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Original Article

The Relationship Between Leucocytes and Bacterial Count in The Wound Swab Sample with Colony Counting In Wound Tissue Infection

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ABSTRACT

Background: Wound tissue infection is a problem in developing countries. The cause of the infection must be known, so that appropriate antibiotics are given for that required microbiological examination. The number of Polymorphonuclear leukocytes (PMN) >25/HPF and the number of colonies >10^5/Colony Forming Unit (CFU) determine the occurrence of infection. Proving the relationship between the number of leukocytes and bacteria in the wound swab sample to the number of colonies in superficial Surgical site infection (SSI).

Methods: This study was an Observational analytical research design with 35 samples of superficial SSI. Microscopic examination: gram stain to see the number of leukocytes and bacteria in a large field of view. Culture examination: number of colonies, identification of microorganisms, and patterns of antibiotic sensitivity with automatic machines. Statistical analysis for the relationship between the number of leukocytes and bacteria to the number of colonies was Chi-square and logistic regression.

Results: Superficial SSI samples were more common at the age <60 years, with malignancy comorbid. The most common cause of infection is *Escherichia coli*. The results of the Chi-square test showed that the number of leukocytes (p=0.017) and the number of bacteria (p=<0.01) were related to the number of colonies, and logistic regression tests found that the number of bacteria was significant to the number of colonies (p=0.010, 95% C1: 0.030–0.624).

Conclusion: The number of bacteria associated with the number of bacterial colonies in patients with superficial SSI.

INTRODUCTION

Surgical site infection (SSI) is a problem worldwide, especially in developing countries. Surgical wound infections lead to prolonged hospital stays, and this increases the cost of treatment. Epidemiological studies are difficult to do because there are many factors cause SSI. The incidence varies widely because it is influenced by several things, such as surgical procedures

between surgeons, between hospitals, and between patients. The incidence of SSI is influenced of patient condition, and by the procedure or action taken.[1,2]

According to the World Health Organization (WHO), from 27 million patients who were operated on, 2-5% had SSI and all of them were nosocomial infections. In the United States, approximately 30 million cases of surgery are performed annually, and 300,000 to 500,000 patients experience infection at the surgical site. However, in 2008 and 2014, the incidence of SSI decreased by 17% from 10 different surgical methods, such as 17% for abdominal hysterectomy and 2% for colon surgery. In Southeast Asia, the incidence of SSI based on a systematic review from 2000 to 2012 was found 7.8%. In Indonesia, the incidence of SSI without differentiating surgical techniques in teaching hospitals, namely in 2016 the incidence of SSI at RSUP dr. Pringadi Medan by 12%, RSUP dr. Sardjito in 2007 was 5.9%, and Adam Malik Hospital was 5.6%. This is also in line with research conducted in Yogyakarta on the risk factors of SSI in digestive surgery at a private hospital in Yogyakarta, where superficial SSI is the most common SSI found in 30% of cases.[3,4]

The cause of surgical wound infection comes from the patient's endogenous and exogenous flora. The presence of infection by microorganisms in the wound causes delays in wound healing. The requires microbiological examinations such as microscopy and culture to identify microorganisms that cause infection and appropriate antibiotics according to the identification of bacteria. The Sample using tissue biopsy, fluid aspiration, and wound swab. Tissue biopsy is the gold standard for identifying the cause of infection in SSI, but a tissue biopsy is expensive and requires invasive procedures.[1,5]

Gram stain helps in assessing the quality of the specimen. The relationship between gram staining and culture is significant. The College of American Pathologists (CAP) applies the Quality Score (Q Score) as a reference for determining the quality of wound swab specimens. The Q score consists of two examinations, namely looking at the number of Polymorphonuclear leukocytes (PMN) cells are an active infection and inflammation, and squamous epithelial cells to determine the presence of infection during the sample collection process. IH Solomer et al. reported that there was a significance between PMN >25/Large power field was significant. [6,7]

Heggers et al. reported that gram staining could predict the presence of large numbers of bacteria $>10^5$ CFU/gram of tissue if one bacterium was found. Bacterial count $>10^5$ bacterial cells/gram tissue is a major determinant of infection and delayed wound healing. This evidence is based on studies of wounds caused by trauma, burns, and other wounds, that the number of bacterial cells $>10^5$ CFU/ml causes a long time in wound healing, both chronic and acute wounds.[8,9,10]

The purpose of this study was to determine the relationship between the number of leukocytes and bacteria in a wound swab sample to the number of bacterial colonies in superficial surgical site infections.

METHOD

This study is an analytical observational study with a cross-sectional design, to determine the relationship between the number of leukocytes and the number of bacteria in a wound swab sample on the number of bacterial colonies in

patients with superficial SSI in a hospital in Central Java. This research was conducted from June to September 2021. The population in this study were all patients who had been superficial SSI.

The research were independent variables of: the number of leukocytes and the number of bacteria. The dependent variable is the number of bacterial colonies. The inclusion criteria in this study were all patients with superficial surgical wound infections who were willing to be research respondents. Q score on gram stain is equal to 0, and age <18 years is not included in this study.

Wound swabs were taken if they met the inclusion criteria. The wound swab sample was carried out two times, the first was for gram staining, and the second was into sterile NaCl (1ml). Samples with gram staining are assessed for their Q score. If the value is Q0 then the sample is not included in the study. Samples that meet the criteria of the Q score are continued to see the number of leukocytes and the number of bacteria in the high-power field.

Samples put into NaCl were carried out with a total plate count technique using the spread plate method. Samples containing microorganisms are transferred to the first dilution tube (1/10 or 10-1) aseptically. 0.1 ml was taken from tube 10-1 with a measuring pipette, then transferred to tube 10-2 aseptically then homogenized using a vortex. Then from the tube. Dopped 0.1 ml into nutrient agar and leveled using L-rods and incubated for 48 hours.

The data can be analyzed using Chi-square and logistic regression to see the relationship between the number of leukocytes and bacteria on the number of colonies in superficial SSI.

RESULT AND DISCUSSION

In this study, there were 35 patients met the inclusion criteria consisting of 22 male patients (62.9%) and 13 female patients (37.1%). The most age group was <60 years old, namely 24 patients (77.1%). The most comorbid malignancy was 21 patients (60%), with the type of laparotomy surgery 27 patients (77.1%).

Table 1. Characteristics of superficial SSI samples

Stage I UTB Value	Frequency (n)	Percentage (%)
Sex		
male	22	62.9
female	13	37.1
Age		
14-45	12	34.3
46-59	12	42.9
≥60	8	22.9
Comorbid		
Nothing	12	34.3
Malignancy	21	60
Chronic Kidney Diseases	1	2.9
Diabetes Mellitus	1	2.9
Types of Operations		
Hernia	2	5.7
Laparatomy	27	77.1
Bones	3	8.6
Laminektomy	3	8.6

Table 2 illustrates that the bacteria that cause infection in IDO are more gram-negative bacteria, namely as many as 31 samples compared to gram-positive bacteria (4 samples). The gram-negative bacteria were gram-negative rods from the Enterobacteriaceae family, namely Escherichia coli as many as 13 samples (37.1%)

Table 2. Types of pathogens in patients with superficial SSI

Stage II UTB Value	Frequency (n)	Percentage (%)
Gram positive (n=4)		
Staphylococcus epidermidis	1	2,9
Enterococcus faecium	1	2,9
Staphylococcus aureus	1	2,9
Staphylococcus hominis	1	2,9
Gram Negative (n=31)		
Escherichia coli	13	37,1
Klebsiella pneumoniae	4	11,4
Enterobacter cloacae	3	8,6
Pseudomonas aeruginosa	2	5,7
Acinetobacter baumannii	2	5,7
Klebsiella aerogenes	2	5,7
Providencia stuartii	1	2,9
Proteus mirabilis	1	2,9
Sternotrophomonas maltophilia	1	2,9
Aeromonas salmonicida	1	2,9
Hafnia alvei	1	2,9

This study was dominated by gram-negative bacteria, and the most *E. coli* were obtained from the wound swab sample (n=13). *E. coli* isolates obtained six *Extended Spectrum Beta Lactamase* (ESBL) isolates and 7 samples of non-ESBL. The sensitivity pattern of *E. coli* bacteria showed that amikacin was still sensitive to all *E.coli* isolates (100%), followed by piperacillin tazobactam in 12 samples (92%), and meropenem in 11 samples (85%)**.**

Gram-positive bacteria in this study were found to have gram-positive cocci, and the most minor samples (n=4) were found. The gram-positive bacteria obtained in this study were S*. aureus, E. faucium, S. epidermidis, and S. hominis*. The resistance pattern in *S. aureus* samples was not *Methicilin Resistant Staphylococcus aureus* (MRSA) and was still sensitive to linezolid and vancomycin (100%).

The analysis was performed using Chi square. Chi-square is a non-parametric test that connects two variables. From table 3 above, the variable number of leukocytes (p=0.017) and the number of bacteria (p=<0.001) were significant to the number of bacterial colonies. Then proceed with multivariate analysis, namely logistic regression, to determine the strength of the relationship between two variables by looking at the value of the correlation coefficient.

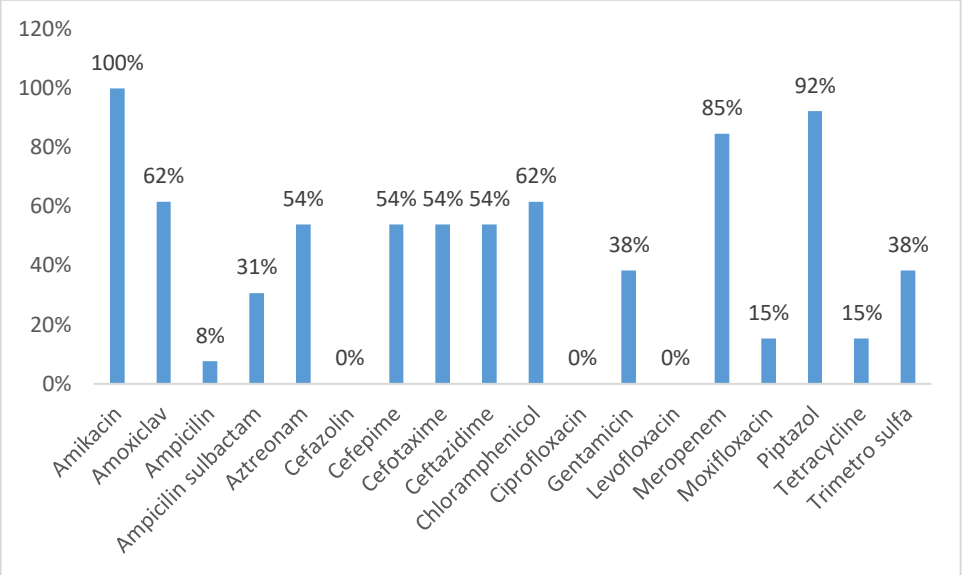

Figure 1. *E.coli* isolate sensitivity pattern in superficial SSI

There was no significant relationship (p = 0.106) between the number of leukocytes and the number of colonies on the incidence of SSI so the hypothesis in this study was rejected. From the results of the number of bacteria, it was found that the number of bacteria affected the number of bacterial colonies (p = 0.010), so the hypothesis was accepted**.**

Table 3. independent variable on the number of bacterial colonies

Variable	Number of bacteria colony (n=35)		*p-value*
	≥ 10^5 CFU/ml	**< 10^5 CFU/ml**	
Number of leucocytes			
1 – 5/HPF	2 (5,7%)	4 (11,4%)	< 0,017*
6 – 15/HPF	0 (0%)	2 (5,7%)	
16 – 24/HPF	6 (17,1%)	2 (5,7%)	
≥ 25/HPF	16 (45,7%)	3 (8,6%)	
Number of bacteriae			
+1	1 (2,9%)	1 (2,9%)	<0.001*
+2	0 (0%)	6 (17,1%)	
+3	10 (28,6%)	4 (11,4%)	
+4	13 (37,1%)	0 (0%)	

DISCUSSION

In this study, superficial SSI was more common in male, aged <60 years, in line with the Brazilian study on SSI in digestive surgical oncology patients where the prevalence of SSI was more in male aged <60 years. This is different from the study of Yang J et al. 2020 on SSI in orthopedics which found that the incidence of SSI (n=25954) had a higher risk of SSI in

male, aged > 60 years with the highest surgical site causing SSI in the upper extremity (n= 8480).[11,12]

Patients with superficial SSI at the Central Java Hospital were affected by the most comorbid malignancies, namely 21 samples (60%), most of which were malignancies in the colorectal area. The action taken on patients with malignancy is in the form of laparotomy surgery so that bacterial contamination during intestinal resection can cause infection. Bacterial counts are very high in the large intestine compared to the upper gastrointestinal system, and contamination of the surgical site can lead to infection. The incidence of SSI is related to the patient's condition, disease, and surgical procedure. The anatomic location the cancer is one of the most important predictors. The risk of superficial SSI is higher in rectal cancer than to colon cancer, therefore bacterial contamination is more common in rectal cancer surgery. Research on the factors of SSI after colorectal cancer surgery in Turkey found that SSI occurred in 81% of rectal cancer and 19% of colon cancer, where there was a significant relationship between SSI and the location of cancer.[13]

All patients identified as superficial SSI underwent culture examination with more results caused by gram-negative bacteria (n=31) than gram-positive bacteria (n=4). The most common bacteria found was Escherichia coli in 13 patients (37.1%), followed by Klebsiella pneumoniae 11.4%. This is in line with research conducted in Spain on rectal surgery, E. coli (42.3%) is the most common cause of SSI. Research conducted in Palembang also found that E. coli (31.2%) was the most common cause of SSI in laparotomy surgery. Research in Ethiopia found that different microorganisms cause SSI. From 66 SSI patients, 37% (n=27) were Staphylococcus aureus, followed by Staphylococcus coagulase-negative bacteria as much as 24.7%, and in third place was E.coli 15.1% (n=11) .[14,15,16]

E.coli isolates obtained six samples of Extended Spectrum Beta Lactamase (ESBL) isolates and seven samples of non-ESBL. The sensitivity pattern of *E.coli* bacteria showed that amikacin was still sensitive to all E.coli isolates (100%), followed by piperacillin tazobactam in 12 samples (92%), and meropenem in 11 samples (85%). This study is in line with the study by Hubab M et al. in the study of antibiotic sensitivity in surgical wound patients where isolates from E. coli were still sensitive to piperacillin tazobactam, meropenem, and imipenem.[17]

Research conducted on the diagnosis of necrotizing soft tissue infection found that PMN >25 in a large visual field was significant for infection in these soft tissues. Different things were found on histopathological examination of periprosthetic tissue to assess acute infection in soft tissues with the finding of PMN >1-10 with a high power field (400x) which was a predictor of acute infection. There is no study explains the number of PMNs on the number of bacterial colonies in the incidence of SSI.[7,18]

Neutrophils, known as PMNs, are the most abundant cell type in human blood and are produced in the bone marrow in large amounts, 10^{11} cells per day. Neutrophils are part of the innate immune system, and these cells perform various functions during the wound repair process. Neutrophils in normal skin are not always present. Neutrophils will be recruited in large numbers after tissue injury. PMNs are inflammatory cells originating from the host with very strong antimicrobial abilities. PMNs produce proteases that help kill infectious pathogens. The presence of PMN in the wound is significant because there is the adequate blood flow that helps in wound healing.[19,20]

From the results of the number of bacteria, it was found that the number of bacteria affected the number of bacterial colonies (p = 0.010), so the hypothesis was accepted. The results of this study are in line with the research of Thompson and Lawrence et al. Thompson's study using a semi-quantitative wound surface swab technique with the number of bacteria 1-4 correlated with biopsy specimens where colony counts were 10^2 to 10^3 CFU/gram and >30 bacteria (+4) corresponded to 10^7 CFU/gram. The same thing was also found in the study of Lawrence et al. It showed that quantitative bacteriology of burn wound infection in superficial swabs where >30 bacteria showed an association with a tissue count of 10^5 CFU/gram.[21,22,23]

CONCLUSION

This study describes that the incidence of superficial SSI is more common in male, aged <60 years with malignancy. The most common bacteria causing superficial SSI are gram-negative bacteria, namely E. coli. In this study, there was no relationship between the number of leukocytes and the number of bacterial colonies in superficial SSI. There is a relationship between the number of bacteria and the number of bacterial colonies in superficial SSI.

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