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Detection of metallo-β-lactamase gene ^{bla}IMP of *Escherichia coli* clinical isolates in Sanglah General Hospital Bali

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ABSTRACT

Background: *Escherichia coli* belong to the family of *Enterobacteriaceae* that are responsible as one of the leading cause of nosocomial infections. The emergence of Carbapenem-resistant *Enterobacteriaceae* presents therapeutic challenges for clinicians on choosing the correct type of antibiotics to prescribe. The Emergence of microorganism capable of producing metallo-β-Lactamase (MBL) causes resistance to several antibiotic, including carbapenem.

Aim: The study aims is to detect whether there are *E.coli* clinical isolates in Sanglah hospital that produce metallo-β-Lactamase (MBL) enzyme and carries ^{bla}IMP metallo-β-Lactamase (MBL) gene.

Methods: Clinical isolates of *E.coli* were collected from patients diagnosed with infection in Sanglah Hospital within January to June of 2018 nd has been subjected to antibiotics susceptibility test using Vitek-2 Compact system. EDTA-DDST was performed to detect MBL producing strains, followed by detection of ^{bla}IMP gene using PCR.

Results: There were no *E.coli* clinical isolates that produce MBL enzyme and carried ^{bla}IMP gene (0.0%). The prevalence of *E.coli* producing ^{bla}IMP gene in Sanglah hospital was 0.0%.

Conclusions: None of the *Escherichia coli* exhibits the ability to produce metallo-β-Lactamase (MBL) enzyme and carried ^{bla}IMP gene. Detecting other genes aside from ^{bla}IMP gene may be necessary to reveal the other causative factors of antibiotics resistance in *Escherichia coli*.

Keywords: *Escherichia coli*, Antibiotic resistance, Carbapenem, Metallo-β-Lactamase Enzyme, ^{bla}IMP gene.
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INTRODUCTION

Escherichia coli are gram negative bacteria belong to the family of *Enterobacteriaceae* that are responsible as one of the main cause of nosocomial infection.[1] Its capability of producing Metallo-β-Lactamase (MBL) causes resistance to several antibiotics of β-lactam, including carbapenem.[3] Carbapenem (meropenem, imipenem, and ertapenem) are broad-spectrum antibiotic commonly prescribed for multidrug-resistant (MDR) bacterial infection.[4] The emergence of Carbapenem-resistant *Enterobacteriaceae* (CRE) presents therapeutic challenges for clinicians in choosing the correct type of antibiotics to prescribe and offer a crisis for public health in global.[5] Detections of microorganism producing β-lactamase would be essential to avoid spread of multidrug-resistant organisms in hospitals and to reduce morbidity and mortality rates among patients.[6]

MBL is an enzyme-mediated carbapenem-resistant. Bacterias that phenotipically produce MBL enzyme can hydrolyze all antibiotics from β-lactam groups.[7] MBLs have several variants of antibiotic resistance genes such as ^{bla}IMP, ^{bla}VIM, ^{bla}NDM, ^{bla}OXA, ^{bla}SPM, etc. Among those genes, ^{bla}IMP gene is found to have the most prevalence in Asia including Indonesia and several countries.[8]

In 2012 it was reported that the prevalence of MBL producing *E. coli* increased in Japan. It was reported that around 90.7% of MBL producing *E.coli* carried ^{bla}IMP-6 gene while 9.3% carried ^{bla}IMP-1 gene.[9] In northern Italy, there were reports of outbreaks of infection caused by *E.coli* and *Klebsiella pneumoniae* producing MBL and NDM in 2011.[11] Several studies conducted in India in 2013 and 2014, showed that around 50% - 81.8% of *E.coli* in India were known as MBL Producer.[18-20] Recent research conducted in Palestine in 2016, showed high prevalence of *E.coli* producing MBL (around 87.4%).[6] Studies on gram negative *Enterobacteriaceae* producing carbapenemase such as *Escherichia coli* were very limited compared to those on non-fermenters bacteria.[6] In our knowledge, studies conducted on multidrug resistance bacteria are still rare in Indonesia.

METHODS

This study was a descriptive observational study

that took place for four months, from March to June of 2019. This research was conducted in Biomedical Laboratory and Microbiology Laboratory Faculty of Medicine Udayana University. Data of *Escherichia coli* and its antimicrobial sensitivity test which were performed using Vitek-2 Compact system in Sanglah Hospital were gathered from clinical specimen from patients in 2018. The study has been approved by the Committee of Ethical Research of Universitas Udayana/Sanglah General Hospital. Data collected in this study were analyzed by descriptive statistic using SPSS version 16 for windows to determine the distributions and frequencies of *E.coli* producing MBL enzyme and carried ^{bla}IMP gene.

Bacterial strains. A total of 160 *Escherichia coli* clinical isolates were collected from patients diagnosed with the infection in Sanglah hospital from January to June of 2018. They had been subjected to sensitivity test for meropenem and ertapenem using Vitek-2 Compact system. *E.coli* isolates were subcultured in McConkey Agar to obtain pure colonies.

Antibiotics susceptibility testing. Susceptibility testing was performed using the microdilution method by Vitek-2 Compact system (Biomeireux) according to the Clinical and Laboratory Standard Institute (CLSI).[10]

Metallo-β-Lactamase phenotyphic test. An EDTA-disk diffusion synergy test (DDST) was used to detect Metallo-β-Lactamase production. The test strain was adjusted to a turbidity of 0.5 McFarland standards and inoculated onto Mueller Hilton Agar plate. A meropenem disk (10μg) and a sterilized blank filter paper disk with 10 μl of a 0.5 M EDTA solution were placed 10 mm apart from edge to edge. After overnight incubation at 37°C, the presence of synergistic inhibition zone was interpreted as EDTA-synergy test positive.[16]

Table 1. Antibiotics susceptibility test result

Antibiotic	*Escherichia coli*		Total (%)
	S n (%)	R n (%)	
MEM	153 (95.6%)	7 (4.4%)	160 (100%)
ETP	153 (95.6%)	7 (4.4%)	

S: Sensitive, R: Resistant, MEM: Meropenem, ETP: Ertapenem.

Table 2. Frequency of MBL enzyme in *E.coli* carbapenem resistant and sensitive

Organism	+ve MBL n (%)	-ve MBL n (%)	Total n (%)
E.coli Carbapenem-resistant	0 (0,0%)	153 (100%)	153 (100%)
E.coli Carbapenem-sensitive	0 (0,0%)	153 (100%)	153 (100%)

DNA Extraction. DNA was extracted from *E.coli* colonies using boiling method. A few colonies from overnight McConkey Agar culture of *E.coli* isolates were suspended in 200 μl of TE buffer (10 mM Tris, 0,5 mM EDTA) using vortex. The suspension was heated in boiling bath at 100°C for 10 min then cooled in ice water for 3-5 minutes. After centrifugation at 8000 rpm for 1 min, the supernatant was used as DNA template for amplification using PCR.

Molecular analysis. Polymerase chain reaction (PCR) was performed for detection of ^{bla}IMP gene on a thermal cycler (Biometra, Germany). Amplification for ^{bla}IMP gene was performed using 740bp primers IMP-F (5'-F: TGAGCAAGTTATCTGTATTC) and IMP-R (3'-R: TAGTTGCTTGGTTTTGATG).[17] PCR was performed under the following conditions: one cycle of initial denaturation at 95°C for 2 minutes, continued by 35 cycles of denaturation at 95°C for 1 min, annealing at 49°C for 1 min, extension at 72°C for 1 min, and one cycle of final extension at 72°C for 5 minutes. The PCR products were loaded onto 1,5% agarose gel and stained using gel red and separated using gel electrophoresis. The separated bands visualized under UV light using UV Transilluminator.

RESULT

A total of 160 isolates of *E.coli* were collected from various specimens from patients diagnosed with the infection in Sanglah hospital. *E.coli* samples were isolated from January to June of 2018. Result of carbapenem antibiotic susceptibility test by Vitek-2 Compact system revealed that only a few (4.4%) of the *E.coli* isolates were resistant to meropenem and ertapenem while most (95.6%) of the *E.coli* clinical isolates showed high sensitivity to meropenem and ertapenem (Table 1).

Metallo-β-Lactamase phenotyphic test. Of all 160 *E.coli* isolates, only 153 grew during subculture in McConkey agar. Out of these 153 samples, the result showed that all (100%) of *E.coli* isolates from Sanglah hospital did not produce MBL enzyme (Table 2). All samples showed EDTA-DDST negative test result since there was no synergistic inhibition zone that appeared between meropenem disk and EDTA disk (Figure 1). This result indicated that *E.coli* isolates did not produce the MBL enzyme phenotypically.

Amplification of ^{bla}IMP metallo-β-lactamase gene. The result of an amplified gene by PCR showed that none (0.0%) of *E.coli* clinical isolates carried the ^{bla}IMP gene (Table 3). The PCR result did not show any band of ^{bla}IMP gene at 740bp (Figure 2). Our negative PCR and EDTA-DDST

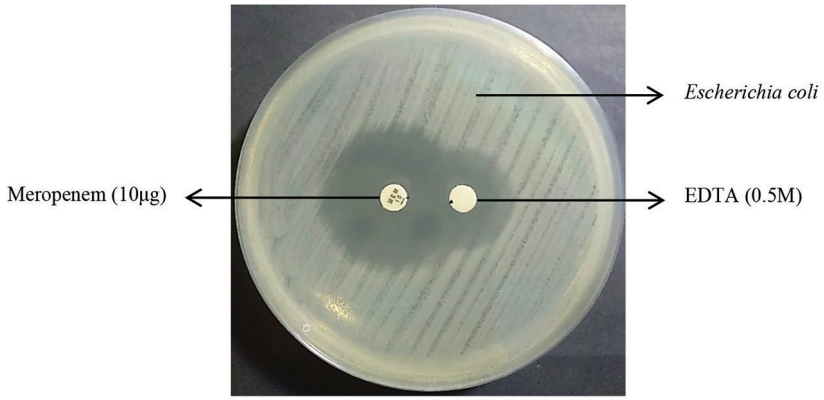

Figure 1. EDTA-disk diffusion synergy test for detection of MBL producer by use of EDTA

Table 3. Frequency of ^{bla}IMP gene in *E.coli* carbapenem resistant and sensitive

Organism	+ve ^{bla}IMP n (%)	-ve ^{bla}IMP n (%)	Total n (%)
E.coli Carbapenem-resistant	0 (0,0%)	153 (100%)	153 (100%)
E.coli Carbapenem-sensitive	0 (0,0%)	153 (100%)	153 (100%)

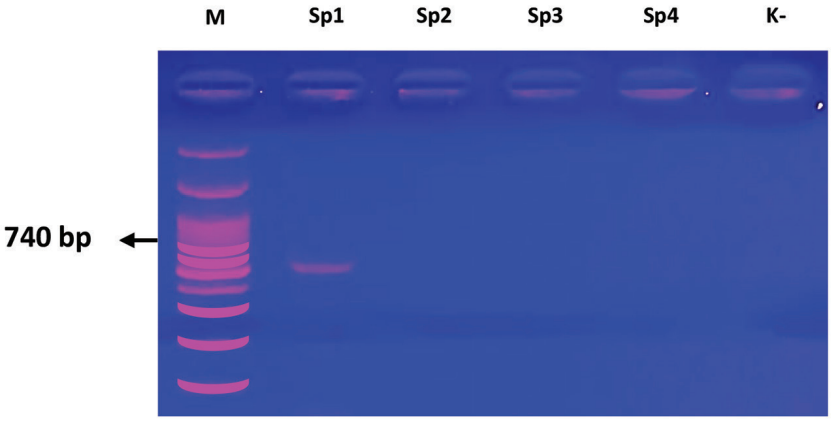

Figure 2. Electroforegram of amplified product from DNA *Escherichia coli*. Lanes M: DNA ladder (100bp), Sp1-4: lanes without amplified product of ^{bla}IMP gene. K-: negative control (contains nuclease free water).

result confirms that the prevalence of *Escherichia coli* producing ^{bla}IMP MBL gene in Sanglah hospital Bali was 0.0%.

DISCUSSION

Escherichia coli are gram negative bacteria belong to family of *Enterobacteriaceae* that are responsible as one of the main cause of nosocomial infection in hospitals.[1] The Emergence of microorganism capable of producing MBL causes resistance to several antibiotic, including carbapenem.[3] Carbapenem are wide spectrum antibiotic commonly prescribed for multidrug resistant (MDR) bacterial infection.[4] The presence of β-lactamase enzyme in bacteria may results in the ineffectiveness of carbapenem antibiotic. MBLs belong to group B β-lactamase enzyme which has the ability to inactivated antibiotics β-lactam by cleaving the amino bond of the β-lactam ring structure.[2] MBLs has unique characteristic that has metal ion site on enzyme active site which it is sensitive to chelating agent such as EDTA, sodium mercapto acetic acid (SMA), and dipicolinic acid.[7] The catalysis activity of MBL enzyme cannot be inhibited by the presents of β-lactamase inhibitors such as clavulanic acid, tazobactam, and sulbactam.[12]

In this study, MBL phenotyphic test results showed that all of *E.coli* isolates both resistant and sensitive to carbapenem did not produce MBL enzyme. This result was similar with study conducted by Rasyid and Suharti (2012) where detection of MBL enzyme in *E.coli* carbapenem-resistant by the same method showed that none of the *E.coli* isolates produced MBL enzyme.[5] This result was different compared to study conducted by Chika et al. (2017) where it is shown that 15 (31%) of the *E.coli* isolates were detected as MBLs producer.[13] The variation in results among these studies may be due to several factors such as various sample types, different sample location, and different sample collection time.

The absence of MBL enzyme means that phenotipically *E.coli* showed sensitivity to carbapenem. However, some of *E.coli* carbapenem-resistant isolates used in this study which were subjected using Vitek-2 Compact system showed sensitivity to carbapenem. This result indicated that Vitek-2 Compact System is not entirely accurate.[23] Despite the absence of MBL enzyme in *E.coli* carbapenem-resistant in this study, *E.coli* still has other antibiotics resistance mechanisms such as efflux pump, modified cell wall, and alteration of drug target. Stuart et al. (2013) found that in *E.coli* carbapenem-resistant from patients in one of the hospitals in Beijing, showed an increase of activity of the efflux pump which resulted in resistance to carbapenem despite the absence of the production of inactivator enzyme carbapenemase.[14]

In this study, we found that none of *E.coli* isolates both resistant and sensitive to carbapenem carried ^{bla}IMP gene. This result was similar with study conducted by Nairoukh et al. (2018), in which none of *E.coli* multidrug resistant carried ^{bla}IMP gene.[15] The absence of ^{bla}IMP genotypically means that *E.coli* did not produce MBL enzyme and showed sensitivity to carbapenem antibiotic phenotypically. The result of this study was different compared to a study conducted by Adwan et al. (2016) where it is found that the ^{bla}IMP gene were detected in 41.4% of the *E.coli* isolates.[6]

The absence of blaIMP gene in *E.coli* carbapenem-resistant in this study did not close the possibility that *E.coli* has another gene aside from blaIMP such as blaVIM, blaNDM, blaOXA, blaCTX, blaSPM, etc that can result in carbapenem resistance. A study conducted by Nairoukh et al. (2018) to identify the MBLs gene of *E.coli* MDR showed that none of the isolates carried blaIMP gene. The isolates instead carried blaCTX-M-1 and blaCTX-M-15, and blaKPC-2.[15] This result indicated that the absence of blaIMP gene in *E.coli* did not means that the isolates did not encode another antibiotics resistance gene.

Our study showed the absence of *Escherichia coli* producing blaIMP MBL gene in Sanglah hospital Bali. Study conducted in Turkey, reported none (0.0%) of multidrug resistant *E.coli* producing MBL in a tertiary care hospital.[21] In other study in North Palestine which showed the prevalence of *E.coli* isolated from Thabet Hospital-Tulkarm producing blaIMP gene to be 41.4%.[6] In other countries, the prevalence of *E.coli* producing MBL range around 1.7% - 45.2%.[6] Several study in Japan showed the highest prevalence of MBL gene was attributed to blaIMP gene.[9] These high prevalence of *E.coli* producing MBLs may be the result of several factors such as irrational use of antibiotics, intrahospital dissemination, incorrect patients therapy, movement of medical staff in hospital, and geographical condition that can trigger the spread of multidrug resistant organism (MDRO).[6] The low prevalence of *E.coli* producing blaIMP gene in Sanglah hospital may be explained due to several factors such as prudent use of antibiotics, correct therapy, and good hospital practice management.

This study will help to provide new data about the prevalence of multidrug resistant bacteria in Indonesia and may be used as additional information to determine empirical therapy for patient treatment caused by *Escherichia coli* multidrug resistant infection. The detection of other genes aside blaIMP gene may deem necessary to reveal the other causative factors of antibiotics resistance in *Escherichia coli*.

CONCLUSION

None of the *Escherichia coli* exhibit the ability to produce MBL enzyme and carried blaIMP gene. Detecting other genes aside blaIMP gene may deem necessary to reveal the other causative factors of antibiotics resistance in *Escherichia coli*.

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DISCLOSURE

The authors declare no conflict of interests. There is no funding adequately from any grand sources.

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