Effects of Zinc Chelator TPEN in Bacterial Susceptibility to Antibiotics Beta-Lactam and Aminoglycoside

Betty **Suryawati**[1)], Salsabila Resaputri **Mulyono**[2)], Salsabilla Karunia **Rahmah**[3)], Yusuf Ari **Mashuri**[4)]

[1)]Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret
[2)] Undergraduate Program Faculty of Medicine, Universitas Sebelas Maret
[3)]Undergraduate Program Faculty of Medicine, Universitas Sebelas Maret
[4)]Department of Public Health, Faculty of Medicine, Universitas Sebelas Maret

ABSTRACT

Background: Previous research showed that zinc plays significant roles in bacterial virulence, including bacterial resistance to antibiotics which has been increasing over the year. This research aims to elucidate the effect of the addition of zinc chelator TPEN on bacterial susceptibility towards beta-lactam and aminoglycoside antibiotics.

Methods: This was an experimental study using 30 clinical isolates of Gram positive and negative bacteria. Bacteria were inoculated into Mueller Hinton agar as control and Mueller Hinton with addition of 30 µM and 20 µM zinc chelator TPEN. Antibiotic susceptibility test was conducted using Kirby Bauer method. The difference of inhibition zone diameter was compared and analyzed using Mann-Whitney test.

Results: There was a significant difference in inhibition zone diameter of meropene ($p<0.05$) while no significant differences was observed in ceftriaxone, cefotaxime, ampicillin, imipenem, kanamycin, amikacin, gentamicin, and tobramycin ($p>0.05$). Statistical test using the whole pair of data, showed no significant difference in inhibition diameter of control and experimental group in both beta-lactam ($p>0.05$) and aminoglycoside antibiotics ($p>0.05$).

Conclusion: The addition of zinc chelator TPEN in Mueller Hinton agar increase bacterial susceptibility to meropenem significantly. Meanwhile, it did not influence bacterial susceptibility to the other beta-lactam and aminoglycoside antibiotics.

Key words – zinc chelator, TPEN, bacterial susceptibility, beta-lactam, aminoglycoside

Correspondence:
Betty Suryawati. Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia. Email: betty.suryawati@staff.uns.ac.id.

INTRODUCTION

Antimicrobial resistance (AMR) is the biggest threat to global health because it is a significant cause of morbidity and mortality[1]. Inappropriate duration and doses of use can lead to resistance[2]. AMR decreases the effectiveness of antibiotics and the emergence of multidrug resistant organisms (MDROs) and extensively drug-resistant (XDR)[3]. The cases of infection due to MDROs have increased and caused several effects such as limited choice of antibiotics, prolonging treatment time, increasing the risk of death, and increasing nocosomial infections[4]. With the increasing number of MDROs and the limited choice of antibiotics, research is needed for the development of antibiotics.

There are several mechanisms for the emergence of resistance in bacteria to antibiotics, for example in beta-lactams and aminoglycosides. The mechanisms of resistance to beta-lactam antibiotics include decreased beta- lactam penetration and target site alteration, efflux mechanisms, and beta-lactam inactivation by beta-lactamase enzymes[5]. Meanwhile, the mechanism of resistance to aminoglycoside are enzymatic modification (acetylation, phosphorylation, and adenylation), modification of the attachment target through enzyme or chromosomal mutations, and antibiotic efflux[6].

Bacteria have strict regulation of the bioavailability of transition metals which play an important role in host and pathogen interactions, one of them is zinc[7,8]. Zinc is a fundamental metal micronutrient for bacterial growth. In bacteria, zinc has roles in the function of catalysts or structural cofactors of enzymes and proteins involved in various processes such as DNA replication and protein synthesis, as well as regulators[9]. Excess intracellular zinc levels are toxic to organism because it can interfere with the redox potential[7,10]. Previous study showed that the addition of zinc actually inhibited the growth of *Acinetobacter baumannii* and *Escherichia coli* which were given aminoglycoside antibiotics through inhibition of the activity of the enzyme AAC(6 ')–Ib[11,12]. Meanwhile, a study showed that intracellular zinc depletion induced by the addition of zinc chelator can cause oxidative stress, DNA damage and cell apoptosis[13].

The effect of zinc can be investigated through the susceptibility of bacteria to antibiotics whose zinc is eliminated with zinc chelating agent, one of which is TPEN (N,N,N′,N′-tetrakis(2-pyridylmethyl) ethylene diamine). TPEN is an ion chelator that is fat soluble and has a high affinity for zinc[14]. Previous studies have shown that TPEN has the potential to have a role as a carbapenem adjuvant against bacteria that produce MBL/Metallo-β-Lactamase (zinc dependent β-lactamase[15,16]. With the addition of TPEN in the antibiotic susceptibility test, zinc is expected to bind to TPEN resulting in the limitation of zinc and causes disruption of zinc homeostasis in bacteria. The effect of this limitation process will be observed on the susceptibility of bacteria to beta-lactam and aminoglycoside antibiotics.

This study aimed to to elucidate the effect of the addition of zinc chelator TPEN on bacterial susceptibility towards beta-lactam and aminoglycoside antibiotics.

METHODS

This is an experimental study using pre-experimental static group comparison methods. The samples used were 30 clinical isolates of Gram positive and Gram negative bacteria which were the collection of Microbiology Laboratory Faculty of Medicine, Universitas Sebelas Maret and identified using the bioMérieux API NE20 and API E20.

In this study, the effect of TPEN on bacterial susceptibility is assessed by inoculating sample into four types of Mueller Hinton agar, two for beta-lactam susceptibility testing and two for aminoglycoside susceptibility testing. First, TPEN Sigma Aldrich 50 mg was dissolved in 96% ethanol to reach a concentration of 10 mM.TPEN concentration was measured with micropippette and 50 ml centrifuge tube. The susceptibility test conducted in this study used the Kirby Bauer disk diffusion method. Bacteria were inoculated into Mueller Hinton agar as control (MH) and Mueller Hinton with the addition of 30 µM TPEN for beta-lactam and 20 µM for

aminoglycoside (MH+TPEN). The beta- lactam antibiotics used in this study were ceftriaxone, cefotaxime, ampicillin, imipenem, and meropenem. While the aminoglycoside antibiotics used in this study were kanamycin, amikacin, gentamicin, and tobramycin.

Each data were recorded into the susceptibility status were classified using CLSI 2015 classification standard into sensitive, intermediate, and resistant bacteria. CLSI 2013 and EUCAST classification standard were used for bacteria that were not included in CLSI 2015. The diameter of the inhibition zone that has been obtained is then averaged and statistical tests are carried out to determine whether TPEN zinc chelator affects the susceptibility of bacteria to beta-lactam and aminoglycoside antibiotics.

RESULTS

Different profile of 30 clinical isolates of bacterial samples were used in this study. Four samples did not grow on beta-lactam antibiotic's experimental agar (Mueller Hinton + 30 µM TPEN), hence only 26 samples were used in the effect of TPEN on bacterial susceptibility to beta-lactam antibiotics study The results of the antibiotics susceptibility testing are shown on Table 1.

Table 1. The effect of 30 µM TPEN to bacterial susceptibility to beta-Lactam antibiotic.

Sample	Mueller Hinton					Mueller Hinton + 30 µM TPEN				
	CRO	CTX	AMP	IPM	MEM	CRO	CTX	AMP	IPM	MEM
E.coli (5)	10 (R)	7 (R)	6 (R)	32 (S)	32.8 (S)	12 (R)	12 (R)	6 (R)	40 (S)	40 (S)
E.coli (S13)	9.1 (R)	9.8 (R)	6 (R)	31 (S)	33.2 (S)	9.4 (R)	10.1(R)	6 (R)	36.5 (S)	36.5 (S)
P aeruginosa (225)	24 (S)	24 (S)	6.9 (R)	34 (S)	45 (S)	27 (S)	26.7 (S)	8.2 (R)	41.9 (S)	41.9 (S)
P.aeruginosa (226)	24.7 (S)	22.9 (S)	6 (R)	26.6 (S)	36.7 (S)	24.5 (S)	25 (S)	6 (R)	33.8 (S)	33.8 (S)
P.aeruginosa (227)	32.3 (S)	28 (S)	6 (R)	40.5 (S)	51 (S)	37 (S)	37 (S)	10.6 (R)	45 (S)	45 (S)
P.aeruginosa (189)	32 (S)	26 (S)	8.1 (R)	24 (S)	32 (S)	34 (S)	30 (S)	6 (R)	26 (S)	26 (S)
P. aeruginosa (2)	21.2 (S)	17 (R)	6 (R)	26 (S)	31 (S)	23 (S)	19 (R)	6 (R)	26 (S)	26 (S)
P. luteola (S9)	6 ((R))	6 (R)	6 (R)	9.9 (R)	8.6 (R)	6 (R)	6 (R)	6 (R)	21.4 (S)	21.4 (R)
P. aeruginosa (S11)	21.7 (S)	19 (R)	6 (R)	27.3 (S)	34.6 (S)	21.3 (S)	18.9(R)	6 (R)	26.3 (S)	26.3 (S)
P. aeruginosa (S15)	21.6 (S)	20.2 (I)	6 (R)	26.5 (S)	36 (S)	21.6 (S)	20.2 (I)	6 (R)	27.2 (S)	27.2 (S)
Klebsiella sp. (S6)	36 (S)	38 (S)	10.5 (R)	34 (S)	35.5 (S)	42.5 (S)	46 (S)	11.2 (R)	38 (S)	38 (S)
Klebsiella sp. 199	33 (S)	35 (S)	9 (R)	29.5 (S)	34 (S)	38 (S)	38 (S)	16 (S)	30.3 (S)	30.3 (S)
Klebsiella sp. (S14)	32 (S)	34 (S)	7 (R)	31.1 (S)	32.9 (S)	31.1 (S)	31 (S)	7 (R)	31.7 (S)	31.7 (S)
Klebsiella sp. (S16)	30.5 (S)	30.9 (S)	6 (R)	29 (S)	32.1 (S)	35.4 (S)	37.8 (S)	6 (R)	36.6 (S)	36.6 (S)
K.pneumoniae(243)	30 (S)	33 (S)	9.7 (R)	27.5 (S)	29.5 (S)	32.7 (S)	34 (S)	6 (R)	25.7 (S)	25.7 (S)
K.pneumoniae (S8)	29.6 (S)	29.7 (S)	6 (R)	27.3 (S)	31.3 (S)	37 (S)	36 (S)	6 (R)	38.1 (S)	38.1 (S)
K.pneumoniae (10)	32.9 (S)	33.3 (S)	10.3 (R)	29.3 (S)	30.8 (S)	49.7 (S)	51.2 (S)	Clear	43.8 (S)	43.8 (S)
Acinetobacter sp.	6 (R)	6 (R)	6 (R)	12.8 (R)	10 (R)	6 (R)	6 (R)	8 (R)	24.3 (S)	24.3 (S)
A.baumannii (239)	6 (R)	6 (R)	6 (R)	12 (R)	9 (R)	8.3 (R)	6 (R)	6 (R)	23.6 (R)	23.6 (S)
Acinetobacter sp.(2)	6 (R)	6 (R)	6 (R)	11.5 (R)	9 (R)	9.3 (R)	8 (R)	8.4 (R)	21.6 (R)	21.6 (S)
Moraxella sp.	19 (R)	11.9 (R)	6 (R)	22 (R)	31.8 (R)	25 (S)	20 (S)	6 (R)	24.7 (R)	24.7 (S)
Enterobacter loacae	29.6 (S)	30.2 (S)	7 (R)	28.5 (S)	29.4 (S)	29.9 (S)	30 (S)	7 (R)	27 (S)	27 (S)
S. aureus (4)	24 (S)	26 (S)	20 (R)	46 (S)	37 (S)	25.5 (I)	26.5(S)	Clear	47 (S)	47 (S)
S. aureus (1)	28.2 (S)	29 (S)	17.7 (R)	49.6 (S)	37 (S)	27.7 (S)	28.3(S)	28.4 (R)	43.7 (S)	43.7 (S)
S. aureus (6)	27 (S)	25.6 (S)	20 (R)	51.7 (S)	40.9 (S)	31.2 (S)	32.3 (S)	Clear	53.5 (S)	53.5 (S)
S. aureus 277 (7)	12.2 (R)	12.3 (R)	11.6 (R)	29.6 (S)	17.7 (S)	16.4 (I)	18 (I)	20 (R)	46.9 (S)	46.9 (S)

AMP= Ampicillin; CRO= Ceftriaxone; CTX= Cefotaxime; IPM= Imipenem; MEM= Meropenem; (S)= Sensitive; (I)= Intermediate; (R)= Resistant

The highest increase of mean inhibition zone diameter of beta-lactam antibiotics appears on meropenem, followed by imipenem, ceftriaxone, cefotaxime, and ampicillin.

Table 2. The effect of 30 µM TPEN to Bacterial Susceptibility to Aminoglycoside Antibiotics

Sampel	Mueller Hinton				Mueller Hinton + 20 µM TPEN			
	K	AK	TOB	CN	K	AK	TOB	CN
E. coli (5)	$14^{(I)}$	$20^{(S)}$	$9^{(R)}$	$8^{(R)}$	$10^{(R)}$	$16^{(I)}$	$8^{(R)}$	$6^{(R)}$
E. coli (S13)	$12^{(R)}$	$17^{(S)}$	$8^{(R)}$	$7^{(R)}$	$9^{(R)}$	$15^{(I)}$	$7^{(R)}$	$6^{(R)}$
P. aeruginosa (225)	$7^{(R)}$	$21^{(S)}$	$22^{(S)}$	$16^{(S)}$	$7^{(R)}$	$21^{(S)}$	$21^{(S)}$	$16^{(S)}$
P. aeruginosa (226)	$9^{(R)}$	$20^{(S)}$	$21^{(S)}$	$16^{(S)}$	$7^{(R)}$	$18^{(S)}$	$19^{(S)}$	$13^{(I)}$
P. aeruginosa (227)	$11^{(R)}$	$22^{(S)}$	$24^{(S)}$	$17^{(S)}$	$9^{(R)}$	$20^{(S)}$	$24^{(S)}$	$17^{(S)}$
P. aeruginosa (KU 189)	$6^{(R)}$	$26^{(S)}$	$23^{(S)}$	$23^{(S)}$	$6^{(R)}$	$24^{(S)}$	$23^{(S)}$	$24^{(S)}$
P. aeruginosa (2)	$12^{(R)}$	$23^{(S)}$	$23^{(S)}$	$20^{(S)}$	$10^{(R)}$	$21^{(S)}$	$22^{(S)}$	$18^{(S)}$
P. luteola (S9)	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$
P. aeruginosa (S11)	$10^{(R)}$	$9^{(S)}$	$21^{(S)}$	$17^{(S)}$	$9^{(R)}$	$22^{(S)}$	$21^{(S)}$	$17^{(S)}$
P. aeruginosa (S15)	$10^{(R)}$	$21^{(S)}$	$20^{(S)}$	$17^{(S)}$	$10^{(R)}$	$25^{(S)}$	$25^{(S)}$	$19^{(S)}$
Pseudomonas sp. (T5)	$35^{(S)}$	$33^{(S)}$	$31^{(S)}$	$32^{(S)}$	$32^{(S)}$	$30^{(S)}$	$30^{(S)}$	$27^{(S)}$
Klebsiella sp. (S6)	$25^{(S)}$	$25^{(S)}$	$24^{(S)}$	$25^{(S)}$	$23^{(S)}$	$23^{(S)}$	$22^{(S)}$	$22^{(S)}$
Klebsiella sp. 199	$6^{(R)}$	$23^{(S)}$	$21^{(S)}$	$21^{(S)}$	$6^{(R)}$	$21^{(S)}$	$18^{(S)}$	$19^{(S)}$
Klebsiella sp. (S14)	$20^{(S)}$	$20^{(S)}$	$17^{(S)}$	$19^{(S)}$	$18^{(S)}$	$19^{(S)}$	$19^{(S)}$	$1^{(S)}$
Klebsiella sp. (S16)	$6^{(R)}$	$23^{(S)}$	$19^{(S)}$	$20^{(S)}$	$6^{(R)}$	$20^{(S)}$	$17^{(S)}$	$17^{(S)}$
K. pneumoniae (243)	$21^{(S)}$	$22^{(S)}$	$19^{(S)}$	$21^{(S)}$	$18^{(S)}$	$19^{(S)}$	$17^{(S)}$	$18^{(S)}$
K. pneumoniae (S8)	$6^{(R)}$	$22^{(S)}$	$19^{(S)}$	$21^{(S)}$	$6^{(R)}$	$20^{(S)}$	$17^{(S)}$	$18^{(S)}$
K. pneumoniae (S10)	$21^{(S)}$	$24^{(S)}$	$20^{(S)}$	$20^{(S)}$	$21^{(S)}$	$22^{(S)}$	$19^{(S)}$	$19^{(S)}$
Acinetobacter sp. (199)	$6^{(R)}$	$16^{(I)}$	$7^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$
A. baumannii (KU 239)	$6^{(R)}$	$18^{(S)}$	$6^{(R)}$	$13^{(I)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$
Acinetobacter sp. (228)	$6^{(R)}$	$16^{(I)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$
Moraxella sp.	$9^{(R)}$	$19^{(S)}$	$19^{(S)}$	$15^{(S)}$	$9^{(R)}$	$19^{(S)}$	$21^{(S)}$	$16^{(S)}$
Enterobacter cloacae	$20^{(S)}$	$21^{(S)}$	$19^{(S)}$	$19^{(S)}$	$19^{(S)}$	$19^{(S)}$	$17^{(S)}$	$18^{(S)}$
S. aureus (4)	$25^{(S)}$	$22^{(S)}$	$25^{(S)}$	$23^{(S)}$	$22^{(S)}$	$21^{(S)}$	$23^{(S)}$	$22^{(S)}$
S. aureus (1)	$22^{(S)}$	$23^{(S)}$	$24^{(S)}$	$23^{(S)}$	$22^{(S)}$	$21^{(S)}$	$24^{(S)}$	$22^{(S)}$
S. aureus (6)	$22^{(S)}$	$22^{(S)}$	$22^{(S)}$	$21^{(S)}$	$21^{(S)}$	$21^{(S)}$	$22^{(S)}$	$20^{(S)}$
S. aureus 277 (7)	$7^{(R)}$	$24^{(S)}$	$25^{(S)}$	$25^{(S)}$	$6^{(R)}$	$23^{(S)}$	$24^{(S)}$	$22^{(S)}$
S. pyogenes (2)	$13^{(R)}$	$8^{(S)}$	$10^{(R)}$	$17^{(S)}$	$10^{(R)}$	$10^{(R)}$	$10^{(R)}$	$13^{(I)}$
Enterococcus (221 S4)	$7^{(R)}$	$7^{(S)}$	$7^{(R)}$	$7^{(R)}$	$7^{(R)}$	$8^{(R)}$	$7^{(R)}$	$7^{(R)}$
Enterococcus faecalis	$6^{(R)}$	$7^{(S)}$	$6^{(R)}$	$6^{(R)}$	$6^{(R)}$	$7^{(R)}$	$6^{(R)}$	$6^{(R)}$

K= Kanamycin; AK= Amikacin; CN= Gentamicin; TOB= Tobramycin; $^{(S)}$= Sensitive; $^{(I)}$= Intermediate; $^{(R)}$= Resistant.

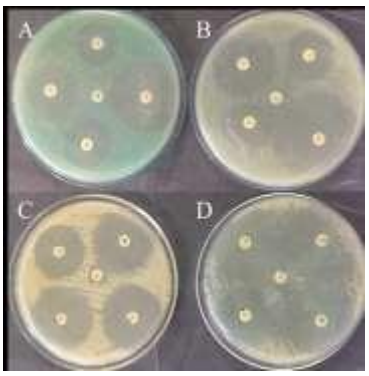

Figure 1. (A) and Mueller Hinton + 30 µM TPEN (B), and Klebsiella sp. on control Mueller Hinton agar (C) and Mueller Hinton + 30 µM TPEN (D).

Figure 2. Aminoglycoside susceptibility test using disk diffusion method on Acinetobacter baumanii.

Figure2 shows *Acinetobacter baumanii* on Mueller Hinton agar without TPEN (A) and the bacteria on Mueller Hinton agar with 20µM of TPEN (B). This susceptibility showed that there was a decrease in inhibition zone diameter around the antibiotics

Effects of Zinc Chelator TPEN in Bacterial Susceptibility towards Beta-Lactam antibiotics.

Statistical study showed a significant difference in inhibition zone diameter of control and experimental group of meropenem (p= 0.038), while no significant differences was observed in ceftriaxone, cefotaxime, ampicillin, and imipenem (p= 0.323; p= 0.341; p= 0.597; p= 0.249 respectively). Statistical study using the whole pair of data showed no significant difference in inhibition zone diameter of control and experimental group in beta-lactam and aminoglycoside antibiotics (p= 0.051) Therefore, there is no overall effect of zinc chelator TPEN on bacterial susceptibility to beta-lactam.

Effects of Zinc Chelator TPEN in Bacterial Susceptibility towards Aminoglycoside

Statistical study showed no significant differences was observed in kanamycin, amikacin, gentamicin, and tobramycin (p= 0.522; p= 0.112; p=0.640; p= 0.330 respectively). Statistical study using the whole pair of data showed no significant difference in inhibition zone diameter of control and experimental group in beta-lactam and aminoglycoside antibiotics (p= 0.104). Based on this statistical analysis, there is no overall effect of zinc chelator TPEN on bacterial susceptibility to aminoglycoside.

DISCUSSION

Previous study stated that metal chelator including TPEN is a potential inhibitor of metallo-beta-lactamase by decreasing meropenem MIC of *Eschericia coli* that produce NDM, VIM, and IDM-8 beta- lactamase[15]. This state matched the result of this study where there is a significant difference of mean inhibitory zone of bacterial susceptibility between control and experimental group on meropenem.

Another study showed that 25 µM TPEN can resensitize *Acinetobacter baumannii* to imipenem, and EDTA can resensitize VIM-1 producing *Klebsiella pneumoniae* to ceftriaxon[17,18]. These research shows the effect of metal chelator on bacterial susceptibility in class-B beta-lactamase producing microorganism, which are metallo-beta-lactamase that require zinc as its covalent to hydrolyze beta-lactam ring[19]. Meanwhile, the samples used in this study have different susceptibility to each antibiotics, most of it were resistant to ampicillin and sensitive to carbapenem (meropenem and imipenem). There is no relevant literature that explain the effect of zinc deficiency on bacterial susceptibility to non class-B beta-lactamase- producing bacteria, hence the mechanism underlying this result is unknown.

Bacterial resistance mechanism in the sample used in this study is not further identified. But, the main resistance mechanism of bacteria to beta-lactam antibiotics is by producing beta-lactamase. Bacteria that are resistant to cephalosporin mainly produce AmpC beta-lactamase and ESBL, and ampicillin resistant bacteria produce TEM beta-lactamase [20,21]. These enzymes are serine-hydrolase which did not require zinc in hydrolyzing beta-lactam ring. This may explain why there are no significant effect of zinc chelator TPEN on bacterial susceptibility to cefotaxime, ceftriaxone, and ampicillin antibiotic.

Zinc chelator TPEN can penetrate through cell membranes. It also has high affinity and often used as selective chelator of zinc. However, in the previous study, TPEN has low affinity for magnesium (Mg), calcium (Ca) and also known having chelation ability of iron (Fe), and copper (Co) [14,22]. This study shows that the addition of 20 µM zinc chelator TPEN does not affect the susceptibility of bacteria toward aminoglycoside due to the presence of other metal ions, including extracellular zinc which can reduce the number of molecules and the affinity of TPEN to bind with bacteria's intracellular zinc.

Zinc or Zn^{2+} is known as inhibitor of several enzymes, one of which is aminglycoside N-acetyltransferases. However, not every bacteria have aminoglycoside modifying enzymes (AMEs), especially aminoglycoside acetyltransferases (AAC(6′)-Ib). In this study, *Escherichia coli* and *Acinetobacter baumanii* turned out matched with the previous studies which stated that the addition of zinc can reduce the level of resistance of *Acinetobacter baumanii* and *Escherichia coli* to several aminoglycoside

antibiotics[11]. The addition of 20 µM TPEN in the susceptibility test of *Acinetobacter baumanii* and *Escherichia coli* towards aminoglycoside antibiotics in this study showed a narrowing of the inhibition zone diameter to change the susceptibility status of bacteria to be more resistant. Therefore, this study supports previous studies because the presence of zinc depletion or limitation conditions can affect the activity of AMEs, especially aminoglycosid eacetyltransferases (AAC(6′)-Ib).

In this study, all samples had different levels of susceptibility to each antibiotic with a tendency to be more sensitive to amikacin and more resistant to kanamycin. The largest decrease in the mean diameter of the inhibition zone occurred in amikacin, followed by kanamycin, gentamicin, and tobramycin. Amikacin is usually given to bacteria that are resistant to enzymes that can inactivate gentamicin and tobramycin [23,24]. The decrease in the mean diameter of the inhibition zone in amikacin was greatest in Gram negative bacteria. However, this is in contrast to Gram positive bacteria which experience the smallest decrease in inhibition zone diameter in amikacin. Resistance to Gram positive bacteria towards aminoglycoside is mediated by the bifunctional enzyme aac(6′)-Ie-aph (2″)-Ia [23]. However, there is no relevant literature that explain the effect of zinc deficiency on this bifunctional enzyme.

Bacterial resistance mechanism through enzymes other than N-acetyltransferases or other mechanisms related to zinc has not been widely studied. The bacterial samples used in this study also have different profiles of resistance properties. This may also explain why there is no effect of TPEN zinc chelator on bacterial susceptibility to aminoglycoside antibiotics in this study such as kanamycin, amikacin, gentamicin, and tobramycin.

CONCLUSION

In conclusion, the addition of 30 µM zinc chelator TPEN in Mueller Hinton agar increase bacterial susceptibility to meropenem significantly. Meanwhile it did not influence bacterial susceptibility to ceftriaxone, cefotaxime, ampicillin, and imipenem. The addition of 20 µM zinc chelator TPEN also did not influence bacterial susceptibility to kanamycin, amikacin, gentamicin, and tobramycin.

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