Classical Enterotoxin Genes of *Staphylococcus aureus* Isolated from the Raw Milk of Cows and Goats in Yogyakarta Indonesia

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ABSTRACT

Milk is a highly nutritious food containing several essential nutrients. Consequently, it can be contaminated by pathogenic bacteria, including *Staphylococcus aureus*. This study aimed to analyze the genes encoding nine classical enterotoxins of *S. aureus* isolated from cow and goat raw milk. A total of 75 *S. aureus* isolates were obtained from milk samples of dairy cows (46 isolates) and Peranakan Etawa (PE) goats (29 isolates) from different farms in Yogyakarta Indonesia. Based on the cultural and biochemical properties and the 23S rRNA occurrence, all isolates could be identified as *S. aureus*. The seh gene was the most frequently observed in 21 isolates (45.65%) of cows, followed by the sea gene (in 8 isolates, 17.39%), the seg and the sei genes (each in 4 isolates, 8.69%). The sec is the only staphylococcal enterotoxin gene observed in *S. aureus* isolated from goat milk with the prevalence of 6.89%. The genes encoding four out of nine classical enterotoxins (seh, sea, seg, and sei) are commonly isolated from *S. aureus* in cow milk, but only sec gene can be found in the isolates from goat milk.

Keywords: *Staphylococcus aureus*, enterotoxin gene, dairy raw milk, multiplex PCR

1. INTRODUCTION

*Staphylococcus aureus* is an important agent of subclinical mastitis in dairy animals. It is known as one of the leading causes of foodborne illness and food poisoning outbreak worldwide [1]. The presence of *S. aureus* and genes encoding staphylococcal enterotoxin (SE) in livestock products is hazardous for consumer health [2]. Foods containing SE are the main cause of foodborne diseases [3,4]. *S. aureus* strains from mastitis cases were reported to be capable of producing toxic shock syndrome toxin-1 (TSST-1) that cause toxic shock syndrome (TSS) [5]. In many countries, the incidence of foodborne disease and food poisoning outbreaks is related to different enterotoxin genes [6,7,8,9].

Initially, the division of classical SE genes consists of sea-see. After the findings of seg, seh, sei, sej, and other SEs, the division of gene develops [10]. Based on this, it is necessary to identify the distribution pattern of SE genes in farm products as an effort to identify and anticipate the treatment and control.

2. MATERIALS AND METHODS

This study used *S. aureus* isolated from cow milk (46 isolates) and Etawa goat milk (29 isolates) which collected from several dairy farms in Yogyakarta, Indonesia. The strains were identified as *S. aureus* based on phenotypic and genotypic identifications in the Laboratory of Clinical Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Phenotypic identifications included mannitol salt agar (MSA), coagulase, Gram staining, catalase,
and Voges-Proskauer (VP) tests [11]. Genotyping was performed based on the presence of the 23S rRNA, nuc, and coa genes, as described in previous studies by Windria et al. [12].

The detection of enterotoxin encoding genes (sea-sej) was carried out using two sets of multiplex polymerase chain reaction (PCR) method (Set 1: sea, seb, sec, sed, see; Set 2: seh, sei, sej) [13]. The primers and programs used are presented in Table 1. The PCR products were analyzed by electrophoresis in 3% agarose gel (Cambrex Bio Science Rockland, Inc., Rockland, ME) stained with 0.5 μg/ml of EtBr, in 0.5xTBE buffer. The PCR products were visualized using a transilluminator.

3. RESULTS AND DISCUSSION

Molecular identification confirmed all of S. aureus isolates have 23S rRNA, nuc, and coa genes. The results of the analysis of enterotoxin coding genes are presented in Table 2.

<table>
<thead>
<tr>
<th>Genes (bp)</th>
<th>Oligonucleotides sequence (5’-3’)</th>
<th>PCR condition</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 23S rRNA (1250 bp) | f: ACG GAG TTA CAA AGG ACG AC  
  r: AGC TCA GCC TTA ACG AGT AC | 37 cycles, 94°C for 5 min, 94°C for 40 sec, 64°C for 60 sec, 72°C for 75 sec, 72°C for 5 min | [14] |
| nuc (279) | f: GCG ATT GAT GGT GAT ACG GTT  
  r: ACG CAA GCC TTG ACG AAC TAA AGC | 37 cycles, 94°C for 5 min, 94°C for 1 min, 55°C for 30 sec, 72°C for 30 sec, 72°C for 5 min | [15] |
| coa | f: ATA GAG ATG CTG GTA CAG G  
  r: GCT TCC GAT TGT TCG ATG C | 30 cycles, 94°C for 5 min, 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and 72°C for 5 min | [16] |
| sea (127) | f: CTTTTGGAAACCGTTAAAACG  
  r: TCTGAACCTTCCCATCAAAAAAC | | [17] |
| seb (477) | f: TCGCATCAAACGTGACAAACG  
  r: GCAGGTACTCTATAAAGTGCTGC | | [17] |
| sec (271) | f: CTCAAGAACTAGACATAAAAAGCTAGG  
  r: TCAAAATCGGATTAACATTATCC | | [17] |
| sed (319) | f: CTTTGTGGATAATATCTCCTTATAACCCG  
  r: TCTATGCTATATCTTTATAGGGTACCATAC | 35 cycles, 95°C for 15 min, 95°C for 30 sec, 57°C for 90 sec, and 72°C for 90 sec, 72°C for 10 min | [17] |
| see (178) | f: CAGTACCTATAGATAAAAGTTAAAAACAGC  
  r: TACAGACGTGACCTTGACCCCCC | | [17] |
| seg (287) | f: AAGTAGACATTTTTGCGCTCC  
  r: AGAACCATCAAACCTGATAGC | | [13] |
| seh (213) | f: GTCTATAGGATACAAACT  
  r: GACC TTTACTTATTTGCTGC | | [13] |
| sei (454) | f: GGTGATTTTGTTAGTAAC  
  r: ATCCATATTCTTGCCCTTACAG | | [13] |
| sej (152) | f: ATAGCATCAGAAGCTTGTGTC  
  r: CTTTCTGAATTTACCACCAAGG | | [10] |
Table 2. Data of staphylococcus enterotoxin genes from raw milk of cow and goat

<table>
<thead>
<tr>
<th>Animal</th>
<th>N</th>
<th>sea</th>
<th>seb</th>
<th>sec</th>
<th>sed</th>
<th>see</th>
<th>seg</th>
<th>seh</th>
<th>sei</th>
<th>sej</th>
<th>SE gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>46</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>21</td>
<td>4</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(17.39%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(8.69%)</td>
<td>(45.65%)</td>
<td>(8.69%)</td>
<td>(0%)</td>
<td>(80.43%)</td>
</tr>
<tr>
<td>Goat</td>
<td>29</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0%)</td>
<td>(6.89%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(6.89%)</td>
</tr>
</tbody>
</table>

Data in Table 2 showed the distribution of S. aureus enterotoxin genes was different in cow and goat. The prevalence of seh, sea, seg and sei genes was 45.65% (21/46), 17.39% (8/46), 8.69% (4/46), and 8.69% (4/46), respectively (Figure 1). The seh and sea were the most frequent genes observed in the cow isolates. Different from those found in cows isolates, sec was the only S. aureus enterotoxin gene found in goat isolate.

The identification of classical enterotoxin genes is important as a basis for determining the enterotoxin gene related familial relationships. Studies on the overall super antigenic toxin genes can be used to know the pathogenesis of S. aureus and can give some advantages in diagnostic, epidemiological, and medical purposes [10,18]. The virulence factors of S. aureus, such as β-hemolysin and SEs can be applied in constructing the appropriate steps of treatment [19].

The early outbreaks of food poisoning were mostly caused by classical enterotoxin genes (sea-see and seh) [20]. In this study, the most commonly se gene in S. aureus isolates from cows was seh gene, followed by the sea gene. This result is different from the information about the enterotoxin genes found in other countries, where sea gene is the dominant gene and frequently found in foodborne disease cases. The food poisoning outbreak occurred in Japan in 2000 was known to be caused by the sea gene that resistant to high temperatures [6]. The sec, sea, sed, ser, and sej where the mostly found enterotoxin gene in Italy [7] and Iran [8].

The seh gene produces cytotoxic effects and induces apoptosis on bovine mammary epithelial cells (bMECs) in mastitis cases [21]. Negative effect due to seh gene can emerge in low bacterial concentration. Therefore, in an environmental condition that is not optimum for the growth of S. aureus, the potential health risks of seh gene is still exist [22].

In several recent cases of poisoning caused by S. aureus, it is known that the dominant causative toxin are seg, sei, sem, sen, sea, seu, but not the sea-see genes [23]. Similar finding is also reported in China, where the seg-seu toxin genes were found more frequently than the sea-sed whilst see and seh gene were absence in all isolates [9]. However, from the harmful effects, the occurrence of seg and sei genes is more important due to their potential in stimulating T-cell proliferation and inhibiting K562 and B16 cells proliferations. In the future, proteins with this kind of function can be used in drug development and cancer therapy [24].

A different pattern of enterotoxin distribution reported by Mehli et al. [25], where 87.5% of S. aureus isolated from cow milk and its processed products have sec gene, followed by seg and seh genes. In our study, the sec gene was only found in two S. aureus isolates from goat milk. This gene is of concern because it is commonly found in goat mastitis [26] and in the methicillin-resistant S. aureus group isolated from patients with prosthetic valve endocarditis (PVE) [27]. Basically, there are five genes with the highest virulence in goat milk, including Panton-Valentine Leukocidin (pvl) (29.5%), sec (23.2%), ser (16.8%), tst (14.7%), and seb (12.6%) [28].

4. CONCLUSION

The genes encoding four out of nine classical enterotoxins (seh, sea, seg, and sei) are commonly isolated from S. aureus in cow milk, but only sec gene can be found in the isolates from goat milk.

AUTHORS’ CONTRIBUTIONS

DCW, SW, FA, and SIOS are equally contributed to this work.
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