Supporting Information

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

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Table S1. Primers used in this study

Primer Name	Sequence
CNS_551_F	gctcgacg TTAHTRTGYTTYCCRTTTACRCC
CNS_rexB_R3_T72	ttgtaatacgactcactatagggg GATTTNCCNGTMCCWGCTC
CNS_dnaJ_F	cgtcgcgt TGGCCAAAAGAGAYTATTATGA
CNS_dnaJ_R_T72	ttgtaatacgactcactatagggg CGGAATTGTTTACCHGTTTGWGTACC

Primers for amplification of *dnaJ* are based on modification of those used previously (manuscript Ref. 27). Nucleotide degeneracies are indicated by the IUPAC labels. 5' extensions were added to increase annealing temperature or for sequencing and are presented in lower case lettering.

Table S2. Accession numbers of DNA sequences used in the phylogenetic analysis of CNS species

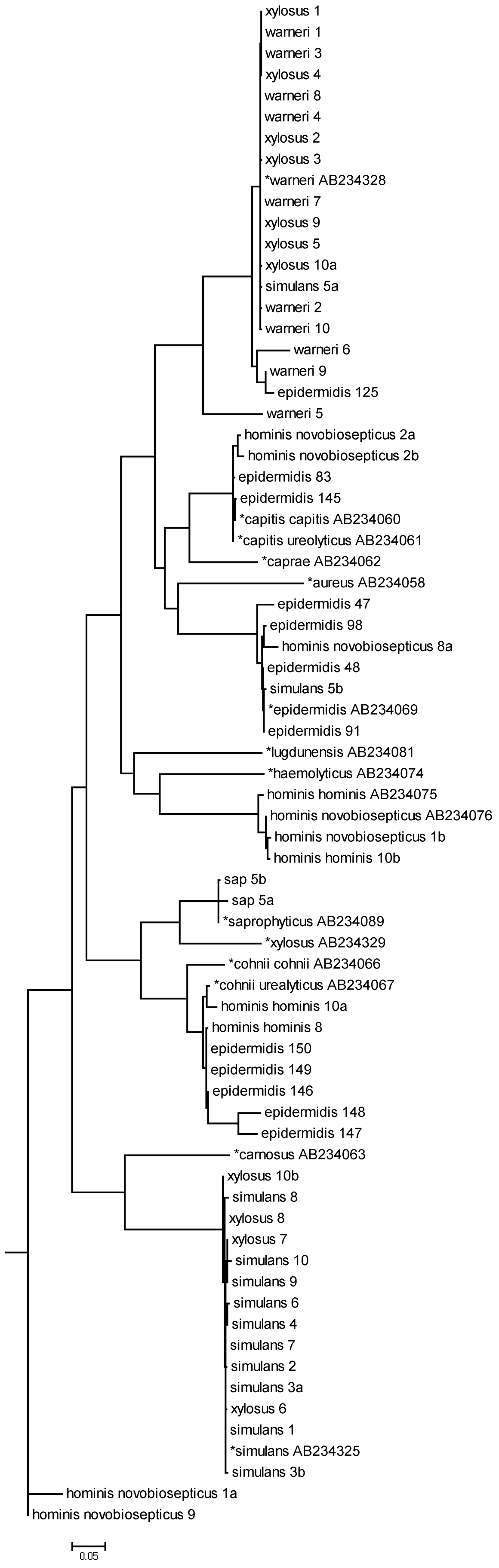
Species	*dnaJ*	*groEL*	*rpoB*	16S rDNA
S. capitis	AB234060	AF036322	AF325885	FJ357585
S. caprae	AB234062	AF053574	AF325868	AB009935
S. cohnii	AB234066	AF053582	AF325893	D83361
S. epidermidis	AB234069	AF029245	AF325872	CP000029
S. haemolyticus	AB234074	U92809	AF325888	D83367
S. hominis	AB234075	AF053572	AF325875	AY167805
S. lugdunensis	AB234081	AF053570	AF325870	AB009941
S. saprophyticus	AB234089	AF053578	AF325873	AP008934
S. simulans	AB234325	AF053584	AF325877	D83373
S. warneri	AB234328	AF053569	AF325887	L37603
S. xylosus	AB234329	AF053573	AF325883	D83374
M. caseolyticus	222150250	AF053577	222152109	D83359

Table S3. Inventory of active SPase proteins within Staphylococci species. The amino acid regions corresponding to that between Ser^{29} and catalytic Ser^{36} in *S. epidermidis* is shown.

Species	Protein	GI#	Sequence
S. epidermidis	SpsIB	57866486	`SYSIKGDS`
S. lugdunensis	SpsIB	289551204	`SYTIKGDS`
S. hominis	SpsIB	228474322	`SYTIKGDS`
S. haemolyticus	SpsIB	70726986	`SYTIKGDS`
S. simulans	SpsIB		`SYTVRGDS`
S. caprae	SpsIB		`PYTIKGDS`
S. cohnii	SpsIB		`SYTVKGDS`
S. capitis	SpsIB	223042998	`PYTIKGDS`
S. warneri	SpsIB	239636508	`PYTIKGDS`
S. saprophyticus	SpsIB	73663119	`PYTVKGDS`
S. xylosus	SpsIB	94958269	`SYTVKGDS`
S. aureus	SpsIB	88194660	`PYTIKGES`
S. carnosus	SpsIB	224476066	`SYTVRGDS`
S. epidermidis	SpsI	57865986	`SYTVKGAS`
S. lugdunensis	SpsI	289551814	`TYSVSGDS`
S. hominis	SpsI	228474183	`SYTVSGSS`
S. haemolyticus	SpsI	70727661	`SYTVSGSS`
S. simulans	SpsI		–[b]
S. caprae	SpsI		–[b]
S. cohnii	SpsI		–[b]
S. capitis	SpsI	223043379	`SYTVSGES`
S. warneri	SpsI	239637342	`SYTVSGES`
S. saprophyticus	SpsI		–[c]
S. xylosus	SpsI		–[c]
S. aureus	SpsI		–[c]
S. carnosus	SpsI		–[c]

[a] Sequence determined in this study. [b] Unknown if protein is encoded by this species. [c] Protein not encoded by this species

Figure S1. Phylogenetic analysis of *dnaJ* genes used to confirm or reassign the speciation of select CNS isolates. The tree is rooted using *Macrococcus caseolyticus* as an outgroup. *Database sequences used as references to determine speciation.



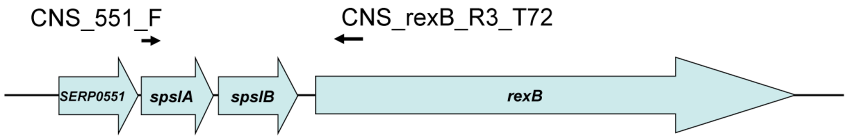


Figure S2. Diagram of the *S*. *epidermidis* genomic region that encodes SpsIB. The syntany of these genes is conserved in all of the examined CNS species. Location of the primers used to PCR amplify the spsIB gene are indicated. The CNS_rexB_R3_T72 primer contains a tag allowing sequencing of the *spsIB* with T7 universal 2 primer.

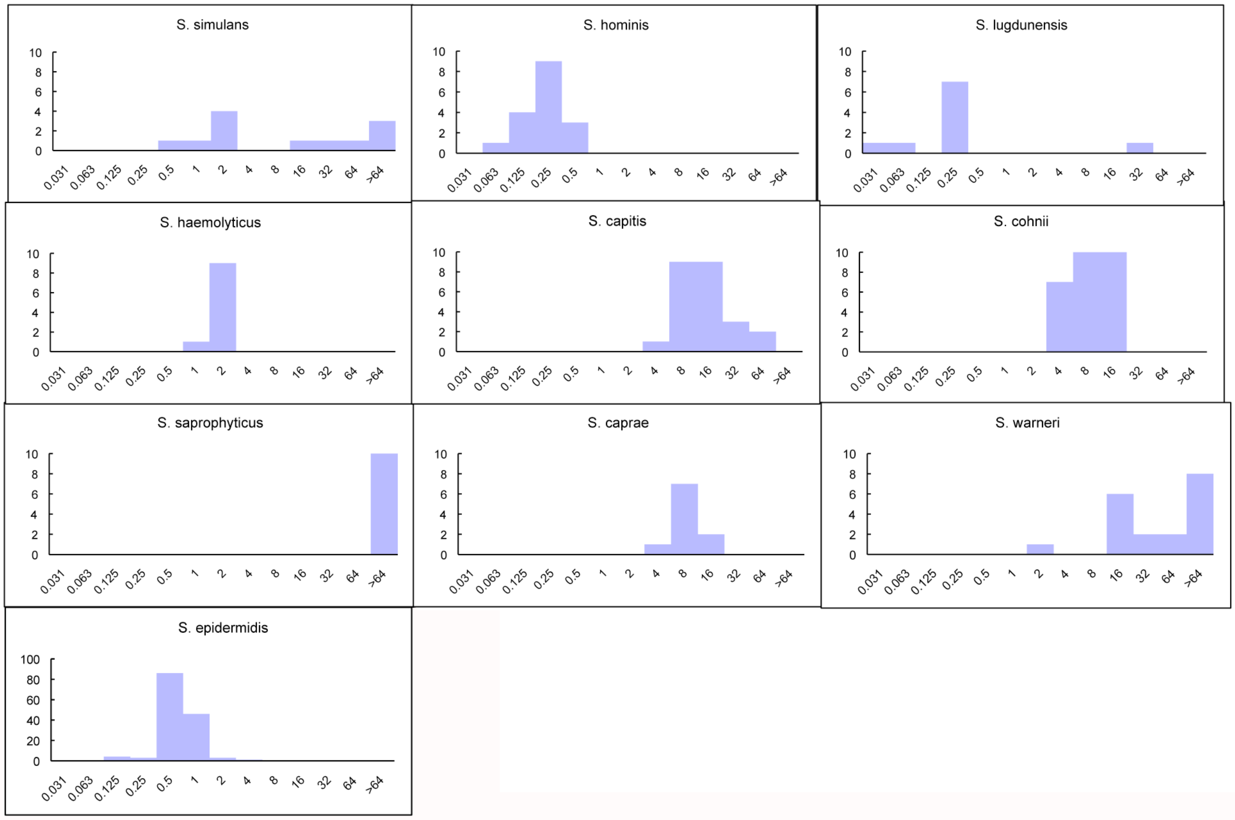
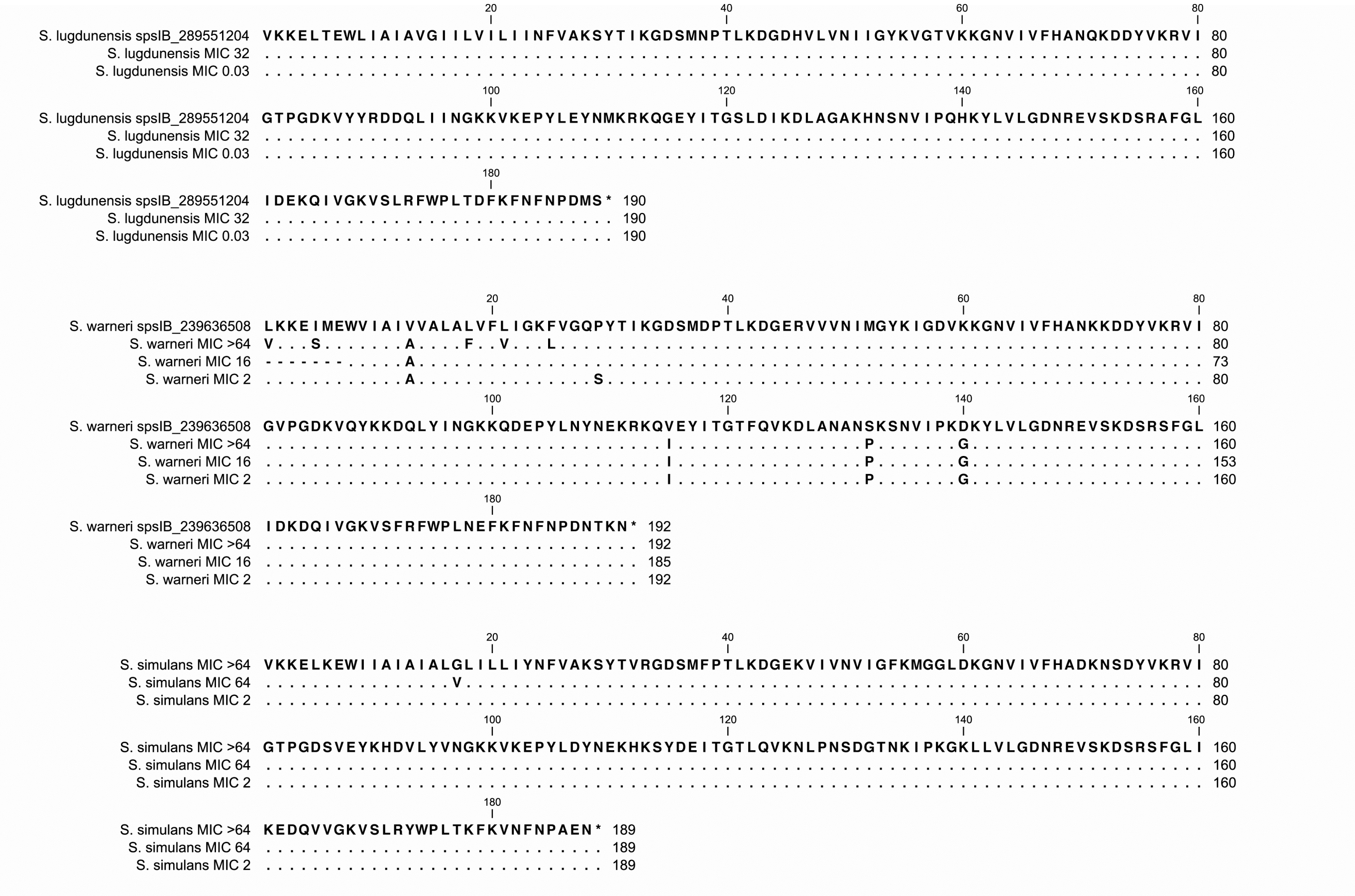


Figure S3. Distribution of arylomycin sensitivities for the examined CNS species shown as the number of isolates (y-axes) observed at each MIC in µg/ml (x-axes).



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                                                  20                  40                  60                  80
                                                   |                   |                   |                   |
S. lugdunensis spsIB_289551204 VKKELTEWLIAIAVGIILVILIINFVAKSYTIKGDSMNPTLKDGDHVLVNIIGYKVGTVKKGNVIVFHANQKDDYVKRVI 80
         S. lugdunensis MIC 32 ................................................................................ 80
       S. lugdunensis MIC 0.03 ................................................................................ 80
                                                 100                 120                 140                 160
                                                   |                   |                   |                   |
S. lugdunensis spsIB_289551204 GTPGDKVYYRDDQLIINGKKVKEPYLEYNMKRKQGEYITGSLDIKDLAGAKHNSNVIPQHKYLVLGDNREVSKDSRAFGL 160
         S. lugdunensis MIC 32 ................................................................................ 160
       S. lugdunensis MIC 0.03 ................................................................................ 160
                                                 180
                                                   |
S. lugdunensis spsIB_289551204 IDEKQIVGKVSLRFWPLTDFKFNFNPDMS* 190
         S. lugdunensis MIC 32 .............................. 190
       S. lugdunensis MIC 0.03 .............................. 190

                                                  20                  40                  60                  80
                                                   |                   |                   |                   |
    S. warneri spsIB_239636508 LKKEIMEWVIAIVVALALVFLIGKFVGQPYTIKGDSMDPTLKDGERVVVNIMGYKIGDVKKGNVIVFHANKKDDYVKRVI 80
            S. warneri MIC >64 V...S.......A....F..V...L....................................................... 80
             S. warneri MIC 16 -------.....A................................................................... 73
              S. warneri MIC 2 ............A...............S................................................... 80
                                                 100                 120                 140                 160
                                                   |                   |                   |                   |
    S. warneri spsIB_239636508 GVPGDKVQYKKDQLYINGKKQDEPYLNYNEKRKQVEYITGTFQVKDLANANSKSNVIPKDKYLVLGDNREVSKDSRSFGL 160
            S. warneri MIC >64 ..................................I................P.......G.................... 160
             S. warneri MIC 16 ..................................I................P.......G.................... 153
              S. warneri MIC 2 ..................................I................P.......G.................... 160
                                                 180
                                                   |
    S. warneri spsIB_239636508 IDKDQIVGKVSFRFWPLNEFKFNFNPDNTKN* 192
            S. warneri MIC >64 ................................ 192
             S. warneri MIC 16 ................................ 185
              S. warneri MIC 2 ................................ 192

                                                  20                  40                  60                  80
                                                   |                   |                   |                   |
           S. simulans MIC >64 VKKELKEWIIAIAIALGLILLIYNFVAKSYTVRGDSMFPTLKDGEKVIVNVIGFKMGGLDKGNVIVFHADKNSDYVKRVI 80
            S. simulans MIC 64 ................V............................................................... 80
             S. simulans MIC 2 ................................................................................ 80
                                                 100                 120                 140                 160
                                                   |                   |                   |                   |
           S. simulans MIC >64 GTPGDSVEYKHDVLYVNGKKVKEPYLDYNEKHKSYDEITGTLQVKNLPNSDGTNKIPKGKLLVLGDNREVSKDSRSFGLI 160
            S. simulans MIC 64 ................................................................................ 160
             S. simulans MIC 2 ................................................................................ 160
                                                 180
                                                   |
           S. simulans MIC >64 KEDQVVGKVSLRYWPLTKFKVNFNPAEN* 189
            S. simulans MIC 64 ............................. 189
             S. simulans MIC 2 ............................. 189
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Figure S4. Alignment of SPase proteins from representative CNS isolates displaying unusual sensitivities. Reference SPase sequences are included where available and are identified by their GI number. Identical amino acids are indicated by dots. MICs of the isolates whose SPase sequence are shown are listed in the sequence label.