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RESEARCH ARTICLE
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Detection and Characterization of the Toxic Shock Syndrome (TSST-1) Toxin Gene *Staphylococcus aureus* in Isolates of Patients with Urinary Tract Infections

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ABSTRACT

Staphylococcus aureus is a bacteria that causes urinary tract infections. One of the toxins produced by *S. aureus* is toxic shock syndrome toxin-1 (TSST-1), which can cause multi-organ abnormalities. This disorder is characterized by the onset of fever, hypotension, disorders of the digestive organs, endothelial cells and vascular muscles. The aim of this study was to detect the presence of the TSST-1 gene encoding S. aureus isolates in isolates of patients with urinary tract infections. This research began with the conventional re-identification stage of S. aureus, followed by molecular-based identification using the PCR method. A total of 25 *S. aureus* isolates were cultured aerobically, followed by gram staining, catalase, coagulase, MSA, MR, Indole and VP tests. Molecular-based identification of S. aureus was carried out by amplifying the 16S rRNA gene, followed by amplifying the gene encoding TSST-1 as the target gene. PCR product of the gene encoding TSST-1. The results showed that conventional re-identification of *S. aureus* resulted in 100% visibility of the position of *S. aureus*. Molecular-based identification by amplifying the 16S rRNA gene gave good results, with the discovery of a DNA fragment measuring 745 bp, according to the target gene of 2 isolates (8%). As conclusion, 8% of *S. aureus* bacteria were positive for the tsst-1 gene.

Keywords: *Staphylococcus aureus*; toxic shock syndrome toxin-1 (TSST-1); urinary tract infections

INTRODUCTION

Infectious diseases are one of the problems in the world of health.(1) Urinary tract (UTI) is the second most common type of infection that occurs in the body. UTIs are one of the most common infections worldwide, but little is known about their global scale and long-term trends.(2-5) Urinary tract infection is described as the presence of microbes in significant numbers in the urinary tract.(6,7) *Staphylococcus aureus* is also a bacteria that causes chronic and urinary tract infections in cattle. Infection caused by *Staphylococcus aureus* is very difficult to control with treatment.(8) The number of UTIs due to *Staphylococcus aureus* is a high incidence.(9) Variations in *S. aureus* infection are associated with virulence factors, such as adhesion and exo-protein factors consisting of exotoxins and enzymes, including nuclease, protease, lipase, hyaluronidase, and collagenase. The exotoxins produced by S. aureus are haemolysin, -haemolysin, -haemolysin, Panton Valentine leukocidin (PVL), Toxic Shock Syndrome Toxin-1 (TSST-1), Staphylococcal Enterotoxin (SE) and Exfoliative Toxins (EF).(10-12)

TSST-1 is an example of the SAgs exotoxin group which has the ability of pyrogenicity, super-antigenicity and crosses mucosal surfaces. The gene encoding tsst-1 is present in the pathogenicity island produced in the post-responsive phase. TSST-1 is a small, nonglycosylated, polypeptide molecule weighing approximately 22kD. The toxin is stable against chemicals, resistant to hot and dry conditions.(13) TSST-1 is one of the main virulence factors and the main cause of Toxic Shock Syndrome (TSS).(14-16)

TSST-1 has the ability to activate T lymphocytes through direct binding between T Cell Receptor (TCR) Vβ with Major Histocompatibility Complex class II (MHC II) molecules. Activation results in the production of proinflammatory cytokines, including Interleukin (IL2), Interferon (IFN) and Tumor Necrosis Factor (TNF) causing symptoms of high fever, red rash, desquamation, hypotension, and acute, life-threatening multi-organ failure which is a symptom of TSS.(10-12) The ability of bacteria to cause infection depends on the number of virulence factors of the colonizing bacterium.

Research on *Stapylococcus aureus* producing TSST-1 toxin from clinical patient isolates in Indonesia is still rare. This study aimed to detect the presence of the TSST-1 gene encoding S. aureus isolates in isolates of patients with urinary tract infections.

METHODS

Several tests were carried out for the identification of *S. aureus*. Initially Gram's staining was performed to identify the morphology of the organism, and various biochemical tests such as catalase test, oxidase test, Mannitol salt agar test, slide coagulase, motility, citrate, and fermentation of carbohidrat (fructose, galactose, lactose, maltosel, mannose, sucrose) test were carried out.

Genomic DNA from the bacteria was isolated from the samples with chloroform-phenol method. Initially 1.5ml of 24 hours bacterial culture was taken and subjected to centrifugation. The resulting pellet was treated with 1000 µl of DNAzol and vortex was incubated for 20 minutes at 25°C, add 200µl of kloroform and vortex and centrifuged. Later 600 µl of isopropanol was added and centrifuged. The pellet was washed with 75% ethanol and dried. Finally, the DNA pellet was stored in nuclease free water. Then the DNA fragments were Measured by Nanodrop ™ 2000 Thermo scientific spectrophotometer.

The TSST01 gene was amplified by using two sequence of the primers 3′. *S. aureus* isolates were tested for the presence of the 745-bp PCR product of the TSST-1 gene. The initial denaturation was at 94°C for 45 second. Denaturation of 94°C for 20 minute, annealing at 57°C for 30 second and elongation temperature of 72°C for 30 minute was maintained for 30 cycles. The final elongation was at 72°C for 3 minutes and the reaction was held at 4°C. The amplified products were subjected to 2% agarose gel electrophoresis.

RESULTS

The results of bacteriological culture examination of the twenty-five samples showed that all samples (100%) were positive for *S. aureus* (Table 1). The results of the PCR examination are shown in Figure 1. Results Bacteriological culture results

Table 1. Bacteriological culture results

Sample	*S. aureus*	
	Positive	Negative
M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, M25	100% (25 samples)	-

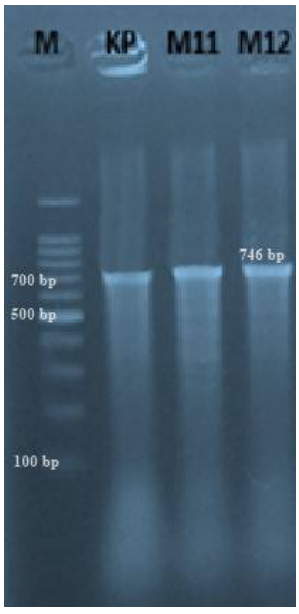

Figure 1. Agarose gel electrophoresis of PCR products obtained from amplification of the TSST-1 gene. Lane M: 100bp DNA ladder marker. Lane KP: Positive control. Lane M11 and M12: sample Lanes: PCR products with the expected size of approximately 745 bp.

DISCUSSION

Twenty five different samples were collected from persons. Among these samples, 25 isolates clinical samples are spherical colonies of greyish white colour. The isolates are spherical, greyish white colonies were again subcultured in a new blood agar medium and MSA (mannitol salt agar) were incubated for 24 hours. The results of bacteriological examination are then followed by comparing the bacterial characters of *S. aureus*. Observation on *S. aureus* bacterial characterization is conducted using a conventional method.[17] Based on the bacterial comparison of *S. aureus* characters, it can be concluded that samples are *S. aureus* bacteria.

TSST-1 is a small, nonglycosylated, polypeptide molecule weighing approximately 22kD. The toxin is stable against chemicals, resistant to hot and dry conditions.[14] TSST-1 is one of the main virulence factors and the main cause of TSS.[14-16] TSST-1 has the ability to activate T lymphocytes through direct binding between T Cell Receptor (TCR) Vβ with Major Histocompatibility Complex class II (MHC II) molecules. Activation results in the production of proinflammatory cytokines, including Interleukin (IL2), Interferon (IFN) and Tumor Necrosis Factor (TNF) causing symptoms of high fever, red rash, desquamation, hypotension, and acute, life-threatening multi-organ failure which is a symptom of TSS.[10-12] The ability of bacteria to cause infection depends on the number of virulence factors of the colonizing bacterium. Research on *Stapylococcus aureus* producing TSST-1 toxin from clinical patient isolates in Indonesia is still rare.

The visualization results of agarose gel electrophoresis in Figure 1 show that the DNA bands in the positive control obtained were very good. The electrophoresis results showed that the size of the positive control band obtained was 745 bp. The visualization results of the TSST-1 gene amplication showed that only 2 samples had bands out, which indicated that the 2 isolates were positive for the TSST-1 gene. The inability of *S. aureus* to produce this toxin reduces the virulence factors of *S. aureus*.[16,18-21]

Staphylococcus aureus is also a bacteria that causes infections of the urinary tract and chronic in cattle. Infection caused by *Staphylococcus aureus* is very difficult to controlled by treatment[8], and the number of UTIs due to *Staphylococcus aureus* is a high incidence rate. Variations in *S. aureus* infection are related to factors virulence, such as adhesion and exo-protein factors consisting of exotoxins and enzymes, including nuclease, protease, lipase, hyaluronidase, and collagenase. The exotoxin produced by S. aureus are haemolysin, -haemolysin, -haemolysin, PantonValentine leucocidin (PVL), TSST-1, Staphylococcal Enterotoxin (SE) and Exfoliative Toxins (EF).[10-12] TSST-1 is an example of the exotoxin SAgs group has the ability of pyrogenicity, super-antigenicity and crosses mucosal surfaces. Gen tsst-1 encoding is present in pathogenicity islands produced in the post-responsive phase. Toxic Shock Syndrome Toxin (TSST-1) is a small, nonglycosylated, polypeptide molecule weighing approximately 22kD. The toxin is stable against chemicals, resistant to heat conditions and dry.[13]

CONCLUSION

Based on the results of the study, showed that 8% (2 samples) of *S. aureus* bacteria were positive for the tsst-1 gene.

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