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Incidence of *Klebsiella pneumoniae* producing Metallo Beta-Lactamase (MBL) at RSUP Dr. Wahidin Sudirohusodo Makassar

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Abstract: Bacterial resistance to antibiotic is one of the factors triggering infection therapy failure. This study was conducted to determine the prevalence of carbapenem-resistance Klebsiella pneumoniae infection and the phenotype of *carbapenem-resistant Metallo-Beta-Lactamase (MBL)-producing Klebsiella pneumoniae isolates* at RSUP Dr. Wahidin Sudirohusodo Makassar. This study included *Klebsiella pneumoniae* identification on each infectious patient's isolates. The sensitivity test of antibiotics, phenotype confirmatory test, and MBL phenotypic test were conducted using agar diffusion Kirby-Bauer, Vitek-2-Compact, and Double Disc Synergy Test (DDST) method, respectively. As the result, the antibiotic sensitivity test using the Vitek-2-Compact method on 50 clinical samples (pus, sputum, blood. tissue, urine, brain fluid, and feces) found that 10 isolates (20%) were resistant to carbapenem. The phenotypic test using the Double Disc Synergy Test (DDST) method found that carbapenem-resistant isolates caused by the production of *Metallo Beta Lactamase (MBL) enzymes* were 2 isolates or 20% of the total carbapenem-resistant isolates.

Keywords: Carbapenem, Double Disc Synergy Test (DDST), *Klebsiella pneumoniae*, Metallo-Beta-Lactamase

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Introduction

Antimicrobial resistance has been identified as one of the greatest threats to human health in the future, with increasing numbers of resistant microbial strains being reported annually across human and animal populations in both developed and developing countries. Health organizations and research institutions promote for tighter control over the distribution and use of antibiotics in the community, with an emphasis on prescribing and dispensing front-line antibiotics. Moreover, efforts have been made over the years to promote rational use of drugs, for Example The Rational Drug Use Programs (INRUD) held by the WHO international network (Chandler, 2017).

Members of the Enterobacteriaceae that are resistant to several classes of antimicrobials have been growing. Clinicians are increasingly turning to the carbapenem class agents as the last option for the effective treatment of serious infections caused by this pathogen. The mechanisms underlying carbapenem resistance in this complex Enterobacteriaceae include the production of carbapenem-hydrolysis-β-lactamase (CRE [CP-CRE] carbapenemase-producing) and the resistance due to the presence of a combination of other factors (non-CP-CRE), such as hyperproduction of Ampc beta-lactamase or extended-spectrum-beta-lactamase (ESBL) combined with changing membrane permeability (Pierce et al., 2017).

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During the last decade, the emergence of broad-spectrum beta-lactamase-producing bacteria and carbapenem-resistant Enterobacteriaceae have become a global threat to public health. From 2001 to 2012, the rate of resistance to imipenem reached 30% in some epidemic areas in the Middle East, while the top two Asian countries with the highest levels of resistance to imipenem were Indonesia (6%) and the Philippines (4%). 2009 New Delhi Metallo-β-lactamase (blaNDM-1) genes were found in Klebsiella pneumoniae samples in Indonesia (Parathon et al., 2017).

Metallo beta lactamase (MBL) was originally discovered more than forty years ago but was not initially considered a serious problem for antibiotic therapy because MBL was found in the chromosomes of non-pathogenic organisms. However, this situation changed in the 1990s, with the spread of Metallo beta-lactamase-type IMP (Imepenemase) and VIM (Verona Imipenemase) enzymes in gram-negative pathogens, including Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii (Laraki N. et al, 1999; Lauretti L. et al, 1999).

Metallo beta-lactamase (MBL) is a carbapenemase that provides resistance to all beta-lactam antibiotics except monobacteria. MBL is not inhibited by clavulanic acid, tazobactam, or sulbactam, because the combination of beta-lactam with beta-lactamase inhibitors available today is ineffectual. Apart from the broad spectrum of activity, another concerning factor is that there were many MBL genes can be found in plasmids with genes encoding other antibiotic resistance determinants, namely aminoglycoside resistance genes. These positive MBL strains are usually resistant to beta-lactam, aminoglycosides, and fluoroquinolones (Viswamohanan et al., 2016).

Based on the description above, researchers were interested in conducting study on the detection of phenotype isolates of *carbapenem-resistant Metallo-Beta-Lactamase (MBL)-producing Klebsiella pneumoniae isolates* among infectious patients in the treatment room of RSUP DR. Wahidin Sudirohusudo Makassar. The Klebsiella pneumoniae bacteria was investigated because as the species of Enterobacteriaceae, it was widely found as the cause of nosocomial infection in RSUP DR. Wahidin Sudirohusodo. This study aimed to determine the prevalence of Klebsiella pneumoniae producing Metallo beta-lactamase (MBL) enzymes that are resistant to carbapenem type antibiotics in infectious patients at the Central General Hospital Dr. Wahidin Sudirohusodo Makassar.

Materials and Methods

a. Equipments and Materials

Equipments used in this research were BSC Type II, Bunsen, petri dish, gloves, hot plate, incubator, needle, laminar water flow, refrigerator, micropipette (1000 µl, 100 µl, 20 µl, 10 µl), microtube, serological pipette, test tube, analytical scales, tips (1000 µl, 100 µl, 20 µl, 10 µl), Turbidimeter (McFarland) vortex, water bath, Vitex 2 Compact®, and other glassware. The materials used in this study are: Buffer TEA, Ethidium bromide, Medium TSIA MacConkey agar, Mueller Hinton Agar, McFarland standard suspension, sodium EDTA, 0.9% sodium chloride, and antibiotic paper disk (Imipenem/HDI 10µg), *Klebsiella pneumoniae* Bacterial isolate.

b. Procedures of Data Collection

1. Identification of Bacteria.

Suspension of Colonies growing on MacConkey medium were made by NaCl 0.45% combined with 0.5 McFarland suspension. Next, 145 µl of the suspension were injected into Vitek 2 Compact® devices. These bacteria's suspension should not take more than 30 minutes to be inoculated into the VITEK 2 Compact®. For identification of Klebsiella pneumoniae bacteria, the GN card was used on VITEK 2 Compact® machine.

2. Phenotype Confirmation Test using the Double Disk Synergy Test Method (DDST)

The test was conducted by preparing the identified *Klebsiella pneumoniae* bacterial suspension, equivalent to 0.5 McFarland, then swab them on Mueller Hinton Agar's medium. 10 µg Imipenem disc and blank disc were placed on Mueller Hinton agar (which has been treated with bacterial suspense) 20 mm from the center of the agar plate and 10 µL of 0.5 M EDTA solution was added to the blank disc. Imipenem disc inhibition zone and EDTA disc were compared after overnight incubation at 35°C. A positive result of MBL was interpreted interpreted if there is an increase in the inhibition zone on a Blank disk containing EDTA compared to an Imepenem disk.

Result and Discussion

This research was conducted at the Clinical Pathology Laboratory, the subdivision of Tropical Infectious Diseases and Medical Record Installation, Dr. Wahidin Sudirohusodo Makassar General Hospital. Research sample were collected on isolates of patients diagnosed with infectious diseases in the treatment ward from April to July 2019.

The subjects were all patients diagnosed with infectious disease based on laboratory examination

results including higher value of WBC (White Blood Cell) values, Neutrophil values, lymphocytes, monocytes, LEDs (blood sediment rate), PCT (Procalcitonin). In addition, the patient's isolates showed *Klebsiella pneumoniae* as the only infection-causing bacteria, without contamination of other infection-causing bacteria.

Table 1. Diagnosis of disease-based distribution subjects.

No.	Types of Diseases	Number of Patient (n)	Percentage (%)
1.	Sepsis	15	30
2.	Lung TB	10	20
3.	Urinary tract infections	5	10
4.	Gastrointestinal infections	5	10
5.	Upper respiratory tract infection	4	8
6.	Pneumonia	4	8
7.	Cerebral Infection	3	6
8.	Cytomegalovirus infection	2	4
9.	Leukemia	2	4
	Total	50	100

Based on **Table 1**, it showed that most of patients were diagnosed with sepsis (30%) as the source of Klebsiella pneumoniae bacterial isolate. Sepsis was defined as a systemic response to confirmed or suspected infections, including pneumonia, intra-abdominal infections, and urinary tract infections (Sawano et al., 2016). Severe sepsis can be defined as sepsis with sepsis-induced organ dysfunction or tissue hypoperfusion (hypotension, increased lactate, or decreased urinary output), while sepsis shock is defined as severe sepsis plus persistent low blood pressure after intravenous fluid administration (Dellinger et al., 2013). Klebsiella pneumoniae is a rare sepsis-causing bacterium but is known for its severe outcome with high mortality rate. Bacteremia caused by Klebsiella pneumoniae occured more in patients with potentially damage to the immune system, such as Elderly, Diabetes mellitus, with a worse prognosis (Sawano et al., 2016).

Table 2. Identification of *Klebsiella pneumoniae* bacteria

No.	Sample	Total	Percentage (%)	Type bacteria	Probability
1	Blood	11	22	*Klebsiella pneumonia*	93 – 99%
2	Sputum	11	22	*Klebsiella pneumonia*	93 – 99%
3	Feces	8	16	*Klebsiella pneumonia*	99%
4	Urine	6	12	*Klebsiella pneumonia*	99%
5	Bronchus	3	6	*Klebsiella pneumonia*	99%
6	Stomach	3	6	*Klebsiella pneumonia*	99%
7	Tissue	1	2	*Klebsiella pneumonia*	99%
8	Pleura	1	2	*Klebsiella pneumonia*	99%

The results in **Table 2** described that the level of turbidity Vitek 2 Compact® to identify *Klebsiella pneumoniae* bacteria is in the range of 93% - 99%. Blood and sputum samples have a probability level range of 93% - 99%, very good levels, and samples of feces, urine, bronchial lavage, gastric lavage, tissue, and pleural fluid have a probability level of 99%. This indicates that the probability of identifying *Klebsiella pneumoniae* bacteria in the sample using Vitek 2 Compact® still has a high probability level. The probability value of Vitek 2 Compact® according to Pincus is divided as: excellent (96% - 99%), very good (93% - 95%), good (89%- 92%), acceptable (85% - 88%), and the presence of two to three taxa that have the same pattern is stated as low discrimination (Pincus, 2013).

Table 3. Confirmation test results of phenotype *Klebsiella Pneumoniae*

Isolates	Resistance	Percentage (%)	Intermediate	Percentage (%)	Sensitivity	Percentage (%)
50	10	20	1	2	39	78

Antibiotics	Resistance	Percentage (%)	Intermediate	Percentage (%)	Sensitivity	Percentage (%)
Imipenem	7	14	1	2	42	84
Meropenem	7	14	1	2	42	84
Doripenem	6	12	0	0	44	88

Table 3 showed that sensitivity test of 50 by Vitek 2 Compact® against various types of antibiotics carbapenem group indicates that Imipenem had 7 (14%) resistant isolates and 1 (2%) intermediate, Meropenem had 7 (14%) resistant isolates and 1 (2%) intermediate, and Doripenem has 6 (12%) resistant isolates. Resistance to carbapenem antibiotics is also called Carbapenem-Resistant Klebsiella pneumoniae (CRKP). This resistance was first reported in the United States in 2001 (Saidel-Odes & Borer, 2013). Mechanisms of resistance to carbapenem include the production of beta-lactamase (carbapenemase), efflux systems (pumps), and mutations that alter the expression or function of porins and penicillin-binding proteins (PBP) (Papp-Wallace et al., 2011).

Table 4. Confirmation MBL test from phenotype *Klebsiella Pneumoniae*

Isolates *Klebsiella pneumoniae*	MBL Positive	Percentage (%)	MBL Negative	Percentage (%)
10	2	20	8	80

Table 4 described that from 10 carbapenem-resistant isolates tested using the dual disk synergy test method (DDST), there were 2 (20%) isolates with positive Metallo Beta-Lactamase (MBL) and 8 (80%) isolates with negative Metallo Beta-Lactamase (MBL). According to Chinjal A, in the study of one of India's hospitals, from 32 isolates of Klebsiella pneumoniae tested, there were 6 (18.75%) MBL positive isolates (Panchal et al., 2017). The percentage is lower than the results of this study. In the Central Nepal it was found that from 185 isolates of *Klebsiella pneumoniae*, 39 isolates (21.08%) were MBL positive and 71.79% Klebsiella pneumoniae producing MBL were resistant to some antibiotics (Bora et al., 2014). This is higher than this study. In other studies conducted in various countries, MBL production levels of the isolate Klebsiella pneumoniae ranged from 33-36% (Nepal et al., 2017). This study showed that the production rate of MBL from the Klebsiella pneumoniae isolate is still relatively low.

Conclusion

According to the result, it can be concluded that during the research period, the prevalence of Klebsiella pneumoniae resistance to carbapenem antibiotic group was 10 isolates (20%) of the total isolates of infectious patients caused by Klebsiella pneumoniae bacteria at RSUP Dr. Wahidin Sudirohusodo Makassar. It also concluded that there were 2 (20%) of clinical carbapenem-resistance Klebsiella pneumoniae bacteria isolates at RSUP Dr. Wahidin Sudirohusodo Makassar that caused by the production of Metallo beta-lactamase (MBL) enzymes.

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