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Antibiotic Susceptibility Patterns of Gram-Negative Bacteria in the Pediatric Hematology-Oncology Ward of RSUD Dr. Soetomo, Surabaya

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***Abstract*---**Infection is one of the causes of death in children with malignancy. Gram-negative bacteria with broad resistance to antibiotics are the cause of invasive infections in patients with malignancy. This prospective observational study was conducted by collecting medical record data, including the identity of patients, their diagnosis results, and the antibiotic susceptibility test results, from January to December 2020 in the pediatric hematology-oncology ward of Dr. Soetomo Regional General Hospital (hereinafter referred to as RSUD Dr. Soetomo), Surabaya. Positive cultures were obtained in 20.9% of the 196 samples. The growth of gram-negative bacteria was found in 48.8% of the positive culture test results, with a percentage of 50% in blood cultures, 30% in pus cultures, and 15% in urine cultures. The most common gram-negative bacteria isolates obtained in general were E. coli (40%) and K. pneumoniae (30%). The most commonly found non-fermenting gram-negative bacteria was P. aeruginosa (15%). The lowest antibiotic susceptibility was obtained to ampicillin (0%) and ampicillin-sulbactam 10%. The lowest antibiotic susceptibility was obtained to amikacin (85%) and cefepime (80%). The MDR resistance pattern was found in 80% of samples, 40% of which was found in ESBL-producing bacteria. In addition, The AmpC resistance pattern was found in one out of three E. cloacae isolates, and the antipseudomonal cephalosporin resistance pattern was found in one out of three P. aeruginosa isolates. The culture test results indicated a high proportion of MDR bacteria. The most identified multidrug resistance mechanism included ESBL beta-lactamase and obtained AmpC resistance patterns and pseudomonas multidrug resistance.

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Introduction

Cases of malignancy in children are estimated to be diagnosed in 400 thousand children per year worldwide. Hematologic malignancies and solid tumors are the most common causes of these cases. The survival rate of the patients in low to middle-income countries ranges from 15-45% (Steliarova-Foucher et al., 2017). One of the main causes of mortality in children with cancer is infection. Children with hematological malignancies and solid tumors are susceptible to infection due to impaired immune systems resulting from the malignancy itself, chemotherapy complications, and healthcare-associated infections(Zajac-Spychala et al., 2021).

Many infectious complications are caused by invasive bacteria in hospitalized patients. Gram-negative bacilli such as *Escherichia coli*, *Klebsiella spp.*, and *Pseudomonas aeruginosa* were the main causes of most infections in neutropenic patients in the 1960-1970s. Such invasion occurs within the first 2-3 weeks after the administration of chemotherapy due to a rapid decrease in the neutrophil count. This infection is characterized by acute febrile episodes, which can progress to sepsis if not treated immediately. However, since the 1980s, studies have noted a relative decline in the number of gram-negative bacteremias. A significant increase in infection is caused by gram-positive aerobic bacteria, namely staphylococci and streptococci. It is considered to be caused by quinolone prophylaxis and increased intravenous cannulation, which facilitates access for and colonization of germs from the skin (Zajac-Spychala et al., 2021).

This pattern continues today with coagulase-negative staphylococci as the most common isolates from blood cultures. A study conducted in the pediatric hematology-oncology inpatient ward of RSUD Dr. Soetomo in 2013 indicated that coagulase-negative *Staphylococcus* and *P. aeruginosa* were the most growing bacteria based on blood cultures and urine cultures, respectively (Putrawan et al., 2013). However, several therapeutic centers found an increase in infections of Enterobacteriaceae (e.g., *E. coli* and *Klebsiella* sp.) and non-fermenting gram-negative rods (e.g., *P. aeruginosa* and *Stenotrophomonas maltophilia*) (Zajac-Spychala et al., 2021; Kapoor et al., 2014; Trehan et al., 2015).

It is exacerbated by the multidrug resistance (MDR) profile of gram-negative bacteria that infect children with malignancy. *E. coli* and *Klebsiella pneumoniae* bacteria, with acquired extended-spectrum beta-lactamase (ESBL) gene, demonstrate broad resistance to beta-lactam antibiotics. ESBL-producing bacteria are often susceptible only to carbapenem antibiotics (Zajac-Spychala et al., 2021). Carbapenem antibiotics have a broad spectrum of action but with the risk of selecting carbapenemase-producing bacteria in their use. Another MDR profile encountered was the overproduction of the AmpC cephalosporins. The AmpC gene is commonly found in the inducible *Enterobacter cloacae* bacteria. Expression of this enzyme will inactivate broad-spectrum cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone (Jacoby, 2009). Meanwhile, *P. aeruginosa* MDR was also found to increase in pediatric patients with malignancy. These bacteria are resistant to antipseudomonal cephalosporins, carbapenems, and aminoglycosides, thereby narrowing the choice of infection therapy in patients)(Zajac-Spychala et al., 2021).

This study aimed to determine the growth pattern of bacterial culture in pediatric patients with malignancy in the hematology-oncology ward of RSUD Dr. Soetomo. In addition, this study is expected to provide an overview of the antibiotic susceptibility of gram-negative bacteria.

Methods

This observational study was conducted by tracing the medical record documents in the pediatric hemato-oncology inpatient room at Dr. Hospital. Soetomo Surabaya in January 2020-December 2020. The researchers collected data on the identities of patients who underwent culture tests, their diagnosis results, as well as the results of the culture tests and the antibiotic susceptibility tests. This study received an ethical certificate from the ethics committee of RSUD Dr. Soetomo, Surabaya

Results

A total of 41 positive culture test results were obtained from 196 samples (Table 1). Besides, as many as 20 culture test results indicated the growth of gram-negative bacteria in 15 patients, hematological malignancies in 12 patients, and solid tumors in 3 patients.

Table 1
Characteristics of patients and gram-negative bacteria growth based on the results of culture tests

Characteristics of Patients	%
Sex	
Female	46.7%
Male	53.3%
Diagnosis	
Blood malignancy	80%
Solid tumor	20%

The positive culture test results indicated gram-negative bacteria in the blood (50%), pus (30%), urine (15%), and rectal (5%) samples (Table 2). Polymicrobial growth was found in 2 samples. The most common gram-negative bacteria isolates obtained in general were *E. coli* (40%) and Klebsiella pneumoniae (20%). In the blood culture, the gram-negative bacteria isolates found, from the most common to the least common, were Pseudomonas aeruginosa (30%), *E. coli* and *K. pneumoniae* (20%), and *Salmonella* sp., *E. cloacae*, and *Acinetobacter baumanii* (10%). Meanwhile, the most common gram-negative bacteria isolate found in pus and urine cultures were E. coli, with a percentage of 66.7% in both cultures.

Table 2
Characteristics of culture samples

Culture Types	n
Blood	10
Pus	6
Urine	3
Rectal	1
Isolates	
E. coli	8
K. pneumoniae	4
E. cloacae	3
Salmonella sp.	1
A. baumanii	1
P. aeruginosa	3

The obtained antibiotic susceptibility profile of gram-negative bacteria indicated the lowest susceptibility to ampicillin (0%), followed by ampicillin-sulbactam (10%) and cefazolin (15.8%). The antibiotic susceptibility to the cephalosporin class reached a peak of 35% for cefotaxime and ceftriaxone and 80% for cefepime. Meanwhile, the highest antibiotic susceptibility was obtained to amikacin (85%), followed by fosfomycin (77.8%) and meropenem (70%) (Table 3).

The culture results indicated that the gram-negative bacteria isolates were multidrug-resistant to 80% of the above antibiotics. The results of blood, pus, and urine cultures indicated the growth of multidrug-resistant bacteria with a percentage of 70%, 83.3%, 67.7%, respectively. In addition, *E. coli*, *E. cloacae*, and *P. aeruginosa* had a 100% multidrug resistance level, while *K. pneumoniae* had only a 25% multidrug resistance level. *E. coli* had an ESBL level of 87.5%, including 1 isolate that was pan-resistant, and a carbapenem resistance level of 25%. *K. pneumoniae* had a carbapenem resistance level of 50% and an ESBL level of 25%. *E. cloacae* had a resistance level of 33.3% to beta-lactams, beta-lactam inhibitors, and third-generation cephalosporins. *P. aeruginosa* had a resistance level of 33.3% to antipseudomonal cephalosporins.

Table 3
Antibiotic susceptibility characteristics of gram-negative bacteria

Antibiotic susceptibility proportion	%
Aminoglycosides	
Gentamicin	60%
Amikacin	85%
Beta-lactam, Monobactam	
Ampicillin	0%
Aztreonam	44.4%
Ampicillin-Sulbactam	10%
Amoxicillin-clavulanic acid	20%
Piperacillin	10%
Piperacillin/Tazobactam	60%

Cephalosporins	
Cefazolin	15.8%
Ceftazidime	40%
Cefotaxime	35%
Ceftriaxone	35%
Cefoperazone-sulbactam	65%
Cefepime	80%
Tetracycline	36.8%
Tigecycline	66.7%
Chloramphenicol	35.3%
Cotrimoxazole	40%
Quinolones	
Ciprofloxacin	42.9%
Levofloxacin	61.5%
Moxifloxacin	58.3%
Carbapenem	
Meropenem	70%
Imipenem	68.4%
Fosfomycin	77.8%

Discussion

Positive culture results were obtained in 20.9% of the samples. From the obtained positive results, the growth of gram-negative bacteria reached a percentage of 48.8%. Gram-negative bacteria growth in children with malignancy was also found in studies in India, Taiwan, and Poland) (Zajac-Spychala et al., 2021; Kapoor et al., 2014; Kuo et al., 2017). However, several studies found the dominance of gram-positive bacteria. Most positive culture results were obtained in patients with blood malignancies. Patients with blood malignancies are at risk for impaired function and quantity of leukocytes that play a crucial role in fighting bacterial infections. The most commonly found gram-negative bacteria was E. coli, while the most commonly found non-fermenting gram-negative bacteria was *P. aeruginosa*, which was in line with a study in India (Kapoor et al., 2014; Trehan et al., 2014).

Gram-negative bacteria indicated a low antibiotic susceptibility level to beta-lactam and combinations of beta-lactamase inhibitors. Only 10% of the isolates had antibiotic susceptibility to ampicillin-sulbactam, an empirical antibiotic in the hematology-oncology of RSUD Surabaya. The antibiotic susceptibility of gram-negative bacteria to cephalosporin class antibiotics ranged from 15-65% and reached a peak of 70% to carbapenems. A similar pattern was found in India, with antibiotic susceptibility to cephalosporins ranging from 20-60% and to carbapenems ranging from 70-80%. The highest antibiotic susceptibility was found to aminoglycoside antibiotics. However, aminoglycosides require careful administration in children due to the nephrotoxic and ototoxic side effects.

The growth of multidrug-resistant bacteria was found in 80% of the isolates in this study. The main antibiotic resistance mechanism of gram-negative bacteria was beta-lactamase synthesis. The most commonly found beta-lactamase type was ESBL. This enzyme hydrolyzes the beta-lactam ring of the penicillin and first to third-generation cephalosporins and is inhibited by clavulanic acid. Based on the 2021 data of the Clinical and Laboratory Standards Institute (CLSI), three ESBL species were identified, namely *E. coli*, *Klebsiella* sp., and *Proteus* sp. (James et al, 2022). ESBL-producing bacteria are also capable of producing other beta-lactamases and carbapenemases. In this study, *E. coli* and *K. pneumoniae* were obtained in 40% of the positive culture results. Based on these results, carbapenem-resistant bacteria were found, which was indicated by the presence of carbapenemases in ESBL-producing bacteria.

AmpC Beta-lactamases hydrolyze beta-lactams and first to third-generation cephalosporins and are not inhibited by beta-lactamase inhibitors. AmpC Beta-lactamases can be encoded by chromosomal genes and plasmid genes. Some bacteria with the AmpC chromosomal gene, such as *E. cloacae* and *Morganella morganii*, show inducible properties (Jacoby, 2009). Clavulanic acid and some cephalosporins can increase AmpC synthesis. Excessive synthesis can expand the substrate of this enzyme to the carbapenem class antibiotics. It is a crucial consideration in selecting antibiotic therapy for AmpC bacteria. The originally susceptible beta-lactam antibiotic therapy, beta-lactamase inhibitors, and cephalosporins can become resistant to antibiotics due to the induction of the AmpC gene (Jacoby, 2009). This study showed the growth of one of three *E. cloacae* with an AmpC resistance pattern. Two isolates of *E. cloacae* showed susceptibility to third-generation cephalosporins but resistance to amoxicillin-clavulanate.

P. aeruginosa, a non-fermenting gram-negative bacteria, had an intrinsic resistance to ampicillin, amoxicillin, ampicillin-sulbactam, ceftriaxone, cefotaxime, tetracycline, chloramphenicol, cotrimoxazole, and ertapenem (James et al., 2002). In addition to a broad intrinsic resistance profile, these bacteria also produce cephalosporins, beta-lactamase, and carbapenemase enzymes. The results of the culture test on one of the isolates of *P. aeruginosa* indicated a resistance profile to the antipseudomonal cephalosporin, namely ceftazidime.

Conclusions

The results of the culture tests revealed the highest proportion of gram-negative bacteria growth. Antibiotic susceptibility patterns of multidrug-resistant bacteria were found in 80% of the isolates. The most identified multidrug resistance mechanism included ESBL beta-lactamase and obtained AmpC resistance patterns and pseudomonas multidrug resistance.

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