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**Biogeographic assessment of Gorgonian associated
bacteria with antipathogenic Urinary Tract Infections
(UTIs) in Karimunjawa Marine National Park, Java Sea,
Indonesia**

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1 Biogeographic assessment of Gorgonian-associated bacteria with antipathogenic Urinary
2 Tract Infections (UTIs) in Karimunjawa Marine National Park, Java Sea, Indonesia

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14
15 **Abstract**

16 Gorgonian corals of Karimunjawa are impacted by anthropogenic activities, such as
17 increasingly high mariculture intensity with consequent eutrophication, overfishing, tourism,
18 sewage, and other pollutant discharges, which result in changes in the microbial community
19 structure. In this study, bacterial communities associated with six species of Gorgonian,
20 *Viminella sp.*, *Ellisella sp.*, *Antipathes sp.*, *Melithaea sp.*, *Astrogorgia sp.*, and *Junceella sp.*
21 from both the Marine Protected Area (MPA) and non-Marine Protected Area (non-MPA) zones
22 were screened for their antipathogenic potential against Urinary Tract Infections (UTIs)
23 pathogens. The selected bacterial isolates were identified and compared for their abundance
24 and diversity between the two zones. A total of 156 bacterial strains were assayed for their
25 prospective antipathogenic compounds against seven UTI pathogens, including
26 *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus*
27 *saprophyticus*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Candida albicans*. The
28 results showed that 17 of 92 (18.48%) and 6 of 64 (9.37%) bacterial isolates from MPA and
29 non-MPA, respectively, exhibited antimicrobial activity in at least one of the UTI pathogens.
30 By analyzing the gene of 16S rRNA, it was discovered that the 17 isolates of MPA were
31 associated with phyla Actinobacteria, Firmicutes and Proteobacteria, including *Streptomyces*
32 *zhaozhoue*, *Nocardiopsis salina*, *Micrococcus endophyticus*, *Brevibacterium casei*,
33 *Micrococcus yunnanensis*, *Saccharopolyspora coralli*, *Bacillus paramycooides*, *Virgibacillus*
34 *salarius*, *Oceanobacillus iheyensis*, and *Vibrio alginolyticus*. In contrast, only six selected
35 isolates of non-MPA were associated with the phyla Actinobacteria and Proteobacteria,
36 including *Nocardiopsis salina*, *Micrococcus yunnanensis*, and *Acinetobacter soli*. The
37 Diversity Index (H'), SPECIES RICHNESS (S), and Relative Abundance of the MPA zone
38 were higher than those of non-MPA. These results demonstrated that Gorgonian octocoral
39 species in the MPA region harbor varied bacteria and we propose that many Gorgonian-
40 associated bacteria have the prospective for advancing broad-spectrum antibiotics.

41
42 **Keywords:** antimicrobial activity; diversity; Gorgonian-associated bacteria; Marine
43 Protected Area; UTIs pathogens.

44
45 **Introduction**

46 Karimunjawa National Park (KNP) is a mini archipelago with 27 small islands, located
47 in the Java Sea. This Archipelago was among the first maritime areas recognized in Indonesia
48 as being necessary for marine biodiversity conservation. This Park is a precious and diverse

49 tropical water ecosystem that is composed of tranquil white beaches, hard corals, soft coral,
50 Gorgonian corals, seaweeds, mangrove, seagrass beds, birds, turtles, and many kinds of sea
51 creatures, from crabs, anchovy, starfish, sharks, stingray, jellyfish, red snappers, etc. However,
52 anthropogenic pressures, such as high population growth, demands for living space, the
53 development of marine tourism, and increasing sea transport/traffic have affected marine life
54 and its vicinity. Under the Decree of the Director-General of PHKA no. 127/KPTS/DJ-
55 VI/1989, the Islands were zoned into 3 (three) management zones, including a marine
56 protection area (MPA) zone, a utilization zone, and a non-marine protected (non-MPA) area
57 (Campbell et al. 2013). The MPA includes protected areas in the sea that restrict some human
58 activity for conservation purposes, typically to protect natural and cultural resources. The MPA
59 has proven to effectively conserve marine biodiversity and restore ecosystem functioning
60 (Giakoumi and Pey 2017). However, the role of MPA in providing resilience to global threats,
61 such as biological diversity, is poorly understood. Hence, assessing the effects of MPA on
62 biodiversity is crucial for effective MPA planning and management. This study investigated
63 the bacterial diversity of Gorgonian-associated bacteria with antipathogenic potential UTIs in
64 MPA and non-MPA of Karimunjawa.

65 In Indonesia, UTI incidence is around 180,000 new cases per year (Sugianli et al. 2020).
66 *Escherichia coli* is the most dominant pathogen acquired nosocomially (Ejrnms 2011).
67 Nosocomial infections are infections acquired during the receipt of health services and may
68 also appear after discharge from the hospital (Grasselli et al. 2017). Infection occurs when a
69 pathogen spreads to a susceptible patient. UTIs describe microbial colonization and infection
70 of urinary tract structures and are grouped by the site of infection as kidneys, bladder, and
71 urethra (Sheerin 2011). In this study, the bacteria causing UTI, *Staphylococcus saprophyticus*,
72 *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*,
73 and *Candida albicans* were used as test bacteria (Orhan et al. 2010; Ochada et al. 2015). The
74 emergence of multidrug-resistant organisms (MDR) is another complication seen in
75 nosocomial infections, a serious problem that needs to be resolved immediately in many
76 developing countries, including Indonesia (Lee et al. 2014; Fernández et al. 2019). Therefore,
77 it is necessary to find new antibiotics to combat the developing MDR infectious diseases.

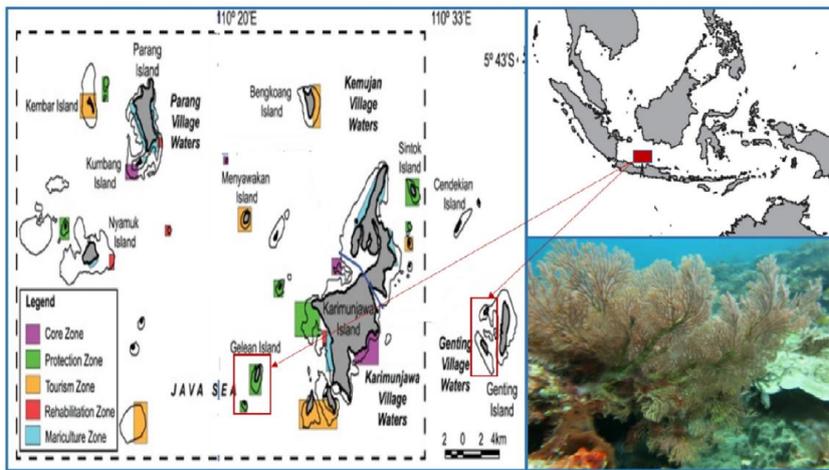
78 Octocoral gorgonian is part of a family of Gorgoniidae belonging to the order
79 Alcyonacea, subclass Anthozoans, and phylum Cnidaria (Almeida et al. 2014; Horvath 2019).
80 This organism is ubiquitous in Karimunjawa and has been observed on some islands, from
81 inshore to seabed (Sabdono et al. 2022). Gorgonian corals (sea fans) are characterized by their
82 fan-like shape, providing nutrition and protection for other marine animals (Matulja et al. 2020;
83 Sanchez et al. 2021). Gorgonian corals do not have a CaCO₃ framework for self-protection,
84 unlike hard corals. To survive, they produce various secondary metabolites to maintain their
85 stability (Matulja et al. 2021). However, the main obstacle faced in utilizing these secondary
86 metabolites for drugs is the problem of supply (Lindequist 2016). In contrast, marine
87 microorganisms have recently attracted greater attention because it is known that the
88 association of microbes with Gorgonians also synthesizes the same secondary metabolites as
89 their hosts (Modolon et al. 2020; Liu et al. 2021). Therefore, through fermentation and genetic
90 engineering systems, microbes can supply a large number of active compounds. Performing
91 rapid regeneration can also overcome supply problems (Wang et al. 2017). It is well known
92 that the Gorgonian Octocoral harbors many symbiotic microorganisms and produces a variety
93 of bioactive compounds that are very important for drug discovery (Sang et al. 2019; Modolon
94 et al. 2020). This study investigated the diversity of Gorgonian-associated bacteria with
95 antipathogenic potential against nosocomial pathogens of UTI infections from the MPA and
96 non-MPA zones of Karimunjawa, Java Sea.

97
98

99 **Materials and Methods**

100 **2.1. Sampling and bacterial isolation**

101 This study was conducted at four islands across Karimunjawa National Park on March
 102 2021, under SIMAKSI Permit no.: 1470/T.34/TU/SIMAKSI/03/2021. Sampling was carried
 103 out at the MPA zones, Burung Island (S 06° 37' 16.9"; E 110° 38' 07.2"), Geleang Island (S
 104 05°52'56,0" E 110°21'29,5"), and the non-MPA zones, Sambangan Island (S 06° 35' 08.5'', E
 105 110° 38' 24.8'') and Seruni Island (S 05°51'13,3" E 110°34'36,8'') of Karimunjawa, Java Sea,
 106 Indonesia by scuba diving (Figure 1). Six octocoral Gorgonian *Viminella sp.*, *Ellisella sp.*,
 107 *Antipathes sp.*, *Melithaea sp.*, *Astrogorgia sp.*, and *Junceella sp.* were photographed *in situ*
 108 underwater, sampled, placed in a ziplock, and brought to the laboratory (Figure 2).
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 110



111
 112 Figure 1. Map of Karimunjawa (Campbell et al. 2013)
 113
 114



115
 116 Figure 2. Gorgonian of Karimunjawa Archipelago (Notes: **A.** *Melithaea sp.*; **B.** *Astrogorgia*
 117 *sp.*; **C.** *Antipathes*; **D.** *Ellisella sp.*; **E.** *Junceella sp.*; **F.** *Viminella sp.*
 118

119 Bacterial isolation was carried out by using the serial dilution method. The sample was diluted
120 to the concentrations of 10^{-0} , 10^{-1} and 10^{-2} ; 100 μ l of each concentration was inoculated into a
121 Petri dish containing marine agar Zobell 2214E media. Inoculated samples were spread evenly
122 and incubated at room temperature. After 2 x 24 h incubation, purification was performed by
123 inoculating bacterial colonies using the streak method and incubating at 36°C.

124

125 **2.2. Screening of antimicrobial activity**

126 The antimicrobial activity screening was carried out using the agar plug method against
127 UTI pathogens, such as *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. saprophyticus*, *A.*
128 *baumannii*, and *C. albicans*. The pure bacterial cultures were spread evenly into the Zobell
129 medium and incubated for 3 x 24 hours. Pathogenic bacteria were refreshed on Nutrient Agar
130 (NA) for 1 x 24 hours. Pathogens were inoculated into a test tube containing Nutrient Broth to
131 match their density with the McFarland 0.5 standard. Pathogenic bacteria were swabbed evenly
132 into Mueller Hinton Agar (MHA) medium, followed by placing the plugs on the media and
133 incubated for 3 x 24 hours. The clear zone around the plug indicated the presence of
134 antimicrobial activity.

135

136 **2.3. 16S rRNA gen-PCR amplification**

137 PCR amplification of partial 16S rRNA gene of selected Gorgonian-associated bacteria,
138 purification of PCR products, and subsequent sequencing analysis were performed according
139 to Wijaya et al. (2022) methods.

140

141 **2.4. Data analyses**

142 The biological indexes, such as Species Richness (SR), Relative Abundance (RA), the
143 Shannon Diversity Index (H'), the Pielou's Evenness Index (E), and the Jaccard Index were
144 used to analyze the data obtained in the laboratory.

145

146 **2.5. Nucleotide sequence accession numbers**

147 The accession numbers of the 16S rRNA sequences of the prospective strains were
148 deposited in GenBank, including OL831129, OL831140, OL831143, -, OL944616, OL831230,
149 OL832059, OL830778, OL832112, OL832113, OL824939, OL825016, OL824940,
150 OL825006, OL825002, OL830784, OL862993, OL830811, OM108167, OM108139 and
151 OM108168 for the isolates GL.6.5, GL.7.3, GL.7.5, GL.9.1, GL.9.2, GL.17.13, GL.17.16,
152 GL.17.15, GL.17.21, GL.17.34, BU.2.5, BU.6.2, BU.7.3, BU.19.2, BU.20.1, SA.16.3,
153 SA.19.2, SA.19.3, SE.10.2, SE.10.3 and SE.12.2

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157 **Results**

158 **Antimicrobial screening of Gorgonian-associated bacteria**

159 Out of 156 isolates, 14.7% (n = 23) showed antimicrobial activity against UTI
160 pathogens (Tables 1 and 2). The total active isolates isolated from the MPA area (18.48%) were
161 higher than those from the non-MPA area (9.37%). Amongst 23 active isolates, three isolates
162 can inhibit the growth of more than one pathogen, whereas the remaining isolates (n = 20)
163 inhibited only one pathogen tested. Most of the active isolates (43.4%) were capable of
164 inhibiting the growth of *E. coli*, followed by *P. aeruginosa* (26.1%), *S. aureus* (17.4%), *A.*
165 *baumannii* (17.4%) and *K. pneumoniae* (8.7%), respectively. No isolates could inhibit the
166 growth of *S. saprophyticus* and *C. albicans*.

167

168 Table 1. Total of Gorgonian-associated bacteria and active isolates in MPA and non-MPA

Genus	MPA		Non-MPA		Σ isolate/ active
	Σ isolate	Σ active	Σ isolate	Σ active	
<i>Viminella</i> sp.	20	2	7	0	27/2
<i>Ellisella</i> sp.	27	8	19	1	46/9
<i>Antipathes</i> sp.	11	2	7	0	18/2
<i>Melithaea</i> sp.	15	1	10	2	25/3
<i>Astrogorgia</i> sp.	14	3	12	2	26/5
<i>Junceella</i> sp.	5	1	9	1	14/2
T o t a l	92	17(18.5%)	64	6(9.4%)	156/23

169

170 Table 2. Antipathogenic assay of selected bacterial active against UTIs pathogens

Host	Isolate Code	Identification	Indicator Test:						
			A	B	C	D	E	F	G
<i>Viminella</i> sp.	GL6.5	<i>Streptomyces zhaozhoue</i>	-	+	-	-	-	-	-
	GL7.3	<i>Nocardiopsis salina</i>	+	-	+	-	-	-	-
<i>Ellisella</i> sp.	SA16.3	<i>Nocardiopsis salina</i>	-	-	-	-	+	-	-
	GL9.1	<i>Nocardiopsis salina</i>	-	+	-	-	-	-	-
	GL9.2	<i>Nocardiopsis salina</i>	-	-	+	-	-	-	-
	GL17.13	<i>Oceanobacillus iheyensis</i>	-	-	+	-	-	-	-
	GL17.16	<i>Micrococcus endophyticus</i>	-	-	+	-	-	-	-
	GL17.15	<i>Nocardiopsis salina</i>	-	+	-	-	-	-	-
	GL17.18	<i>Kocuria palustris</i>	-	-	-	-	+	-	-
	GL17.21	<i>Bacillus paramycoides</i>	+	-	-	-	-	-	-
<i>Junceella</i> sp.	GL17.34	<i>Virgibacillus salarius</i>	-	+	-	-	-	-	-
	SA19.2	<i>Micrococcus yunnanensis</i>	-	+	-	-	-	-	-
<i>Melithaea</i> sp.	SA19.3	<i>Micrococcus yunnanensis</i>	-	-	-	-	-	+	-
	BU2.5	<i>Brevibacterium casei</i>	-	+	-	-	-	-	-
	BU6.2	<i>Vibrio alginolyticus</i>	-	+	-	-	-	-	-
<i>Antipathes</i> sp.	SE10.2	<i>Micrococcus yunnanensis</i>	+	-	-	-	-	-	-
	GL14.22	<i>Marinococcus halophilus</i>	-	+	-	-	-	-	-
<i>Astrogorgia</i> sp.	GL222	<i>Micrococcus yunnanensis</i>	-	-	-	-	+	-	-
	BU14.4	<i>Pseudomonas stutzeri</i>	+	+	-	-	-	-	-
	BU14.6	<i>Saccharopolyspora cebuensis</i>	-	-	+	-	-	+	-
	BU16.8	<i>Salinicola salarius</i>	-	+	-	-	-	-	-
	SA7.6	<i>Acinetobacter soli</i>	-	-	-	-	+	-	-
	SA7.7	<i>Micrococcus yunnanensis</i>	-	-	+	-	-	-	-
T o t a l	156	23	4	10	6	0	4	2	0

171 Note: **A:** *S. aureus*; **B:** *E. coli*; **C:** *P. aeruginosa*; **D:** *S. saprophyticus*; **E:** *A. baumannii*; **F:**
 172 *K. pneumonia*; **G:** *C. albicans*.

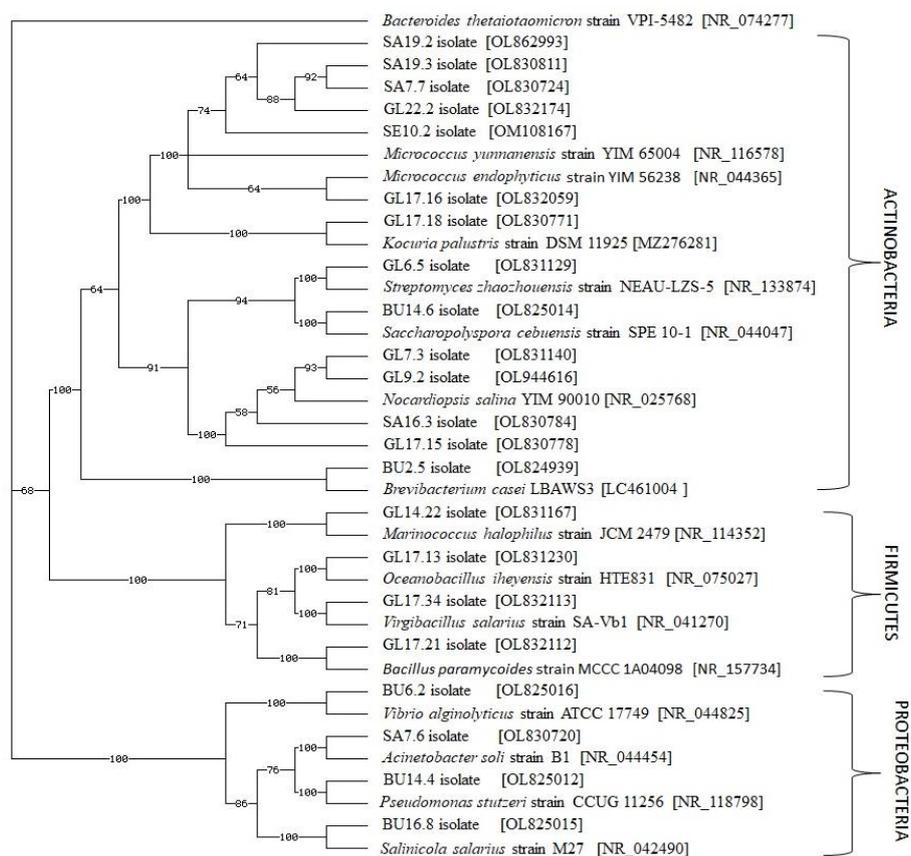
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175 **16S rDNA gene and phylogenetic analysis**

176 The 16S rRNA gene sequencing analyses showed that these 23 isolates could be
 177 assigned to 14 different species within the three phyla: Actinobacteria (*Streptomyces*
 178 *zhaozhoue*, *Nocardiopsis salina*, *Micrococcus endophyticus*, *Brevibacterium casei*,
 179 *Micrococcus yunnanensis*, *Saccharopolyspora coralli*, *Kocuria salina*), Firmicutes (*Bacillus*
 180 *paramycoides*, *Virgibacillus salarius*, *Oceanobacillus iheyensis*, *Marinococcus halophilus*)

181 and Proteobacteria (*Vibrio alginolyticus*, *Acinetobacter soli*, *Salinicola salarius*). Six of the 23
 182 isolates (26.08%) were members of the genus *Marinecoccus*, followed by *Nocardiopsis* with
 183 five isolates (21.7%). The remaining 12 of the 23 isolates (52.1%) were a genus of the
 184 *Streptomyces*, *Brevibacterium*, *Saccharopolyspora*, *Bacillus*, *Virgibacillus*, *Oceanobacillus*,
 185 *Vibrio*, *Acinetobacter*, *Salinicola*, *Marinecoccus*, *Pseudomonas* and *Kocuria*. Identification of
 186 pairwise 16S rRNA gene similarities was analyzed by using NCBI-BLAST homology. The
 187 PAUP v.05 (Swofford 1998) and CLUSTAL_X (Thompson et al. 1997) were used to construct
 188 phylogenetic trees (Figure 3).
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 192
 193 Figure 3. Phylogenetic tree of Gorgonian-associated bacteria with antipathogen properties.
 194

195 **Distribution of bacteria with antibacterial activity**

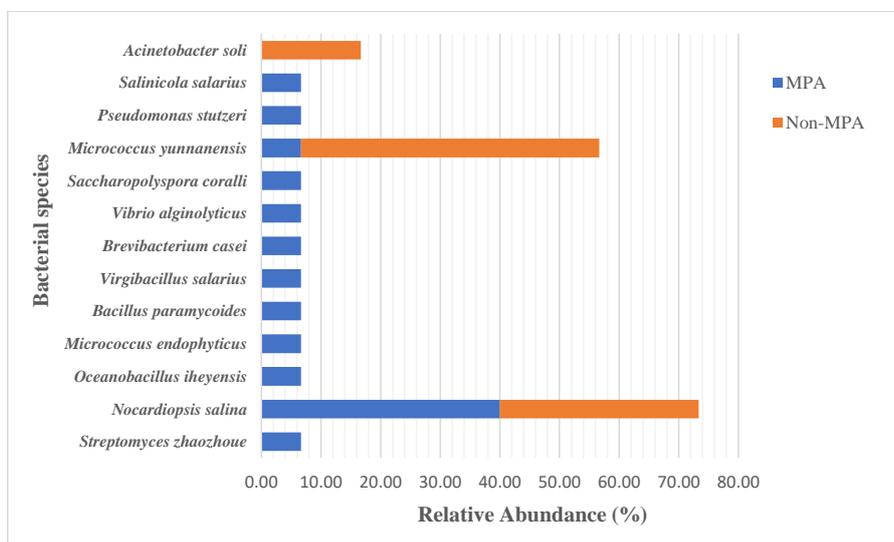
196 The distribution, diversity index, species richness, and evenness of antibacterial isolates are
 197 presented in Table 3, Figures 4 and 5. The number of antibacterial strains isolated from MPA
 198 and non-MPA areas was 17 (73.9%) and six isolates (26.08%), respectively. Amongst these
 199 anti-bacterial isolates, *Micrococcus* had the highest proportion (26.08%) followed by
 200 *Nocardiopsis* (21.7%), *Streptomyces* (4.34%), *Brevibacterium* (4.34%), *Saccharopolyspora*

201 (4.34%), *Bacillus* (4.34%), *Virgibacillus* (4.34%), *Oceanobacillus* (4.34%), *Vibrio* (4.34%),
 202 *Acinetobacter* (4.34%), *Salinicola* (4.34%), *Marinecoccus* (4.34%), *Pseudomonas* (4.34%)
 203 and *Kocuria* isolates (4.34%).

204
 205 Table 3. Relative abundance, species diversity and evenness of antipathogenic isolates

Phylum	Species	MPA		Non-MPA	
		N	RA	N	RA
Actinobacteria	<i>Streptomyces zhaozhoue</i>	1	6.67	0	0
	<i>Nocardiopsis salina</i>	5	40.00	2	33.33
	<i>Micrococcus endophyticus</i>	1	6.67	0	0
	<i>Brevibacterium casei</i>	1	6.67	0	0
	<i>Micrococcus yunnanensis</i>	1	6.67	3	50.00
	<i>Kocuria palustris</i>	1	6.67	0	0
	<i>Saccharopolyspora coralli</i>	1	6.67	0	0
Firmicutes	<i>Bacillus paramycoides</i>	1	6.67	0	0
	<i>Virgibacillus salarius</i>	1	6.67	0	0
	<i>Oceanobacillus iheyensis</i>	1	6.67	0	0
Proteobacteria	<i>Vibrio alginolyticus</i>	1	6.67	0	0
	<i>Pseudomonas stutzeri</i>	1	6.67	0	0
	<i>Salinicola salarius</i>	1	6.67	0	0
	<i>Acinetobacter soli</i>	0	6.67	1	16.66
Total		17		6	
Species		13		3	
Richness					
Diversity Index		1.99		1.01	
Evenness		0.86		0.92	

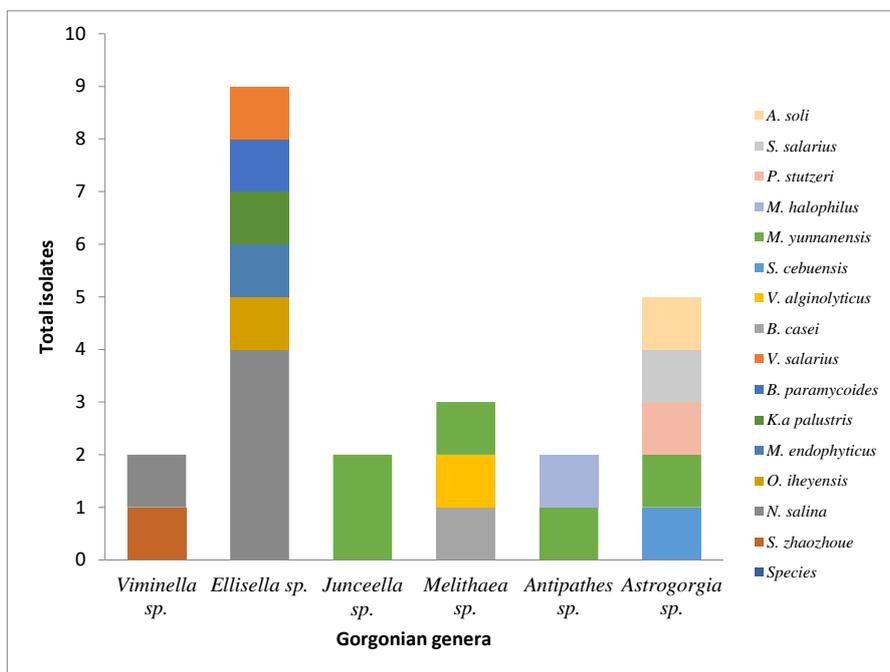
206 Note: Number of individuals (N); Relative abundance, % (RA).
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Figure 4. Relative abundance (RA) of Gorgonian-associated bacteria.

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Figure 5. The abundance of antibacterial isolates on Gorgonian genera.

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Furthermore, 43.4% (10 isolates) of antibacterial isolates displayed strong activity against *E. coli* and about 29.09% (six isolates) of antibacterial isolates displayed activity against the pathogenic bacteria *P. aeruginosa*. Four bacterial isolates (17.4%) showed activity against *S. aureus* and *A. baumannii*. No bacterial isolate displayed activity against *S. saprophyticus* and *C. albicans*.

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Comparison of bacterial communities in the MPA and non-MPA and amongst Gorgonian genera were analyzed by the Bray-Curtis formula. The analysis results demonstrated that the bacterial community's dissimilarity between MPA and non-MPA and amongst Gorgonian genera were significantly different (Tables 4 and 5). The results indicated that the bacterial communities varied significantly.

229
230

Table 4. The dissimilarity of antimicrobial strains between MPA and non-MPA.

231
232

Sampling sites	MPA	Non-MPA
MPA	-	73.91 %
Non-MPA	73.91 %	-

233

234 Table 5. The dissimilarity of antimicrobial communities amongst Gorgonian genera.

235

	A	B	C	D	E	F
A	-	81.82%	100%	100%	100%	100%
B	81.82%	-	100%	100%	100%	100%
C	100%	100%	-	60%	50%	77.43%
D	100%	100%	60%	-	60%	71.43%
E	100%	100%	50%	60%	-	77.43%
F	100%	100%	77.43%	77.43%	77.43%	-

236 Note: **A:** *Viminella sp.*; **B:** *Ellisella sp.*; **C:** *Antipathes sp.*; **D:** *Melithaea sp.*; **E:** *Astrogorgia*
 237 *sp.*; **F:** *Junceella sp.*

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239

240 **Discussion**

241 Gorgonian is a benthic community that harbors distinct microbial symbionts in
 242 seawater (van de Water et al. 2017). Their populations have suffered from mass mortality
 243 events related to anthropogenic disturbances. Changes in environmental conditions due to
 244 anthropogenic and natural disturbances can alter the microbial composition (McCauley et al.
 245 2020). In this study, the six species of Gorgonian- (*Viminella sp.*, *Ellisella sp.*, *Antipathes sp.*,
 246 *Melithaea sp.*, *Astrogorgia sp.*, and *Junceella sp.*) associated bacteria from the MPA and non-
 247 MPA zones of Karimunjawa, Java Sea were investigated for their diversity and antibacterial
 248 activity. It is well-known that marine invertebrate-associated bacteria were abundant and
 249 prolific in novel active compound production (Bibi et al. 2017; Varijakzhan et al. 2021). The
 250 156 Gorgonian-associated bacteria were assayed for their prospective antipathogenic
 251 compounds against seven UTI pathogens, including *S. aureus*, *E. coli*, *P. aeruginosa*, *S.*
 252 *saptophyticus*, *A. baumannii*, *K. pneumonia*, and *C. albicans*. The results showed that 23
 253 isolates demonstrated inhibition of the growth of UTI pathogens (Table 1 and Table 2). Their
 254 genomic DNA was extracted and sequenced for molecular identification. The 23 anti-bacterial
 255 isolates were identified as 14 genera, including Micrococcus, Nocardiosis, Streptomyces,
 256 Brevibacterium, Saccharopolyspora, Bacillus, Virgibacillus, Oceanobacillus, Vibrio,
 257 Acinetobacter, Salinicola, Marinococcus, Pseudomonas and Kocuria. Phylogenetic analysis
 258 showed that the 23 isolates analyzed in the present study could be classified into three clades
 259 (I–III): Clade I consisted of the isolates of phyla Actinomycetes; Group II of the isolates is
 260 Firmicutes; Group III of the isolates consisted of Proteobacteria (Table 2 and Figure 3). Some
 261 previous studies have identified the Gorgonian-associated bacteria with antimicrobial activity.
 262 Zhang et al. (2013) demonstrated that Streptomyces and the Micromonospora genus dominated
 263 Gorgonian-associated antibacterial isolates from five species of Gorgonian of the South China
 264 Sea. Jiang et al. (2013) reported seven genera, including Bacillus, Staphylococcus,
 265 Halobacillus, Geobacillus, Jeotgalicoccus, Psychrobacter, and Vibrio, isolated from the four
 266 species of South China Sea Gorgonians. In addition, the Bacillus genus was the most diverse
 267 and displayed antibacterial activities. These results indicate that each Gorgonian species has
 268 varying numbers and types of bacterial groups. On the contrary, Gray et al. (2011) showed that
 269 the same Gorgonian corals at different locations have different kinds of bacteria. So far, little
 270 is known about how the diversity of Gorgonian-associated bacteria diverges amongst
 271 Gorgonian coral species, particularly those on how environmental factors influence this
 272 relationship. Hence, a study of Gorgonian-bacterial diversity is urgently needed to help
 273 understand more about microbial diversity-function relationships.

274 During the last decade, MPAs have been used to conserve marine resources worldwide,
 275 although little is known about their effectiveness and success. A comparison of antibacterial
 276 composition between MPA and non-MPA showed that 17 of 92 (18.48%) and six of 64

277 (9.37%) bacterial isolates from MPA and non-MPA, respectively, exhibited antimicrobial
278 activity in at least one of the UTIs pathogens. The analysis results also showed the relative
279 abundance, species richness, and diversity index of antibacterial isolates in the MPA was higher
280 than those of non-MPA zones. (Table 3, Figures 4 and 5). Bourne et al. (2013) stated that the
281 species richness, evenness, and phylogenetic diversity of invertebrate-associated microbiomes
282 were not influenced by the bacterial composition. However, these results demonstrated that
283 Gorgonian octocoral species in the MPA region harbor varied bacteria and we propose that
284 many Gorgonian-associated bacteria have the prospective for advancing broad-spectrum
285 antibiotics. Our results have significant implications for PA management being effectively
286 managed and thus contributing to biodiversity protection.

287 The MPA and non-MPA antibacterial isolates were dominated by the same phyla
288 Actinobacteria (Table 3, Figures 4 and 5); however, the relative abundance, species richness,
289 and diversity index exhibited significant differences. *Micrococcus* genera dominated both the
290 MPA and the non-MPA samples. Some previous studies reported the diversity of Gorgonian
291 coral-associated bacteria. This study found that the number and genera of bacterial isolates
292 from MPA and non-MPA were significantly different (Tables 4 and 5). The abundance and
293 diversity of antimicrobial isolates in MPA were higher than in non-MPA. On the contrary,
294 Nogales et al. (2010) stated that microbial communities increase diversity and variability in
295 polluted areas. Due to these differences, it is obvious that little is known regarding the effect
296 of anthropogenic activities on the composition of microbial communities. Even the
297 anthropogenic stress covered in this study only modified the environmental conditions by
298 zoning marine areas; however, it is a complex situation.

299 The Gorgonian coral *Ellisella* sp. concealed the most antibacterial activities of isolates
300 and the highest diversity of antibacterial activity genera (Figures 4 and 5). At the same time,
301 Bayer et al. (2015) reported that the Gorgonian coral *Eunicella cavolini* was dominated by the
302 genus *Endozoicomonas* sp. It seems that the totals and kinds of bacterial groups varied amongst
303 Gorgonian species. In this study, only about 15% (23 isolates) of Gorgonian-associated bacteria
304 were active against at least one of the pathogenic UTIs, which means that only a few
305 Gorgonian-associated bacteria produced active antimicrobial compounds. Recently, marine
306 invertebrate-associated microorganisms have become a potential source of new active
307 compounds. Several recent studies have shown that bacteria isolated from tunicates,
308 nudibranchs, sponges, and soft corals produce promising antibacterial active compounds (Putra
309 et al. 2016; Cita et al. 2017; Ayuningrum et al. 2019; Kristiana et al. 2019). This study
310 demonstrated that culturable Gorgonian-associated bacteria could produce antibiotics and
311 inhibit the growth of UTI nosocomial pathogenic bacteria. The diversity and abundance of
312 Gorgonian-associated bacteria with antibacterial properties were higher in MPA than in non-
313 MPA zones, indicating effective and efficient management in biodiversity protection.
314 Maintenance of trophic structure and diversity of functions is the necessary effort that must be
315 undertaken as a management priority to enable ecosystem resilience.

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