

## ***MICROBIOLOGICAL ASPECT OF STAPHYLOCOCCAL INFECTION IN SEVERE DEGREE OF BURNS***

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### ***ABSTRACT***

***Introduction:*** Burns serve as one of the most common devastating forms of trauma. Significant thermal injuries induce skin disruption and immunosuppression that predispose patients to infectious complication. The importance of sampling, detection, and proper treatment for wound infections will significantly increase the likelihood of clinical improvement of the patients. ***Case:*** A 44-years-old male came to hospital due to electrical shock. There were serious burns to his face, chest, arms, and legs, some of which were second- and third-degree burns. The patient was conscious, without problems on airway and breathing. Leukocytosis with neutrophilia was presented from laboratory examination, with an increase in liver enzymes and serum creatinine. The gram-positive cocci with leucocytes were revealed from gram examination, and colonization of *Staphylococcus aureus* was found. Intravenous cefazolin was administered according to the sensitivity test. ***Discussion:*** Infection causes the increase in morbidity and mortality of burn patients. Clinical diagnosis of infection must be supported microbiologically by gram examination and culture. One of the most common infectious agents in severe burns is *Staphylococcus aureus*. The significance of *S.aureus* as the leading cause of infection must be supported by microbiological data. Dominant growth in culture media and induction of host inflammatory response were indicated as significance of infection. ***Conclusion:*** Clinical and microbiological diagnosis of burns infection play important role to prevent complications in severe degree of burns.

***Keywords:*** *Staphylococcus aureus, Burns, Microbiology, Antibiotics.*

### ***ABSTRAK***

***Pendahuluan:*** Luka bakar merupakan salah satu bentuk trauma yang serius. Cedera termal berat dapat menyebabkan rusaknya barier kulit dan imunosupresi, yang dapat menyebabkan komplikasi infeksi. Pentingnya pengambilan sampel, deteksi, dan penanganan infeksi luka bakar yang benar akan meningkatkan

kemungkinan perbaikan klinis penderita. **Kasus:** Seorang laki-laki usia 44 tahun datang ke rumah sakit karena tersengat listrik. Luka bakar yang berat ditemukan pada wajah, dada, lengan, dan kaki, baik luka bakar derajat dua maupun derajat tiga. Kesadaran pasien didapatkan baik, tidak ada masalah pada jalan napas maupun respirasi. Lekositosis dengan netrofilia didapatkan pada pemeriksaan laboratorium, dengan peningkatan enzim liver dan kreatinin serum. Pada pemeriksaan gram didapatkan kokus gram positif dengan lekositosis, dan kolonisasi *Staphylococcus aureus* ditemukan dari kultur. Cefazolin intravena diberikan sesuai pemeriksaan sensitivitas antibiotik. **Diskusi:** Infeksi meningkatkan morbiditas dan mortalitas pasien luka bakar. Diagnosis infeksi harus didukung secara mikrobiologi dengan pemeriksaan gram dan kultur. Salah satu agen infeksi yang sering ditemukan pada luka bakar berat adalah *Staphylococcus aureus*. Signifikansi *S.aureus* sebagai penyebab infeksi harus didukung oleh data mikrobiologi. Pertumbuhan dominan pada media kultur dan induksi respon inflamasi menentukan signifikansi tersebut. **Kesimpulan:** Diagnosis klinis dan mikrobiologis infeksi luka bakar berperan penting untuk mencegah komplikasi pada luka bakar derajat berat.

**Kata Kunci:** *Staphylococcus aureus*, Luka Bakar, Mikrobiologi, Antibiotik.

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## INTRODUCTION

Severe burns are the most serious form of trauma, causing anatomical, physiological, endocrine, and immunological disorders, especially in burns that involve more than 20% of the body surface area.<sup>1</sup> Burns cause thermal damage to the skin, with 3 characteristic areas. First, the coagulation zone closest to the thermal source, includes the dead

tissue that forms the eschar wound. Near the necrotic tissue there is a second area called the stasis zone, which is still viable but has a risk of ischemia due to impaired perfusion. The hyperemia zone is the third, consisting of relatively normal skin tissue with increased blood flow and vasodilation, with minimal cellular damage.<sup>2</sup> Overall, the main concern for burns is the eschar wounds,

because they are avascular, so that immune cells and systemic antibiotics can't reach.

Management of burns includes primary resuscitation of airway, breathing, and circulation, with secondary management of nutrition, wound care and infection control. The six most common complications of burns are: pneumonia 4.6%, sepsis 2.7%, cellulitis 2.6%, respiratory disorders 2.5%, wound infection 2.2%, other infections 2%.<sup>2</sup> The source of infection in burns could be endogenous or exogenous, and could be changed over time. Severe burns cause mechanical damage to the skin, allowing microbes to penetrate the deeper tissues. In addition, there is a moist and protein-rich avascular tissue in burns that is ideal for the growth of microorganisms. In the initial phase of burns, the wound is sterile, but in 48 hours it will be colonized with skin pathogens from the sweat glands or hair follicles. After 5-7 days, the wound will be colonized by fungi or bacteria, adapted from the gastrointestinal, upper respiratory tract, or from the environment.

Although *Streptococcus pyogenes* was the most

common pathogen in the past, it has now been replaced by *Staphylococcus aureus* and gram-negative pathogens, such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*.<sup>2</sup>

## CASE DESCRIPTION

A 44 years-old male, a construction laborer, came to the hospital due to electrical shock. While working on the 2<sup>nd</sup> floor, the patient was accidentally exposed to electricity and was knocked into the canopy and then fell to the first floor ( $\pm$  5 meter). When found, the shirt and pants had been burnt, and wounds were found on the face, chest, arms, and legs. There was no active bleeding and the patient was conscious. History of hypertension, diabetes, and blood clotting disorders were denied.

In primary survey, the airway and breathing was clear, with the breath rate of 24 times/ minute. The blood pressure was 90/70 mmHg with pulse rate of 110 bpm. The patient was conscious (E4V5M6) and the pupil reflex was symmetrical 3/3 mm. In secondary survey, there were wounds on the face, chest, upper and lower limbs, with 33% grade IIAB

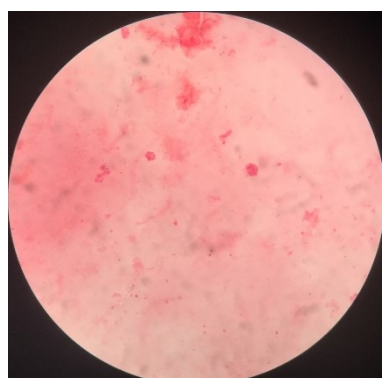
burns and 15% grade III burns (Figure 1).

The patient was diagnosed with 48% second and third-degree of burns. Fluid resuscitation was given

according to the Baxter formula with the total of 11,5 L, of which was divided into 5,7 L in the first 8 hours of onset and 5,7 L in the following 16 hours.



**Figure 1.** The patient with second-third degree of burns on clinical manifestation



**Figure 2.** Gram positive cocci on gram examination

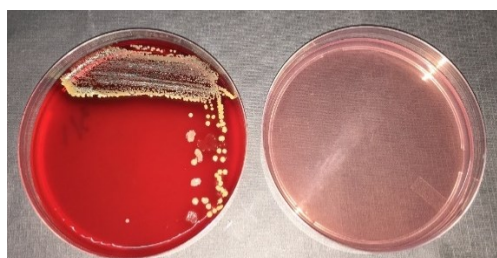
Blood examination showed leucocytosis 11,43 ( $4,10-11,00 \times 10^3/\mu\text{L}$ ) with neutrophilia 9,52 ( $2,50-7,50 \times 10^3/\mu\text{L}$ ); elevated liver enzyme SGOT 713,2 (11-33 U/L), SGPT 169,6 (11-50 U/L); hypoalbuminemia 1,9 (3,4-4,8 g/dL); and decreased renal function BUN 61 (8-23 mg/dL), kreatinin 4,95 (0,70-

1,20 mg/dL). Electrocardiography showed normal sinus rhythm 97 x/min, and chest radiology was within normal limit. There was gram positive cocci found on direct gram examination from the wound base (Fig 2).

Culture on blood agar from wound base showed 2 types of

colonization. The first was white-yellowish colony, bigger in size and grew dominantly, with buttery consistency. The another grew only on second quadrant, smaller in size with transparent white color. Culture on mac conkey agar did not reveal any growth (Fig 3). The first colony was catalase + (Fig 4) and coagulase + (Fig 5), but the second colony was negative for both.

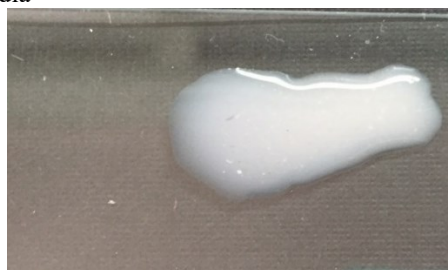
Identification and antibiotic sensitivity test were done with Vitek2 system. The first colony was identified as *Staphylococcus aureus*, with negative cefoxitin screening. The preferred antibiotic was first generation of Cephalosporin (cefazolin) (Table 1). The second colony was identified as *Streptococcus dysgalactiae ssp equisimilis*



**Figure 3.** Culture on blood agar and mac conkey media



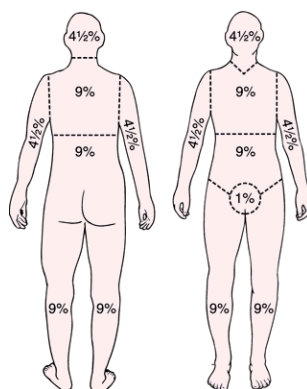
**Figure 4.** Positive catalase test for the first colony



**Figure 5.** Positive coagulase test for the first colony

**Table 1.** Antibiotic sensitivity test for *S.aureus*

Antimicrobial	Interpretation	Antimicrobial	Interpretation
Cefoxitin screening	Negative	Ceftriaxone	S
Nafcilin	Sensitive (S)	Cefepime	S
Amoxicilin	R	Gentamycin	S
Cloxacillin	S	Ciprofloxacin	S
Dicloxacillin	S	Levofloxacin	S
Flucloxacillin	S	Erythromycin	S
Cefadroxil	S	Clindamycin	S
Cefalothin	S	Azithromycin	S
Cefazolin	S	Tetracycline	R
Cefuroxime	S	Trimethoprim/Sulfamethoxazole	S



**Figure 6.** Rule of Nine

## DISCUSSION

Burns due to heat, electrical injury, chemical injury, and lightning cause disruption of the skin, mucous membrane, and deeper tissues. Diagnosis is made clinically based on history taking and physical examination. The burn area was determined by the *Rule of Nine* (Fig 6), but the degree of burns is classified as first-, second-, or third-degree, depending on how deep and severe the burns penetrate the skin surface. First degree burns affect only the epidermis, the burn site is red and painful, but long-term tissue damage is rare. Second-degree burns involve the epidermis and part of the dermis as well. The burn site appears red, blistered, may be swollen and painful. The third degree burns destroy the

epidermis and dermis. It may also damage the underlying bones, muscles, and tendons. The burn site appears white or charred. There is no sensation in the area since the nerve endings are destroyed.<sup>1</sup>

Diagnosing infection of burns is taken from several criteria, one of which is ABA (*American Burn Association*, table 2). Changes in the eschar tissue to blackish brown or purplish color and the formation of edema at the edges of the wound are the clinical signs of ongoing infection. Although the definitive diagnosis of infection is made histologically, but this procedure is not done routinely since it takes a long time and the correlation between culture and systemic infection is still unclear.<sup>2</sup>

**Table 2.** Infection of burns (*American Burn Association*)<sup>2</sup>

<b>Syndrome</b>	<b>Clinical and pathological criteria</b>
<b>Wound colonisation</b>	Bacteria on the wound surfaces at low concentrations (< 10 <sup>5</sup> /g tissue); no invasive infection
<b>Wound infection</b>	Bacteria on wounds and eschar at high concentrations (>10 <sup>5</sup> /g tissue); no invasive infection
<b>Invasive infection</b>	Bacteria on wounds at high concentration (>10 <sup>5</sup> /g tissue), may cause separation of the eschar tissue, invasion of healthy tissues, or sepsis
<b>Cellulitis</b>	Bacteria on wounds at high concentration (>10 <sup>5</sup> /g tissue), with erythema, induration, pain and inflammation of surrounding tissues.
<b>Necrotizing fasciitis</b>	Agresive infection with the necrosis of deeper tissue

In this case, diagnosis of burns was made since the skin damage appeared immediately after contact with high-voltage electrical injury. There were multiple bullae on physical examination with some of the skin appeared whitish in color, indicating the second- and third-degree burns. The total body surface area of burns was 48%; 3% on the face, 17% on the chest, 4% on the back, 11% on upper extremities, 12% on lower extremities and 1% on genitalia. Some of the burns was clinically infected, noted by the changes of the eschar into blackish brown in color on the face and chest and the formation of edema on the edge of the burns. Based on ABA criteria, this case was already invasively infected with bacteria, characterized by the presence of leucocytosis with neutrophilia on the complete blood count examination.

The multiorgan dysfunction was already noted since there were increment of liver enzyme and impaired kidney function.

Gram stain is a simple and inexpensive laboratory examination carried out in cases with suspicion of bacterial and fungal infection. The main difference between gram positive and gram negative bacteria is that gram positive bacteria contain a thick peptidoglycan cell wall along with teichoic acid, allowing the bacteria to stain in purple during gram staining whereas gram negative bacteria contain a thin peptidoglycan cell wall with no teichoic acid, allowing the cell wall to stain in pink during counter staining.<sup>5</sup> Staphylococci are gram positive bacteria, arranged in cluster on gram stain, while Streptococci arranged in

pairs or in rows to form chain on gram stain.<sup>4,5,6</sup>

Gram results in this case showed leukocytes +1 (1-5/field of view), no epithelium, and gram-positive cocci +2 (11-25/field of view). This result shows that the specimens taken were quite good, because there was no epithelium, and the inflammatory response to infection was positive because of leukocytes +1. However, the morphology of the cocci arrangement that appeared in clusters or in rows was not clearly visible.

There are several categories in culture media: (1) Non-selective media that support the growth of nonfastidious bacteria, for example blood agar media; (2) selective media that support the growth of one type or group of microbes but inhibit the growth of other microbes, such as mac conkey agar which is selective for gram-negative bacteria, and CNA (Columbia agar with colistin and nalidixic acid) which is a selective medium for gram-positive bacteria; (3) differential media that show the characteristics of microbial growth in the media. This type of media can be differential and nonselective such as

sheep blood agar (can differentiate organisms based on hemolysis characteristics), or differential and selective such as mac conkey which inhibits gram positive but selective for gram negative based on lactose fermentation; (4) enriched media, which are media containing ingredients that can support the growth of fastidious bacteria, for example, chocolate agar; (5) broth media used as a supplement to detect small amounts of aerobic and anaerobic bacteria, an example of this media is THIO (thioglycolate broth).<sup>7</sup> The medium selected for culture is determined by the type of specimen and the organism that is likely to cause the infectious process. Specimens from the wound base usually require non-selective media, selective and differential media for gram-negative, selective media for gram-positive, and broth media used as a supplement if only a few bacteria are growing. The culture media were incubated at 35-37oC for 48-72 hours.<sup>7</sup>

In this case, culture was performed on 2 media, namely blood agar which is a non-selective medium, and Mac Conkey's agar



which is a selective medium for gram-negative. Meanwhile, selective culture of gram-positive and broth media was not carried out because the two media (blood agar and mac conkey) were able to analyze the growth of gram-positive bacteria, gram-negative bacteria and fungi. Colony growth on blood agar can show gram-positive, gram-negative, and fungi. While the colony growth on Mac Conkey only showed the growth of gram negative bacteria.

The most common gram-positive bacteria in burns are Staphylococci and Streptococci, and catalase test can distinguish both.<sup>2</sup> Staphylococci are gram-positive cocci that produce catalase, are non-motile, do not form spores, and are aerobic or facultatively anaerobic. Colonies formed after incubation for 18-24 hours at a temperature of 35-37°C had the following characteristics: colonies were round, medium in size (4-8 mm), cream-white to yellowish in color, smooth surface, with buttery looking. *S.aureus* can produce a hemolytic zone around the colony and produce a yellow pigment on longer incubation. This organism is a normal flora found

on the skin and mucous membranes of humans. Staphylococcal species are distinguished by a coagulase test, in which a positive result will form a clot in the plasma due to the presence of clumping factor, which causes agglutination in human, rabbit, or pig plasma, and is a major marker for *S.aureus*. Coagulase positive staphylococci consisted of *S.aureus*, *S.intermedius*, *S.delphini*, *S.lutrae*, and *S.hyicus*. These species are frequently found in animals and are rarely isolated from human specimens, except for *S.aureus*.<sup>5,8</sup>

Streptococci are gram-positive cocci that do not produce catalase, and on culture they will show small, transparent colonies. *S.dysgalactiae* subspecies *equisimilis* is a group C hemolytic streptococcus.<sup>6,9</sup> Infections caused by *S.dysgalactiae* subspecies *equisimilis* are rare, but can involve several areas of the body. The spectrum of infection may resemble that of *S.pyogenes* and may include upper respiratory tract infections, skin and soft tissue infections, and invasive infections such as necrotizing fasciitis. Group C streptococci are common pathogens

found in domestic animals, birds, rabbits.<sup>10</sup>

In this case, 2 colonies grew on blood agar media. The first colony grew dominantly in 2 quadrants and was larger in size, and yellowish white in color, soft solid texture like butter. The second colony was smaller and transparent white. The catalase test was positive on the first bacterial colony, which means that the first colony was a staphylococcal. Then proceed with a coagulase test on the same colony, and the test was coagulase positive, which mean that the staphylococci were subspecies of aureus. While the catalase test on the second colony was negative, which means that the second colony that did not grow dominantly was a streptococcus. Then the two colonies were identified on the vitek and the results of the analysis showed that the two colonies were *Staphylococcus aureus* and *Streptococcus dysgalactiae ssp equisimilis*.

*S.aureus* can be isolated in a number of mild to severe infectious diseases, also in burns. It has many virulence factors such as enterotoxins, cytolytic toxins, exfoliative toxins, and cellular

components of protein A. These bacteria also have high natural resistance, so they are called opportunistic pathogens. Infection can be suppurative or toxin-mediated, and the severity was determined based on the virulence of the bacteria, the amount of inoculum, and the immune system of the host. Infection begins with a breakdown of the skin/mucosa barrier so that Staphylococci can enter deeper tissues and blood vessels, activate an acute inflammatory response, and proliferate polymorphonuclear cells. Opportunistic infections usually occur as a result of existing skin damage, such as lacerations, burns, surgical wounds.<sup>5,8</sup>

In this case, the causative agent that considered significant was *Staphylococcus aureus* because this bacterium was found to be dominantly growing on blood agar, accompanied by gram examination which showed an inflammatory response to infection (leukocytes +1, epithelium absent, cocci +2). *S.aureus* is often found in burns of less than 5 days, especially in burns with an area of >20% of the body surface. In addition, these bacteria

have various virulence factors that can accelerate the worsening of infection. Meanwhile, *Streptococcus dysgalactiae ssp equisimilis* is not considered significant as the causative agent of infection because this bacterium is only found in a small amount in culture media, and this bacterium is a normal flora in animals and rarely causes serious infections in humans..

Treatment of infection in patients with burns depends on the site of infection and the isolated pathogen. Empirical therapy is given depending on the resistance profile. The pharmacokinetics of antibiotics in patient with burns may be impaired, so serum creatinine examination should be performed.<sup>1,2</sup>  $\beta$ -lactams inhibit bacterial growth by interfering cell wall formation. These drugs bind to enzymes involved in the formation of peptidoglycan into cell walls.<sup>12</sup> Penicillinase-resistant penicillins have residues on the side chain that inhibit the binding of  $\beta$ -lactamases from Staphylococci, so these penicillins are very useful as a therapy for infections caused by *S.aureus*. Antibiotics of this class include nafcillin, oxacillin, and

dicloxacillin. However, currently many strains of *S. aureus* are resistant to methicillin.<sup>11</sup> This resistance mechanism is not mediated by penicillinase, but by PBP2A (penicillin-binding protein 2A) which is encoded by the *mecA* gene. PBP2A can take over cell wall formation when normal PBPs from staphylococci are inhibited by  $\beta$ -lactam agents.<sup>5,12</sup> To determine resistance to methicillin, a screening test using cefoxitin can be performed. However, definitive detection for the MRSA (methicillin-resistant *Staphylococcus aureus*) group was carried out using PCR (Polymerase Chain Reaction) to detect the presence of the *mecA* gene.<sup>13</sup>

In this case, the isolated *S. aureus* was still MSSA (methicillin-sensitive *Staphylococcus aureus*) because the results of the cefoxitin screening were negative. The recommended antibiotics are antistaphylococcal penicillins such as cloxacillin, dicloxacillin, but these antibiotics are not yet available in Indonesia. Another option is other  $\beta$ -lactams such as cephalosporins. From the results of antibiotic sensitivity, the patient was still sensitive to cefazolin,

cephalothin, cefadroxil. Considering the condition of the patient being treated at the Burn Unit, the patient requires more systemic antibiotic therapy. Therefore, the recommended treatment option is a first-generation cephalosporin injection, namely cefazolin.

### CONCLUSION

Burn infection is one of the causes of morbidity and mortality in burn patients. Clinically, the diagnosis of infection can be made based on the criteria of the American Burn Association and supported microbiologically by gram and culture examination. One of the infectious agents that are often found in burns with a surface area of > 20% is *Staphylococcus aureus*. Because *S.aureus* is a normal flora of the skin, the significance of these bacteria as the causative agent of infection must be supported by the dominant growth of bacteria in the culture media and the inflammatory response on gram examination and peripheral blood leukocytosis. Treatment with definitive antibiotics according to the sensitivity of the bacteria should be given to accelerate clinical

improvement and prevent prolonged administration of antibiotics.

### INFORMED CONSENT

Informed consent was given from the patient's parent for publication of this case.

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### CONFLICT OF INTEREST

The authors have no conflict of interest.

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