the other hand, *S. aureus* subsp. *aureus* ST288 isolates 10338, 10497, 15532, 16035, 18341, 18412 and 18583 are known as enterotoxin-A producer. Complement of the genome for the enterotoxin-A gene (entA) of *S. aureus* subsp. *aureus* ST288 isolate 10338, 10497, 15532, 16035, 18341, 18412 and 18583 are shown in Table 4. Since all isolates were detected carrying *sea* gene (Table 2), the isolates were in agreeing to *S. aureus* subsp. *aureus* ST288 that able to form enterotoxin-A. The present study has been indicating that sequencing with 16S rRNA as gene target has been successfully identifying the isolates to specific strains. Additional PCR analysis employing SEA1/SEA2 primers increased accuracy of characterization of the isolates.

### Antibiotic Resistance Among the Isolates

Four isolates showed resistance to streptomycin (Table 5). All isolates, however, were susceptible to gentamycin and oxytetracycline. Interestingly, the resistant strains to streptomycin were isolated from different food sources, i.e. from milk (S10), egg dishes (TB1), sautéed chicken cuts (UA13) and chicken cuts satay (SJ1). Next to resistance to streptomycin, isolate TB1 showed also intermediate resistance to kanamycin, chloramphenicol and tetracycline.

## DISCUSSION

Among the SEs, SEA is reported as the most common cause of staphylococcal food poisoning worldwide, but the involvement of other SEs has been also found. SEA is considered as the main cause of staphylococcal food poisoning, probably due to its high resistance to proteolytic enzymes (Argudin *et al*., 2010). In the present study, all (eight) isolates were identified carrying *sea* gene, suggesting the potential risk of these strains due to production of SEA. Another study conducted in Indonesia by Salasia *et al.* (2011), however, did not find this gene in 11 food isolates (i.e. fermented milk product, sausage, meat ball, cakes and cheese), but found *seb, sec, see* and *seg* genes. They found, however, *sea* gene in 5 isolates of 19 milk isolates.

Regarding the sequencing results, as predicted, the use of 3 different primers resulted in various identity percentages. Sequencing by primer 16sF (5′-CCGCCTGGGGAGTACG-3′) resulted in higher identity percentages (almost all achieved 99%) towards the existing genome of *S. aureus* strains in comparison to the sequencing by 63F (5′-CAGGCCTAACACATGCAAGTC-3′) and 1387R (5′-GGGCGGWGTGTACAA GGC-3′). As was presented in Table 3, sequencing by 16sF also resulted in higher query coverage than that achieved by 63F and 1387R, except for isolate SJ4. These results highlighted that 16S rRNA sequencing by short sequence could provide sufficient identification amongst *S. aureus* strain. The 16sF has been used before to detect 16S rRNA genes of *S. aureus* isolates from food sample (Lee *et al*., 2007; Lee & Park 2016).

This study found that all isolates demonstrated 99% homology to the sequence genome of 4 strains of *S. aureus* by sequencing using primer 16sF. Based on the highest total score of the BLAST results, all isolates showed similarity to the genome of *S. aureus* strain MSSA476 and *S. aureus* subsp. *aureus* ST772-MRSA-V strain DAR4145. Strain MSSA476 is an invasive community acquired methicillin-susceptible *S. aureus* (Holden *et al.* 2004). On the other hand strain DAR4145 is a multidrug resistant strain of ST772-MRSA-V (Steinig *et*

Table 3. Query length, E value, identity percentages, query coverage and total score of the sequencing results towards homologous sequences found in the NCBI GenBank database after the BLAST process

| Isolates | Sequenced by 63F | | | | | Sequenced by 1387R | | | | | Sequenced by 16sF | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Query length (bp) | E Value | % Identity | Query cover (%) | Total score | Query length (bp) | E Value | % Identity | Query cover (%) | Total score | Query length (bp) | E Value | % Identity | Query cover (%) | Total score |
| **Homologous sequence: *S. aureus* strain MSSA476, complete genome** | | | | | | | | | | | | | | | |
| SJ1 | 1263 | 0 | 94 | 91 | 10820 | 1232 | 0 | 90 | 90 | 8793 | 210 | 2e-97 | 99 | 93 | 2117 |
| SJ4 | 1126 | 0 | 97 | 96 | 10798 | 1272 | 0 | 97 | 98 | 12349 | 216 | 2e-99 | 99 | 94 | 2161 |
| S1 | 1573 | 0 | 98 | 82 | 12853 | 1509 | 0 | 98 | 82 | 12853 | 215 | 4e-101 | 99 | 95 | 2195 |
| S4 | 1251 | 0 | 98 | 92 | 11906 | 938 | 0 | 99 | 90 | 9075 | 217 | 4e-101 | 99 | 94 | 2195 |
| S10 | 1504 | 0 | 98 | 87 | 13679 | 1489 | 0 | 99 | 86 | 13529 | 215 | 9e-102 | 99 | 95 | 2206 |
| TB1 | 1514 | 0 | 97 | 87 | 13424 | 1353 | 0 | 98 | 95 | 13618 | 212 | 2e-98 | 99 | 95 | 2139 |
| UA2 | 1331 | 0 | 97 | 93 | 12560 | 1372 | 0 | 98 | 93 | 13119 | 217 | 4e-101 | 99 | 94 | 2195 |
| UA13 | 1350 | 0 | 99 | 94 | 13590 | 1345 | 0 | 98 | 95 | 13507 | 212 | 2e-98 | 99 | 95 | 2139 |
| **Homologous sequence: *S. aureus* subsp. *aureus* ST772-MRSA-V, strain DAR4145, complete genome** | | | | | | | | | | | | | | | |
| SJ1 | 1263 | 0 | 94 | 91 | 10820 | 1232 | 0 | 90 | 90 | 8787 | 210 | 2e-97 | 99 | 93 | 2117 |
| SJ4 | 1126 | 0 | 97 | 96 | 10793 | 1272 | 0 | 97 | 98 | 12344 | 216 | 2e-99 | 99 | 94 | 2161 |
| S1 | 1573 | 0 | 97 | 84 | 13330 | 1509 | 0 | 98 | 82 | 12853 | 215 | 4e-101 | 99 | 95 | 2195 |
| S4 | 1251 | 0 | 98 | 92 | 11912 | 938 | 0 | 99 | 90 | 9070 | 217 | 4e-101 | 99 | 94 | 2195 |
| S10 | 1504 | 0 | 98 | 87 | 13684 | 1489 | 0 | 99 | 86 | 13529 | 215 | 9-e102 | 99 | 95 | 2206 |
| TB1 | 1514 | 0 | 97 | 87 | 13430 | 1353 | 0 | 98 | 95 | 13618 | 212 | 2e-98 | 99 | 95 | 2139 |
| UA2 | 1331 | 0 | 97 | 93 | 12565 | 1372 | 0 | 98 | 93 | 13119 | 217 | 4e-101 | 99 | 94 | 2195 |
| UA13 | 1350 | 0 | 99 | 94 | 13596 | 1345 | 0 | 98 | 95 | 13507 | 212 | 2e-98 | 99 | 95 | 2139 |
| **Homologous sequence: *S. aureus* subsp. *aureus* ST288, isolate 10338, 10497, 15532, 16035, 18341, 18412 and 18583, complete genome** | | | | | | | | | | | | | | | |
| SJ1 | 1263 | 0 | 94 | 91 | 9009 | 1232 | 0 | 90 | 90 | 7329 | 210 | 2e-97 | 99 | 93 | 1756 |
| SJ4 | 1126 | 0 | 97 | 96 | 8991 | 1272 | 0 | 96 | 98 | 10274 | 216 | 2e-99 | 99 | 94 | 1793 |
| S1 | 1573 | 0 | 97 | 84 | 11092 | 1509 | 0 | 98 | 82 | 10699 | 215 | 4e-101 | 99 | 95 | 1820 |
| S4 | 1251 | 0 | 98 | 92 | 9911 | 938 | 0 | 99 | 90 | 7558 | 217 | 4e-101 | 99 | 94 | 1820 |
| S10 | 1504 | 0 | 98 | 87 | 11388 | 1489 | 0 | 99 | 86 | 11262 | 215 | 9e-102 | 99 | 95 | 1830 |
| TB1 | 1514 | 0 | 97 | 87 | 11175 | 1353 | 0 | 98 | 95 | 11336 | 212 | 2e-98 | 99 | 95 | 1774 |
| UA2 | 1331 | 0 | 97 | 93 | 10455 | 1372 | 0 | 98 | 93 | 10921 | 217 | 4e-101 | 99 | 94 | 1820 |
| UA13 | 1350 | 0 | 99 | 94 | 11314 | 1345 | 0 | 98 | 95 | 11244 | 212 | 2e-98 | 99 | 95 | 1774 |
| **Homologous sequence: *S. aureus* subsp. *aureus* DSM 20231, complete genome** | | | | | | | | | | | | | | | |
| SJ1 | 1263 | 0 | 94 | 91 | 10820 | 1232 | 0 | 90 | 90 | 8794 | 210 | 2e-97 | 99 | 93 | 2106 |
| SJ4 | 1126 | 0 | 97 | 96 | 10798 | 1272 | 0 | 96 | 98 | 12338 | 216 | 2e-99 | 99 | 94 | 2150 |
| S1 | 1573 | 0 | 97 | 84 | 13319 | 1509 | 0 | 98 | 82 | 12848 | 215 | 4e-101 | 99 | 95 | 2183 |
| S4 | 1251 | 0 | 99 | 92 | 11900 | 938 | 0 | 99 | 90 | 9070 | 217 | 4e-101 | 99 | 94 | 2183 |
| S10 | 1504 | 0 | 98 | 87 | 13673 | 1489 | 0 | 99 | 86 | 13524 | 215 | 9e-102 | 99 | 95 | 2195 |
| TB1 | 1514 | 0 | 97 | 87 | 13418 | 1353 | 0 | 98 | 95 | 13612 | 212 | 2e-98 | 99 | 95 | 2128 |
| UA2 | 1331 | 0 | 97 | 93 | 12554 | 1372 | 0 | 98 | 93 | 13114 | 217 | 4e-101 | 99 | 94 | 2183 |
| UA13 | 1350 | 0 | 99 | 94 | 13585 | 1345 | 0 | 98 | 95 | 13502 | 212 | 2e-98 | 99 | 95 | 2128 |
| **Homologous sequence: *S. aureus* subsp. *aureus* strain ATCC25923, complete genome** | | | | | | | | | | | | | | | |
| SJ1 | 1263 | 0 | 94 | 91 | 10826 | 1232 | 0 | 90 | 90 | 8782 | 210 | 10e-96 | 98 | 93 | 2095 |
| SJ4 | 1126 | 0 | 97 | 96 | 10804 | 1272 | 0 | 96 | 98 | 12338 | 216 | 8e-98 | 98 | 94 | 2139 |
| S1 | 1573 | 0 | 97 | 84 | 13313 | 1509 | 0 | 98 | 82 | 12848 | 215 | 2e-99 | 99 | 95 | 2172 |
| S4 | 1251 | 0 | 98 | 92 | 11895 | 938 | 0 | 99 | 90 | 9058 | 217 | 2e-99 | 99 | 94 | 2172 |
| S10 | 1504 | 0 | 98 | 87 | 13668 | 1489 | 0 | 99 | 86 | 13524 | 215 | 4e-100 | 99 | 95 | 2183 |
| TB1 | 1514 | 0 | 97 | 87 | 13413 | 1353 | 0 | 98 | 95 | 13612 | 212 | 10e-97 | 98 | 95 | 2117 |
| UA2 | 1331 | 0 | 97 | 93 | 12549 | 1372 | 0 | 98 | 93 | 13114 | 217 | 2e-99 | 99 | 94 | 2172 |
| UA13 | 1350 | 0 | 99 | 94 | 13579 | 1345 | 0 | 98 | 95 | 13502 | 212 | 10e-97 | 98 | 95 | 2117 |

*al*., 2015). These results suggested that the isolates in this study showed equal likelihood whether they were corresponding to the MRSA or the MSSA. This finding is possible since these strains also occupied many people in Indonesia. Recent epidemiological study showed MRSA carriage rate of 4.3%, and MSSA carriage rate of 1.5% among 1,502 patients in hospitals in Java and Bali (Santosaningsih *et al*., 2014).

Furthermore, all isolates which were sequenced by 16sF also showed 99% homology to the sequence genome of *S. aureus* subsp. *aureus* ST288, a highly transmissible methicillin-resistant *S. aureus* (Vogel *et al*., 2012) that produce enterotoxin-A. The occurrence of strain ST228 were notified for long periods. Conceicao *et al*., (2007) reported that strain ST239-MRSA-III was replaced by both strain ST5-MRSA-II and ST228-MRSA-I between

Table 4. Accession and complement of the genome for the enterotoxin-A gene (entA) of *S. aureus* subsp. *aureus* ST288, isolate 10338, 10497, 15532, 16035, 18341, 18412 and 18583

| Isolate | Accession | Complement for the enterotoxin A gene | Source* |
|---|---|---|---|
| 10388 | HE579059.1 | 1988966..1989738 | http://www.ncbi.nlm.nih.gov/nuccore/HE579059 |
| 10497 | HE579061.1 | 1988953..1989726 | http://www.ncbi.nlm.nih.gov/nuccore/HE579061 |
| 15532 | HE579063.1 | 1989266..1990039 | http://www.ncbi.nlm.nih.gov/nuccore/HE579063 |
| 16035 | HE579065.1 | 1988898..1989671 | http://www.ncbi.nlm.nih.gov/nuccore/HE579065 |
| 18341 | HE579069.1 | 1988952..1989725 | http://www.ncbi.nlm.nih.gov/nuccore/HE579069 |
| 18412 | HE579071.1 | 1988718..1989491 | http://www.ncbi.nlm.nih.gov/nuccore/HE579071 |
| 18583 | HE579073.1 | 1988770..1989543 | http://www.ncbi.nlm.nih.gov/nuccore/HE579073 |

Note: * Last accessed [5 April 2016]

Table 5. Antibiotic susceptibility of *S. aureus* isolates

| Isolate | Sources | Gentamicin | Streptomycin | Kanamycin | Chloramphenicol | Tetracycline | Oxytetracycline |
|---|---|---|---|---|---|---|---|
| S1 | raw milk | S | S | S | S | S | S |
| S4 | raw milk | S | S | I | I | S | S |
| S10 | raw milk | S | R | I | S | S | S |
| TB1 | egg dishes | S | R | I | I | I | S |
| UA1 | sautéed chicken cuts | S | S | S | S | S | S |
| UA2 | sautéed chicken cuts | S | S | S | S | S | S |
| UA13 | sautéed chicken cuts | S | R | S | S | S | S |
| SJ1 | chicken cuts satay | S | R | I | S | S | S |
| SJ4 | chicken cuts satay | S | S | S | S | S | S |

Note: S: Sensitive, I: Intermediate, R: Resistance, determined by diameter zone of inhibition in mm. Gentamicin: S ≥15, I 13–14, R ≤12; Kanamycin: S ≥18, I 14–17, R ≤13; Streptomycin S ≥15, I 12–14, R ≤11; Chloramphenicol S ≥18, I 13–17, R ≤12; Tetracyclines S ≥19, I 15–18, R ≤14 (CLSI, 2014).

1994 and 2004 in Hungary. A study conducted in hospitals in a relative small geographic area in Switzerland also observed that several MRSA clones (ST5-MRSA-II, ST45-MRSA-IV, ST228-MRSA-I and ST247-MRSA-I) were present over a period of 8 years from 1997 to 2004 (Blanc *et al*., 2007). More recently, Vogel *et al.* (2012) compared the whole genome of eight ST228 isolates recovered between 2001 and 2008 that spread over ten years in a tertiary care hospital in Switzerland. These reports suggested that the spreading of ST228 strain was confirmed.

As discused above, when sequenced by 16sF all isolates showed good homology (99%) to some strains found in the NCBI GenBank data base with different characteristics on its antibiotic resistance. Next to this characteristic, the coresponding strains also showed differences on capability to form enterotoxin. Strain MSSA476 and DAR4145, as well as strain DSM 20231 did not produce enterotoxin-A (NCBI, 2016). On the other hand, *S. aureus* subsp. *aureus* ST288 isolate 10338, 10497, 15532, 16035, 18341, 18412 and 18583 are known as enterotoxin-A producers. Therefore, additional information to confirm the presence of a gene that responsible for formation of enterotoxin-A is considerably important. Since all selected isolates were confirmed carrying *sea* gene, indicated by the presence of 120 bp amplicon after PCR amplification using primer SEA1 and SEA2 as presented in Table 2, all isolates were most in agreeing with *S. aureus* subsp. *aureus* ST288. The *sea* gene is 771 bp in size encoding an enterotoxin A precursor of 257 amino acid residues (Huang *et al*., 1987). Specific primers SEA1 and SEA2 were frequently used in PCR analysis to detect the presence of *sea* gene in *S. aureus* isolates from food, such as in raw and pasteurized milk (Rall *et al*., 2008); Kérouanton *et al*., 2007) and ready-to-eat Kimbap (Lee *et al*., 2007). Specific PCR primers have commonly been employed to confirm the presence or absence of specific characteristics associated with target microorganisms such as virulence factors.

Next to genotypic characteristic, information on antibiotic resistance amongst *S. aureus* strains found in food is also important for surveillance and epidemiology study. This study found that four isolates from different food sources (from milk, egg dishes, sautéed chicken cuts and chicken cuts satay) were resistant to streptomycin. Streptomycin resistance among *S. aureus* isolates was also reported in other study. Jamali *et al.* (2015) found that amongst *S. aureus* isolates from raw milk and dairy products (n=328), 5.8% demonstrated resistance to streptomycin, 4% to kanamycin, 3.7% to chloramphenicol, and 2.1% to gentamicin. Most isolates were resistant to tetracycline (56.1%) followed by to penicillin (47.3%). The high percentage of resistant isolates to these last two antibiotics could be due to the widespread use of these antibiotics to control and treat infections on dairy farms (Jamali *et al.*, 2015). Moreover, the fact that streptomycin resistant strains were found in milk, egg dishes and chicken dishes indicated possible

occurrence of cross contamination from human or food vendor. Schmitz *et al.* (1999) found that 21% of the *S. aureus* isolates (n=699) collected from different hospitals in Europe were resistant to streptomycin. They also found, however, 23% of the *S. aureus* isolates were resistant to gentamycin, 29% to tobramycin, and 31% to kanamycin. In more recent study, Onwubiko & Sadiq (2011) also found that 55.8% of *S. aureus* from clinical isolates in a tertiary health institution in North-western Nigeria (n= 129) showed resistance to streptomycin, 68.8% to tetracycline, 38.1% to chloramphenicol, and 7.6% to gentamycin. The spreading of *S. aureus* strains that resistant to antibiotics has become a global concern. Continued surveillance of *S. aureus* producing enterotoxin-A in milk, egg and poultry food products at genotypic levels is necessary to understand and limit further increases of staphylococcal food poisoning incidences.

## CONCLUSION

This study has demonstrated that in order to increase the accuracy of the identification results, next to the sequencing of *S. aureus* targeting 16S rRNA gene, PCR analysis using specific primer is considerably important. All eight isolates were carrying *sea* gene, detected by PCR analysis, indicating that they can produce staphylococcal enterotoxin-A. Genotypic characterization of the selected strains by sequencing using 16sF, showed agreeing to the sequence genome of *S. aureus* subsp. *aureus* ST288 that also produce enterotoxin-A. This study also found that 4 of 8 selected isolates were resistant to streptomycin.

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