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SENSITIVITY AND SPECIFICITY OF BACTERIAL CULTURE USING VITEK-2 FROM SPUTUM FOR DETECTING KLEBSIELLA PNEUMONIA AND ACINETOBACTER BAUMANII BACTERIA IN PNEUMONIA PATIENTS AT HAJI ADAM MALIK GENERAL HOSPITAL MEDAN

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Abstract
Introduction: Nosocomial infections are a cause of worldwide morbidity and mortality, a problem is getting worsen when nosocomial pathogens get antibiotic resistance. In Asian countries, antimicrobial resistance to common pathogens of Hospital-Acquired Pneumonia (HAP). Based on Microbiological culture data reports at H. Adam Malik General Hospital in 2017 within 2622 sputum isolates, the most common was Klebsiella pneumonia (16.55%) and Acinetobacter baumanii (17.6%). Detection of bacteria that produce extended-spectrum betalactamase (ESBL) is very important for infection control and epidemiological surveillance. The method used is VITEK-2 and PCR (Polymerase Chain Reaction).
Aim: To evaluate the sensitivity and specificity values in the diagnostic test of bacterial sputum culture examination with VITEK-2 and PCR in detecting *K. pneumonia* and *A.baumanii* bacterias.
Methods: The diagnostic test uses primary data based on the results of sputum culture and secondary data based on medical records in pneumonia patients who are hospitalized in H. Adam Malik General Hospital Medan. The sampling technique uses consecutive sampling method on 50 respondents. Data were analyzed using SPSS version 20.
Result: The diagnostic test results using the VITEK-2 test in detecting both K. pneumonia and A. baumanii showed sensitivity (93%) and specificity (100%). The evaluation of sensitivity and specificity in detecting K. pneumonia was (93.3%) and (100%) higher than in detecting A.baumanii was (76.4%) and (100%).
Conclusion: VITEK-2 method can be used in diagnosis and screening in detecting bacteria *K. pneumonia* and *A.baumanii* in pneumonia. The sensitivity value of this method is higher in detection of *K. pneumonia* than *A.baumanii*.

Introduction
Antimicrobial resistance (AMR) is a growing public health crisis throughout the world and has been recognized as an important global health problem for the past several decades. Antibiotic resistance occurs when bacteria develop the ability to defeat drugs designed to kill these bacteria. It is estimated that mortality rate in 50,000 lives per year are estimated due to infection problems and antibiotic resistance in the US and Europe.[1] The Centers for Disease Control and Prevention in the United States estimates that every year there are 23,000 deaths caused of AMR.[2] Based on Microbiological culture data reports obtained from H. Adam Malik General Hospital from January to December in 2017, there were 2622 sputum isolates where the highest prevalence of *K. pneumonia* was 16.55% and *A. baumanii* was 17.6%.

Nowadays in Asian countries, antimicrobial resistance with multidrug resistant (MDR) found most common in hospital-acquired pneumonia (HAP) patients including *Acinetobacter spp*. and *K. pneumoniae species*.[3] Data from the United States the percentage of *A. baumannii* isolates that are resistant to imipenem has increased from an average of 10% between 1999 and 2005 to 48% in 2008, and meropenem resistance has increased from 19% to 57.4% over the same time period.[4] Study in Nepal (2018) of the species *A. baumanii* (41%) was followed by *K. pneumoniae* (28%). In Singapore, ESBL-producing Enterobacteriaceae, especially *Klebsiella spp*, has increased

by 35% - 40%. Study in Brazil in (2019) found isolates of *K. pneumoniae* (n = 21, 84%) classified as MDR. Research in Brazil in 2018 as many as 25 isolates of *K. pneumoniae* produced ESBL, carbapenemase, and carried the 72% blaCTX-M gene.[5]

Another study in Indonesia at Dr Soetomo Hospital in Surabaya, the detection of ESBLs gene, the CTX-M gene, was isolated (55.6%) of *K. pneumoniae*.[6] Detection of bacteria that produce ESBL is very important for infection control and epidemiological surveillance. PCR is considered the gold standard for evaluation of testing methods. Study with Vitek2 about ESBL identification is based on the inhibitory effect of cefepime, cefotaxime, and ceftazidime and/or with clavulanic acid, the highest sensitivity was (73%-79%).[7,8] Study in 2008 found the sensitivity and specificity ESBL detection was (78.8% and 80.6%).[9] Research in France states that the sensitivity and specificity values for the Vitek 2 confirmation test for identification of *K. pneumoniae* are (91.8% /100%) and (91.8% / 100%) ESBL.[10]

Method

This study is an observational analytic study with a diagnostic test design. This study was carried out among 50 pneumonia patients that hospitalized in the internal medicine and lung wards of H. Adam Malik General Hospital Medan who fulfilled the inclusion and exclusion criteria since August 2019. The inclusion criteria were age ≥18 years, diagnosed pnemonia according to clinical, laboratory and radiology based on medical record. Exclusion criteria was inadequate sputum samples.

The data was collected through primary data based on result from determination bacterial gene mutations that cause resistance in pneumonia using the molecular PCR method carried out in the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra and secondary data using medical record data included age, sex and diagnosed pneumonia. Data were analyzed using SPSS 20th in evaluating the sensitivity and specificity of bacterial culture using VITEK-2 in detecting *A. baumanii* and *K. pneumonia*.

Result

The average age of respondents in this study was 55.50 years, of which were (70%) men and women (30%). All of samples from 50 sputum showed that result based on cultured bacteria with Vitek 2 found a bacterial growth of (90%) samples, consisting of *K. pneumonia* (33.3%), *A. baumanii* (28.8%), *Pseudomonas aeroiginosa* (20%), *Acinetobacter iwofii* (4.4%), *E.coli* (2.2%), *Staphylococcus hemolyticus* (2%), *Streptococcus crista* (6.6%) and *Enterobacter cloaca* (2.2%).

The data from PCR found there were *K. pneumoniaes* detected (48.4%) and *A. baumanii* 17 (51.5%).

The prevalence of ESBL was found as many (35.5%), where (93.7%) were found in the bacteria *K. pneumonia*, *A.baumanii* (0.03%), and *E. coli* (6%). The *Klabsiella pneumonia* bacteria had the CTX M gene (93.7%) and the OXA gene (31.2%) and (25%) had the CTXM and OXA genes. Whereas Acinetobacter baumanii has the CTX M gene (17.6%), the OXA gene (23.5%) and the CTXM and OXA genes together (5.8%). (Table 4.1)

Based on diagnostic test results using VITEK-2 in detecting *K. pneumonia* and/or *A.baumanii* found sensitivity (93%) and specificity (100%). This shows that VITEK-2 method is more accurate in terms of diagnostic than *K. pneumonia* and/or *A.baumanii* bacterial. (Table 2)

The result of VITEK-2 for diagnostic test in detecting *K. pneumonia* found sensitivity (93.3%) and specificity (100%). This shows that the VITEK-2 method is more accurate in diagnostic terms than screening for *K. pneumonia* bacterial. (Table 3)

The result of VITEK-2 diagnostic test results in detecting *A. baumanii* found sensitivity (76.4%) and specificity (100%). This shows that the VITEK-2 method is more accurate in terms of diagnostics than screening for *A.baumanii* bacterial. (Table 4)

Table 1. Distribution characteristics subject.

Variable	n (%)
Age (Years)	55,5 (25-86)
Sex	
Man	35 (70%)
Women	15 (30%)
Bacterial Identification using Vitek2	
Klebsiella pneumonia	15 (33,3%)
Acinetobacter baumanii	13 (28,8%)
Pseudomonas aeroginosa	9 (20%)
Acinetobacter iwofii	2 (4,4%)
E. coli	1 (2,2%)
Staphylococcus hemolyticus	1 (2,2%)
Streptococcus crista	3 (6,6%)
Enterococcus cloaca	1 (2,2%)
Identification ESBL using Vitek2	16 (35,5%)
Klebsiella pneumonia	15 (93,7%)
Acinetobacter baumanii	0
E.coli	1 (6,2%)
Bacterial identification using PCR	
Klebsiella pneumonia	16 (48,4%)
Acinetobacter baumanii	17(51,5%)
Genotipe ESBL *K.pneumonia*	
CTX-M	15 (93,7%)
OXA	5 (31,2%)
CTX-M + *OXA*	4 (25%)
Genotipe ESBL *A. baumanii*	
CTX-M	3(17,6%)
OXA	4(23,5%)
CTX-M + *OXA*	1(5,8%)

Table 2. The sensitivity and specificity test of bacterial culture in detecting K. pneumonia and/or A. baumanii

		Positive	Negative	Total
Bacteria Cultured	**Positive**	28	0	28
	Negative	2	20	22
	Total	30	20	50

Table 3. The sensitivity and specificity test of bacterial culture in detecting K. pneumonia

		Positive	Negative	Total
Bacteria Cultured	**Positive**	15	0	15
	Negative	1	34	35
	Total	16	34	50

Table 4. The sensitivity and specificity test of bacterial culture in detecting A. baumanii

		Positive	Negative	Total
Bacteria Cultured	**Positive**	13	0	13
	Negative	4	33	37
	Total	17	33	50

Discussion

In this study, VITEK-2 method in detecting the bacteria *K. pneumonia* and/or *A. baumanii* was found sensitivity (93%) and specificity (100%) in patients with pneumonia, which specificly showed that ability of VITEK-2 in detecting *A. baumanii* had sensitivity (76.4%) and specificity (100%) and *K. pneumonia* had sensitivity (93.3%) and specificity (100%). This study is in line with the study of Joyanes et al., was using 198 clinical isolates in which it identified *A. baumanii* with sensitivity (76%).[11] The same result is also found in Bobenchik study (2015) at Los Angeles where a value of sensitivity (92.7%) was obtained in detecting *A. baumanii*.[12] Other studies on the VITEK-2 method show satisfactory results in detecting gram-negative bacteria such as *A. baumanii* and *K. pneumonia*. Ling et al, of 281 isolates found sensitivity (95%). Nakasone et al. and Gherardi et al. analyzed 181 and 95 gram-negatives and obtained sensitivity (96.7%) and (100%) with the VITEK-2 system.[13,14] Study in Switzerland showed a high specificity value (90.4%) in detecting *K. pneumonia*.[15]

Extended-spectrum beta-lactamases (ESBLs) are a large groups bacterial, rapidly growing group of enzymes that provide resistance to oxymino cephalosporins and monobactams and are inhibited by clavulanates. Early detection that show accurate and reliable result tesr is needed of management infection. In this study found a lower prevalence of ESBL (35.5%), even though study Horie et al. showed higher prevalence of ESBL (41.9%) in Europe and the Mediterranean, (52.9%) in Latin America and (47.2%) in the Asia Pacific region.[16] Other researchers in Ethiopia from 426 samples identified as much ESBL (57.7%) also survey conducted at a hospital in East Africa reported the overall proportion of ESBL was collected (42%).[17,18]

Klebsiella pneumoniae is one of the pathogens that cause pneumonia and results in a wide range of infections causing major morbidity and mortality. Novel study found that the potential of many antimicrobial agents has been resistent to this bacterium. ESBL is known to be an enzyme produced by bacteria to fight betalactam antibiotics.[19] In the last decade, ESBL type CTX-M has replaced the TEM and SHV types and has become the dominant isolate in Enterobacteriaceae clinically. We found a high prevalence of CTXM in *K. pneumonia* (100%) bacterial. Alongside with study in Lebanon (2014) confirmed that 68 ESBL isolates *K.pneumonia* (86.7%) containing CTX-M.[20] Research in the United States in 1990 showed that the prevalence of blaCTX-M was still rare (25%) in 2000 but increased rapidly in 2005 (90%).[21] Research in New York also shows the alignment of the increase in the prevalence of blaCTX-M *K. pneumonia* showed significant increased in 2005 to 2009 (1.7%) and in 2010 to 2012 (26.4%) ($p < 0.0001$).[22]

Carbapenem has been the drug of choice for the treatment of serious infections caused by strains of Enterobacteriaceae that produce β-lactamase (ESBL). The emergence of carbapenem resistance has been increasingly reported among Enterobacteriaceae and is a major clinical problem. The most common mechanism for carbapenem resistance in *K. pneumoniae* is the production of carbapenemases.[23] Previous study have been reported Class D carbapenemases obtained mainly in *Acinetobacter spp*. Oxacillinase was first identified from isolates of *K. pneumonia* in Istanbul, Turkey.[24] *Acinetobacter baumannii* is an opportunistic pathogen that is often involved in outbreaks of infection. Isolates resistant to several *A. baumannii* drugs have been reported to increase over the past decade, possibly as a consequence of extensive use of antimicrobial agents.[25] Several studies focusing on carbapenem resistance in *A. baumannii* have been reported, mostly related to β-lactamase production. *A. baumannii* also produces intrinsic β-lactamase which is an oxasilinase represented by the OXA variant.[26,27]

However, in our study, the prevalence of the OXA gene in *A. baumanii* was still low (23.5%) but contained CTXM gene expression (17.6%) that in line with study in Iran that found CTXM gene expression also appeared (20%) in 100 *A. baumanii* isolates.[28] Studies in Haiti and India also showed the low prevalence of CTXM gene expression. Despite the high prevalence of blaCTX-M-15 in Enterobacteriaceae, the gene is still rarely identified in *A. baumannii*. This may be due to the fact that plasmids carrying blaCTX-M-15 have a narrow host range and

cannot replicate within *A. baumannii*, it is possible that the integration of the ISEcp1-blaCTX-M-15 element into the chromosome *A. baumannii* may have occurred.[29]

Conclusion

This study concluded that VITEK-2 is a diagnostic support tool with good sensitivity and specificity, so it can use to identify the *Klebsiella pneumonia* and *Acinetobacter baumanii* bacteria that cause pneumonia. It is known about the spread of ESBL genotypes that are still low, especially CTX-M and OXA which are found in the bacteria *K. pneumonia* and *A. Baumanii,* so should be concern in the management of infectious patients where the high prevalence of ESBL genotypes will affect the morbidity, mortality, period care, and high maintenance costs in inpatients pneumonia

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