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1 **Title: Low-generation cationic phosphorus dendrimers: Novel approach to tackle**
2 **drug-resistant *S. aureus in vitro* and *in vivo***

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30

31 **Keywords:** Polycationic-phosphorous dendrimers, AMR, MRSA, fluorescence microscopy, resistance, SEM.

32 **Abstract**

33 The incessant, global increase in antimicrobial resistance (AMR) is a very big challenge for healthcare systems. AMR is
34 predicted to grow at an alarming pace, with a dramatic increase in morbidity, mortality and US\$ 100 trillion loss to the
35 global economy by 2050. The mortality rate caused due to methicillin-resistant *S. aureus* (MRSA) is much higher as
36 compared to infections caused due to drug-susceptible *S. aureus*. Additionally, there is a big paucity of therapeutics
37 available for treatment of serious infections caused due to MRSA. Thus, urgent discovery and development of novel
38 therapies is an urgent, unmet medical need. In this context, we synthesized AE4G0, a low-generation cationic-
39 phosphorus dendrimer expressing potent antimicrobial activity against *S. aureus* and *Enterococcus* sp., and
40 demonstrating a broad selectivity index against eukaryotic cells. AE4G0 exhibits concentration-dependent, bactericidal
41 activity and synergizes with gentamicin, especially against gentamicin-resistant MRSA NRS119. Fluorescence and
42 scanning electron microscopy demonstrate that treatment with AE4G0 led to the utter destruction of *S. aureus* ATCC
43 29213 without inducing resistance, despite repeated exposure. When tested *in vivo*, AE4G0 demonstrates significant
44 efficacy against *S. aureus* ATCC 29213, alone and in combination with gentamicin against gentamicin-resistant *S. aureus*
45 NRS119 in the murine skin model of infection. Taken together, AE4G0 demonstrates the potential to be translated as a
46 novel therapeutic option for the treatment of topical, drug-resistant *S. aureus* infections.

47 **Introduction**

48 AMR is emerging and spreading at an alarming pace, thus adversely affecting healthcare systems globally. Uncontrolled
49 and inappropriate antibiotic use are some of the leading causes of the expansion of multidrug-resistant (MDR) strains
50 predicted to be responsible for ~700,000 annual deaths and a US\$ 100 trillion loss to the global economy by 2050[1].
51 Among the top World Health Organisation (WHO) priority pathogens for which novel therapeutic options are urgently
52 required, *Staphylococcus aureus*, a gram-positive bacteria that mainly inhabits human skin and mucous membranes, is
53 one of the main causative agents for community and hospital-acquired infections including endocarditis, skin and soft
54 tissue infections, bacteraemia, osteomyelitis and life-threatening pneumonia[2]. In fact, infection with MRSA leads to

55 64% more chance of death as compared to infection with drug-susceptible *S. aureus*[3]. Since MRSA and vancomycin-
56 resistant *S. aureus* (VRSA) are WHO high priority pathogens, thus, discovery and development of new antibiotics is an
57 urgent, unmet medical need[4].

58 In this context, highly branched tree-like polymers, called dendrimers, are characterized by precisely defined structures
59 and molecular weights. Due to their multivalency, polyvalent interactions with cellular and molecular targets have been
60 observed[5-7]. Many publications report neutral, anionic or cationic phosphorus dendrimers as exhibiting anti-
61 tumoral[8-10], anti-tubercular[11], anti-inflammatory[12-16],effect on prion or Alzheimer's diseases[17] or for the
62 formation of a variety of hydrogels[18]. Surprisingly, anti-bacterial activities of these phosphorus dendrimers have not
63 been investigated in detail. In an exception, a recent report describes synergistic effects of a polyanionic phosphorus
64 dendrimer of generation 4 bearing 96-end carboxylic groups to synergize with levofloxacin, potentially leading to
65 reduced antibiotic dose for treatment of bacterial infection[19]. Therefore, it was of great interest to explore the
66 antimicrobial properties of original, polycationic phosphorus dendrimers of low generation and more intensively, of
67 AE4G0. AE4G0 was tested against highly drug-resistant MRSA, VRSA along with *Enterococcus spp.* Various pre-clinical
68 parameters of AE4G0 were determined, followed by *in vivo* potential in a murine model of skin infection alone and in
69 combination with gentamicin against *S. aureus* ATCC 29213 and gentamicin-resistant MRSA NRS119.

70

71 **Materials and Methods**

72 **Materials**

73 All manipulations were carried out using a standard dry argon-high vacuum technique. Organic solvents were dried and
74 freshly distilled under argon prior to use. Reagents were obtained from commercial sources and used as received.
75 Compound **(1)** was synthesized from chloroacetic acid by standard esterification upon acid catalysis. Compounds **(3a,b)**
76 (so-called Girard reagents P and T) were obtained from commercial sources and used as received.

77 **Analytical and spectroscopic techniques**

78 ^1H , $^{13}\text{C}\{^1\text{H}\}$ and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were recorded on Bruker AV400PAS and AV300PAS (Bruker, Karlsruhe, Germany)
79 instruments. ^1H and ^{13}C chemical shifts (δ , ppm) were measured relative to residual resonances of solvents.
80 High-resolution mass spectra were recorded using GCT Premier (Waters, Milford, MA, USA) and UPLC Xevo G2 Q TOF
81 (Waters, Milford, MA, USA) mass spectrometers for chemical ionization and electrospray ionization, respectively.

82 **Dendrimers synthesis**

83 Aldehyde-terminated dendrimer (**4**) (0.46 mmol) was dissolved in 20 mL CHCl₃, then solution of an acetohydrazide (2.83
84 mmol) in 20 mL methanol was added. Reaction mixture was acidified to pH~5 with acetic acid and stirred overnight at
85 45 °C. Once ¹H NMR confirmed the completeness of aldehyde conversion into hydrazone, the solvent was removed,
86 and the solid residue was washed with CHCl₃ and diethyl ether, then dried.

87 *Pyridinium-terminated dendrimer PG0*

88 Pale powder, yield: 95%. ¹H NMR (400 MHz, DMSO-d₆) δ, ppm: 5.76, 6.18 (2d, J = 13.8 Hz, 12H, C(O)-CH₂-N), 7.08 (m,
89 12H, CH-C-CH=N), 7.63 (m, 12H, O-C-CH), 8.21 (q, J = 7.1 Hz, 12H, pyridinium), 8.30 – 8.54 (m, 6H, CH=N), 8.68 (q, J = 7.7
90 Hz, 6H, pyridinium), 9.23 (m, 12H, pyridinium), 12.49, 12.63, 13.29 (3br s, 6H, N-NH). ¹³{¹H}C NMR (101 MHz, DMSO) δ,
91 ppm: 61.36, 61.74 (d, J = 11.4 Hz), 121.40, 127.94 (dd, J = 17.8, 7.7 Hz), 129.12 (d, J = 18.2 Hz), 131.70, 144.71 (d, J =
92 11.6 Hz), 146.50 – 146.89 (m), 147.06, 147.50 (d, J = 16.1 Hz), 151.21, 161.88 (d, J = 11.7 Hz), 166.77 (d, J = 18.9 Hz),
93 172.44. ³¹P{¹H} NMR (162 MHz, DMSO-d₆) δ, ppm: 8.5 (m).

94 *Trimethylammonium-terminated dendrimer AE4G0*

95 Pale powder, yield: 95%. ¹H NMR (400 MHz, DMSO-d₆) δ, ppm: 3.30 – 3.46 (m, 54H, N-CH₃), 4.48, 4.90 (2d, J = 11.9 Hz,
96 12H, C(O)-CH₂-N), 6.94 – 7.21 (m, 12H, CH-C-CH=N), 7.50 – 7.74 (m, 12H, O-C-CH), 8.28 – 8.50 (m, 6H, CH=N), 12.11 –
97 13.40 (m, 6H, N-NH). ¹³{¹H}C NMR (101 MHz, DMSO) δ, ppm: 53.3 – 54.4 (m), 62.5, 63.8, 65.4, 79.8, 120.7 – 122.0 (m),
98 128.9 – 129.7 (m), 131.1 – 132.1 (m), 144.9 (d, J = 15.0 Hz), 148.2 (d, J = 15.8 Hz), 151.4 (d, J = 25.6 Hz), 160.4, 165.2 –
99 166.0 (m), 172.4. ³¹P{¹H} NMR (162 MHz, DMSO-d₆) δ, ppm: 8.4 (m). ESI-TOF HRMS: [M]²⁺ 770.8468 (calcd 770.8458),
100 [M]³⁺ 514.2347 (calcd 514.2332), [M]⁴⁺ 385.9276 (calcd 385.9268), [M]⁵⁺ 308.9439 (calcd 308.9430), [M]⁶⁺ 257.9548
101 (calcd 257.9548).

102 *Triethylammonium-terminated dendrimer NEt₃G0*

103 Pale powder, yield: 90%. ¹H NMR (400 MHz, DMSO-d₆) δ, ppm: 1.28 (dt, J = 19.1, 7.2 Hz, 54H, -CH₃), 3.52, 3.62 (2d, J =
104 7.1 Hz, 36H), 4.31, 4.61 (2s, 12H, C(O)-CH₂-N), 7.06 (p, J = 10.9, 9.6 Hz, 12H, CH-C-CH=N), 7.48 – 7.78 (m, 12H, O-C-CH),
105 8.18 – 8.47 (m, 6H, CH=N), 12.32 – 13.63 (m, 6H, N-NH). ¹³{¹H}C NMR (101 MHz, DMSO) δ, ppm: 8.05, 53.92, 54.43,
106 54.76, 55.93, 121.28 (d, J = 13.8 Hz), 129.37 (d, J = 10.9 Hz), 131.52, 144.95, 148.15, 151.49, 160.17, 165.46. ³¹P{¹H}

107 NMR (162 MHz, DMSO-d6) δ , ppm: 8.1 (m). ESI-TOF HRMS: $[M]^{2+}$ 897.9897 (calcd 897.9896), $[M]^{3+}$ 598.6618 (calcd
108 598.6614), $[M]^{4+}$ 449.2483 (calcd 449.2480), $[M]^{5+}$ 359.5996 (calcd 359.5999), $[M]^{6+}$ 299.8333 (calcd 299.8346).

109 *1-methyl-piperidinium-terminated dendrimer C6MeG0*

110 Pale powder, yield: 90%. ^1H NMR (400 MHz, DMSO-d6) δ , ppm: 1.53 (m, 12H, N-CH₂-CH₂-CH₂-), 1.90 (m, 24H, N-CH₂-
111 CH₂-), 3.36 (s, 18H, CH₃-N), 3.43 – 3.87 (dm, 24H, 4H, N-CH₂-CH₂-), 4.58, 4.87 (2m, 12H, C(O)-CH₂-N), 7.03 (m, 12H, CH-C-
112 CH=N), 7.62 (m, 12H, O-C-CH), 8.20 – 8.56 (m, 6H, CH=N), 12.16 – 12.60, 13.08 – 13.46 (2m, 6H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR
113 (101 MHz, DMSO) δ , ppm: 19.38 – 20.09 (m), 20.67 – 21.32 (m), 50.46, 61.38 – 62.31 (m), 121.22 (d, J = 38.8 Hz), 129.28
114 (d, J = 15.1 Hz), 131.45 (d, J = 12.7 Hz), 144.83, 148.29 (d, J = 19.0 Hz), 151.44 (d, J = 15.5 Hz), 160.22, 162.47, 165.43 (t,
115 J = 9.4 Hz), 166.75, 168.47, 172.42. $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d6) δ , ppm: 8.2 (m). ESI-TOF HRMS: $[M]^{2+}$ 891.4425
116 (calcd 891.4412), $[M]^{3+}$ 594.9662 (calcd 594.9644), $[M]^{4+}$ 446.2261 (calcd 446.2245), $[M]^{5+}$ 357.1830 (calcd 357.1812),
117 $[M]^{6+}$ 297.8186 (calcd 297.8189).

118 *Piperidinyl-terminated dendrimer C6HG0*

119 Pale powder, yield: 80%. ^1H NMR (400 MHz, DMSO-d6) δ , ppm: 1.41 (m, 12H, N-CH₂-CH₂-CH₂-), 1.55 (q, J = 5.7 Hz, 24H,
120 N-CH₂-CH₂-), 2.43 (d, J = 5.9 Hz, 24H, N-CH₂-CH₂-), 3.06, 3.48 (2m, 12H, C(O)-CH₂-N), 7.00 (m, 12H, CH-C-CH=N), 7.55 (m,
121 12H, O-C-CH), 7.93, 8.33 (2m, 6H, CH=N), 11.12 (m, 6H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO-d6) δ , ppm: 21.6, 24.0
122 and 24.2 (2s), 25.8 and 26.1 (2s), 54.4 and 54.7 (2s), 58.4, 61.9, 121.3 and 128.6 (2d), 132.2 (m), 141.9 (m), 146.6 (t, J =
123 11.9 Hz), 151.0 (d, J = 18.8 Hz), 166.7 and 171.4 (2s). $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d6) δ , ppm: 8.4 (m). ESI-TOF HRMS:
124 $[M+2H]^{2+}$ 806.8448 (calcd 806.8458), $[M+3H]^{3+}$ 538.2329 (calcd 538.2332), $[M+4H]^{4+}$ 403.9258 (calcd 403.9268),
125 $[M+5H]^{5+}$ 323.3423 (calcd 323.3430), $[M+6H]^{6+}$ 269.6193 (calcd 269.6205).

126 *Pyrrolidinyl-terminated dendrimer C5HG0*

127 Pale powder, yield: 85%. ^1H NMR (400 MHz, DMSO-d6) δ , ppm: 1.70 (dt, J = 24.1, 5.4, 24H, N-CH₂-CH₂-), 2.58 (m, 24H,
128 N-CH₂-CH₂-), 3.21, 3.61 (2s, 12H, C(O)-CH₂-N), 7.02 (m, 12H, CH-C-CH=N), 7.55 (m, 12H, O-C-CH), 7.87 – 8.36 (m, 6H,
129 CH=N), 11.17 (m, 6H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO-d6) δ , ppm: 23.86 (d, J = 9.8 Hz), 54.11 (d, J = 30.9 Hz),
130 55.30, 58.67 (d, J = 4.6 Hz), 121.30, 128.78 (d, J = 41.1 Hz), 132.17, 141.94, 146.60 (d, J = 11.0 Hz), 151.10, 166.87,
131 171.69. $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d6) δ , ppm: 8.5 (m). ESI-TOF HRMS: $[M+2H]^{2+}$ 848.8912 (calcd 848.8850),

132 [M+3H]³⁺ 566.5989 (calcd 566.5988), [M+4H]⁴⁺ 425.2006 (calcd 425.2010), [M+5H]⁵⁺ 340.3614 (calcd 340.3624),
133 [M+6H]⁶⁺ 283.6346 (calcd 283.8033).

134 *Biphenyl-blocked aldehyde-terminated dendrimer (6)*

135 Hexachlorocyclotriphosphazene (4 mmol) and K₂CO₃ (12 mmol) were mixed in 20 mL of distilled CH₃CN and cooled
136 down with an ice bath. Solution of 2,2'-dihydroxybiphenyl (4 mmol) in 10 mL distilled CH₃CN was added dropwise, and
137 reaction mixture was stirred overnight at 0 °C to yield *P,P*-dihydroxybiphenyl-*P',P'',P''*-tetrachlorocyclotriphosphazene
138 (**5**) that was used at the next stage without further purification. ³¹P{¹H} NMR (162 MHz, CDCl₃) δ, ppm: 12.8 (dd), 25.3
139 (d). 4-hydroxybenzaldehyde (16.8 mmol) and K₂CO₃ (20 mmol) were added, and the reaction mixture was stirred
140 overnight at r.t. Once ³¹P{¹H} NMR showed complete conversion of (**5**) into biphenyl-blocked aldehyde-terminated
141 dendrimer (**6**), the solvent was removed. The solid residue was dissolved in CH₂Cl₂, and washed 3 times with 1M KOH
142 and 3 times with brine. The organic layer was dried over Na₂SO₄ and concentrated into a solid residue that was washed
143 with diethyl ether to give (**6**) as white crystals that were recovered on a filter and dried. Yield: 80%.

144 ¹H NMR (400 MHz, DMSO-d₆) δ, ppm: 6.89 (dt, J = 7.7, 1.7 Hz, 2H, biphenyl), 7.36 (d, J = 8.6 Hz, 8H, -CH-C-CHO), 7.41 –
145 7.52 (m, 4H, biphenyl), 7.67 (dd, J = 7.3, 2.1 Hz, 2H, biphenyl), 7.95 (d, J = 8.6 Hz, 8H, P-O-C-CH), 9.99 (s, 4H, -CHO).
146 ¹³C{¹H} NMR (101 MHz, DMSO-d₆) δ, ppm: 121.8 (t, J = 2.6 Hz), 127.3, 128.1 (d, J = 1.6 Hz), 130.5, 130.8, 132.1, 134.2,
147 147.3, 147.4, 154.3 (t, J = 3.6 Hz), 192.3. ³¹P{¹H} NMR (162 MHz, DMSO-d₆) δ, ppm: 8.5 (d, J = 94.1 Hz), 24.3 (dd, J =
148 95.8, 92.6 Hz). ESI-TOF HRMS: [M] 804.1075 (calcd 804.1066)

149 ***2-Amino-substituted acetohydrazone-terminated biphenyl-blocked dendrimers***

150 Aldehyde-terminated biphenyl-blocked dendrimer (**6**) (0.25 mmol) was dissolved in 10 mL CHCl₃, then solution of an
151 acetohydrazide (1.025 mmol) in 10 mL methanol was added. Reaction mixture was acidified to pH~5 with acetic acid
152 and stirred overnight at r.t. Once the completeness of aldehyde conversion into hydrazone was confirmed by ¹H NMR,
153 the solvent was removed, and the solid residue was washed with CHCl₃ and diethyl ether, then dried.

154 *Pyridinium-terminated dendrimer PGOL*

155 Pale powder, yield: 90%. ¹H NMR (400 MHz, DMSO-d₆) δ, ppm: 5.69, 6.11 (2d, J = 7.7 Hz, 8H, C(O)-CH₂-N), 6.86 (t, J =
156 8.9 Hz, 2H, biphenyl), 7.21 (m, 8H, CH-C-CH=N), 7.48 (m, 4H, biphenyl), 7.66 – 7.86 (m, 10H, O-C-CH, biphenyl), 8.22 (q, J
157 = 9.6, 8.1 Hz, 8H, pyridinium), 8.30 - 8.52 (m, 4H, CH=N), 8.69 (q, J = 6.4, 4.9 Hz, 4H, pyridinium), 9.08 – 9.19 (m, 8H,

158 pyridinium), 12.43, 13.21 (2m, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO-d6) δ , ppm: 61.39, 61.83, 121.72 (d, J = 20.9 Hz),
159 127.28, 127.85 – 128.35 (m), 129.24 (d, J = 16.7 Hz), 130.66 (d, J = 30.4 Hz), 131.86, 144.58, 146.76 (d, J = 10.8 Hz),
160 146.99, 147.47 (d, J = 11.5 Hz), 151.44, 161.86, 166.96 (d, J = 13.9 Hz), 172.44. $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d6) δ ,
161 ppm: 9.1 (dd, J = 93.4, 7.2 Hz), 24.8 (dd, J = 94.8, 91.7 Hz). ESI-TOF HRMS: $[\text{M}]^{2+}$ 668.6871 (calcd 668.6852), $[\text{M}]^{3+}$
162 446.1274 (calcd 446.1261), $[\text{M}]^{4+}$ 334.8477 (calcd 334.8465).

163 *Trimethylammonium-terminated dendrimer* TGOL

164 Pale powder, yield: 99%. ^1H NMR (400 MHz, DMSO-d6) δ , ppm: 3.39 (s, 36H, N-CH₃), 4.46, 4.86 (2d, J = 11.6 Hz, 8H,
165 C(O)-CH₂-N), 6.91 (dd, J = 53.4, 7.9 Hz, 2H, biphenyl), 7.18 (m, 8H, CH-C-CH=N), 7.43 – 7.58 (m, 4H, biphenyl), 7.64 –
166 7.80 (m, 10H, O-C-CH, biphenyl), 8.19 – 8.50 (m, 4H, CH=N), 12.12 – 12.54, 12.96 – 13.30 (2m, 4H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR
167 (101 MHz, DMSO-d6) δ , ppm: 53.50 – 54.16 (m), 62.65 (d, J = 9.3 Hz), 63.83, 121.01 – 122.09 (m), 127.27, 128.21,
168 129.13 – 129.62 (m), 130.50, 130.85 (d, J = 9.4 Hz), 131.44 – 131.90 (m), 144.81 (d, J = 13.8 Hz), 147.44 (d, J = 9.6 Hz),
169 148.13 (d, J = 11.4 Hz), 151.50 (d, J = 16.5 Hz), 160.41 (d, J = 6.3 Hz), 162.59 (d, J = 21.0 Hz), 165.79 (d, J = 15.3 Hz).
170 $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d6) δ , ppm: 9.0 (d, J = 93.7 Hz), 24.6 (m). ESI-TOF HRMS: $[\text{M}]^{2+}$ 628.7490 (calcd
171 628.7478), $[\text{M}]^{3+}$ 419.5015 (calcd 419.5011), $[\text{M}]^{4+}$ 314.8788 (calcd 314.8778).

172 *Triethylammonium-terminated dendrimer* NEt₃GOL

173 Pale powder, yield: 90%. ^1H NMR (400 MHz, DMSO-d6) δ , ppm: 1.27 (dt, J = 20.6, 7.1 Hz, 36H, N-CH₂-CH₃), 3.52, 3.61
174 (2d, J = 7.5 Hz, 24H, N-CH₂-CH₃), 4.35, 4.60 (2m, 8H, C(O)-CH₂-N), 6.85 (m, 2H, biphenyl), 7.21 (m, 8H, CH-C-CH=N), 7.46
175 (p, J = 7.3 Hz, 4H, biphenyl), 7.63 – 7.78 (m, 8H, O-C-CH), 7.84 (m, 2H, biphenyl), 8.25, 8.46 (2m, 4H, CH=N), 12.28, 13.44
176 (2m, 4H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO-d6) δ , ppm: 8.02 (d, J = 2.1 Hz), 53.39 – 56.32 (m), 121.68 (d, J = 27.0
177 Hz), 127.25, 128.19, 129.48 (d, J = 8.4 Hz), 130.64 (d, J = 27.1 Hz), 131.68 (d, J = 7.5 Hz), 144.94, 147.44 (d, J = 9.5 Hz),
178 148.20 (d, J = 13.3 Hz), 151.61, 160.14, 162.16, 165.52. $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d6) δ , ppm: 8.9 (m), 24.6 (m). ESI-
179 TOF HRMS: $[\text{M}]^{2+}$ 712.8425 (calcd 712.8417), $[\text{M}]^{3+}$ 475.5643 (calcd 475.5638), $[\text{M}]^{4+}$ 356.9242 (calcd 356.9248).

180 *1-methyl-piperidinium-terminated dendrimer* C6MeGOL

181 Pale powder, yield: 90%. ^1H NMR (400 MHz, DMSO-d6) δ , ppm: 1.56 (m, 8H, N-CH₂-CH₂-CH₂-), 1.87 (m, 16H, N-CH₂-CH₂-
182), 3.35 (s, 12H, CH₃-N), 3.37 – 3.81 (m, 16H, N-CH₂-CH₂-), 4.54, 4.83 (2d, J = 5.3 Hz, 8H, C(O)-CH₂-N), 6.87 (m, 2H,
183 biphenyl), 7.20 (m, 8H, CH-C-CH=N), 7.47 (m, 4H, biphenyl), 7.73 (m, 10H, O-C-CH, biphenyl), 8.19 – 8.51 (m, 4H, CH=N),

184 12.32, 13.31 (2m, 4H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO-d₆) δ , ppm: 19.85 (d, J = 3.0 Hz), 20.94 – 21.33 (m), 47.45,
185 47.87, 50.43, 60.10, 61.52 – 62.18 (m), 121.60 (q, J = 16.1, 13.3 Hz), 127.26, 128.20, 129.43 (d, J = 6.7 Hz), 130.65 (d, J =
186 30.6 Hz), 131.67 (d, J = 5.7 Hz), 144.79 (d, J = 10.1 Hz), 147.44 (d, J = 9.2 Hz), 148.21 (d, J = 10.8 Hz), 151.53 (d, J = 16.0
187 Hz). $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d₆) δ , ppm: 9.0 (m), 24.7 (m). ESI-TOF HRMS: $[\text{M}]^{2+}$ 708.8105 (calcd 708.8104), $[\text{M}]^{3+}$
188 472.8761 (calcd 472.8762), $[\text{M}]^{4+}$ 354.9090 (calcd 354.9091).

189 *1-methyl-pyrrolidinium-terminated dendrimer C5MeGOL*

190 Pale powder, yield: 90%. ^1H NMR (400 MHz, DMSO-d₆) δ , ppm: 2.13 (m, 16H, N-CH₂-CH₂-), 3.36 (s, 12H, CH₃-N), 3.75 (m,
191 16H, N-CH₂-CH₂-), 4.48, 4.91 (2d, J = 6.4 Hz, 8H, C(O)-CH₂-N), 6.88 (m, 2H, biphenyl), 7.20 (m, 8H, CH-C-CH=N), 7.48 (m,
192 4H, biphenyl), 7.72 (m, 10H, O-C-CH, biphenyl), 8.16 – 8.50 (m, 4H, CH=N), 12.31, 13.21 (2m, 4H, N-NH). $^{13}\text{C}\{^1\text{H}\}$ NMR
193 (101 MHz, DMSO-d₆) δ , ppm: 20.48 – 22.99 (m), 48.28 – 50.45 (m), 62.24 (d, J = 22.5 Hz), 65.37, 121.04 – 122.18 (m),
194 127.28, 128.21, 129.36 (d, J = 12.4 Hz), 130.66 (d, J = 30.6 Hz), 131.64, 144.63 (d, J = 9.8 Hz), 147.45 (d, J = 9.4 Hz),
195 148.03 (d, J = 11.6 Hz), 151.50 (d, J = 15.0 Hz), 160.70, 165.88 – 166.54 (m). $^{31}\text{P}\{^1\text{H}\}$ NMR (162 MHz, DMSO-d₆) δ , ppm:
196 9.0 (d, J = 93.2 Hz), 24.7 (m). ESI-TOF HRMS: $[\text{M}]^{2+}$ 680.7794 (calcd 680.7791), $[\text{M}]^{3+}$ 454.1890 (calcd 454.1887), $[\text{M}]^{4+}$
197 340.8929 (calcd 340.8935).

198 *Piperidiny-terminated dendrimer C6HGOL*

199 Pale powder, yield: 80%. ^1H NMR (400 MHz, DMSO-d₆) δ , ppm: 1.41 (m, 8H, N-CH₂-CH₂-CH₂-), 1.55 (q, J = 5.7 Hz, 16H,
200 N-CH₂-CH₂-), 2.43 (d, J = 5.9 Hz, 16H, N-CH₂-CH₂-), 3.04, 3.36 (2m, 8H, C(O)-CH₂-N), 6.78 (m, 2H, biphenyl), 7.18 (m, 8H,
201 CH-C-CH=N), 7.44 (m, 4H, biphenyl), 7.68 (m, 10H, O-C-CH, biphenyl), 7.84 – 8.43 (m, 4H, CH=N), 11.20 (m, 4H, N-NH).
202 $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO-d₆) δ , ppm: 21.54, 24.09 (d, J = 14.8 Hz), 25.91 (d, J = 17.6 Hz), 54.51 (d, J = 26.1 Hz),
203 58.40, 61.94, 121.71 (d, J = 23.9 Hz), 127.15, 128.21, 128.73, 129.12, 130.53 (d, J = 21.2 Hz), 132.37, 141.95 (d, J = 19.1
204 Hz), 146.54 (d, J = 14.1 Hz), 147.47 (d, J = 9.3 Hz), 151.15 (d, J = 21.1 Hz), 166.57, 171.35, 172.44 (d, J = 6.8 Hz). $^{31}\text{P}\{^1\text{H}\}$
205 NMR (162 MHz, DMSO-d₆) δ , ppm: 9.2 (dt, J = 94.6, 11.1 Hz), 24.9 (m). ESI-TOF HRMS: $[\text{M}+2\text{H}]^{2+}$ 680.7798 (calcd
206 680.7800), $[\text{M}+3\text{H}]^{3+}$ 454.1888 (calcd 454.1887), $[\text{M}+4\text{H}]^{4+}$ 340.8928 (calcd 340.8935).

207 **Bacterial Strains**

208 All the dendrimers synthesized were primarily screened by following Clinical and Laboratory Standard Institute (CLSI)
209 guidelines[20] against *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *K. pneumoniae* BAA 1705, *A. baumannii* BAA 1605, *P.*
210 *aeruginosa* ATCC 27853, *Enterobacter* sp and *Enterococcus* spp., collectively known as ESKAPE pathogens. The screening

211 includes the drug-resistant clinical strains of *S. aureus* and *Enterococcus* spp, including those resistant to vancomycin
212 and other clinically utilized antibiotics. These strains were procured from Biodefense and Emerging Infections Research
213 Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection
214 (BEI/NARSA/ATCC, USA) and routinely cultivated on MHA and MHBII. To obtain the starter culture, a single colony was
215 picked from the MHA plate, inoculated in MHBII, and incubated overnight at 37°C with shaking for 18–24h.

216

217 **Growth media and reagents**

218 All bacterial media and supplements, including Cation supplemented Mueller–Hinton broth II (MHBII), Mueller–Hinton
219 agar (MHA) and tryptic soy broth (TSB) were purchased from Becton-Dickinson (Franklin Lakes, NJ, USA). All other
220 chemicals and antibiotics were procured from Sigma-Aldrich (St. Louis, MO, USA). While performing the experiments,
221 all the relevant guidelines and regulations were followed.

222

223 **Antibiotic susceptibility testing**

224 CLSI guidelines for microdilution assay were followed to conduct the antibiotic susceptibility testing[20]. 10 mg/ml
225 stock solution of tested compounds were prepared in dimethyl sulfoxide (DMSO)/water. MHBII was used to inoculate
226 bacterial culture, and optical density (OD) was measured at 600 nm, followed by dilution to achieve $\sim 10^5$ colony-
227 forming units (CFU)/mL. The compounds were tested from 64 to 0.5 $\mu\text{g}/\text{mL}$ in a two-fold serial diluted fashion, with 2.5
228 μL of each concentration added to the wells of a 96-well round-bottomed microtiter plate. Later, 97.5 μL of bacterial
229 suspension was added to each well containing either the test compound or appropriate control. The plates were
230 incubated at 37 °C for 18-24 h, and minimum inhibitory concentration (MIC) was determined. The MIC is defined as the
231 lowest concentration of the test compounds at which no visible growth was observed. MIC determination for each test
232 compound was carried out independently three times using duplicate samples.

233

234 **PMBN Assay**

235 Polymyxin B nonapeptide (PMBN) is a derivative of Polymyxin B, that lacks direct bactericidal activity but increases
236 outer membrane (OM) permeabilization, thus permitting increased entry of xenobiotics in GNB[21, 22]. Previous
237 studies were followed to determine the MIC of antimicrobials in the presence of PMBN[22]. The MIC of AE4G0,
238 vancomycin, Levofloxacin and rifampicin were determined against *E. coli* ATCC 25922 and *A. baumannii* BAA 1605 in the
239 presence of PMBN at a concentration of 10 $\mu\text{g}/\text{ml}$.

240

241 **Cytotoxicity against Vero cells (ATCC CCL-81)**

242 The cytotoxicity of all synthesized dendrimers were checked against eukaryotic Vero cells using MTT assay, CC_{50} was
243 determined and the selectivity index was calculated[23]. Doxorubicin was used as a positive control and each
244 experiment was repeated in triplicate.

245

246 **Bacterial time-kill kinetics**

247 Time kill kinetics was used to assess the bactericidal activity of the AE4G0[24, 25]. Briefly, *S. aureus* ATCC 29213 was
248 diluted $\sim 10^5$ CFU/mL in MHBII, treated with 1x and 10x MIC of AE4G0 and vancomycin and incubated at 37°C with
249 shaking for 24 h. 100 μ L samples were collected at 0 h, 1 h, 6 h and 24 h, serially diluted in PBS and plated on MHA,
250 followed by incubation at 37 °C for 18–20 h. Time-kill curves were constructed by counting the colonies from plates and
251 plotting the CFU/mL of surviving bacteria at each time point in the presence and absence of the compound. Each
252 experiment was repeated three times in duplicate, and the mean data were plotted.

253

254 **Drug Interaction with FDA-approved drugs**

255 Interaction of AE4G0 with standard drugs approved by FDA was determined by chequerboard method[26]. As
256 recommended by CLSI, serial two-fold dilutions of each drug were prepared before testing. AE4G0 was diluted two-fold
257 along ordinate ranging from 64 to 0.5 μ g/mL (8 dilutions). In contrast, antibiotics were serially diluted (twelve dilutions),
258 ranging from 64-0.03 μ g/mL for ceftazidime, 4–0.0019 μ g/mL for daptomycin and vancomycin, 2-0.0009 μ g/mL for
259 levofloxacin, gentamicin, minocycline and meropenem, 0.06-0.000014 μ g/mL for rifampicin and 16-0.0078 μ g/mL for
260 linezolid in 96-well microtiter plates. Ninety-five microlitres of $\sim 10^5$ CFU/mL were added to each well and plates were
261 incubated at 37°C for 18-24 h. After incubation, FICs (fractional inhibitory concentrations) were calculated as follows:
262 $\sum FIC = FIC A + FIC B$, where FIC A is MIC of Drug A in combination/MIC of Drug A alone and FIC B is MIC of Drug B in
263 combination/MIC of Drug B alone. The combination is synergistic when $\sum FIC$ is ≤ 0.5 , indifferent when $\sum FIC$ is >0.5 to 4,
264 and antagonistic when $\sum FIC$ is >4 [26].

265

266 **AE4G0 activity against pre-formed *S. aureus* biofilm**

267 Antibiofilm activity of AE4G0 was determined as described previously[27]. Briefly, in 1% TSB, *S. aureus* ATCC 29213 was
268 cultured overnight at 37°C and 180 rpm. The overnight culture was diluted at 1:100 in TSB supplemented with 1%

269 glucose and 0.2 mL/well was transferred into 96-well polystyrene flat-bottom tissue culture plates. To increase biofilm
270 formation, low oxygen concentration was maintained and kept at 37°C for 48h. Post-incubation media were decanted,
271 and plates were rinsed gently three times with 1xPBS (pH=7.4) to remove the planktonic bacteria. Plates were refilled
272 with TSB with different drug concentrations and incubated for 24 h at 37°C. After drug treatment, the media was
273 decanted, washed three times with 1x PBS (pH 7.4), and biofilm was fixed by incubating the plate at 60°C for 1 h. After
274 fixing, the biofilm is stained with 0.06% crystal violet for 10 min, rinsed with PBS, and dried at room temperature. The
275 bound crystal violet was eluted with 30% acetic acid (0.2 mL) for biofilm quantification. Absorbance was taken on a
276 microtiter plate reader at 600 nm for biofilm quantification.

277

278 **Determination of post-antibiotic effect (PAE)**

279 Post antibiotic effect (PAE) of AE4G0 was determined as reported earlier[28]. Overnight culture of *S. aureus* ATCC 29213
280 was diluted in MHBII $\sim 10^5$ CFU/mL, exposed to 1x MIC and 10x MIC of AE4G0 and vancomycin and incubated at 37 °C
281 for 1 h. Following incubation, the culture was centrifuged and washed twice with pre-warmed MHBII to remove any
282 traces of antibiotics. Finally, cells were resuspended in drug-free MHBII and incubated further at 37°C. Samples were
283 taken every 1 h, serially diluted, and plated on TSA for enumeration of CFU. PAE was calculated as $PAE = T - C$, where T
284 is the difference in time required for 1 log₁₀ increase in CFU vs. CFU observed immediately after removing the drug and
285 C in a similarly treated drug-free control.

286

287 **Induction of resistance in *S. aureus* ATCC 29213 against AE4G0**

288 The emergence of resistant bacteria to AE4G0 was assessed, and the frequency of resistant mutants was evaluated by
289 serial exposure of the organism to an antimicrobial agent[29]. *S. aureus* ATCC 29213 was exposed to serial passage of
290 AE4G0 and Levofloxacin and monitored for changes in MIC for 35 days, following which antibiogram was determined.

291

292 **Scanning Electron Microscopy with AE4G0 against *S. aureus* ATCC 29213**

293 Scanning Electron Microscopy (SEM) was utilized to determine any compound-induced topological changes in *S. aureus*
294 ATCC 29213 following treatment with AE4G0. The mid-log phase grown bacteria (10^8 cfu/mL) were treated with 10x
295 MIC of AE4G0 for 1 h, whereas untreated control was treated with vehicle control DMSO and processed in the same
296 manner. After treatment, the cells were washed twice with phosphate buffer saline (PBS) pH 7.2. Subsequently, cells

297 were fixed in aldehyde fixative 2% glutaraldehyde and 4% paraformaldehyde in PBS pH 7.2 overnight. The cells were
298 adhered on poly-L-lysine coated glass coverslips and osmicated in 1% OsO₄. Then the cells were gradually dehydrated in
299 an ascending graded series of ethanol, critical point dried, and sputter coated with Au-Pd (80:20) using a Polaron E5000
300 sputter coater. Bacterial morphology was examined in an FEI Quanta 250 SEM using an SE detector at an accelerating
301 voltage of 30 kV.

302

303 **Fluorescence Microscopy with AE4G0 against *S. aureus* ATCC 29213**

304 *S. aureus* viability in the presence of AE4G0 was evaluated using the LIVE/DEAD *BacLight* bacterial viability kit
305 (Invitrogen, catalogue no. L7007). *S. aureus* ATCC 29213 cells were grown to an OD₆₀₀ 0.2 were treated with AE4G0 at
306 1x and 10x MIC for 1 h. After incubation, cells were washed three times and resuspended in saline. 3 µl of SYTO9 and PI
307 (mixed in equal volumes) provided in the kit were added to each sample with the final volume of 1 ml and incubated at
308 room temperature in the dark for 15 minutes. Fluorescent images of stained bacteria were obtained using a
309 Fluorescence microscope (EVOS FLc). The Emission/Excitation wavelength for Syto9 is 485/498, whereas for PI
310 Emission/Excitation wavelength was 535/617 nm.

311

312 **Murine skin infection model**

313 The *in vivo* potential of AE4G0 to eliminate *S. aureus* infection was determined by following the superficial skin
314 infection model and experiments were performed as described previously with slight modifications[30]. Briefly, male
315 swiss mice 4-6 weeks old, weighing ~22–24 g, were used throughout the study and were caged alone in an Individually
316 vented cage (IVC) to check cross-contamination and to maintain aseptic conditions throughout the experiment.
317 Ketamine and xylazine were prepared in distilled water 40mg/kg and 8mg/kg of body weight, respectively, and injected
318 intraperitoneally (IP) as a mixture of 100 µl in each mouse for anaesthesia. Then, we proceeded with the removal of fur
319 by applying depilatory cream; the area was cleaned with the help of sterile distilled water. Approximately, 2 cm² of skin
320 area was scratched till it became visibly damaged and was characterized by reddening and glistening without any
321 bleeding. For bacterial infection, a 10 µl droplet containing 10⁷ CFU/mL of *S. aureus* ATCC 29213 and/or gentamicin-
322 resistant MRSA NRS119 was applied to the reddened area of skin. To confirm the infection, post 4 h, untreated mice
323 were sacrificed. Various dilutions were plated on TSA plate at the exact time dosing of the treatment groups, i.e., 2%

324 fusidic acid (positive control), 1% AE4G0, and untreated (vehicle) was started, followed by a second dose post 16 hours
325 from the first dose and henceforth, drug regimen comprised of twice daily application (in the morning and the evening,
326 with an 8 h interval) for 4 days. The mice were treated with 25-30 mg of each-2% Fusidic acid (LEO Pharma, Ballerup,
327 Denmark), 1% AE4G0 and untreated (vehicle) regularly. All groups were sacrificed post 18 h of the last dose to avoid the
328 carryover effects of the treatment group. Around ~ 2 cm² of wounded skin post sacrifice were excised and homogenized
329 with 500 μ l phosphate-buffered saline (1x PBS) in 2ml MP tissue grinding Lysing Matrix F tubes using MP FastPrep[®]-24
330 set at 4.0 M/S for 30 s (3 cycles). The dilutions were plated post-homogenization on TSA plates, followed by overnight
331 incubation at 37°C to determine the bacterial CFU count in each treatment group. Each experiment was repeated three
332 times in duplicate and the mean and SEM of Log₁₀ CFU/gm were plotted.

333

334 **Ethics approval**

335 Animal experiments were performed on six-eight-week-old swiss mice procured from the National Laboratory Animal
336 Facility of CSIR-Central Drug Research Institute, Lucknow. The experimental protocols were reviewed and approved by
337 the Institutional Animal Ethics Committee of CSIR-Central Drug Research Institute, Lucknow (IAEC/2019/2/Renew-1/
338 Dated-22.06.2020). Animal experiments were performed per the guidelines provided by the Committee for Control and
339 Supervision of Experiments on Animals (CPCSEA, Govt. of India)

340

341 **Statistical analysis**

342 Statistical analysis was performed using GraphPad Prism 8.0.2 software (GraphPad Software, La Jolla, CA, USA).
343 Comparison between three or more groups was analyzed using a one-way analysis of variance with Dunnett's multiple
344 comparison test. P-values<0.0001 were considered to indicate significance.

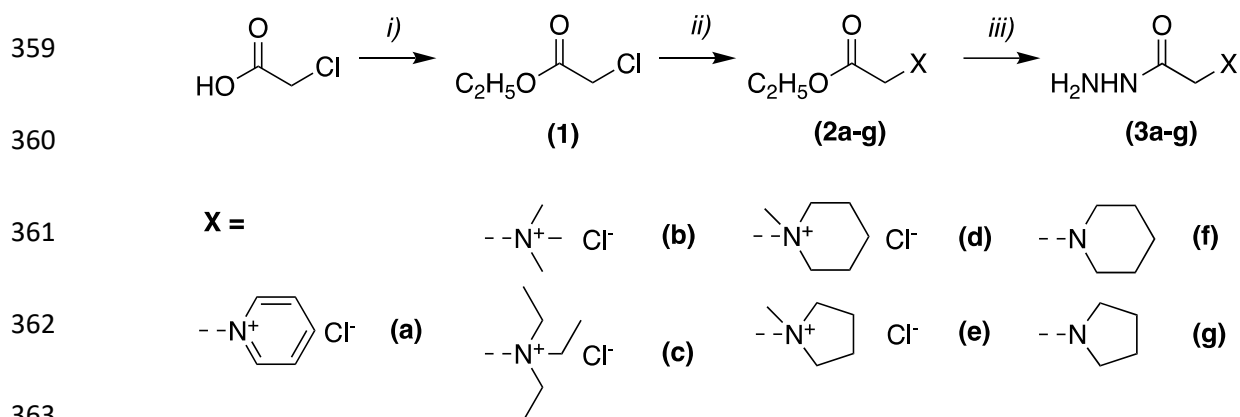
345

346 **Results and discussion**

347 **Synthesis of cationic dendrimer species**

348 Cationic acetohydrazides are considered useful tools for functionalizing surfaces to produce devices for biomedical use.
349 In particular, ammonium and pyridinium acetohydrazides (so-called Girard reagents T and P) have found wide use.
350 Remarkably, when grafted onto dendrimer scaffolds, acetohydrazides bring new modalities to the macromolecules. For
351 example, phosphorus dendrimers bearing Girard T, P moieties (as acetohydrazones) on the surface are able to form
352 single-component, physical hydrogels through the interdigitation of dendrimer branches stabilized by hydrogen

353 bonding between hydrazone fragments on the surface[17, 31-33]. Furthermore, acetohydrazone-functionalized
 354 dendrimers help to modulate the rate of drug release from macroscale biomaterials. In view of this, it is interesting to
 355 assess the biological activity of acetohydrazone-terminated phosphorus dendrimers. The series of cationic
 356 acetohydrazides (**3a-g**) differing by structure of a cation were synthesized in high yields, based on the methyl
 357 chloroacetate by sequential substitution of chlorine with different amines to form cationic esters (**2a-g**) followed by
 358 hydrazinolysis of an ester bond. (Scheme 1)



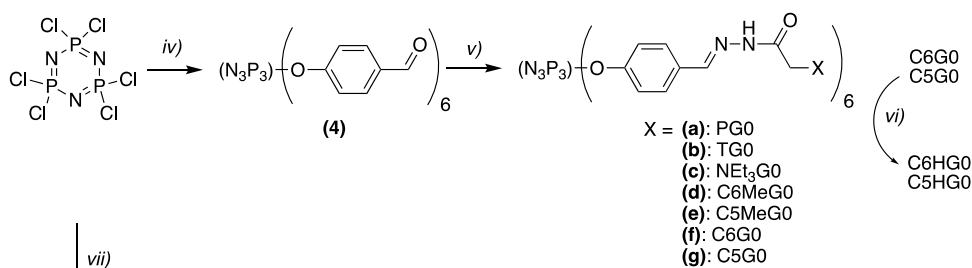
364 **Scheme 1:** Synthesis of cationic acetohydrazides **3a-g** Conditions: *i*) HCl, ethanol; *ii*) amine, CH₃CN, 90 °C; *iii*) N₂H₄,
 365 methanol, 90°C, 60-95%.

366

367 The dendritic scaffold used was either hexafunctional (hexa(hydroxybenzaldehyde) cyclotriphosphazene) or
 368 tetrafunctional one (*P,P*-dihydroxybiphenyl-*P',P'',P''*-tetra (hydroxybenzaldehyde) cyclotriphosphazene). The
 369 functionalization of dendrimer surface with cationic moieties was done by a simple reaction between acetohydrazides
 370 and aldehyde-terminated dendrimers upon acidic catalysis to form corresponding acetohydrazones (Scheme 2). The
 371 completeness of conversion was monitored by the disappearance of aldehyde signal in ¹H NMR spectra. The resulting
 372 products (see Fig 1) were obtained in high yields. In the case of dendrimer species bearing tertiary amines, the
 373 protonation with HCl was done at the last stage.

374

375



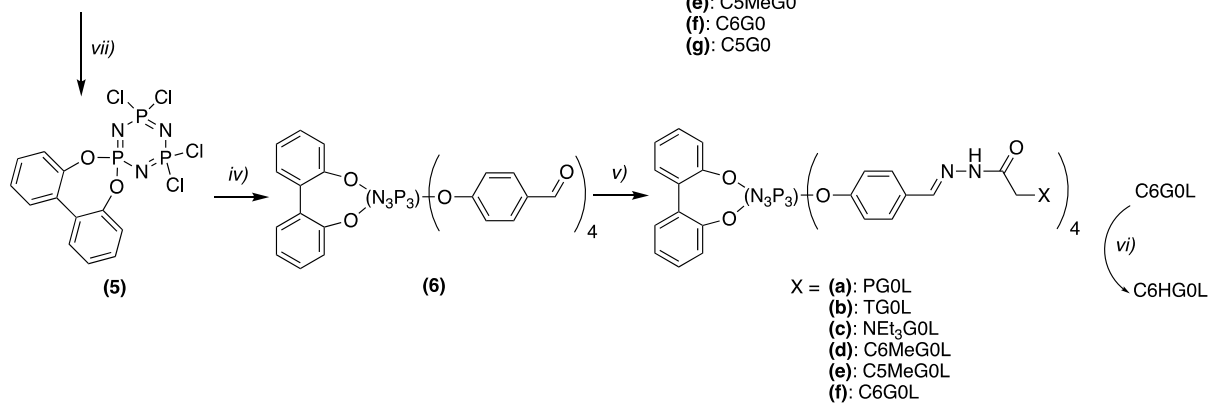
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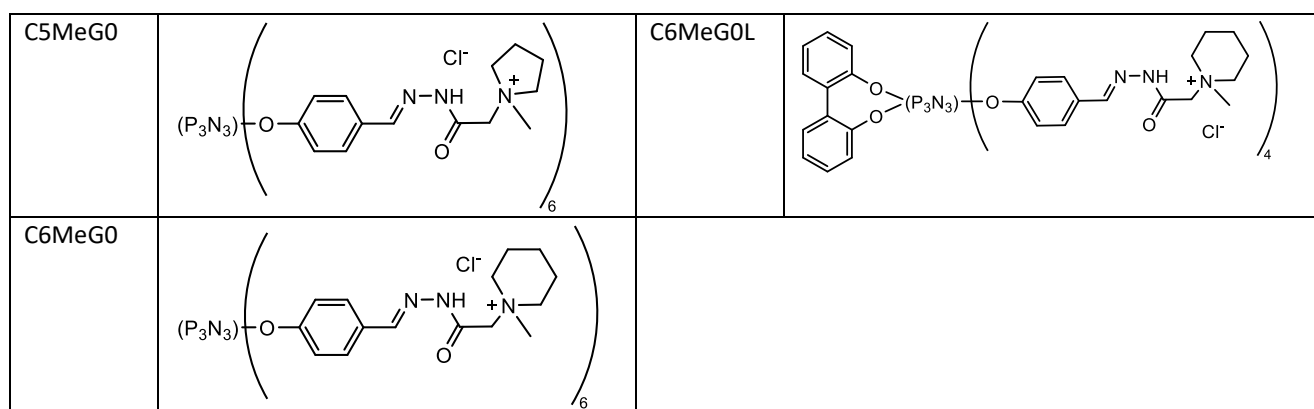
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380

381 **Scheme 2:** Synthesis of the cationic phosphorus dendrimers generation 0. Conditions: *iv*) 4-hydroxybenzaldehyde,382 CH₃CN, K₂CO₃, 92%; *v*) (3a-g), CHCl₃:methanol (1:1), acetic acid to pH~5, 85+%; *vi*) HCl/diethyl ether, THF, 0 °C; *vii*) 2,2'-383 dihydroxybiphenyl, CH₃CN, K₂CO₃.384 **Fig 1: Structures of various dendrimers synthesized and tested.**

Dendrimer	Structure	Dendrimer	Structure
AE4G0		PG0L	
PG0		TG0L	
NEt3G0		NEt3G0L	
C5HG0		C6HG0L	
C6HG0		C5MeG0L	



385

386

387 **AE4G0 is potently active against *S. aureus* ATCC 29213 and *Enterococcus* sp**

388 After synthesis and purification, the antimicrobial activity of the synthesized low-generation phosphorus dendrimers
 389 was determined against bacterial pathogen panel (S2 SI). As seen, except for piperidiny-terminated dendrimers, all
 390 other low-generation dendrimers were active against gram-positive *S. aureus* ATCC 29213 (MIC 4-32 mg/L). PG0L and
 391 C5HG0 were active against GNB but at higher MIC 16-64 mg/L (S2 SI). From all these, AE4G0 was selected to be
 392 screened further due to its low MIC against *S. aureus*, i.e., 4-8 mg/L coupled with ease of synthesis of AE4G0 and more
 393 eco friendly. On the other hand, AE4G0 is inactive against GNB including *E. coli*, *A. baumannii*, *K. pneumoniae* and *P.*
 394 *aeruginosa* (Table 1).

395 Furthermore, AE4G0's antibacterial property was tested against clinical drug-resistant strains of *S. aureus* and
 396 *Enterococcus* sp, including MRSA and VRSA, where it showed almost equipotent activity against all the tested strains,
 397 irrespective of their resistance mechanism, suggesting a new mechanism of action and lack of cross-resistance with
 398 existing drugs.

399

400 **PMBN does not influence the entry of AE4G0 into GNB pathogens**

401 Typically, the OM of GNB presents a very significant barrier to the entry of antibiotics, thus PMBN, a delipidated version
 402 of polymyxin B is utilized as reagent to study the entry of molecules under investigation into GNB. As seen in S3(SI),
 403 vancomycin and rifampicin MIC decreased by several folds in the presence of PMBN as expected whereas no change in
 404 MIC was observed for AE4G0, against *E. coli* ATCC 25922 and *A. baumannii* BAA 1605 (S3 SI), suggesting that intact OM
 405 of GNB has negligible impact on AE4G0 activity against GNB pathogens.

406

407 **AE4G0 is not cytotoxic against eukaryotic Vero cells**

408 When tested for cytotoxicity against Vero cells, AE4G0 demonstrated CC_{50} of >250 mg/L, which gives a selectivity index
409 of >31.25 (Table 2). This very good selectivity index of AE4G0 supported taking it further for pre-clinical development.

410

411 **AE4G0 exhibits concentration-dependent time-kill kinetics against *S. aureus* ATCC 29213**

412 Time kill kinetics are a very important parameter to determine the efficacy of a compound under investigation. In this
413 context, the bacterial time-kill kinetics of AE4G0 were assessed at 1x and 10x MIC along with vancomycin as control
414 against *S. aureus* ATCC 29213 and the results are plotted in Fig 2a. At 10x MIC in 24 h, AE4G0 reduced >7 \log_{10} CFU/mL
415 as compared to untreated *S. aureus* ATCC 29213, which is comparable to vancomycin. Thus, AE4G0 exhibits
416 concentration-dependent, bactericidal activity against *S. aureus* ATCC 29213 which is comparable to vancomycin.

417 **AE4G0 synergizes with gentamicin against *S. aureus* ATCC 29213 and gentamicin-resistant MRSA NRS119**

418 Drug-combinations are an **integral** part of treatment plans for many infectious diseases; thus the combination potential
419 of any new investigational molecule is determination with approved FDA drugs. Thus, AE4G0 was tested for synergy
420 with FDA-approved drugs such as daptomycin, ceftazidime, linezolid, gentamicin, levofloxacin, meropenem,
421 minocycline, rifampicin and vancomycin **using chequerboard method** as seen in Table 3. As can be seen, AE4G0 **partially**
422 synergized with gentamicin against *S. aureus* ATCC 29213[34], which was further confirmed using time-kill analysis
423 using both compounds at 1x MIC. As seen in Fig 2b, combination of AE4G0 and gentamicin demonstrate a reduction **of**
424 **>2 \log_{10} CFU/mL of bacterial load compared with initial inoculum at 24h, also in combination it reduces the bacterial**
425 **load >2 \log_{10} CFU/mL compared with the most potent drug alone i.e. AE4G0 alone, which is the criteria for synergy in**
426 **time -kill kinetics[35, 36]. Next, the effect of the combination was tested against gentamicin-resistant MRSA NRS119,**
427 **and AE4G0 partially synergises with gentamicin as it reduced the bacterial load >1 \log_{10} CFU/mL compared with initial**
428 **inoculum at 24h, in combination it reduces bacterial load ~1 \log_{10} CFU/mL compared with the most potent drug alone**
429 **i.e. AE4G0 , Interestingly in combination it reduces the bacterial load ~6.0 \log_{10} CFU/mL compared with untreated at**
430 24h, AE4G0 in combination with gentamicin significantly better than either drug alone (Fig 2c). Thus, taken together
431 **chequerboard method and time kill-kinetics assay**, AE4G0 combines spectacularly with gentamicin against *S. aureus*
432 ATCC 29213 and **partially synergizes against** gentamicin-resistant MRSA NRS119 and exhibits superior killing kinetics a
433 compared to individual drugs.

434 **AE4G0 annihilates pre-formed *S. aureus* biofilm**

435 Bacteria under stressful conditions make biofilms to negate the action of xenobiotic molecules, including antibiotics,
436 often leading to recurrent infections and consequently, therapeutic failure. As a consequence, many FDA-approved
437 antibiotics demonstrate limited activity against bacterial biofilms, so we investigated the activity of AE4G0 against pre-
438 formed *S. aureus* biofilms along with vancomycin and levofloxacin. As seen in Fig 3, AE4G0 eradicates biofilm
439 significantly better than levofloxacin and vancomycin at 1x MIC. Thus, AE4G0 exhibits potent activity against bacteria
440 under in different physiological conditions, which is an essential part of an antimicrobial, as stated earlier¹⁶.

441

442 **AE4G0 exhibits a prolonged Post antibiotic effect (PAE)**

443 PAE is an important pharmacokinetic parameter that influences the dosing regimen as well as the emergence of
444 resistance; thus, it is an important parameter to be determined in pre-clinical assessment. The PAE of AE4G0 was
445 determined along with vancomycin against *S. aureus* ATCC 29213. As seen in Table 4, AE4G0 exhibits a PAE of ~15.5 h,
446 which is much more than that of vancomycin, thus exhibiting an essential characteristic distinguishing it from
447 vancomycin. All combined, AE4G0 exhibits concentration-dependent bactericidal activity with long PAE.

448

449

450 **AE4G0 does not induce resistance in *S. aureus* ATCC 29213.**

451 Emergence of resistance is inevitable to any molecule in clinical use, thus the frequency of resistance of AE4G0 was
452 determined by serial exposure to *S. aureus* ATCC 29213 over 35 days along with levofloxacin and the MIC is plotted in
453 Fig 4. As seen, despite consistent exposure, AE4G0 fails to induce resistance in *S. aureus* ATCC 29213, whereas, under
454 the same conditions, *S. aureus* generates stable, high resistance to levofloxacin. **Antibiogram of *S.aureus* ATCC 29213
455 grown in presence of AE4G0 for 35 passages does not shows cross-resistance to approved antibiotics can be seen in S4
456 (SI).** Thus, AE4G0 exhibits concentration dependent bactericidal kinetics with long PAE and does not induce resistance
457 in *S. aureus*.

458

459

460 **AE4G0 causes cellular lysis of *S. aureus* ATCC 29213 as seen by Fluorescence and Scanning electron microscopy (SEM)**

461 To decipher the mechanism of action of AE4G0 on *S. aureus*, AE4G0 treated *S. aureus* were stained with LIVE/DEAD
462 BacLight bacterial viability kit (Invitrogen, catalog no. L7007) as per manufacturer's instructions and visualized by
463 Fluorescence microscopy using EVOS FLc microscope using 100x oil immersion objective. As visualized in Fig 5,

464 treatment with 10x MIC of AE4G0 for 1 h led to dead bacterial cells (stained red) compared to untreated viable cells
465 (stained green).

466 This cellular destruction was further probed by visualizing the samples treated with 10x MIC of AE4G0 for 1 h with
467 scanning electron microscopy (SEM) at 30,000x magnification, and the results are shown in Fig 5. As compared to
468 untreated cells, widespread cell destruction is visible in AE4G0-treated *S. aureus* cells, with lots of intracellular debris
469 oozing out from lysed cells. Together, these results clearly demonstrate the cellular disruption of *S. aureus* ATCC 29213
470 upon treatment with AE4G0.

471

472 **AE4G0 exhibits potent *in vivo* activity in murine skin model**

473 Since *in vitro* AE4G0 exhibits promising antimicrobial activity against clinical drug-resistant isolates of *S. aureus*, its *in*
474 *vivo* activity against *S. aureus* ATCC 29213 was determined in a murine skin infection model. AE4G0 demonstrated ~ 1.2
475 \log_{10} CFU/gm reduction in bacterial load compared to untreated, while fusidic acid reduced $\sim 2.5 \log_{10}$ CFU/gm
476 compared to untreated. Statistical analysis upholds the significance ($p < 0.0001$) of the AE4G0 efficacy in reducing
477 bacterial load in skin infection mode, as seen in Fig 6a. In addition, when the combination of AE4G0 and gentamicin was
478 tested against gentamicin-resistant MRSA NRS119, combination was more efficacious than either drug alone and
479 reduced $\sim 0.7 \log_{10}$ CFU/gm ($p < 0.01$) as compared to untreated, whereas AE4G0 reduced $\sim 0.55 \log_{10}$ CFU/gm alone
480 while gentamicin reduced $\sim 0.3 \log_{10}$ CFU/gm, thus further demonstrating the power of the combination against drug-
481 resistant *S. aureus* (Fig 6b). All combined, the potent *in vitro* activity of AE4G0 is exquisitely translated *in vivo* as well
482 alone and in combination with gentamicin.

483 All combined, AE4G0 is a novel, phosphorus dendrimer exhibiting direct equipotent activity against drug-resistant panel
484 of *S. aureus* (MIC 8 mg/L) and *Enterococcus spp* (32 mg/L) and exhibits concentration-dependent bacterial killing.
485 AE4G0 synergized with gentamicin, showing better bacterial load reduction than individual treatment groups against *S.*
486 *aureus* ATCC 29213 and gentamicin-resistant MRSA NRS119. As reported earlier, *S. aureus* can potentially become
487 resistant to all classes of antibiotics currently in therapeutic regime, either by mutation or by acquiring horizontally
488 transferred resistance factors[37]. AE4G0 fails to induce resistance in *S. aureus* ATCC 29213 whereas levofloxacin under
489 the same conditions, develops stable, high-level resistance. In addition, AE4G0 has a long PAE of ~ 15.5 h, an essential
490 asset for a new molecule as it reduces the dosages required for therapeutic clearance.

491 Biofilm formation is a natural phenomenon found in most bacteria; strong adhesion and drug resistance are one of the
492 main features of biofilms, which help bacteria to neutralize the host immune response and escape antibiotic killing[38].

493 We report AE4G0 has the potential to eradicate the *S. aureus* biofilm significantly, which is an essential aspect for a new
494 drug entity. When analysed by fluorescence microscopy, dead cells were more prominent as compared to untreated
495 after the treatment of *S. aureus* ATCC 29213 with 10x MIC of AE4G0 for 1h, and in the case of SEM, intracellular debris
496 coming out of the lysed cells visible when *S. aureus* ATCC 29213 treated with 10x MIC of AE4G0 for 1h. When tested in
497 the murine skin infection model, AE4G0 reduced bacterial load by $\sim 1.2 \log_{10}$ CFU/gm compared to untreated *S. aureus*
498 ATCC 29213. When AE4G0 was combined with gentamicin, the combination showed better reduction than the
499 individual treatments and reduced $\sim 0.7 \log_{10}$ CFU/gm bacterial load compared to untreated gentamicin-resistant *S.*
500 *aureus* NRS119. When used in combination, AE4G0 reduces the Gentamicin dose for treatment, thus further helping in
501 reduction of gentamicin dosages in treatment of topical infections.

502

503 **Conclusion**

504 **Discovery** and development of new antimicrobials is an urgent medical need calling for significant innovation.
505 Dendrimers represent an unconventional **strategy** since their most common **utilization** is as carrier or cargo of drugs
506 and or other **molecules** such as dendriplexes with siRNA, mRNA, etc. In marked contrast, *active per se* antimicrobial
507 properties of dendrimers are poorly documented. Although phosphorus dendrimers are very active against various
508 diseases, very few reported dendrimers **demonstrating** activity against *S. aureus*, but none were from generation 0[39,
509 40]. The problem associated with polycationic dendrimers is their toxicity to eukaryotic cells[41]; dendrimers with low
510 molecular mass are known for their **lower** toxicity than dendrimers with high molecular mass[42]. Thus, in this context,
511 this work reports the first example of direct antimicrobial activity *per se* of a small dendrimer of generation 0 against *S.*
512 *aureus* ATCC 29213 along with in vivo activity alone and in combination with gentamicin against gentamicin-resistant
513 MRSA NRS119. Considering its excellent antimicrobial properties, AE4G0 has the potential to be translated into a novel
514 therapeutic strategy targeting drug-resistant *S. aureus* topical infections.

515

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523 NR31884, NR31885, NR31886, NR31887, NR31888, NR31903, NR31909 and NR31912. This manuscript bears CSIR-CDRI
524 communication number

525 **Ethics approval:** The use of mice for infectious studies (IAEC/2019/2/Renew-1/Dated-22.06.2020) was approved by
526 Institutional Animal Ethics Committee at CSIR-CDRI, Lucknow.

527 **Competing financial interests:** The authors declare no competing financial interests.

528 **Transparency declarations:** None to declare

529 **Supporting information files:**

530 S1 synthesis of monomers, ¹H, ¹³C NMR spectra, HRMS of compound AE4G0

531 S2 MIC of dendrimers against gram-positive and gram-negative bacteria.

532 S3 PMBN assay for AE4G0.

533 S4 Antibiogram of *S. aureus* ATCC 29213 grown in presence of AE4G0 and Levofloxacin for 35th passage.

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536 **References:**

- 537 1. Kaul, G., et al., *Update on drug-repurposing: is it useful for tackling antimicrobial resistance?* 2019,
538 Future Medicine. p. 829-831.
- 539 2. Guo, Y., et al., *Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus*. *Frontiers*
540 *in cellular and infection microbiology*, 2020. **10**: p. 107.
- 541 3. World Health Organisation. *Antimicrobial resistance*. 2021 [cited 2023 February 04]; Available from:
542 www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
- 543 4. World Health Organisation. *WHO publishes list of bacteria for which new antibiotics are urgently*
544 *needed*. 2017 [cited 2023 February 5]; Available from: [www.who.int/news/item/27-02-2017-who-](http://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed)
545 [publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed](http://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed).

- 546 5. Worley, B.V., D.L. Slomberg, and M.H. Schoenfisch, *Nitric oxide-releasing quaternary ammonium-*
547 *modified poly (amidoamine) dendrimers as dual action antibacterial agents*. *Bioconjugate chemistry*,
548 2014. **25**(5): p. 918-927.
- 549 6. Meyers, S.R., et al., *Anionic amphiphilic dendrimers as antibacterial agents*. *Journal of the American*
550 *Chemical Society*, 2008. **130**(44): p. 14444-14445.
- 551 7. Galanakou, C., D. Dhumal, and L. Peng, *Amphiphilic dendrimers against antibiotic resistance: light at*
552 *the end of the tunnel?* *Biomaterials Science*, 2023.
- 553 8. Mignani, S., et al., *Multivalent Copper (II)-Conjugated Phosphorus Dendrimers with Noteworthy In*
554 *Vitro and In Vivo Antitumor Activities: A Concise Overview*. *Molecular Pharmaceutics*, 2021. **18**(1): p.
555 65-73.
- 556 9. Mignani, S., et al., *In vivo therapeutic applications of phosphorus dendrimers: State of the art*. *Drug*
557 *Discovery Today*, 2021. **26**(3): p. 677-689.
- 558 10. Chen, L., et al., *Engineered Stable Bioactive Per Se Amphiphilic Phosphorus Dendron Nanomicelles as*
559 *a Highly Efficient Drug Delivery System To Take Down Breast Cancer In Vivo*. *Biomacromolecules*,
560 2022. **23**(7): p. 2827-2837.
- 561 11. Mignani, S., et al., *Safe Polycationic Dendrimers as Potent Oral In Vivo Inhibitors of Mycobacterium*
562 *tuberculosis: A New Therapy to Take Down Tuberculosis*. *Biomacromolecules*, 2021. **22**(6): p. 2659-
563 2675.
- 564 12. Blanzat, M., et al., *Dendritic catanionic assemblies: in vitro anti-HIV activity of phosphorus-containing*
565 *dendrimers bearing Gal β 1cer analogues*. *ChemBioChem*, 2005. **6**(12): p. 2207-2213.
- 566 13. Blattes, E., et al., *Mannodendrimers prevent acute lung inflammation by inhibiting neutrophil*
567 *recruitment*. *Proceedings of the National Academy of Sciences*, 2013. **110**(22): p. 8795-8800.
- 568 14. Fruchon, S., et al., *Anti-inflammatory and immunosuppressive activation of human monocytes by a*
569 *bioactive dendrimer*. *Journal of Leucocyte Biology*, 2009. **85**(3): p. 553-562.
- 570 15. Hayder, M., et al., *A phosphorus-based dendrimer targets inflammation and osteoclastogenesis in*
571 *experimental arthritis*. *Science translational medicine*, 2011. **3**(81): p. 81ra35-81ra35.

- 572 16. Posadas, I., et al., *Neutral high-generation phosphorus dendrimers inhibit macrophage-mediated*
573 *inflammatory response in vitro and in vivo*. Proceedings of the National Academy of Sciences, 2017.
574 **114**(37): p. E7660-E7669.
- 575 17. Klajnert, B., et al., *EPR study of the interactions between dendrimers and peptides involved in*
576 *Alzheimer's and prion diseases*. Macromolecular bioscience, 2007. **7**(8): p. 1065-1074.
- 577 18. Apartsin, E., et al., *Dendriplex-Impregnated Hydrogels with Programmed Release Rate*. Frontiers in
578 Chemistry, 2022. **9**: p. 1195.
- 579 19. Wrońska, N., et al., *Synergistic effects of anionic/cationic dendrimers and levofloxacin on*
580 *antibacterial activities*. Molecules, 2019. **24**(16): p. 2894.
- 581 20. Wayne, P., *Clinical and Laboratory Standards Institute: Performance standards for antimicrobial*
582 *susceptibility testing: 20th informational supplement*. CLSI document M100-S21, 2012.
- 583 21. Ferrer-Espada, R., et al., *A permeability-increasing drug synergizes with bacterial efflux pump*
584 *inhibitors and restores susceptibility to antibiotics in multi-drug resistant Pseudomonas aeruginosa*
585 *strains*. Scientific reports, 2019. **9**(1): p. 3452.
- 586 22. Vaara, M., *Polymyxin derivatives that sensitize Gram-negative bacteria to other antibiotics*.
587 Molecules, 2019. **24**(2): p. 249.
- 588 23. Twentyman, P.R. and M. Luscombe, *A study of some variables in a tetrazolium dye (MTT) based*
589 *assay for cell growth and chemosensitivity*. British journal of cancer, 1987. **56**(3): p. 279-285.
- 590 24. Thakare, R., et al., *Repurposing disulfiram for treatment of Staphylococcus aureus infections*.
591 International journal of antimicrobial agents, 2019. **53**(6): p. 709-715.
- 592 25. Thakare, R., et al., *Repurposing Ivacaftor for treatment of Staphylococcus aureus infections*.
593 International journal of antimicrobial agents, 2017. **50**(3): p. 389-392.
- 594 26. Odds, F.C., *Synergy, antagonism, and what the chequerboard puts between them*. Journal of
595 Antimicrobial Chemotherapy, 2003. **52**(1): p. 1-1.

- 596 27. Kwasny, S.M. and T.J. Opperman, *Static biofilm cultures of Gram-positive pathogens grown in a*
597 *microtiter format used for anti-biofilm drug discovery*. Current protocols in pharmacology, 2010.
598 **50**(1): p. 13A. 8.1-13A. 8.23.
- 599 28. Suller, M. and D. Lloyd, *The antibacterial activity of vancomycin towards Staphylococcus aureus*
600 *under aerobic and anaerobic conditions*. Journal of applied microbiology, 2002. **92**(5): p. 866-872.
- 601 29. Tambe, S., L. Sampath, and S. Modak, *In vitro evaluation of the risk of developing bacterial resistance*
602 *to antiseptics and antibiotics used in medical devices*. Journal of Antimicrobial Chemotherapy, 2001.
603 **47**(5): p. 589-598.
- 604 30. Kugelberg, E., et al., *Establishment of a superficial skin infection model in mice by using*
605 *Staphylococcus aureus and Streptococcus pyogenes*. Antimicrobial agents and chemotherapy, 2005.
606 **49**(8): p. 3435-3441.
- 607 31. Apartsin, E.K., et al., *Hydrogels of Polycationic Acetohydrazone-Modified Phosphorus Dendrimers for*
608 *Biomedical Applications: Gelation Studies and Nucleic Acid Loading*. Pharmaceutics, 2018. **10**(3): p.
609 120.
- 610 32. El Ghzaoui, A., et al., *Self-assembly of water-soluble dendrimers into thermoreversible hydrogels and*
611 *macroscopic fibers*. Langmuir, 2004. **20**(21): p. 9348-9353.
- 612 33. Majoral, J., et al., *Heterocycles contenant du phosphore? XXIX*. Tetrahedron, 1976. **32**(21): p. 2633-
613 2644.
- 614 34. Flamm, R.K., et al., *Evaluation of the bactericidal activity of fosfomycin in combination with selected*
615 *antimicrobial comparison agents tested against Gram-negative bacterial strains by using time-kill*
616 *curves*. Antimicrobial agents and chemotherapy, 2019. **63**(5): p. e02549-18.
- 617 35. Belley, A., et al., *Assessment by time-kill methodology of the synergistic effects of oritavancin in*
618 *combination with other antimicrobial agents against Staphylococcus aureus*. Antimicrobial agents
619 *and chemotherapy*, 2008. **52**(10): p. 3820-3822.

- 620 36. Petersen, P.J., et al., *In vitro antibacterial activities of tigecycline in combination with other*
621 *antimicrobial agents determined by chequerboard and time-kill kinetic analysis. Journal of*
622 *Antimicrobial Chemotherapy*, 2006. **57**(3): p. 573-576.
- 623 37. Vestergaard, M., D. Frees, and H. Ingmer, *Antibiotic resistance and the MRSA problem. Microbiology*
624 *spectrum*, 2019. **7**(2): p. 7.2. 18.
- 625 38. Kanwar, I., A. K Sah, and P. K Suresh, *Biofilm-mediated antibiotic-resistant oral bacterial infections:*
626 *mechanism and combat strategies. Current pharmaceutical design*, 2017. **23**(14): p. 2084-2095.
- 627 39. Altaher, Y. and M. Kandeel, *Structure-Activity Relationship of Anionic and Cationic Polyamidoamine*
628 *(PAMAM) Dendrimers against Staphylococcus aureus. Journal of Nanomaterials*, 2022. **2022**.
- 629 40. Lopez, A.I., et al., *Antibacterial activity and cytotoxicity of PEGylated poly (amidoamine) dendrimers.*
630 *Molecular BioSystems*, 2009. **5**(10): p. 1148-1156.
- 631 41. Padié, C., et al., *Polycationic phosphorus dendrimers: synthesis, characterization, study of*
632 *cytotoxicity, complexation of DNA, and transfection experiments. New Journal of Chemistry*, 2009.
633 **33**(2): p. 318-326.
- 634 42. Klajnert, B., et al., *Biological properties of low molecular mass peptide dendrimers. International*
635 *journal of pharmaceutics*, 2006. **309**(1-2): p. 208-217.

636

637 **Table 1:** MIC (mg/L) of AE4G0 against ESKAPE Pathogens

Strains			Antibiotics resistant to	Molecular details of strains	MIC of AE4G0 (mg/L)	
Gram-positive bacteria	MSSA	<i>S. aureus</i>	ATCC 29213	None	Type strain	8
	MRSA		NRS100	Methicillin, Tetracycline	Contains subtype I mec cassette & large variety of virulence factors	8
			NRS119	Methicillin, Gentamicin, Linezolid, Trimethoprim / sulfamethaxazole	Contains subtype IV mec cassette & G2576T mutation in domain V in one or more 23S rRNA genes	8

		NRS129	Chloramphenicol	<i>mecA</i> negative	8	
		NRS186	Methicillin, Levofloxacin, Meropenem	USA 300 type CA-MRSA, PVL virulence factor positive & contains <i>mec</i> type IV cassette	8	
		NRS191	Methicillin, Levofloxacin, Meropenem	USA 600 type CA-MRSA, PVL virulence factor negative & contains <i>mec</i> type II cassette	8	
		NRS192	Methicillin, Levofloxacin, Meropenem, Erythromycin	CA-MRSA, PVL virulence factor negative & contains <i>mec</i> type II cassette	8	
		NRS193	Methicillin, Levofloxacin, Meropenem	CA-MRSA, PVL factor negative & contains <i>mec</i> type II cassette	8	
		NRS194	Methicillin, Meropenem	CA-MRSA, PVL virulence factor positive & contains <i>mec</i> type V cassette	8	
		NRS198	Methicillin, Levofloxacin, Meropenem	USA 100 type CA-MRSA, PVL virulence factor negative & contains <i>mec</i> type II cassette	16	
	VRSA	VRS 1	Methicillin, Levofloxacin, Meropenem, Vancomycin, Gentamycin, Teicoplanin & Spectinomycin	USA 100, contains <i>mec</i> subtype II cassette & <i>vanA</i> , Negative for <i>vanB</i> , <i>vanC1</i> , <i>vanC2</i> , <i>vanD</i> , <i>vanE</i> , PVL & ACME	8	
		VRS 4	Methicillin, Levofloxacin, Meropenem, Vancomycin, Gentamycin, Teicoplanin & Spectinomycin	USA 100, contains <i>mec</i> subtype II cassette & <i>vanA</i> , Negative for <i>vanB</i> , <i>vanC1</i> , <i>vanC2</i> , <i>vanD</i> , <i>vanE</i> , PVL & ACME	8	
		VRS 12	Methicillin, Levofloxacin, Meropenem, Vancomycin, Gentamycin, Teicoplanin & Spectinomycin	Data not available	8	
	<i>Enterococcus</i>	NR31884	Methicillin, Gentamicin	Hemolytic, cytolytic isolate	32	
		NR31885	Methicillin, Gentamicin	Cytolytic isolate	32	
		NR31886	Methicillin, Gentamicin	Hemolytic isolate	32	
		NR31887	Methicillin, Gentamicin, Minocycline	None	32	
		NR31888	Methicillin, Gentamicin	Cytolytic isolate	32	
	Gram-negative bacteria	<i>Escherichia coli</i> ATCC 25922		None	Type strain	>64
		<i>Klebsiella pneumoniae</i> BAA-1705		Carbapenem-resistant (Imipenem and Ertapenem)	Type strain	>64
		<i>Acinetobacter baumannii</i> BAA-1605		Ceftazidime, Gentamicin, Ticarcillin, Piperacillin, Aztreonam, Cefepime, Ciprofloxacin, Imipenem and Meropenem	Type strain	>64
		<i>Pseudomonas aeruginosa</i> ATCC 25923		None	Type strain	>64
<i>Enterobacter cloacae</i> complex Strain NR 50394		Azithromycin, Clarithromycin, Phosphomycin and Minocycline	Isolated as nosocomial pathogens	>64		
<i>Enterobacter cloacae</i>		Azithromycin, Ciprofloxacin,	Isolated as nosocomial	>64		

	complex Strain NR 50399	Clarithromycin, Carbenicillin, Levofloxacin, Moxifloxacin, Phosphomycin & Trimethoprim	pathogens	638 639 640 641
	<i>Enterobacter cloacae</i> complex Strain NR 50401	Ceftriaxone, Ciprofloxacin, Levofloxacin, Phosphomycin, Moxifloxacin, Trimethoprim, Cefoprazone & Aztreonam	Isolated as nosocomial pathogens	642 643 >644 645 646

647 MSSA: Methicillin Susceptible *Staphylococcus aureus*, MRSA: Methicillin Resistance *Staphylococcus aureus*, VRSA: Vancomycin Resistance
648 *Staphylococcus aureus*, PVL: Panton-Valentine leucocidin virulence factor, ACME: arginine catabolic mobile element, CA: Community-acquired
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650 Table 2: Cytotoxicity of **Synthesized dendrimers** against Vero cells (ATCC CCL-81).
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Compound	MIC (mg/L)	CC ₅₀ (mg/L)	Selectivity Index (CC ₅₀ /MIC)
AE4G0	8	>250	>31.25
TG0L	4	>200	>50
Net3G0L	4	>200	>50
C5MeG0L	4	>200	>50
PG0L	8	>200	>25
C6MeG0L	4	>200	>50
C5MeG0	16	>200	>12.5
C6MeG0	16	>200	>12.5
C5HG0	32	100	3.125
NEt3G0	8	>200	>25

666 Table 3: Drug interaction of AE4G0 with FDA-approved drugs against *Staphylococcus aureus* ATCC 29213

Drug	MIC of Drug alone (mg/L)	MIC of drug in presence of AE4G0 (mg/L)	MIC of AE4G0 alone (mg/L)	MIC of AE4G0 in presence of drug (mg/L)	FIC Index	Indication
Ceftazidime	8	8	8	8	2	No interaction
Daptomycin	1	2	8	8	3	No interaction
Gentamicin	0.25	0.125	8	2	0.75	No interaction
Linezolid	2	2	8	8	2	No interaction
Levofloxacin	0.25	0.25	8	8	2	No interaction
Meropenem	0.125	0.125	8	8	2	No interaction
Minocycline	0.125	0.125	8	8	2	No interaction

Rifampicin	0.0078	0.0078	8	8	2	No interaction
Vancomycin	1	1	8	8	2	No interaction

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668 Table 4: Post Antibiotic Effect studies for AE4G0

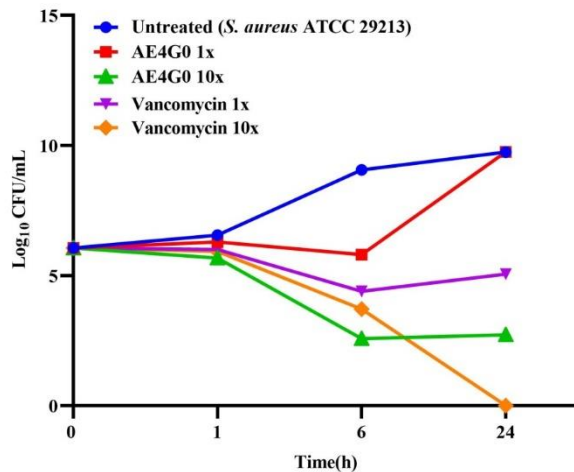
Treatments	Time for 1 log ₁₀ (h)	PAE (h)
Untreated <i>S. aureus</i> ATCC 29213	~2	0
AE4G0 1x	~2	~0
AE4G0 10x	~17.5	~15.5
Vancomycin 1x	~3	~1
Vancomycin 10x	~4	~2

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670 Fig 2: (a) Time Kill kinetics of AE4G0 against *S. aureus* ATCC 29213 along with comparators (b) Time Kill kinetics of
671 AE4G0 + Gentamicin against *S. aureus* ATCC 29213 along with comparators (c) Time Kill kinetics of AE4G0 and
672 Gentamicin against gentamicin resistant *S. aureus* NRS119

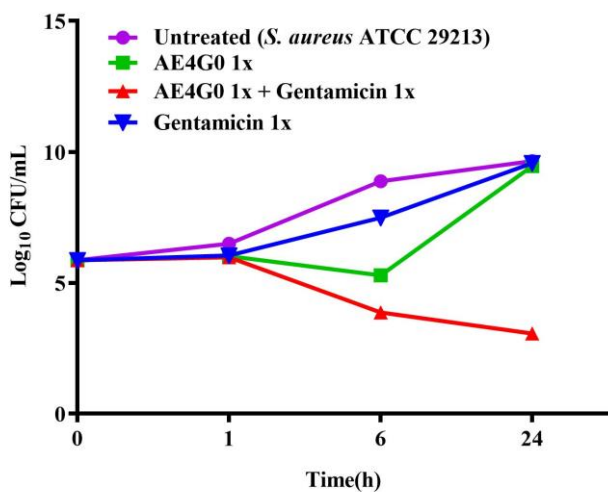
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674 (a)



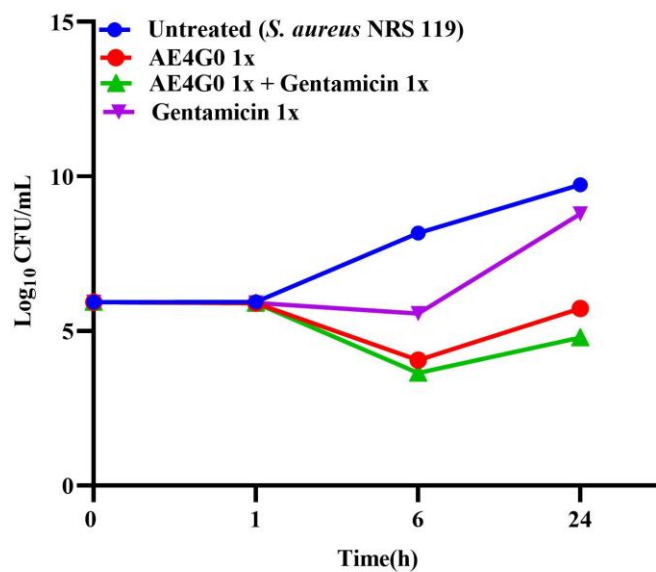
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682 (b)



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684 (c)



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687 Fig 3: Activity of AE4G0, vancomycin and levofloxacin against pre-formed *S. aureus* ATCC 29213 biofilm.

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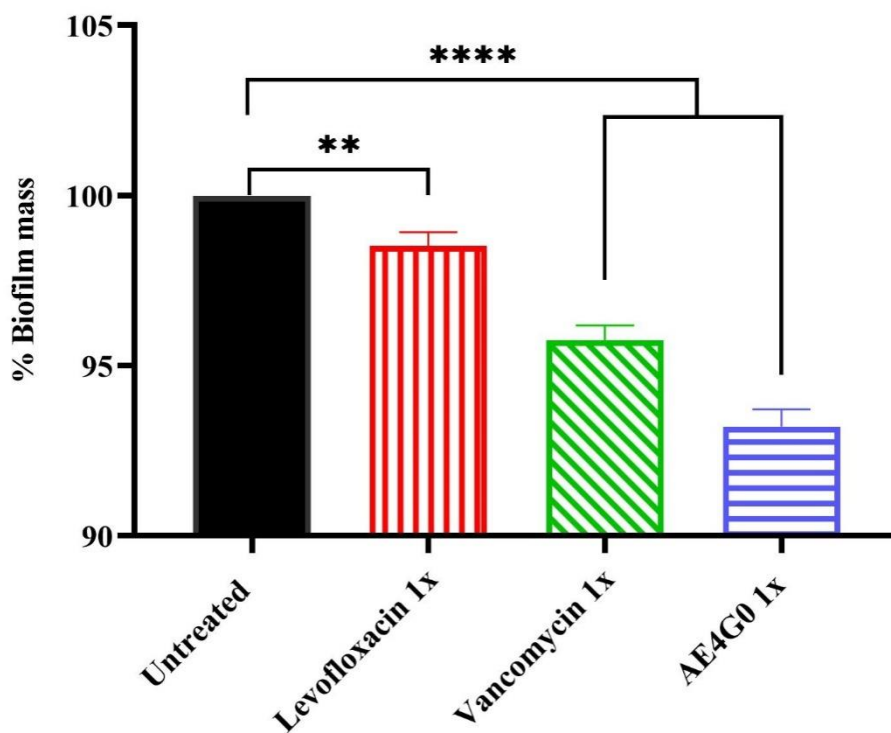
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700 Fig 4: Induction of resistance against AE4G0 and levofloxacin in *S. aureus* ATCC 29213 at sub-inhibitory concentrations.

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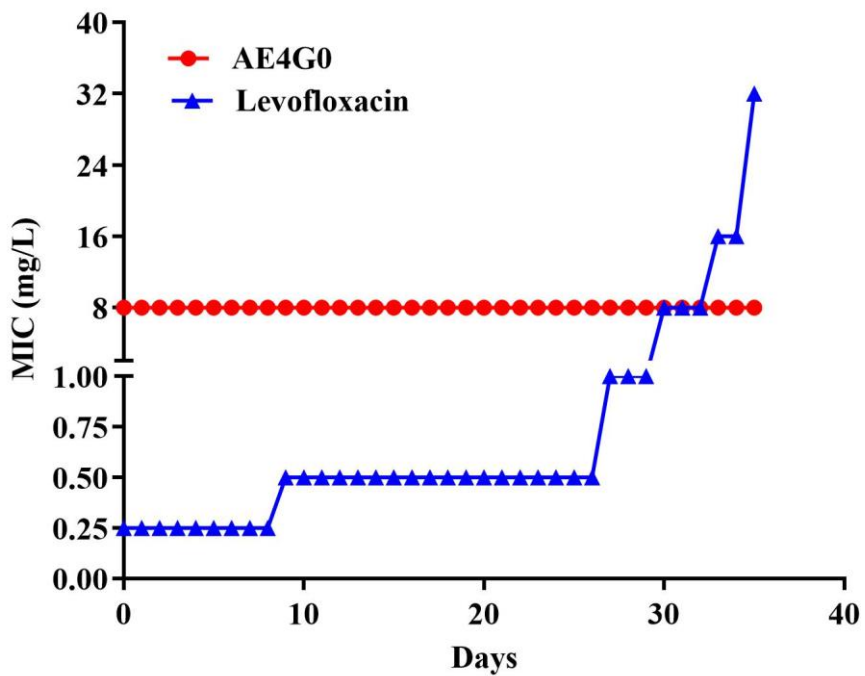
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727 Fig 5: Confocal and SEM analysis of *S. aureus* ATCC 29213 treated with AE4G0 at 10x MIC for 1h. The cells were exposed
728 to AE4G0 and treated with Bacterial viability stain and prepared for SEM. Viable bacterial cells were stained green by
729 SYTO9 whereas dead cells were stained red by PI.

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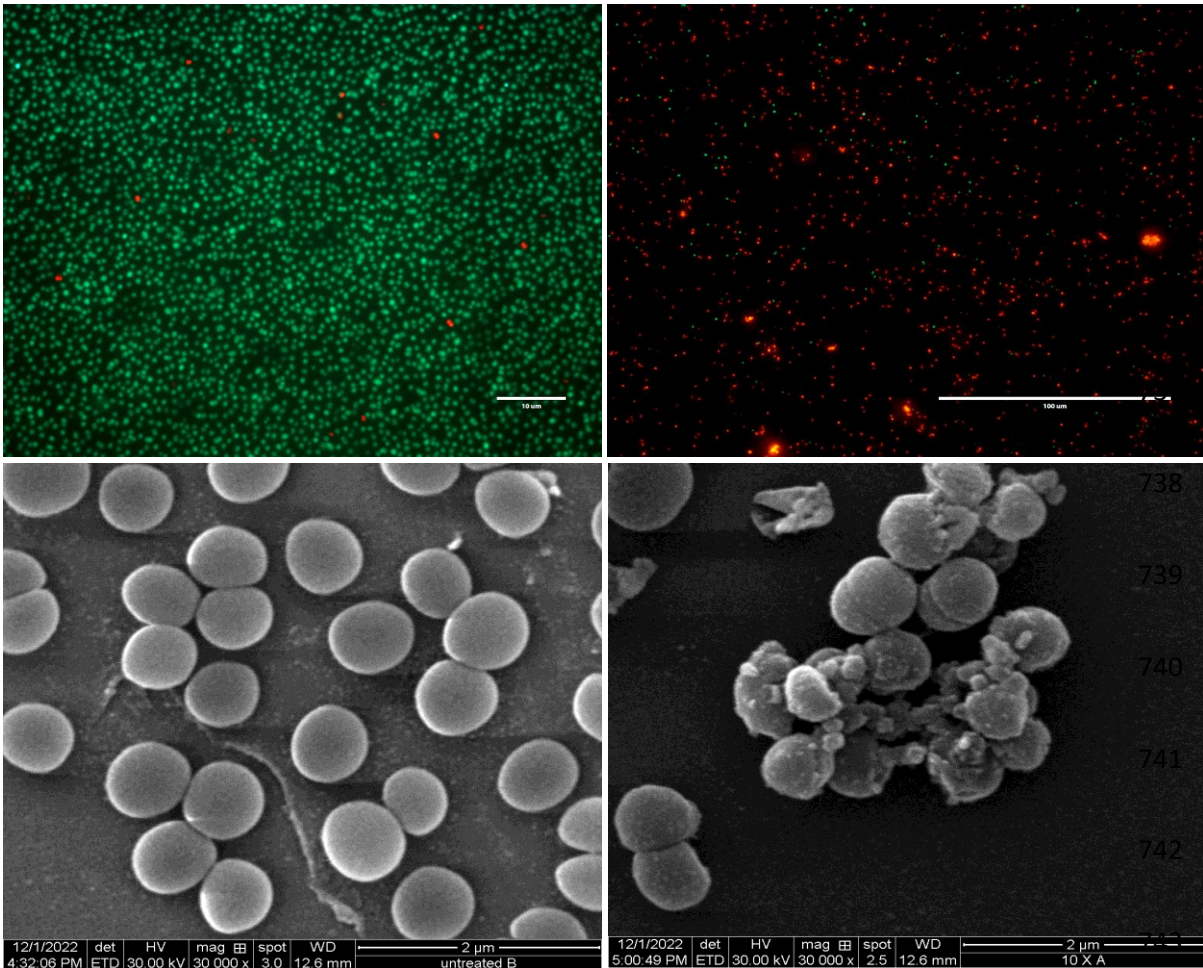
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Untreated *S. aureus*

S. aureus treated with

10x MIC of AE4G0 for 1h



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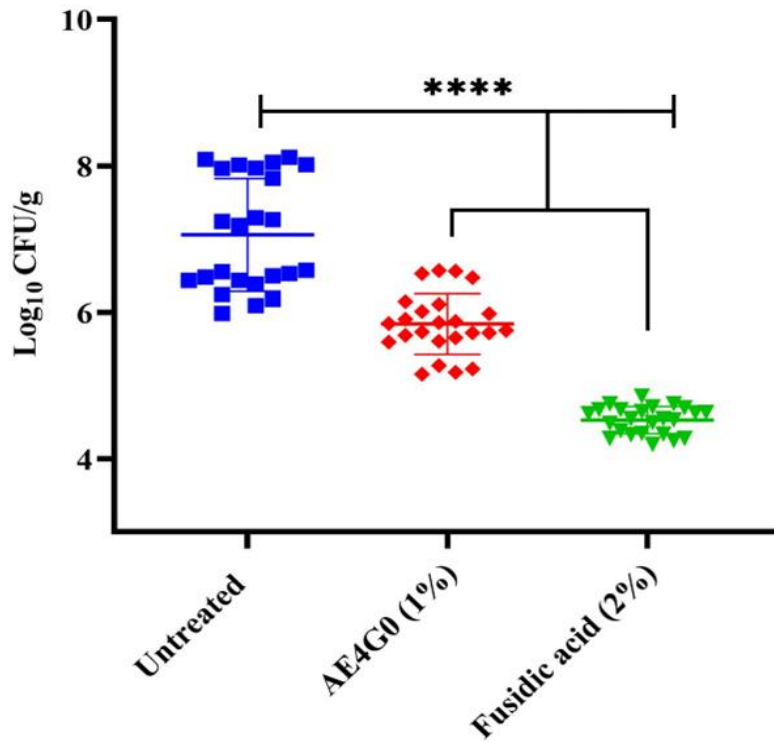
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750 Fig 6: (a) *In vivo* efficacy of AE4G0 against *S. aureus* ATCC 29213 in a murine skin infection model. (b) *In vivo* efficacy of
751 AE4G0 against gentamicin-resistant *S. aureus* ATCC NRS119 in a murine skin infection model.

752 (a)



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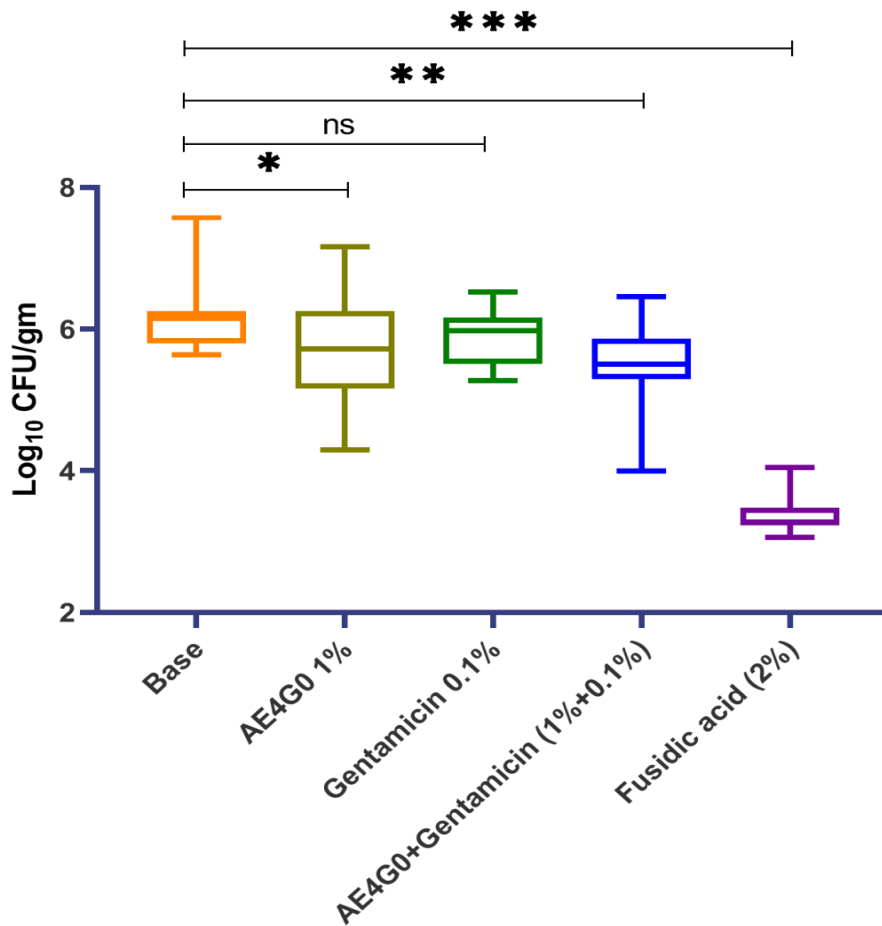
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769 (b)



779

780

781

782

783

784