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Identification and Antimicrobial Susceptibility of *Granulicatella adiacens* Isolated from Periodontal Pocket

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ABSTRACT

Background: Periodontal diseases often occur in developing and developed countries, affecting 20 – 50% of the world population. Periodontitis may also complicate systemic health and have a risk to cause endocarditis infection. *Granulicatella adiacens*, part ofnutritionally variant streptococci (NVS), was proven to cause many diseases, especially endocarditis infection. GA was a part of commensal bacteria but also found to be causing periodontitis by co-aggregating with *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. The frequent used of antibiotics nowadays has led to increased resistance, and this concern led us to examine susceptibilities among several antibiotics. This study was conducted to prove which antibiotic can eliminate *Granulicatella adiacens* for its potential risk.

Methods and Material: Bacteriasamples were isolated from 28 patients with periodontitis. The samples were delivered to the microbiology laboratory to be cultured and purified. After purification, the samples were identified using GP VITEK 2 Compact and tested using the antibiotic disc to find out the susceptibility rates.

Results: From 28 samples, 20 samples were identified containing *Granulicatella adiacens*. Those samples were tested on seven types of antibiotic which is ofloxacin, ceftriaxone, azithromycin, vancomycin, tetracycline, levofloxacin, and clindamycin. Sensitive isolates were found on 90% of ofloxacin and levofloxacin group, 85% of azithromycin group, 50% of vancomycin group, and less than 50% for three other groups. High resistance percentages were found on cefriaxone (55%), vancomycin (45%), clindamycin (40%), tetracycline (30%), and less than 15% for three other groups.

Conclusion: Ofloxacin and levofloxacin were found to be the most sensitive antibiotic tested, while ceftriaxone was the worst antibiotic in eliminating *Granulicatella adiacens*.

Keywords: Antibiotic, *Granulicatella adiacens,* Sensitivity Test, Resistance Test.

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INTRODUCTION

Periodontal disease is the most common oral infectious disease that highly associated with pathogenic biofilm and oral microorganisms that will trigger the host immune response, leading toperiodontal tissue destruction.[1,2]Periodontitis is a chronic inflammatory disease of periodontal tissue caused by groups of microorganisms. It shows progressive destruction of periodontal ligament and alveolar bone, usually followed with pocket periodontal formation, recession, mobility and combination of those conditions.[3,4]"The red complex" of *Porphyromonas gingivalis*, *Tannerella forsythia*, dan *Treponema denticola,* are the main bacteria that identified during conventional culture-based approach in deep periodontal pockets.[1,5–7]These bacteria destruct the periodontal tissue by releasing their products which will activate the host immune response to fight the bacteria, and in the process, the tissue surrounds it will also be destroyed.[8] Periodontitis may also complicate systemic health and have a risk to cause endocarditis infection.[9,10]

Nutritionally variant streptococci (NVS) was proven to cause many cases of bacterial endocarditis, where most of the cases were caused by *Granulicatella adiacens* than *Abiotrophia*, while *Granulicatella elegans*was comparatively rare. This species is part of commensal bacteria and frequently found on dental plaque, endodontic infection, and dental abscess, but also can cause other serious infections.[11] Dhotre et al (2018) found that *Granulicatella adiacens* was isolated from the periodontal pocket on patients with endocarditis bacterial two times higher than from subgingival plaque on patients with endocarditis bacterial. Our pilot study also showed that 77.78% samples isolated from periodontal pocket were identified as *Granulicatella adiacens*.

In developing countries, infectious diseases are a common cause of death.[11] This makes antibiotics the most commonly purchased drugs.[12,13]These drugs are also the most common medicine prescribed.[14]However, frequently used of antibiotic nowadays has led to increased resistance.[15] Antibiotic resistance is a worldwide public health issue. When these drugs used on a susceptible pathogen, it will improve and helps patients condition. However, overuse of these drugs will cause bacterial resistance to emerge.[12,16]Indonesia has a high rate of antimicrobial resistance (AMR) and its AMR keeps rising.[17]The concern of other antibiotic resistance led us to examine susceptibilities among several antibiotics.[18]This study was done to identify the antibiotics sensitivity and resistanceon *Granulicatella adiacens*isolated from periodontitis patients.

METHODS

This study was conducted under the Helsinki Declaration and approved by the Medical Ethics Committee of Dental and Oral Health Hospital of Universitas Hasanuddin. This study's ethical clearance has been approved under the Medical Ethics Committee of Universitas Hasanuddin. Ethical Clearance approval number 0168/PL09/KEFK FKG-RSGM UNHAS/2019. The samples were harvested from the periodontitis patient who came to Dental and Oral Health

Hospital Universitas Hasanuddin and agreed to be a volunteer. The volunteers were explained about the study and they signed the informed consent. A total of 28 bacteria samples were isolated from periodontitis patients. The bacteria samples were taken from the periodontal pockets and transported using AMIES swab collection to Microbiology laboratories in Teaching Hospital Universitas Hasanuddin.

The laboratory process started with enriched and culturing the bacteria samples in brain heart infusion broth (BHIB) for at least 8 hours at temperature 37°C with oxygen concentration 15%, followed by culturing them in the 5% sheep blood agar at temperature 37ºC for 24 hours with oxygen concentration 15%. The cultured bacteria colonies were then identified by finding out the colonies that similar to *Granulicatella* morphology. The colonies were then tested by gram staining, and the suspected colonies were identified biochemically using automated VITEK 2 (bioMérieux, Inc) Compact system to complete the strain identification. After the colonies were proven as *Granulicatella adiacens*, the antimicrobial susceptibility test (AST) were measured manually according to CLSI standards.[17]

The bacteria suspension was prepared and cultured to Mueller Hinton Agar. Seven types of antibiotic disc which were ofloxacin, ceftriaxone, azithromycin, vancomycin, tetracycline, levofloxacin, and clindamycin. The antibiotics used in this study were based on CLSI M100 guidelines.

RESULTS

We identified the bacteria samples using an automated VITEK 2 Compact system. Among 28 samples, the system detected 20 isolates were *Granulicatella adiacens*.AST was done by disc diffusion method for 20 isolated *Granulicatella adiacens*. The number of sensitive, intermediate, and resistant samples of each antimicrobial agent tested was listed in Table 1 (based on guidelines from CLSI M100).

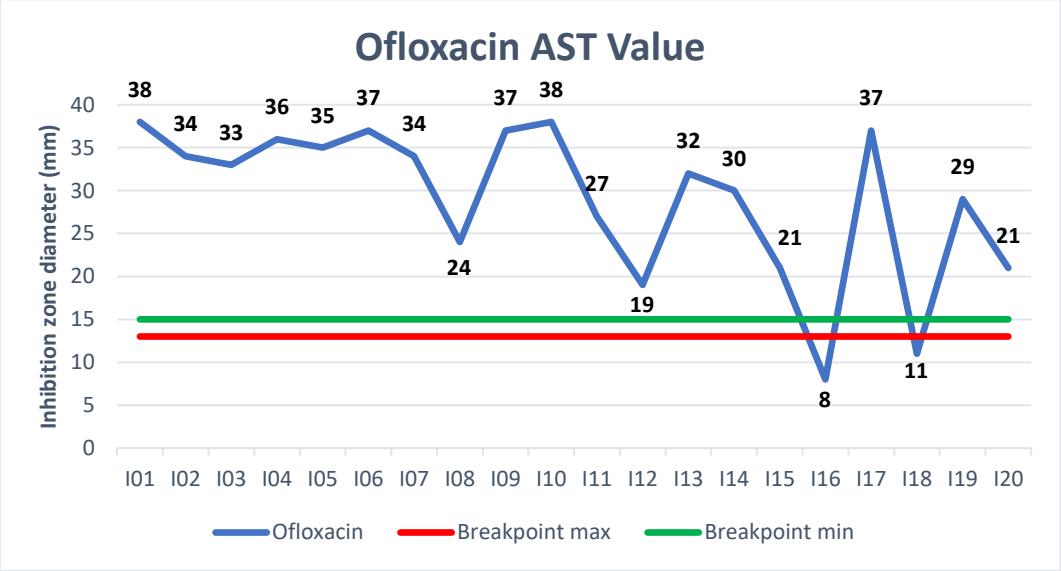

Figure 1: Ofloxacin AST value of*Granulicatella adiacens*.

In figure 1, almost all of the *Granulicatella adiacens* isolates were susceptible to ofloxacin. The breakpoint value for this antibiotic is 13 – 15 mm for the intermediate category. Only 2 of 20 isolates were resistant to this antibiotic.

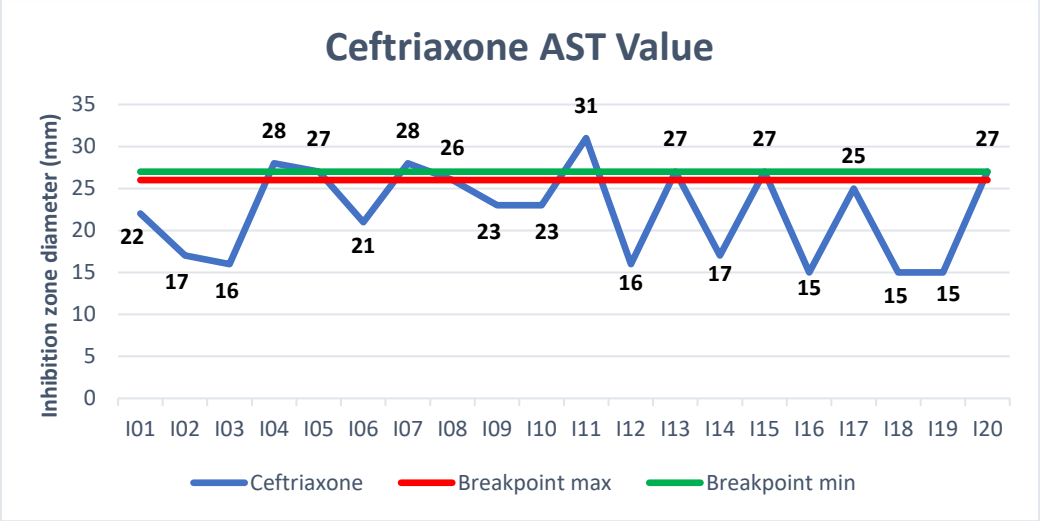

Figure 2: Ceftriaxone AST value of *Granulicatella adiacens*.

In figure 2, most of the *Granulicatella adiacens* isolates were resistant to ceftriaxone. The breakpoint value for this antibiotic is 26 – 27 mm for the intermediate category. Only 7 of 20 samples were sensitive to this antibiotic and 2 of 20 samples were in the intermediate category.

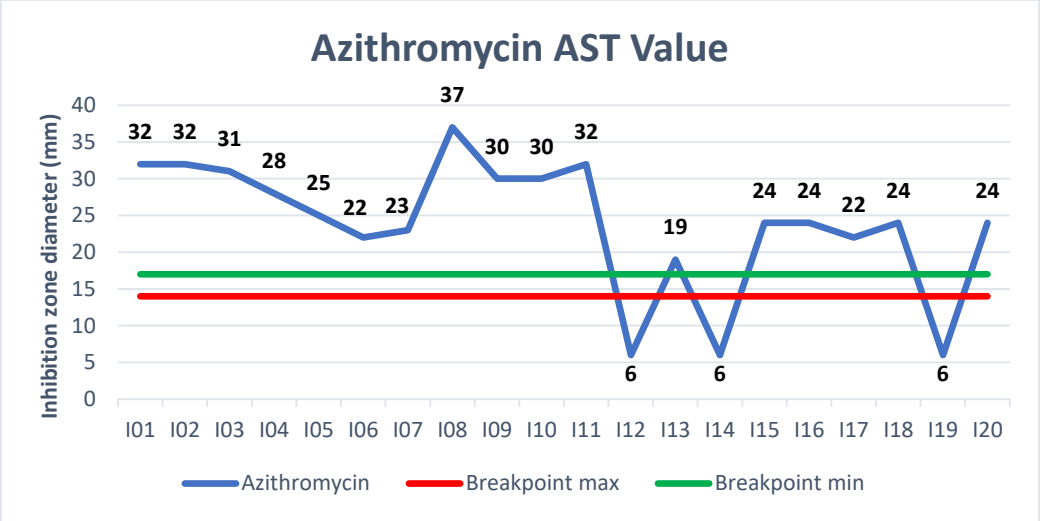

Figure 3: Azithromycin AST value of *Granulicatella adiacens*.

In figure 3, almost all of the *Granulicatella adiacens* isolates were susceptible to ofloxacin. The breakpoint value for this antibiotic is 14 – 17 mm for the intermediate category. Only 3 of 20 isolates were resistant to this antibiotic.

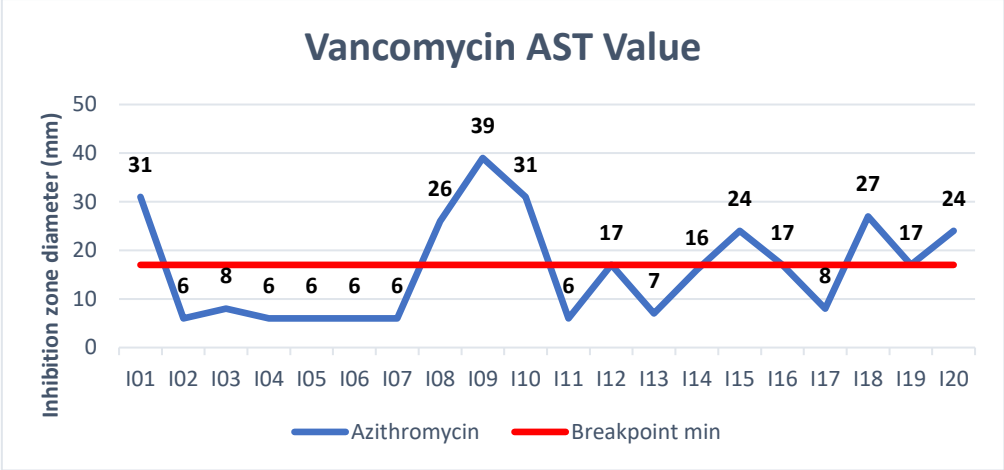

Figure 4: Vancomycin AST value of *Granulicatella adiacens*.

Figure 4 shows that the sensitive *Granulicatella adiacens* isolates were as many as the resistant *Granulicatella adiacens* isolates in vancomycin group. The breakpoint value for this antibiotic is ≥ 17 mm for the sensitive category. Only 1 of 20 isolates were in the intermediate zone.

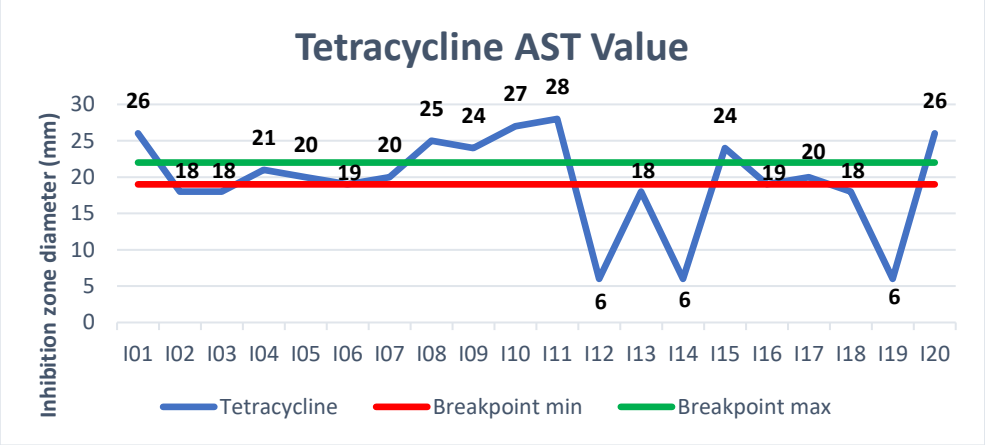

Figure 5: Tetracycline AST value of *Granulicatella adiacens*.

Figure 5 shows that the sensitive *Granulicatella adiacens* isolates were as many as the resistant *Granulicatella adiacens* isolates in tetracycline group. The breakpoint value for this antibiotic is 19 – 22 mm for the intermediate category.

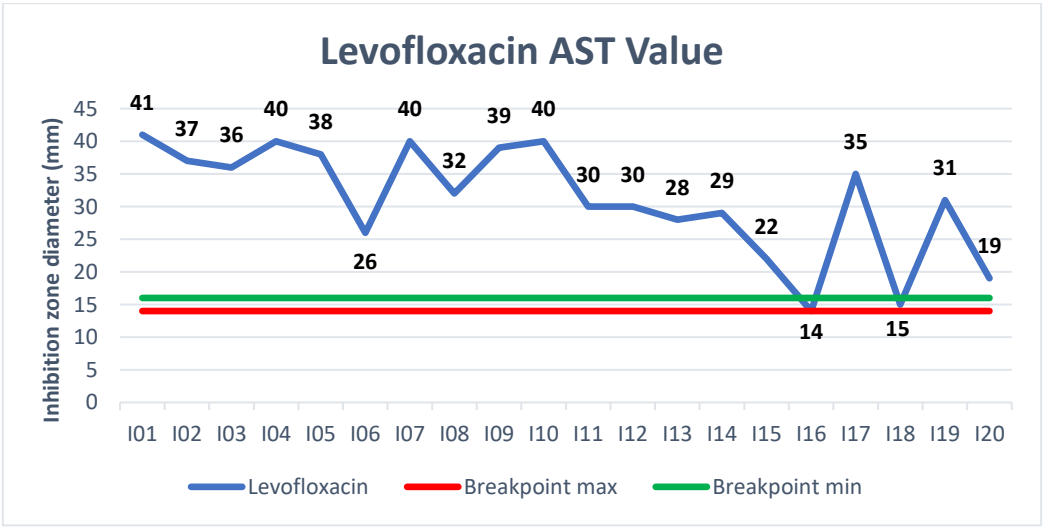

Figure 6: Levofloxacin AST value of *Granulicatella adiacens*.

In figure 6, almost all of the *Granulicatella adiacens* isolates were susceptible to levofloxacin. The breakpoint value for this antibiotic is 14 – 16 mm for the intermediate category. Only 2 of 20 isolates were in the intermediate zone.

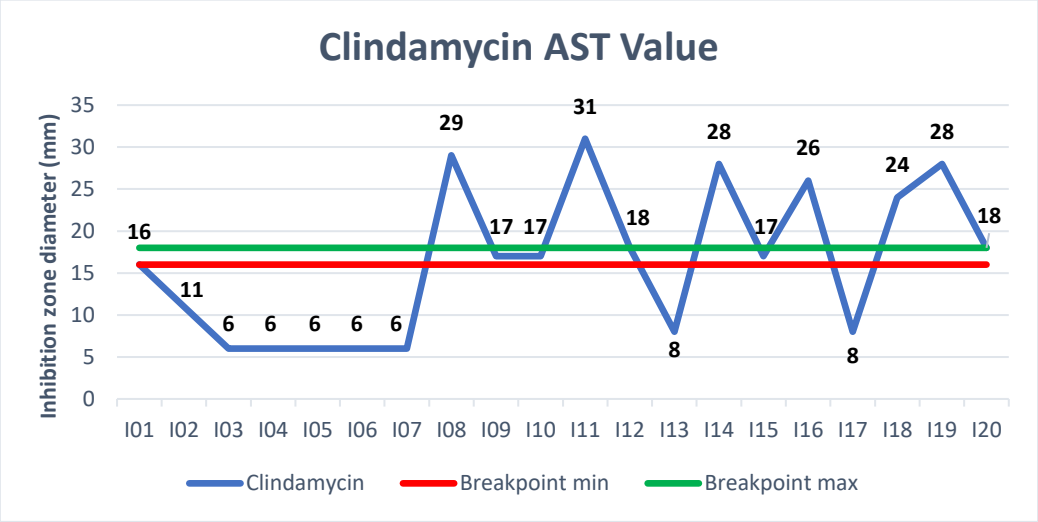

Figure 7: Clindamycin AST value of *Granulicatella adiacens*.

Figure 7 shows that the sensitive *Granulicatella adiacens* isolates were as many as the resistant *Granulicatella adiacens* isolates in clindamycin group. The breakpoint value for this antibiotic is 16 – 18 mm for the intermediate category.

Table 1: Antimicrobial susceptibility testing by disc diffusion

Antimicrobial Agent	Disc Concentration	Number of samples (n/%)		
		Sensitive	Intermediate	Resistant
Ofloxacin	5 µg	18 (90)	0 (0)	2 (10)
Ceftriaxone	30 µg	7 (35)	2 (10)	11 (55)
Azithromycin	15 µg	17 (85)	0 (0)	3 (15)
Vancomycin	30 µg	10 (50)	1 (5)	9 (45)
Tetracycline	30 µg	8 (40)	6 (30)	6 (30)
Levofloxacin	5 µg	18 (90)	2 (10)	0 (0)
Clindamycin	2 µg	6 (30)	6 (30)	8 (40)

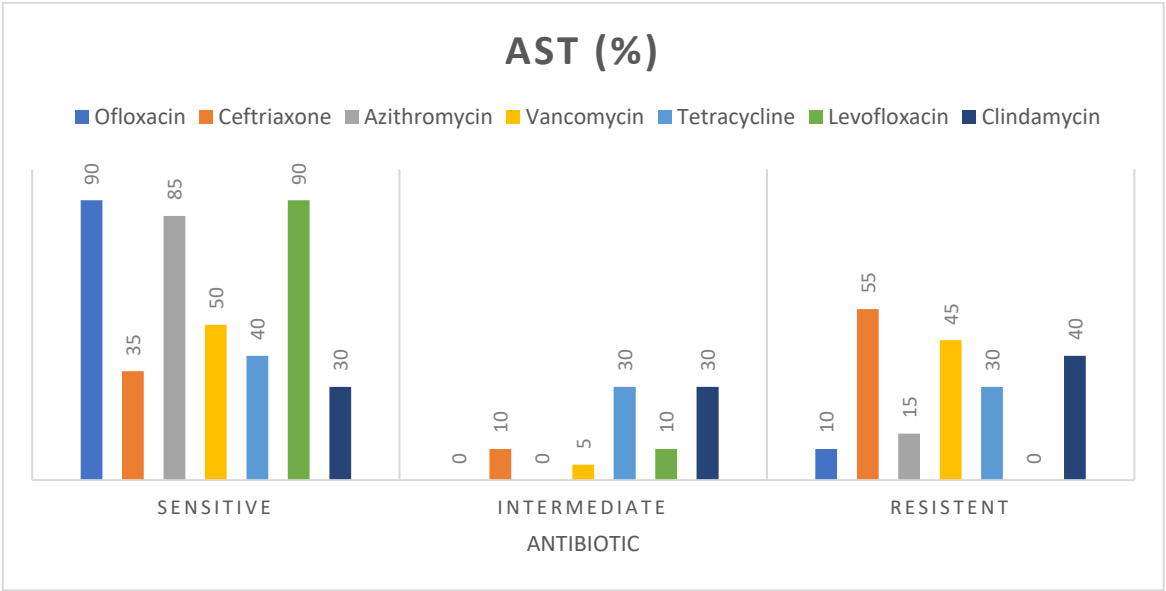

Figure 8: Comparison of susceptibility rate among tested antibiotics.

Figure 8 showed susceptibility rates among tested antibiotics with the highest susceptibility rates were ofloxacin and levofloxacin (90%), followed by azithromycin (85%), vancomycin (50%), tetracycline (40%), ceftriaxone (35%) and clindamycin (30%). While the resistance rates were highest in ceftriaxone (55%), vancomycin (45%), clindamycin (40%), tetracycline (30%), azithromycin (15%), ofloxacin (10%), and the lowest rate in levofloxacin (0%).

DISCUSSION

In this study, 90% of *Granulicatella adiacens* isolates were susceptible to ofloxacin, while 10% of isolates were categorized as resistant. Similar to our study, the study conducted by Harun et al (2019), Dhotre et al (2018), and Tuohy et al (2000) also showed 90 – 100% isolates were susceptible to this antibiotic.[11,20-21]

For ceftriaxone group, 55% of *Granulicatella adiacens* isolates were resistant, 35% of isolates were sensitive, and 10% of isolates were categorized as intermediate. Another study conducted by Zheng et al (2014) showed 100% of isolates were resistant.[20] Conversely, the study that showed resistance rates less than 45% of *Granulicatella adiacens* isolates were Mushtaq et al (2016) , Tuohy et al (2000), Alberti et al (2016), Prasidthrathsint et al (2018), Kanamoto et al (2018), and Dhotre et al (2018).[11,16,22,24–26]

In azithromycin group for this study showed 85% of *Granulicatella adiacens* isolates were sensitive, while 15% of isolates were resistant. In line with our study, Dhotre et al (2018) study showed about 60% of *Granulicatella adiacens* isolates from non-periodontitis patients, and 86% of isolates from periodontitis patients were sensitive to this antibiotic.[11]

For vancomycin group, about 50% of *Granulicatella adiacens* isolates were sensitive, 45% of isolates were resistance, and 5% of isolates were in the intermediate zone. However, all study about antimicrobial susceptibility test on *Granulicatella adiacens* showed 100% isolates were sensitive to this antibiotic, which were study conducted by Alberti et al (2016), Zheng et al (2004), Prasidthratsint et al (2017), Kanamoto et al (2018), Mushtaq et al (2016), Tuohy et al (2000), Dhotre et al (2018), and Harun et al (2019).[11,16,22–27]

Granulicatella adiacens isolates that were tested with tetracycline in our study showed almost similar results among interpretation categories. Forty percent isolates were sensitive, 30% were in intermediate zone, and the last 30% isolates were resistance. Similar to our study, about 50% isolates were sensitive for non periodontitis group in Dhotre et al (2018) study.[9] However, 66,6% isolates were sensitive in Zheng et all (2004) study and 75 – 86% isolates were sensitive for periodontitis group in Dhotre et al (2018).[10,21]

Ninety percent of *Granulicatella adiacens* isolates were sensitive to levofloxacin, while 10% of isolates were in intermediate zone. This results were supported by Alberti et al (2016), Prasidthrathsint et al (2017), Mushtaq et al (2016), and Tuohy et al (2000) that showed about 91 – 100% isolates were sensitive.[14,18,20,21]Dhotre et all (2018) study also showed 83 – 87% isolates were sensitive to this antibiotic, both on periodontitis and non-periodontitis group.[10]

For the last antibiotic tested, clindamycin, there were 30% in sensitive zone, 30% of isolates in intermediate zone, and 40% isolates in resistance zone. In contrast with our study, Alberti et al (2016), Zheng et al (2004), Prasidthrathsint et al (2017), Mushtaq et al (2016), Tuohy et al (2000), and Dhotre et al (2018) study showed more than 70% *Granulicatella adiacens* isolates were sensitive to this antibiotic.[10,15,20–23]

In comparison among each antibiotic, we found that ofloxacin has a more sensitive sample than ceftriaxone ($p \geq 0.05$, Kruskal-Wallis test), azithromycin ($p \geq 0.05$, Kruskal-Wallis test), vancomycin ($p \geq 0.05$, Kruskal-Wallis test), tetracycline ($p \geq 0.05$, Kruskal-Wallis test), and clindamycin($p \leq 0.05$, Kruskal-Wallis test), but less than levofloxacin ($p \leq 0.05$, Kruskal-Wallis test). Ceftriaxone has a less sensitive sample than azithromycin ($p \geq 0.05$, Kruskal-Wallis test), vancomycin ($p \geq 0.05$, Kruskal-Wallis test), tetracycline ($p \geq 0.05$, Kruskal-Wallis test), and levofloxacin ($p \geq 0.05$, Kruskal-Wallis test), but more than clindamycin($p \geq 0.05$, Kruskal-Wallis test). Azithromycin has a more sensitive sample than vancomycin ($p \leq 0.05$, Kruskal-Wallis test), tetracycline ($p \leq 0.05$, Kruskal-Wallis test), and clindamycin($p \leq 0.05$, Kruskal-Wallis test), but less than levofloxacin ($p \geq 0.05$, Kruskal-Wallis test). Vancomycin has a more sensitive sample than tetracycline ($p \leq 0.05$, Kruskal-Wallis test), and clindamycin($p \leq 0.05$, Kruskal-Wallis test), but less than levofloxacin ($p \geq 0.05$, Kruskal-Wallis test). Tetracycline has a more sensitive sample than clindamycin($p \leq 0.05$, Kruskal-Wallis test), but less than levofloxacin ($p \geq 0.05$, Kruskal-Wallis test). Levofloxacine has a more sensitive sample than clindamycin($p < 0.05$, Kruskal-Wallis test).

In study conducted by Pradipta et al (2015) about antibacterial consumption in Indonesia, amoxicillin was proved to be the most used antibiotic, followed by sulphamethoxazol, trimethoprim, isoniazid, tetracycline, ciprofloxacin, rifampicin, pyrazinamide, ethambulbi, chlorampenicol, and doxycycline.[26] This founding can be used as a guidance when prescribed antibiotic for indicated patients. However, the most appropriate ways to prescribe antibiotic is by culturing the bacteria sample and do the AST to make sure which types of antibiotic are suitable to suppress the infection.[27]

We also comparing our study result with other study about AST on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Jacinto et al (2006) study showed that 60% of *Porphyromonas gingivalis* isolates were susceptible to azithromycin and100% of the isolates were susceptible to clindamycin and tetracycline.[28]*Porphyromonas gingivalis* strain was susceptible to tetracycline and clindamycin in Santos et al (2002) study.[29]In a study conducted by Mueller et al (2002), *Aggregatibacter actinomycetemcomitans* was highly susceptible to fluoroquinolones, while the susceptibility for azithromycin was moderate.[31]In Minguez et al (2018) study,58.3% of *Aggregatibacter actinomycetemcomitans* isolates were resistant to azithromycin, while no *Porphyromonas gingivalis* isolates were resistant to azithromycin.[32] In Japoni et al (2011) and Harun et al (2019) study, 96% of *Porphyromonas gingivalis* isolates were susceptible to clindamycin, 60% of the isolates were susceptible to ciprofloxacin, and 100% of isolates were susceptible to azithromycin.[33,34]

CONCLUSION

From the result and discussion mention before, we can conclude that ofloxacin and levofloxacin are the most sensitive antibiotic to eliminate *Granulicatella adiacens*, which can also be used to eliminate *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*Azithromycin, clindamycin, vancomycin, and tetracycline are sensitive enough to eliminate *Granulicatella adiacens*, while the ceftriaxone is the worst antibiotic to eliminate *Granulicatella adiacens*.

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CONFLICT OF INTEREST

There was no conflict of interest

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