Detection of Staphylococcus aureus in wound infection on the skin surface

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Abstract. Wound infection on the skin surface is colonized a wide variety of microorganisms. Microorganisms that cause inflammation of the skin surface is group of pyogenic bacteria. Staphylococcus aureus is one of the class of pyogenic bacteria that produce β-lactamase enzyme and eliminate the antibacterial activity, especially penicillin. The existence of this enzyme will destroy β-lactam ring, so that antibiotics become inactive. This study aimed to detect presence of Staphylococcus aureus on pus from wound infection on the skin surface. This study was descriptive qualitative. Three samples of pus was isolated and identified by culture and biochemical testing using RapID STAPH PLUS. The results of the study identified two isolates of Staphylococcus aureus with a probability >99.9% and 1 isolate of Staphylococcus hyicus with a probability 86.92%.

1. Introduction

The body's main defense against pathogens, toxins, and wounds of the skin. Damage to the skin can cause significant morbidity and even mortality [1]. The process of wound healing of the skin infection is a complex process, involving deposition of collagen in response to tissue injury, and at the final stage produce scar formation. Such mechanisms include inflammation, fibroplasia, and maturation of scar tissue [2].

Wound infection on the skin surface easily in colonization by a wide variety of organisms. Some studies suggest several different kinds of bacteria isolated from patients living in areas with different geographical. Microorganisms that cause inflammation is a group of pyogenic bacteria [3-4]. Skin infections include pyoderma minor to severe necrotizing infections. The skin can be infected by a variety of microorganisms, including bacteria, fungi and parasites. Skin infections are most often caused by bacteria. Gram positive bacteria are the most common causes of skin infection is hemolytic Streptococcus and Staphylococcus aureus. Gram-negative rod bacteria that can cause skin infections such as Pseudomonas aeruginosa, Escherichia coli, Enterobacter sp., Klebsiella sp., and Proteus sp. [5].

Staphylococcus aureus is cosmopolitan and a growing problem in the community and hospitals. This an infection caused by Staphylococcus aureus is usually treated with antibiotics, but in some cases it has been found that some strains of Staphylococcus aureus resistant to antibiotics [6]. Staphylococcus...
Staphylococcus aureus can cause various types of infections, such as skin infections, food poisoning to systemic infection. Skin infections caused by Staphylococcus aureus, i.e impetigo, cellulitis, folliculitis, and abscesses. Staphylococcus aureus has a β-lactamase enzyme capable of breaking the ring of β-lactam antibiotics, so antibiotics become inactive [7]. This study aims to detect of Staphylococcus aureus in the pus taken from the wound on the skin surface.

2. Methods

2.1. Sample Collection
Pus samples were taken from the wound on the skin surface. Samples were taken using a sterile swab and inserted into NaCl broth media. Sample preparation were performed in the Laboratory of Microbiology, Faculty of Health Sciences, Maarif Hasyim Latif University, Sidoarjo, East Java, Indonesia.

2.2. Instruments, Reagents, and Medium
The instrument used in this study is sengkelit, object glass, pipette pasteur, vortex, incubator, BSC, NAS, BAP, MSA, Loeffler serum, Gram stain, Rapid Staph PLUS, Mc Farland 3 standard, RapID STAPH PLUS reagent, RapID Nitrate A, RapID Nitrate B, H2O2 10%, reagent oxidase, plasma citrate.

2.3. Preparation of Samples
Pus in NaCl broth grown in BAP and MSA, then incubated at 370 C for 24 hours. Suspected bacterial colonies on BAP and MSA made preparations and stained using the Gram stain. If found Gram-positive cocci, then planted on Loeffler serum and NAS, incubated 370 C for 24 hours.

2.4. Morphological Observation Bacteria
Mixture of bacteria that has been coloured with Gram stain, observed the shape, structure and properties of the staining bacteria using a microscope [4].

2.5. Catalase Test
One drop PZ daubed on the object glass and inoculate 1-2 bacterial colonies add 2 drops 3% H₂O₂ reagent and then homogenized. A positive result of catalase test if appear bubbles [8,9].

2.6. Coagulase Test
For coagulase test, one drop of plasma citrate on object glass and inoculate 1-2 bacterial colonies and then homogenized. Positive results were characterized by the formation of plasma flocculation [8,9].

2.7. Preparation of Inoculum
Bacteria test must be in pure culture was tested with Gram staining and catalase test. Only catalase test was positive, Gram-positive cocci that was specific traits of stafilokoki tested using the RapID STAPH PLUS. Gram-positive cocci, and catalase test negative cannot be tested by RapID STAPH PLUS, while Gram-positive rod cannot be tested by the RapID STAPH PLUS.

Taken the solid test bacterial colonies in NAS and made the suspension in the inoculation Rapid Fluid 2 (2 mL) and observed turbidity and then synchronized with the Standard Mc Farland 3. If the turbidity level was the same as the Standard Mc Farland 3, hereinafter the mix using a vortex. Suspension of test bacteria can be used more than 15 minutes.

2.8. Panel Inoculation RapID STAPH PLUS
Close the panel on inoculation port is opened by pulling the label marked "peel to inoculate" up and to the left. By using a pipette, the entire inoculum was added to the top right corner of the back panel and close the port inoculation by pressing the lid label back into place. Furthermore, the panel tilt angle 45° toward the test wells. While tilted backwards, the panel is shaken gently from side to side to distribute the inoculum evenly along the back baffle.
In the horizontal position, slowly tilted to the front panel so that the inoculum flows along the bulkhead and into the reaction wells (test wells). In this process, all of inoculum on port inoculation should all move to the reaction wells (test wells) on the panel. And the panel is returned to its original position. If necessary, tap gently on the table panel to remove trapped air in the reaction wells (test wells).

2.9. Incubation Panel RapID STAPH PLUS
Panel RapID STAPH PLUS incubated aerobically at 35-37°C in the incubator for 4-6 hours. For handling easily, the panel can be incubated in incubation trays chipboard provided in the kit RapID STAPH PLUS.

2.10. Assessment Panel RapID STAPH PLUS
RapID STAPH PLUS there are 18 wells reaction (test wells) with 18 test results, the reaction wells (test wells) 13-18 numbers require additional reagents to obtain the test results, which are illustrated as follows:

Holding the RapID Staph Plus panel, lid labels on the reaction wells (test well) was opened. In the first test wells (ADH) to 12 (URE) is read without the addition of reagents. For the reaction wells (test wells) 13 (PYR) to 18 (NIT) need the addition of reagent as follows:
- In the test wells 13 (PYR) to 17 (LGLY) was added 2 drops of Rapid Staph PLUS reagent
- In the wells 18 (NIT) was added 1 drop of reagent Rapid Nitrate A and 1 drop of reagent Rapid Nitrate B

After the addition of the reagents in the reaction wells (test wells) 13 to 18, further reading and assessment of reaction wells (test wells) from left to right using manual interpretation. Values in report form is recorded for subsequent identification of isolates using microcode contained in the ERIC web.

3. Results and Discussion
The results of the isolation and identification of *Staphylococcus aureus* in a sample of pus from the wound on the skin surface using the RapID STAPH PLUS is presented in Table 1 and Figure 1.

| No. | Sample Code | Microcode | Probability Type Bacteria (%) |
|-----|-------------|-----------|-------------------------------|
|     |             |           | *S. aureus* | *S. hyicus* | *S. epidermidis* |
| 1   | Sa1         | 473 004   | >99.9        | -          | -                |
| 2   | Sa2         | 573 014   | >99.9        | -          | -                |
| 3   | Sa3         | 470 004   | 5.77         | 86.92      | 7.27             |

Results identified in 3 samples of pus taken from wound infections of the skin was found three types of pathogens, namely the sample code Sa1 was found *Staphylococcus aureus* with a probability >99.9%, Sa2 was found *Staphylococcus aureus* with a probability >99.9%, and Sa3 was found *Staphylococcus hyicus* with probability 86.92%.
Bacterial inoculum was inoculated in RapID STAPH PLUS after incubated overnight, producing some media reaction biochemical to the RapID STAPH PLUS. The result of reaction bacteria showed physiological properties, which allows us to identify unexpected bacteria *Staphylococcus aureus*.

Complications caused by Staphylococcus aureus is a major clinical problem. Group pyogenic bacteria causes skin infection most common are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Neisseria gonorrhea, Mycobacterium tuberculosis*, etc. [3,4].

*Staphylococcus aureus* infection characterized by tissue damage accompanied by purulent abscess. Infectious disease caused by *Staphylococcus aureus* such as pimples, boils, impetigo and wound infections. More several infections such as pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, seomielitis, and endocarditis. *Staphylococcus aureus* can also cause nosocomial infections, food poisoning and toxic shock syndrome [10,11].

### 4. Conclusion

Identification of bacteria in three samples of pus, found *Staphylococcus aureus* in the sample code Sa1 and Sa2 with probability >99.9%, and *Staphylococcus hyicus* in the sample code Sa3 with probability 86.92%.

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