

1 ***In vitro* activity of the arylomycin natural product antibiotics against *Staphylococcus***
2 ***epidermidis* and other coagulase-negative staphylococci**

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9 Short Title: Anti-CoNS activity of the arylomycins

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15

16 **Abstract**

17

18 The arylomycins are a class of natural product antibiotics that act via the inhibition of type I
19 signal peptidase (SPase), and we have found in diverse bacteria that their activity is limited by
20 the presence of a resistance-conferring Pro residue in SPase that reduces inhibitor binding. We
21 have also demonstrated that *Staphylococcus epidermidis*, which lacks this Pro residue, is
22 extremely susceptible to the arylomycins. Here, to further explore the potential utility of the
23 arylomycins, we report an analysis of the activity of a synthetic arylomycin derivative, arylomycin
24 C₁₆, against clinical isolates of *S. epidermidis* and other coagulase-negative staphylococci
25 (CoNS) from distinct geographical locations. Against many important species of CoNS,
26 including *S. epidermidis*, *S. haemolyticus*, *S. lugdunensis*, and *S. hominis*, we find that
27 arylomycin C₁₆ exhibits activity equal to or greater than that of vancomycin, the antibiotic most
28 commonly used to treat CoNS infections. While the susceptibility was generally correlated with
29 the absence of the previously identified Pro residue, several cases were identified where
30 additional factors appear to also contribute.

31 Introduction

32

33 The coagulase-negative staphylococci (CoNS) are a heterogeneous group of at least 15
34 different species of Gram-positive bacteria that have emerged in recent decades as important
35 nosocomial pathogens (10, 31). A particularly problematic species is *Staphylococcus*
36 *epidermidis*, which is responsible for a growing number of infections among hospital patients
37 with compromised immune systems and is especially notorious for forming biofilms that adhere
38 to surgical equipment and other hospital surfaces and indwelling devices (3, 18). Methicillin was
39 traditionally the first line antibiotic against CoNS, but its widespread use has resulted in
40 resistance in 50% to 80% of CoNS infections and 75 to 90% of nosocomial *S. epidermidis*
41 infections (18). As a result, vancomycin is now the first line agent for treating CoNS infections;
42 however isolates with reduced susceptibility to vancomycin have also been observed (11, 26),
43 and the emergence of enterococci harboring mobile elements that confer vancomycin resistance
44 has raised concerns that resistance might be transferred to *S. epidermidis* and/or other CoNS
45 (17, 28). These concerns continue to motivate the search for new antibiotics that are active
46 against CoNS, and especially against *S. epidermidis*.

47 The arylomycins (Fig. 1) are a novel class of natural product antibiotics that act by
48 inhibiting bacterial type I signal peptidase (SPase) (19, 25). SPase is a Ser-Lys dyad protease
49 that removes N-terminal signal sequences from pre-proteins following their translocation across
50 the cytoplasmic membrane (5, 20). SPase is an attractive target for antibiotic therapy because it
51 is conserved, essential, and located in the relatively accessible outer leaflet of the cytoplasmic
52 membrane. Furthermore, because bacterial SPase acts via a catalytic mechanism that is
53 distinct from its eukaryotic homologues, the arylomycins are unlikely to exhibit mechanistic
54 toxicity in humans (5, 20).

55 Despite the apparent accessibility, essentiality, and conservation of SPase, initial reports
56 suggested that the arylomycins were active against only a few Gram-positive bacteria, including
57 *Streptococcus pneumoniae*, *Rhodococcus opacus*, and *Brevibacillus brevis* (15, 25), and not
58 against other important Gram-positive pathogens or against any Gram-negative bacteria.
59 However, after reporting the first synthesis of an arylomycin, arylomycin A₂, as well as the
60 synthetic derivative arylomycin C₁₆ (Fig. 1), we found that each potently inhibits the growth of *S.*
61 *epidermidis* (24), and that *S. epidermidis* evolves resistance to the arylomycins by mutating
62 residue 29 of one of its two SPases, SpsIB, from Ser (Ser²⁹) to Pro (Pro²⁹) (29). Moreover, a
63 Pro residue is naturally present at the analogous position in the homologous SPases of the
64 pathogens *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and we
65 showed that it imparts resistance by reducing the affinity with which the arylomycins bind.
66 Furthermore, we found that a remarkably diverse range of both Gram-positive and Gram-
67 negative bacteria whose SPases lack a Pro at the analogous position are susceptible to the
68 arylomycins, including *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Helicobacter*
69 *pylori*, and *Chlamydia trachomatis*, and some strains of *Francisella tularensis* (29). In total, the
70 results suggest that the identified SPase polymorphism is a major contributor to naturally
71 occurring arylomycin resistance. However, we also showed that *Yersinia pestis* as well as some
72 strains of *S. aureus* are susceptible to the arylomycins despite the presence of an analogous
73 Pro, while others, such as many of the Lactobacillales, Clostridia and Bacteroidetes, are
74 resistant, despite its absence, implying that in some cases susceptibility must depend on
75 additional factors such as variable levels of toxicity associated with the inhibition of protein
76 secretion.

77 The potent activity of the arylomycins against a strain of *S. epidermidis* (RP62A) suggests
78 that they might be useful in the treatment of this and perhaps other CoNS. Here, to examine the
79 spectrum of activity of the arylomycins against clinical isolates of *S. epidermidis* and other

80 CoNS, we report the activity of arylomycin C₁₆ against two panels of isolates from hospitals in
81 geographically diverse locations and compare the activity to that of vancomycin. The results
82 reveal that the arylomycins have potent antibacterial activity against a range of important CoNS
83 species whose SpsIB orthologs lack the previously identified resistance-conferring Pro, while
84 less activity is observed against species where Pro is present. While we generally observed
85 similar susceptibilities for different isolates within a species, significant differences were
86 observed in several cases, with one atypical susceptibility resulting from the presence of a Ser
87 in place of the resistance-conferring Pro. Significant differences in susceptibility between
88 isolates of the same species are usually observed with clinically deployed antibiotics where
89 selection for resistance has occurred during therapy (2, 9, 14, 21), and therefore these results
90 may be relevant to understanding the natural evolution of arylomycin resistance in nature.

91

92 **Materials and Methods**

93

94 A total of 282 non-duplicate, speciated clinical isolates of CoNS were obtained from the London
95 Health Services Centre, London, Ontario, Canada (13). Of these, 143 isolates were *S.*
96 *epidermidis*, while the remaining 139 consisted of 12 groups comprised of varying numbers of
97 isolates of *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus cohnii*,
98 *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Staphylococcus saprophyticus*,
99 *Staphylococcus simulans*, *Staphylococcus warneri*, and *Staphylococcus hominis*. This panel of
100 CoNS is a subset of a larger panel whose susceptibility to quinupristin/dalfopristin, linezolid,
101 telithromycin, and vancomycin has been reported (13). A second set of non-duplicate, non-
102 speciated clinical isolates of CoNS was obtained from the Department of Microbiology,
103 University of California San Diego Medical Center Hillcrest, San Diego, CA. These strains were

104 collected from patients with various coagulase negative infections between April and June of
105 2008.

106 Arylomycin C₁₆ was synthesized as described previously (24). MICs of arylomycin C₁₆
107 were determined for isolates from both sets of CoNS using a modified CLSI agar dilution
108 method. Bacterial inocula were prepared from log phase cultures or from suspensions of
109 colonies grown on solid media, diluted appropriately, and spotted onto tryptic soy agar at a
110 concentration of 1×10^4 cfu/spot. Spots were then incubated for 24 h at 37 °C, and the MIC was
111 defined as the concentration at which there was no visible growth. MICs of vancomycin were
112 also determined in an identical manner to provide a reference activity. Several of the isolates
113 demonstrated heterogeneous growth on solid media, and in these cases individual colonies of
114 each morphotype were analyzed separately and speciated based on sequencing and
115 phylogenetic analysis of a portion of the *dnaJ* gene, which has previously been demonstrated to
116 serve as a sensitive measure of Staphylococcal speciation (Fig. S1) (27). Speciation was also
117 confirmed by *dnaJ* sequence analysis for isolates whose arylomycin MICs deviated from the
118 MIC₅₀ by greater than 4-fold (Fig. S1). Additionally, the *spsIB* genes from these isolates were
119 sequenced to determine whether any polymorphisms were present that could account for the
120 observed variation in susceptibility. Primers for the PCR amplification of *spsIB* genes were
121 designed based on regions of highly conserved sequence within the upstream ORF SERP0551
122 (*S. epidermidis* RP62A numbering) and the downstream *rexB* gene (Fig. S2). Degeneracies
123 were included at positions that varied within the sequenced strains of the CoNS in order to
124 maximize the likelihood of annealing (Table S1).

125 To determine with high confidence the history of speciation within the CoNS examined in
126 this study, a phylogenetic analysis was performed using regions of four essential genes that
127 have previously been validated as speciation markers (16S rDNA, *rpoB*, *groEL*, and *dnaJ*)
128 (Table S2) (1, 6, 7, 27). For each protein coding gene, published DNA sequences from

129 speciated strains were translated into protein sequence, and aligned using MUSCLE (8). The
130 DNA sequences were then mapped onto the amino acid alignment using the program TranAlign
131 (23). Aligned 16S rDNA sequences were obtained from the Ribosomal Database Project (4).
132 For each organism, the four aligned sequences were concatenated, and phylogenetic analysis
133 was conducted by the method of Maximum Likelihood using PhyML 3.0 (12), with the HKY85
134 nucleotide substitution model, 20 rate categories, and SPR branch improvement. The
135 phylogeny of SPase genes was determined similarly using sequences deposited in the NCBI
136 database as well as sequences obtained in the present study. Trees were displayed using
137 MEGA4 (30).

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139

140 **Results**

141

142 A total of 143 isolates of *S. epidermidis* from the London Health Services Center were analyzed.
143 The MIC₅₀ and MIC₉₀ of arylomycin C₁₆ against these 143 *S. epidermidis* isolates were found to
144 be 0.5 µg/ml and 1 µg/ml, respectively (Table 1). These susceptibilities are similar to that
145 reported previously for the *S. epidermidis* laboratory strain RP62A (MIC = 0.5 µg/ml) (24), and
146 compare favorably to the MIC₅₀ and MIC₉₀ of vancomycin, both of which we found to be 2 µg/ml,
147 in agreement with values reported previously (13). Additionally the range of MICs observed
148 among the 143 isolates was narrow, with the most resistant isolate having an arylomycin C₁₆
149 MIC of 4 µg/ml.

150 The remaining 9 species of CoNS examined displayed arylomycin C₁₆ susceptibilities
151 ranging from highly susceptible to resistant (Table 1). Specifically, we found that *S.*
152 *haemolyticus*, *S. lugdunensis*, and *S. hominis* are highly susceptible to arylomycin C₁₆, with
153 MIC₅₀ and MIC₉₀ values of 0.25 to 2 µg/ml. In contrast, we found that *S. capitis*, *S. caprae*, and

154 *S. cohnii* are only moderately susceptible, with MIC₅₀ values between 8 and 16 µg/ml and MIC₉₀
155 values between 16 and 32 µg/ml, and that *S. warneri* and *S. saprophyticus* are resistant, with
156 MIC₅₀ values equal to or greater than 64 µg/ml and MIC₉₀ values of greater than 64 µg/ml.
157 Finally, while many *S. simulans* isolates were sensitive to the arylomycins (MIC₅₀ of 2 µg/ml), a
158 significant number of isolates were highly resistant, resulting in a MIC₉₀ of >64 µg/ml.

159 Under laboratory conditions, *S. epidermidis* RP62A evolves arylomycin resistance by
160 mutation of Ser²⁹ to Pro²⁹ in SpsIB, one of two active SPases encoded by this organism, and in
161 the related organism *S. aureus*, as well as in the more distantly related *E. coli* and *P.*
162 *aeruginosa*, an analogous Pro²⁹ is naturally present and contributes significantly to arylomycin
163 resistance (29) (here and throughout the remainder of this manuscript, unless otherwise
164 indicated, Ser²⁹ and Pro²⁹ refer to the residue in SpsIB orthologs at the position corresponding
165 to 29 in *S. epidermidis*). In contrast, we did not observe mutations in the second *S. epidermidis*
166 SPase gene, *spsI*, in any of the resistant isolates. To determine whether similar mutations
167 might be responsible for the differences in arylomycin susceptibility observed among the
168 different CoNS, we examined the sequences of their *spsI* and *spsIB* genes (Fig. 2 and Table
169 S3). To identify SPase sequences, we used the NCBI BLAST interface to search the
170 sequenced genomes of *S. capitis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S.*
171 *saprophyticus*, and *S. warneri*, and found that each except *S. saprophyticus* possess both SpsI
172 and SpsIB homologs. We also included the recently reported sequence of the *spsIB* gene from
173 *Staphylococcus xylosus* (22), which does not encode an SpsI SPase (S. Leroy, personal
174 communication). Because no correlation between susceptibility and the sequence of *spsI* was
175 observed, and because *S. epidermidis* evolves resistance by mutation of *spsIB*, we PCR
176 amplified and sequenced the *spsIB* orthologs from *S. caprae*, *S. cohnii*, and *S. simulans*, whose
177 genomes have not been fully sequenced (see Supporting Information). Overall, the data
178 support the model that Pro²⁹ contributes significantly to arylomycin resistance within CoNS. *S.*

179 *lugdunensis*, *S. haemolyticus*, *S. hominis*, and a significant percentage of *S. simulans* isolates
180 are extremely susceptible and these species lack Pro²⁹. In contrast, the CoNS species most
181 resistant to the arylomycins, *S. warneri* and *S. saprophyticus*, each harbor a SPase with Pro²⁹.
182 The correlation between Pro²⁹ and arylomycin susceptibility is less clear for species that display
183 intermediate susceptibilities; for example, while *S. caprae* and *S. capitis* have Pro²⁹, and *S.*
184 *cohnii* has Ser²⁹, all three display MIC₅₀ values between 4 and 16 µg/ml.

185 Most of the species examined have the narrow range of susceptibilities to arylomycin C₁₆
186 expected for an antibiotic that has not been used clinically (2, 9, 14, 21) (Table 1). However, for
187 several species, we found a greater range in susceptibilities, or we found outliers with
188 susceptibilities that differed significantly from those of the other isolates (Table 1 and Fig. S3).
189 For example, one isolate of *S. lugdunensis* is significantly more susceptible than the species
190 average (MIC 8-fold below the MIC₅₀), while a second isolate is unusually resistant (MIC 128-
191 fold above the MIC₅₀). Sequencing of the *spsIB* genes revealed identical sequence at the
192 amino acid level (including at residue 29) (Fig. S4). In addition, a bimodal distribution of MICs
193 was observed for isolates of *S. warneri* and *S. simulans* with peaks at 16 µg/ml and >64 µg/ml
194 and at 2 µg/ml and >64 µg/ml, respectively (Fig. S3). Sequencing the *spsIB* genes from
195 representative isolates of each group, revealed nearly identical SPase sequences and no
196 variation at residue 29 (Fig. S4). Finally, we identified a particularly susceptible isolate of *S.*
197 *warneri* with an MIC that is 32-fold below the species MIC₅₀. Interestingly, the particularly
198 susceptible strain of *S. warneri* encoded Ser²⁹ as opposed to Pro²⁹, which is typically present in
199 *S. warneri* isolates, further supporting the genotype/phenotype correlation.

200 The observed differences in MICs that cannot be accounted for by differences in SpsIB
201 sequence, both within and between species, suggest that additional factors contribute to
202 arylomycin susceptibility. To determine whether these additional factors are shared among
203 related organisms, we examined MICs as a function of phylogeny (Fig. 2). The phylogenetic

204 analysis based on four highly conserved genes (see Materials and Methods) suggests that *S.*
205 *simulans* was the earliest species to diverge from the common ancestor of the examined CoNS,
206 with the other species forming two groups, one comprised of *S. xylosus*, *S. saprophyticus*, and
207 *S. cohnii*, and the second comprised of the remaining species. Notably, SpsI SPases have only
208 been shown to be present in the second group, although their presence cannot be ruled out in
209 the unsequenced species.

210 *S. saprophyticus*, which has Pro²⁹ and is extremely resistant to the arylomycins (MIC₅₀ >
211 64 µg/ml), is most closely related to *S. cohnii*, which lacks Pro²⁹, and yet is only moderately
212 susceptible to the arylomycins (MIC₅₀ = 8 µg/ml). To test whether other species that are closely
213 related to *S. saprophyticus* and *S. cohnii* share this intrinsically lower susceptibility, we
214 determined the MIC of a typed strain of *S. xylosus*, which is closely related to *S. saprophyticus*,
215 but like *S. cohnii* lacks Pro²⁹. As expected, the strain of *S. xylosus* is moderately susceptible to
216 the arylomycin (MIC = 4 µg/ml). Thus, this group of related bacteria appears to have a lower
217 basal level of arylomycin susceptibility. In contrast, *S. epidermidis*, *S. hominis*, *S. lugdunensis*,
218 and *S. haemolyticus*, which lack Pro²⁹ and display extreme arylomycin susceptibility (MIC₅₀ 0.25
219 – 2 µg/ml), as well as *S. capitis* and *S. caprae* which have Pro²⁹ but remain moderately
220 susceptible (MIC₅₀ 8 – 16 µg/ml), are more related to one another than to the other CoNS
221 species examined. Despite the fact that some *S. warneri* isolates are extremely resistant, the
222 remaining isolates have MICs of ~16µg/mL, which is consistent with the level of resistance
223 observed for the other CoNS with Pro²⁹ within the more susceptible phylogenetic group.

224 Finally, we evaluated the activity of arylomycin C₁₆ against strains from an unspiciated
225 CoNS panel from University of California San Diego Medical Center, and observed MIC₅₀ and
226 MIC₉₀ values of 2 µg/ml and >32 µg/ml, respectively (Fig. 3). In addition to revealing a portion of
227 resistant isolates, the distribution of MICs shows significant variations in the susceptibility of the
228 remaining strains. Although interpretation of these results is complicated by the lack of

229 speciation, the MIC distribution is consistent with a model in which both the presence of Pro²⁹
230 and a second yet to be determined factor, combine to yield tiers of arylomycin susceptibility.

231

232 Discussion

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234 Members of the arylomycin class of natural product antibiotics act via a novel mechanism of
235 action, the inhibition of SPase, and thus, the inhibition of the essential process of protein
236 secretion. We found that in addition to excellent activity against *S. epidermidis* (24), arylomycin
237 C₁₆ has potent activity against *S. haemolyticus*, *S. lugdunensis*, and *S. hominis*. Each of these
238 CoNS encodes a SPase with Ser²⁹, consistent with the central role of this residue in determining
239 arylomycin susceptibility that we observed previously with other bacteria (29).

240 The remaining species examined range from only moderately susceptible to extremely
241 resistant, and Pro²⁹ clearly makes a significant contribution to resistance in many of these
242 cases. Interestingly, phylogenetic analysis suggests that Ser²⁹ was prevalent during speciation,
243 but that Pro²⁹ evolved in at least two independent instances, once in *S. saprophyticus* and once
244 in the common ancestor of *S. caprae* and *S. capitis* (Fig. 2). Pro²⁹ in *S. warneri* may represent a
245 third instance, although it might instead result from common ancestry with *S. caprae* and *S.*
246 *capitis*, and this distinction is beyond the resolution of the current analysis. Interestingly, many
247 synonymous and non-synonomous mutations differentiate the *spsIB* genes of the different
248 staphylococcal species, suggesting that ample mutational diversity has been sampled, but only
249 Ser and Pro appear to be tolerated at position 29. Importantly, the multiple, independent
250 instances where Pro²⁹ was introduced during staphylococci speciation suggests that there may
251 be a natural selective pressure for Pro²⁹, and the variation within isolates of an extant species
252 suggests that this pressure may have occurred recently and may even still be present. While

253 the nature of the selection pressure is currently unknown, it is tempting to consider that it may
254 be related to the presence of arylomycins in nature (29).

255 The observed differences in susceptibilities between some of the staphylococcal species
256 reveal that, in addition to SpsIB residue 29, there must be other factors that also contribute to
257 overall susceptibility, and these other factors generally appear to be shared among closely
258 related species, resulting in groups of species having higher or lower basal susceptibilities.
259 One possibility is that the presence of a second SPase gene, orthologs of *S. epidermidis* *spsI*, in
260 some species of CoNS could contribute to their different arylomycin susceptibilities. In principle,
261 expressing two arylomycin sensitive SPase proteins could result in hypersusceptibility if each
262 SPase recognized a different subset of essential pre-protein substrates. Alternatively,
263 expressing one sensitive and one resistant SPase could result in either a susceptible or
264 resistant phenotype, depending on the exact mechanism of arylomycin induced cell death,
265 which is yet to be determined. Interestingly, all of the species with higher basal susceptibility
266 have two SPases while, *S. xylosus* (S. Leroy, personal communication) and *S. saprophyticus*
267 have only one SPase and display lower basal susceptibility. Consistent with a possible role for
268 a second SPase contributing to arylomycin susceptibility, *S. aureus* strain 8325 is highly
269 resistant to arylomycin (MIC >128 µg/ml (24)) despite being closely related to the more
270 susceptible group of CoNS, and this species has only a single SPase, apparently due to a
271 recent deletion of its *spsI* homologue. Further experiments will be required to elucidate the
272 potential role of multiple SPases in CoNS susceptibilities, and/or to identify other factors
273 contributing to susceptibility such as differences in the composition of secreted proteins or the
274 presence of modifying enzymes. Comparison of resistant and susceptible isolates of the same
275 species may provide a valuable approach to identifying additional factors that contribute to
276 arylomycin susceptibility.

277 Regardless of the origins of arylomycin susceptibility and resistance, it is clear that a
278 broad and clinically important (16) range of CoNS strains are susceptible to the arylomycins. In
279 fact, in these cases the level of susceptibility compares favorably with the level of susceptibility
280 to vancomycin, the antibiotic currently recommended for treatment of CoNS infections but for
281 which resistance is a major concern. The availability of a novel antibiotic, with a novel
282 mechanism of action, would significantly improve CoNS therapy. Along with previously reported
283 data (29), the data also suggest that if the arylomycin class of natural products could be
284 optimized to overcome the loss in affinity mediated by the presence of the resistance conferring
285 Pro²⁹, then they would have broad activity against CoNS, and possibly other bacteria as well.

286

287 **Acknowledgements**

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289 We thank S. Leroy and Genoscope (Genomics Institute of CEA, Evry, France) for providing
290 analysis of the unpublished *S. xylosus* genome sequence. This work was supported by the
291 Office of Naval Research (Awards N000140310126 and N000140810478) and the National
292 Institutes of Health (AI081126).

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386 **Figure Captions**

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388 FIG. 1. Structures of arylomycin A₂ (R = *iso*-C₁₂; CH₂(CH₂)₇CH(CH₃)₂) and arylomycin C₁₆ (R =
389 *iso*-C₁₆; CH₂(CH₂)₁₁CH(CH₃)₂).

390

391 FIG. 2. Phylogenetic relationship, SPase sequence, and arylomycin susceptibility of CoNS
392 species. *Macrococcus caseolyticus* was used as an outgroup to root the tree, and confidence
393 values were determined based on the alternative likelihood ratio test. For each species, the
394 amino acids in SpsIB and in SpsI at the positions corresponding to residue 29 in *S. epidermidis*
395 SpsIB are indicated. MIC values reflect the MIC₅₀ except for *S. simulans* and *S. warneri* where
396 the values listed correspond to peaks in a bimodal distribution of MICs. ^aPresence and/or
397 sequence of SpsI homologue is unknown. ^bSpecies does not have an SpsI homologue.

398

399 FIG. 3. Distribution of arylomycin susceptibilities of unspiciated CoNS isolates obtained from
400 the University of California, San Diego Medical Center.

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402

TABLE 1. MICs ($\mu\text{g/mL}$) of speciated coagulase-negative strains from the London Health Services Centre to arylomycin C₁₆ and vancomycin¹

Species	Number of isolates	Arylomycin C ₁₆			Vancomycin		
		MIC ₅₀	MIC ₉₀	MIC Range	MIC ₅₀	MIC ₉₀	MIC Range
<i>S. epidermidis</i>	143	0.5	1	0.12 – 4	2	2	1 – 2
<i>S. lugdunensis</i>	10	0.25	0.25	0.03 – 32	2	2	2
<i>S. hominis</i>	17	0.25	0.5	0.06 – 0.5	2	4	1 – 4
<i>S. haemolyticus</i>	10	2	2	1 – 2	2	2	2 – 4
<i>S. simulans</i>	12	2	>64	2 – >64	1	2	1 – 2
<i>S. caprae</i>	10	8	16	4 – 16	2	2	1 – 2
<i>S. cohnii</i>	27	8	16	4 – 16	2	2	1 – 2
<i>S. capitis</i>	24	16	32	4 – 64	2	2	1 – 2
<i>S. warneri</i>	19	64	>64	2 – >64	4	4	1 – 4
<i>S. saprophyticus</i>	10	>64	>64	>64	1	2	1 – 2
<i>S. xylosus</i> ^a	-	-	-	4	-	-	-
<i>S. aureus</i> ^a	-	-	-	>64 ^b	-	-	-

^aReference strains, *S. xylosus* ATCC 29971 and *S. aureus* NCTC 8325, are included for comparison. ^b Ref. (24).

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