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The Agreement Between of Vitek 2 and HybriSpot 24 in Identification of SHV-Type ESBLs and CTX-M-Type ESBLs from ESBL Producing *Enterobacteriaceae* Isolat

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Abstract

The identification of extended-spectrum beta-lactamase (ESBL) types is important for selecting antibiotics in treating patients infected by ESBL-producing Enterobacteriaceae. This can be done phenotypically and genotypically. Vitek 2 phenotypically identifies ESBL types such as CTX-M and SHV. HybriSpot 24 genotypically identifies CTX-M and SHV-encoding genes (bla_{CTX-M} and bla_{SHV} respectively). The objective of this study was to identify or characterize types of ESBL enzymes and to measure the agreement between Vitek 2 and HybriSpot 24 in detecting SHV and CTX-M ESBL types. The methodology of this research is by taking 30 isolates samples from Dr. Kariadi Hospital's inpatients, which had been phenotypically identified as ESBL-producing Enterobacteriaceae by Vitek 2 in Dr. Kariadi Hospital. The genotypic test was performed using HybriSpot 24 in Indonesia University Hospital, Jakarta. The agreement between these two methods was analyzed using Cohen's Kappa coefficient. The result from this study was obtained from 30 isolates, one was excluded because of contamination, 20 were Escherichia coli and 9 were Klebsiella pneumoniae. Vitek 2 identified enzyme-types in 5 (17%) isolates, namely 3 (10%) CTX-M and 2 (7%) SHV. HybriSpot 24 identified ESBL-encoding genes in 27 (93%) isolates, that is 15 (52%) bla_{CTX-M}, 8 (27%) both bla_{CTX-M} and bla_{SHV}, 2 (7%) bla_{SHV}, and 2 (7%) both bla_{SHV} and bla_{GES} (a carbapenemase-encoding gene). The latter two isolates' MIC for meropenem/ertapenem was ≤0.025. The conclusion of this study is that CTX-M is the most prevalent ESBL-type in Dr. Kariadi Hospital and the agreement between Vitek 2 and HybriSpot 24 in identifying CTX-M type was very poor (κ=3,6%) and poor in SHV type (κ=24,7%).

Keywords: *Vitek2, Hybrispot24, agreement, ESBL types*

1. Introduction

Extended-spectrum beta-lactamase (ESBL) is an enzyme that can hydrolyze beta-lactam antibiotics which can make resistance and fails in treatment with these antibiotics [1]-[3]. This enzyme is often found in *Klebsiella pneumonia*, *Escherichia coli* and other Gram-negative bacilli [1], [4]. Infections caused by *Enterobacteriaceae* bacteria that produce extended-spectrum beta-lactamase have increased in recent decades [5]. At Dr. Kariadi Hospital Semarang, during the period 2004-2005, it was found that the proportion of ESBL-producing bacteria was 50.9% and increased to 79% of *Enterobacteriaceae* in 2016 [6]. Patients who are infected with ESBL will have an increased risk of treatment failure using broad-spectrum beta-lactam antibiotics [4, [5]. Some ESBL-producing strains have over-resistance to broad-spectrum beta-lactam antibiotics, but some are phenotypically "not resistant" according to clinical laboratory standard institute (CLSI) criteria [7], [8].

The detection of ESBL-producing microorganisms accordance to CLSI recommendations in the form of screening using an antibiotic sensitivity test method with disc diffusion and dilution (dilution antimicrobial susceptibility tests) [7]. In addition to the screening test, ESBL detection can be a phenotypic confirmation test for the production of ESBL combination cephalosporin-clavulanate discs, a dual disc diffusion test, a three-dimensional test, a test with agar fermented with clavulanate, and several methods that have been commercially available [7]. In general, ESBL detection methods can be classified into two groups, namely: phenotypic methods using non-molecular techniques, which can detect ESBL enzymes in hydrolyzing various cephalosporins according to CLSI standards; and genotypic methods using molecular techniques that detect the beta-lactamase gene [7]-[9].

One of the form in commercial phenotypic method to detect ESBL is Vitek 2 (bioMerieux Vitek, Hazelton, Missouri), which has the ability to identify bacteria, detect ESBL, and antibiotic sensitivity test based on minimum inhibitory concentration (MIC) of several cephalosporin antibiotics (with or without combination with beta-lactamase inhibitors) according to CLSI standards [10], [11]. The phenotypic method is most widely used in clinical diagnostic laboratories because it is easier and cheaper. Vitek 2 has a 90% accuracy rate for the identification of the *Enterobacteriaceae* family, 95% of sensitivity in detecting ESBL [10]. In addition to identification and detection of ESBL, Vitek 2 is able to detect ESBL types to 100% CTX-M-1 types, 97% CTX-M-9 types and 92% SHV types [10], [11].

The genotypic molecular technique for detecting ESBL is carried out using the most reliable polymerase chain reaction (PCR) for identification and confirmation of ESBL by detecting the presence of a beta-lactamase encoding gene [12], [13]. This method has a significant role in clinical microbiology laboratories and is essential in the determination of therapy. The types and modifications of PCR are real-time PCR, nested PCR, multiplex PCR, and PCR-ELISA [2], [3]. One of the molecular diagnostic procedures is by hybridization, i.e. the ability of two strands of nucleic acids that have homologous complementary base sequences to bind specifically to each other and form double-stranded molecules, or duplex or hybrid. This method is in accordance with the diagnostic procedure on HybriSpot 24 by using sepsis flow chips (SFC) (Master Diagnostics, Granada, Spain) based on DNA microarrays formed by the European Economic Area as a suitable device for in vitro diagnosis. SFC was able to identify 98% of Gram-negative bacteria along with resistance-determining genes, as well as detecting 100% of ESBL-producing strains carrying $bla_{\text{CTX-M}}$ and/or bla_{SHV}. In addition, based on research from Antonia Galiana, SFC can identify monomicrobial compliance with a standard microbiological diagnostic protocol of 96.2% and 89.1% in polymicrobial blood cultures and overall concordance of 92.6% [14].

This study is a research that assesses the agreement between Vitek 2 and HybriSpot 24 in detecting ESBL type SHV and CTX-M and to determine the genetic characteristics of the isolate bacteria *Enterobacteriaceae*-producing ESBL. Research on genetic characteristics in Indonesia reported from Surabaya showed that $bla_{\text{CTX-M-15}}$ was the beta-lactamase gene with the highest prevalence in *Escherichia coli* followed by *Klebsiella pneumonia* [5], [12]. Until now, Dr. Kariadi Hospital Semarang has no genetic data from ESBL-producing *Enterobacteriaceae* that can be used in administering therapy to patients.

2. Methodology

This research is an observational analytic study with a design agreement test (agreement analysis). This research was conducted at the Microbiology Laboratory Dr. Kariadi Semarang and Laboratory of RSUI hospital Depok in May 2019 - July 2019 using *Enterobacteriaceae* clinical isolate from Dr. Kariadi Hospital patients on May 1, 2019 - July 31, 2019. The sample of this study was taken by consecutive sampling, in the form of 30 *Enterobacteriaceae* isolates that cause infection in

patients hospitalized at Dr. Kariadi Hospital on 1 December 2018 - 28 February 2019 from urine, blood, sputum, swabs, and effusion fluids. The clinical specimens were grow on Mc Conkey and Blood Agar media for 18 - 24 hours, then the identification and antibiotic sensitivity testing were further carried out using Vitek 2 (Biomerieux.USA). If the test results show the *Enterobacteriaceae* family and produce the ESBL, then a molecular test is later performed using HybriSpot 24 to detect the presence of genes that encode the ESBL enzyme type. The study was conducted after obtaining the ethical clearance from the Health Research Ethics Committee (KEPK), Faculty of Medicine, Diponegoro University / RSUP Dr. Kariadi Semarang and research permission from the Director of RSUP Dr. Kariadi Semarang.

3. Results and Discussion

From the thirty taken samples, one sample was excluded because two different species were identified, namely *Klebsiella pneumoniae* and *Acinetobacter baumanii* as well as 2 bla_{SHV} and bla_{CTX-M} resistant genes. The test cannot determine which species carry the gene.

Table 1 describes the 29 identified isolates, 20 of them were *Escherichia coli* and 9 isolates were *Klebsiella pneumoniae*. The suitability between Vitek 2 and HybriSpot 24 in the identification of these two species reached 100%. HybriSpot 24 can identify the species of *Escherichia coli* and *Klebsiella pneumoniae* together with the results of Vitek 2 with agreement level $\kappa = 100\%$.

Table 1. Distribution of Bacteria Samples and Species Based on Vitek 2 and HybriSpot 24

Sample Origins	Amount	Vitek 2		HybriSpot 24		Suitability
		E. coli	***K. pneumoniae***	***E. coli***	***K. pneumoniae***	**100%**
Urin	13	10	3	10	3	100%
Sputum	7	3	4	3	4	100%
Darah	4	4	0	4	0	100%
Pus	3	3	0	3	0	100%
Effusion fluid	1	0	1	0	1	100%
Wound swab	1	0	1	0	1	100%
Total	29	20	9	20	9	100%

As for the identification of ESBL types, there are differences between Vitek 2 and HybriSpot 24 as shown in Table 2. Vitek 2 can only identify five (17%) out of 29 isolates. HypbriSpot 24 can identify more types of enzymes through the detection of the encoding gene, which is 27 of 29 isolates (93%). The most type of ESBL obtained from this study was CTX-M which was detected through the presence of the bla_{CTX-M} gene, which was found in eighteen *Escherichia coli* isolates and seven *Klebsiella pneumoniae* isolates. SHV type ESBL was detected by HybriSpot 24 through the presence of the bla_{SHV} gene in ten bacterias, namely nine isolates of *Klebsiella pneumoniae*, and one *Escherichia coli* isolate. HybriSpot 24 can detect the presence of combined types of ESBL CTX-M and SHV in eight isolates, which are seven isolates of *Klebsiella pneumoniae* and one isolate of *Escherichia coli*. A single bla_{SHV} gene was found in two *Klebsiella pneumoniae* bacteria.

In addition to the bla_{SHV} and bla_{CTX-M} genes, HybriSpot 24 was able to detect another resistance gene that was not detected by Vitek 2, the bla_{GES} gene. The gene was carried by 2 isolates of *Escherichia coli* which were also identified as having the bla_{CTX-M} gene. The results of the antibiotic sensitivity test of the two isolates by Vitek 2 were found to still be sensitive to meropenem with MIC values ≤ 0.25.

Table 2. The Comparison of CTX-M and SHV ESBL Type Detection by Vitek 2 and HybriSpot 24

Gen	Vitek 2		HybriSpot 24	
	Detected	Undetected	Detected	Undetected
CTX-M	3	13	15	0
SHV	2	0	2	0
CTX-M SHV	0	8	8	0
CTX-M GES	0	2	2	0
Others genes	0	2	0	2
Total	5	25	27	2

CTX-M type ESBL was detected by HybriSpot 24 in 25 of the 29 ESBL-producing isolates. Three CTX-M ESBL types detected by Vitek 2 were detected by HybriSpot 24, two were single genes and one was a combined gene between $bla_{\text{CTX-M}}$ and bla_{SHV}. The two SHV type ESBL detected by Vitek 2, were also detected by HybriSpot 24, that is as a single gene.

The results of this study also showed that HybriSpot 24 can detect more than one resistance gene. Vitek 2 can only detect the presence of one type of ESBL and cannot detect two types of ESBL simultaneously from one isolate. In this study, there were two ESBL-producing isolates that could not be detected by both Vitek 2 and HybriSpot 24 types.

Table 3 explains the agreement of Vitek 2 in detecting SHV type ESBL in two of ten isolates carrying the gene, while HybriSpot 24 is able to detect overall SHV type ESBL. Kappa test results obtained $\kappa = 24.7\%$, agreement category "less". All SHV type ESBL detected by Vitek2 can be detected by HybriSpot 24.

Table 3. The Kappa Test Results for Detection of ESBL Type SHV by Vitek 2 and bla_{SHV} Gene from HybriSpot 24

SHV Vitek2	bla_{SHV} HybriSpot 24				Total		κ
	Detected		Undetected				
	n	%	N	%	n	%	
Detected	2	6,9	0	0	2	6,9	0,247
Undetected	8	27,6	19	65,5	27	93,1	
Total	10	34,5	19	65,5	29	100	

In Table 4, it was found that Vitek 2 was able to detect CTX-M type ESBL in three out of the 25 gene carrier isolates, while HybriSpot 24 was able to detect the type CTX-M of all (25) gene carrier isolates. Kappa test results obtained a value of $\kappa = 3.6\%$ which means that the agreement level is included in the bad category. All CTX-M ESBL types detected by Vitek 2 can be detected by the HybriSpot 24 gene.

Table 4. The Kappa Test Results for Detection of ESCT typeCTX-M from Vitek 2 and $bla_{\text{CTX-M}}$ by HybriSpot24

CTX-M Vitek 2	$bla_{\text{CTX-M}}$ HybriSpot 24				Total		κ
	Detected		Undetected				
	N	%	N	%	n	%	
Detected	3	10,3	0	0	3	10,3	0,036
Undetected	22	75,9	4	13,8	26	89,7	
Total	25	86,2	4	13,8	29	100	

The beta-lactam antibiotics such as penicillin, cephamycin, carbapenem, and cephalosporin are anti-bacterial drugs that have a high potential to kill Gram-positive or negative bacteria with minimal side effects [1]. These antibiotics are widely used in the

treatment of various infections, such as in the lung, urinary tract, and infections of the bloodstream [3]. However, the widespread use of antibiotics has increased the problem of antibiotic resistance to bacteria [15]. After the emergence of resistance to the early generation of beta-lactam, many new drugs were developed to fight this resistance. This derivative of antibiotics is called broad-spectrum beta-lactam (extended-spectrum beta-lactams). The widespread and inappropriate use of these drugs results in the emergence of strains of bacteria that are resistant to these antibiotics, by producing an extended-spectrum beta-lactamase enzyme [1], [3]. ESBL is an enzyme that can cause resistance to almost all beta-lactam antibiotics, including penicillin, cephalosporins, monobactams, and aztreonam. ESBL enzymes have varying abilities against various beta-lactam oxyimino substrates, but they cannot hydrolyze cefamycin (cefoxitin, cefotetan and cefmetazole) and carbapenem (imipenem, meropenem, doripenem, and ertapenem) [2], [7], [16]. This enzyme can be inhibited by beta-lactamase inhibitors, such as clavulanate, sulbactam, and tazobactam [7], [16].

One of the ESBL tests for bacteria is by using the Vitek 2 tool which uses an AST-GN93 card. Vitek 2 tested ESBL following the standards established by CLSI, using six wells containing cefepime antibiotics at a concentration of 1.0 µg/ml, ceftriaxone 0.5 µg/ml, ceftazidime 0.5 µg/ml, cefepime/clavulanic acid combination 1.0/10 µg/ml, ceftriaxone/clavulanic acid 0.5/4 µg/ml, ceftazidime/clavulanic acid 0.5/4 µg/ml [7]. The growth of bacteria in each well was assessed quantitatively by an optical tracker. The proportion of decreased bacterial growth in the presence of cephalosporins alone compared with a combination of cephalosporins with clavulanic acid shows the production of ESBL [7], [10], [17]. The types of antibiotics tested by Vitek 2 are ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, ceftriaxone, ceftazidim, cefepime, aztreonam, meropenem, ertapenem, gentamicin, amikacin, ciprofloxacin, tigecycline, trimetroprim/sulfametoksazol, and nitrofurantoin. The type of CTX-M and SHV enzymes that are produced based on the pattern of resistance to antibiotics can be known from the results of the output in the form of minimal inhibitory levels (MIC) [16], [18]. MIC was defined as the lowest concentration of antimicrobials that could inhibit the growth of microorganisms that were seen after overnight incubation. AST data was analyzed using a knowledge base of 2000 phenotypes and 20,000 MIC distributions [10], [17]. The interpretive reading of the sensitivity test aims to analyze the pattern of antibiotic sensitivity of the individual so that the underlying resistance mechanism can be predicted. There are also increasing reports of more-complex ESBL phenotypes that include additional mechanisms of resistance, such as AmpC-type enzyme production (both chromosomal and plasmid-mediated), TEM and SHV beta-lactamases with reduced affinities for beta-lactamase inhibitors, hyperproduction of penicillinase, and porin changes [10], [17], [19].

HybriSpot 24 detects ESBL CTX-M and SHV enzyme types based on the presence of the $bla_{\text{CTX-M}}$ and bla_{SHV} genes on the sepsis flow chip (SFC). The operational procedures of this tool include three main things: multiplex-PCR reaction, preparation of hybridization reagents, and flow-through reverse hybridization. Sepsis flow chip (SFC) is conducted based on a method involving simultaneous amplification of at least 40 bacterial and fungal species plus 20 resistance markers by multiplex-PCR, followed by reverse dot blot hybridization to specific DNA probes that are moved to the nylon membrane and based on DNA-Flow. Resistance marker genes contained in these chips such as bla_{mecA}, bla_{vanA}, bla_{vanB}, ESBL carrier resistance genes (bla_{SHV} and $bla_{\text{CTX-M}}$), and carbapenemase genes [14].

The characteristics of the genes obtained from this study were $bla_{\text{CTX-M}}$ (52%), bla_{SHV} (7%), a combination of bla_{SHV} and $bla_{\text{CTX-M}}$ (27%), and a combination of $bla_{\text{CTX-M}}$ and bla_{GES} (7%) and other genes (7%). The $bla_{\text{CTX-M}}$ gene is the greatest gene with a percentage of 86% and the second greatest gene is bla_{SHV} with 34%. This is consistent with the results of research conducted at RSUD Dr. Soetomo Surabaya which has identified 27 of 30 positive *Escherichia coli* isolates (90%) containing the $bla_{\text{CTX-M}}$ gene [12]. Another study which

similar to this results is the AMRIN study which states that Indonesia is a developing country that is affected by the emergence and spread of strains of Escherichia *coli* that carry the $bla_{\text{CTX-M-15}}$ type ESBL gene [5]. The ESBL gene is located in a plasmid that can be easily transferred between and within bacterial species [20], [21]. Some ESBL genes are mutants of plasmid-mediated beta-lactamases (eg, bla_{TEM}/bla_{SHV}), and others are mobilized from environmental bacteria (eg, $bla_{\text{CTX-M}}$) [9], [13], [22]. However, in several studies, globally, it has been mentioned that most of the ESBL carrier genes are $bla_{\text{CTX-M}}$ type genes [4], [12]. The bla_{SHV} gene is the second most gene after $bla_{\text{CTX-M}}$ in this study. It is interesting to note that all *Klebsiella pneumoniae* isolates in this study carried the bla_{SHV} gene, while only one *Escherichia coli* isolate carried the gene. In addition, most of the 27% bla_{SHV} is with other genes. This result is in accordance with the research from Woori J and Tabbaaouche S which states that bla_{SHV} is mostly found in the bacterium *Klebsiella pneumoniae* and it is a gene that is combined with other resistance genes [4], [10]. ESBL type SHV with a characteristic to hydrolyze cefotaxime antibiotics is greater than ceftazidime [7], [16].

In this study, it was found that Vitek 2 was only able to detect 17% ESBL type CTX-M type and 7% ESBL type SHV, this number was very low when compared to research conducted by Woori J, which has detected ESBL type CTX-M type in 96% and 92% for type ESBL SHV and a research conducted by Nathaniel, stating that Vitek 2 can detect the presence of the enzyme CTX-M [10], [19]. The low detection ability of the ESBL type CTX-M and SHV enzymes in this study mainly occurs in isolates that have more than one resistance coding gene, possibly because both genes are expressed, making both enzymes are active and produce MIC patterns that are different from MIC patterns for the CTX-M type and SHV which are recognized by Vitek 2 [18], [19]. The same organism can possess two ESBL $bla_{\text{CTX-M}}$ genes and bla_{SHV} or ESBL $bla_{\text{CTX-M}}$ and other types, this can change the phenotypic antibiotic resistance [19]. With the low detection ability of the ESBL enzyme type, it can be said that Vitek 2 failed to detect the characteristics of the ESBL enzyme type CTX-M and SHV types, partly because many isolates had more than one resistance gene. In addition, it is possible that the enzyme is inducible and the isolate is not producing the ESBL enzyme when it is cultured, so the enzyme activity is not reflected in the MIC of various antibiotics tested [16], [19]. Another factor that can also cause the failure of Vitek 2 in detecting ESBL type CTX-M and SHV enzymes, inadequate levels of dilution, which causing MIC to be lower or higher than it should be (very major error) so that the MIC pattern is not recognized by Vitek 2 [19]. However, in this study, this factor became the smallest factor that might occur, because the making of a colony suspension was in accordance with the standards. In contrast to Vitek 2, HybriSpot 24 can detect 100% of the $bla_{\text{CTX-M}}$ and bla_{SHV} genes. In addition, HybriSpot 24 in this study can also detect another resistance gene, bla_{GES}, which is an enzyme that hydrolyzes the carbapenem class of antibiotics. This is consistent with other studies that found that HybriSpot 24 is also capable of detecting several resistance genes from the same isolate [14].

The results of the agreement test between the two devices in the detection of ESBL type SHV obtained the coefficient $\kappa = 0.247$, included in the category of poor conformity. The agreement test results for the ESBL type CTX-M obtained $\kappa = 0.036$, which is included in the category of very poor (bad). This low level of agreement may be specific in Dr. Kariadi Hospital, or in other hospitals in the same class, relating to the number of strains that have more than one resistance gene, which illustrates the amount of selective pressure due to the high use of antibiotics both in Dr. Kariadi Hospital itself, as well as from hospitals that refer patients to Dr. Kariadi Hospital. Another possibility based on studies of the causes of this discrepancy is related to the low number of inoculums in isolate suspension, which results in inability of Vitek 2 to test carbapenem antibiotics against *Klebsiella pneumoniae*

producing KPC, as well as cephalosporin antibiotics against other types of broad-spectrum beta-lactamase [16], [18], [19].

This research has several important implications, the first is the therapeutic implications. The hospital guidelines in using antibiotics need to be reviewed because gene characteristics are related to antibiotic therapy [1]. This study showed that 86% of isolates had the $bla_{\text{CTX-M}}$ gene, which meant they were able to hydrolyze cefotaxime. Organisms that produce CTX-M type beta-lactamase typically have MIC cefotaxime in the resistant range (64 g/ml), whereas MIC ceftazidime is usually in the seemingly sensitive range (2 to 8 g/ml). However, some CTX-M ESBL types can actually hydrolyze ceftazidime and cause resistance to ceftazidime with MICs up to (256 g / ml) [10], [11]. Even some types of CTX-M can hydrolyze cefepime with high efficiency, with MIC cefepime higher than bacteria that produce other types of ESBL [19]. Tazobactam is a beta-lactamase inhibitor that can have inhibitory activity nearly ten times greater than clavulanic acid against CTX-M type enzymes. The same study also revealed that only (23.3%) isolates of the ESBL gene-carrying bacteria were sensitive to clavulanic acid combined with amoxicillin or ticarcillin antibiotics. The percentage of antibiotic bacterial sensitivity increased significantly (82.2%) when the beta-lactamase inhibitor tazobactam was combined with the antibiotic piperacillin [4]. Thus, cefotaxime should be removed from the antibiotic guidelines at Dr. Kariadi Hospital, whereas ceftazidime and cefepime should be used with extreme caution, for example, very selectively and immediately consider the possibility of replacing antibiotics if within 3-4 days after starting therapy there is no improvement. In addition, the amoxicillin-clavulanate combination also needs to be rigorously evaluated if used in patients because its effectiveness against $bla_{\text{CTX-M}}$ is less strong. Piperacillin-tazobactam is preferred as the selected antibiotic in infections due to CTX-M-producing ESBL.

The second implication is in diagnostic, in hospitals with many ESBL infections, the genotypic examination of ESBL-producing bacteria is needed because different types of ESBL-producing genes require different antibiotic therapy. The $bla_{\text{SHV-2}}$, $bla_{\text{TEM-3}}$, $bla_{\text{CTX-M-15}}$ genes hydrolyze oxyimino-cephalosporin and monobactam but can be inhibited by clavulanic acid, so the combination with clavulanate can be chosen [4]. Whereas $bla_{\text{TEM-30}}$, $bla_{\text{SHV-10}}$ are resistant to clavulanic acid, tazobactam, and sulbactam [4]. Different types of enzymes will have different consequences in the administration of therapy. In order for this information about the aforementioned type of enzyme appear accurately, a genotypic examination by multiplex PCR is needed, considering that many isolates have more than one resistance gene. The detection of resistance enzymes could have very serious consequences, as in the two isolates in this study which turned out to carry the bla_{GES} gene that encodes the carbapenemase enzyme, whereas the MIC on phenotypic examination for meropenem and ertapenem is very low (≤ 0.25). In the absence of genetic information, patients with sensitivity test results like this will be treated with meropenem and result in therapy failure because the bacteria produce the carbapenemase enzyme. This selection is crucial because the effectiveness of the instrument is very dependent on the pattern and distribution of the ESBL enzyme in a hospital. HybriSpot 24 as a diagnostic tool for gene detection might be appropriate in Dr. Kariadi Hospital because most of the ESBL types are $bla_{\text{CTX-M}}$ and bla_{SHV} in the sepsis flow chip panel. However, if the epidemiological pattern shifts with an increase in the proportion of other types of enzymes, or in other hospitals with different types of enzymes that are not in the examination panel, then the tool becomes less useful. On the other hand, Vitek 2 should be able to diagnose more types of ESBL besides CTX-M and SHV, for example, OXA, AmpC precisely. However, its accuracy is highly dependent on the accuracy of dilution of the bacteria suspension which is inserted into the Vitek 2 device and other technical factors. Therefore, the microbiology laboratory must be more strict in carrying out the quality assurance of examinations that carried by its analysts, making the MICs produced are accurate and can be recognized by AES Vitek 2 as a basis for the diagnosis of enzyme types. The genotypic and phenotypic examination has great importance for the clinician. Genotypic testing is used as a form of antibiotic

resistance test (ART) that is needed so that clinicians know which antibiotics should not be used, while antibiotic susceptibility tests (AST) are needed so that clinical clinics know which antibiotics can be used. The phenotypic examination is still useful because it provides an antibiotic sensitivity test in the form of MIC which is not all present in the genotypic examination.

The third implication is the efforts to control transmission through intensive and effective prevention and control of infection. A large number of isolates that have more than one resistance gene implies the existence of "heavy transmission traffic" in the hospital environment. Contact isolation for patients infected or colonized by ESBL-producing bacteria must be carried out consistently and in accordance with minimum standards. In addition, it is necessary to conduct surveillance to look for transmission sources, such as washbasins, washing equipment, medical devices, and so on.

The first limitation of this study is the sample of only 29. Although statistically, it has met the minimum number of samples, in its application, it may still need additional data so that the conclusions obtained are stronger, considering the very large types of ESBL enzymes. The second limitation is the variation of ESBL-producing species including only *Escherichia coli* and *Klebsiella pneumoniae* which may not yet be generalizable to other ESBL-producing species. The broad generalization of this finding is also limited by the dominance of the ESBL $bla_{\text{CTX-M}}$ type in our study sample.

4. Conclusion

In conclusion, the characteristics of ESBL-producing genes in Dr. Kariadi Hospital patients are $bla_{\text{CTX-M}}$, bla_{SHV}, a combination of $bla_{\text{CTX-M}}$ and bla_{SHV}, and a combination of bla_{GES} and $bla_{\text{CTX-M}}$. The largest population of ESBL coding genes in Dr. Kariadi Hospital is the $bla_{\text{CTX-M}}$ type. The $bla_{\text{CTX-M}}$ gene is mostly carried by *Escherichia coli* and the bla_{SHV} gene is carried by *Klebsiella pneumoniae*. There is no agreement between Vitek 2 and HybriSpot 24 in detecting bla_{SHV}-producing *Enterobacteriaceae* and $bla_{\text{CTX-M}}$ bacteria. Suggestions from this study are that further research is needed to look at the characteristics of genes with antibiotic sensitivity patterns, and it is necessary to do research with a larger sample to see the diversity of *Enterobacteriaceae* bacteria types related to gene types and antibiotic sensitivity.

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