Known mechanisms account for less than half of antimicrobial resistance in a diverse collection of non-*aureus* staphylococci.

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Running title: Antimicrobial resistance in non-*aureus* staphylococci

Abstract

Introduction: Non-*aureus* staphylococci (NAS) are implicated in many healthcare-acquired infections and an understanding of the genetics of antimicrobial resistance in NAS is important in relation to both clinical intervention and the role of NAS as a reservoir of resistance genes.

Gap statement: The burden of antimicrobial resistance in NAS, particularly to clinically relevant antimicrobials, is understudied.

Methodology: We sourced 394 NAS isolates from clinical samples, healthy human volunteers, animals and type cultures and subjected them to agar dilution susceptibility testing against eight antimicrobials. We performed whole genome sequencing on 316 isolates and analysed these genotypically for the presence of genetic mechanisms responsible for the phenotypic levels of reduced antimicrobial susceptibility.

Results: Cefoxitin is used to screen for methicillin resistance in *S. aureus*, as it stimulates expression of *mecA*. We observed 174 isolates with an MIC of at least 4 µg/ml to cefoxitin, of which sequencing revealed 47.6% (80/168) did not harbour a known *mec* homologue. Seven clinical NAS isolates displayed high daptomycin minimum inhibitory concentrations (MICs) (>4 µg/ml), with no known mechanism identified. Differences in MICs against erythromycin were attributable to the presence of different resistance genes (*msrA* and *ermC*). In total, 49% (187/394) of isolates displayed reduced susceptibility to three or more of the antimicrobials tested.

Conclusions: The widespread presence of reduced antimicrobial susceptibility in NAS is a concern, with an increased likelihood of (1) harder to treat infections caused directly by NAS, and (2) resistance genes being passed on to other bacteria via horizontal gene transfer, both of which have clinical implications for treatment and management of patients.

43 **Introduction**

44 The non-*aureus* staphylococci (NAS) represent an important source of nosocomial disease,
45 including prosthetic joint infection (PJI), infective endocarditis and infection in pre-term
46 babies (1). In the UK, over 215,000 joint replacements (hip, knee and shoulder) took place in
47 2016, with a year-on-year increase of 4% (2). Of these replacements, 1.5% require surgical
48 revision due to infection (2). These infections are most commonly caused by *Staphylococcus*
49 spp., and attributed to NAS in approximately 31% of cases across Europe (3). In our local
50 hospital, the Norfolk and Norwich University Hospital (NNUH), 50% of isolates identified in
51 suspected PJI are NAS.

52 In clinical microbiology, staphylococci are classified using the coagulase test, with coagulase
53 positive samples overwhelmingly identified as *S. aureus* and coagulase negative samples
54 grouped together under the term coagulase negative staphylococci (CoNS). CoNS is
55 therefore the term found in antimicrobial surveillance data. However, since coagulase
56 negative *S. aureus* strains exist (as do coagulase positive strains of other staphylococcal
57 species), we use the term "non-*aureus* staphylococci" (NAS) to encompass all staphylococci
58 which are not *S. aureus*, regardless of coagulase activity.

59 There is currently an intense focus upon the presence and spread of bacterial antimicrobial
60 resistance, typified in *S. aureus* by methicillin resistance (MRSA). While the body of
61 literature in antimicrobial resistance research is growing for staphylococci, NAS data
62 remains eclipsed by the focus on *S. aureus*. Few studies have investigated antimicrobial
63 resistance (AMR) in NAS. Those that have suggest 45% of NAS harbour methicillin resistance
64 (4), and that NAS may be resistant to a larger number of antimicrobial classes than *S. aureus*
65 (4, 5).

66 An understanding of the genetics of AMR in NAS is important in relation to clinical
67 intervention upon diagnosis of PJI (and other infections) as well as understanding the role of
68 NAS as a reservoir of resistance genes. To address this, we assembled a collection of 400
69 NAS from clinical samples, healthy human volunteers, animals and type cultures and
70 assessed their susceptibility to a range of antimicrobials. We also performed whole genome
71 sequencing to correlate mechanisms of resistance with minimum inhibitory concentrations
72 (MICs).
73
74 **Materials & Methods**
75 *NAS collection*
76 Under NHS Research Ethics Committee approval, the Norwich Biorepository banks blood,
77 solid tissue and bacterial isolates from the NNUH and research institutes on the Norwich
78 Research Park, including the University of East Anglia, and makes these available to the
79 research community. This enabled us to assemble a collection of 380 NAS from a) clinical
80 specimens which were isolated from suspected NAS PJI infections (229, NNUH), b) healthy
81 human volunteers (114, UEA), and c) animal samples (32, UEA) with five having no source
82 recorded. An additional 14 strains of NAS from the National Collection of Type Cultures
83 (NCTC) were supplied by Public Health England.
84 Isolates were identified using MALDI-TOF (Bruker) to the species level (Table 1). All strains
85 were cultured overnight on TSA plates (Oxoid), checked for contamination and purified.
86 Once purified, the NAS collection was stored as glycerol stocks to be screened for their
87 antimicrobial susceptibility. *Staphylococcus aureus* NCTC 12973 was used as a control.
88
89 *Susceptibility testing*

90 To assay the entire NAS collection, five deep well 96-well microplates (VWR) were prepared
91 with 1 ml TSB (Oxoid) per well. Glycerol stocks were used to inoculate the corresponding
92 well. Per plate, one well was designated as a sterility control (TSB only) and one well was
93 inoculated with the *S. aureus* control. After inoculation, plates were sealed and incubated
94 at 37 °C at 180 rpm for a minimum of 10 hours. The experimental design enabled 13.5% of
95 the collection to be tested in duplicate; so MIC data was compared and then tabulated, MIC
96 data is given in Table S1.
97 Iso-sensitest agar (Oxoid) was prepared in 250 ml aliquots and autoclaved. Antimicrobial
98 stocks were added to obtain the desired final concentrations once the media had cooled to
99 < 50 °C. For daptomycin, Ca^{2+} was also added at 50µg/ml. The agar antimicrobial mixture
100 was then poured into sterile rectangular plates (Fisher Scientific) and dried.
101 Per strain, a 1:10 dilution of overnight culture was transferred to a 96 well plate and the
102 OD_{600} was measured. An average OD_{600} was calculated for each column, which was then
103 diluted to approximately OD_{600} 0.6 to generate an inoculum plate for susceptibility testing.
104 Using a 96-pin multi-point inoculator (Denley), ~1 µl of inoculum per isolate was stamped
105 onto the agar containing antimicrobials, from the lowest concentration to the highest.
106 Between inoculum plates, the pins were washed in 70 % ethanol for 30 s and allowed to dry
107 before stamping on an antimicrobial-free plate to confirm sterility. Washes were also
108 carried out between antimicrobials using sterile water. All stamped plates were incubated at
109 37 °C.
110 Isolates found to have reduced susceptibility to daptomycin had their MICs determined for a
111 second time by spotting 10 µl of culture onto TSA plates containing various daptomycin
112 concentrations (supplemented with Ca^{2+} at 50 µg/ml).

113 Test MIC ranges were determined from British Society for Antimicrobial Chemotherapy
114 (BSAC) surveillance MIC data for coagulase negative staphylococci (CoNS) in 2016 (6). Data
115 for daptomycin were taken from 2010. In µg/ml, the ranges tested were: daptomycin 0.25-
116 4, erythromycin 0.125-256, gentamicin 0.016-64, rifampicin 0.004-0.064, teicoplanin 0.25-
117 16, tetracycline 0.25-256 and vancomycin 1-4. No CoNS surveillance data were available for
118 cefoxitin, hence the test range of 0.25-4 µg/ml was based upon published work (7) .
119
120 *Statistical comparison of clinical and non-clinical isolates*
121 Using Prism (GraphPad, San Diego, USA, v 5.04), a Mann-Whitney test was performed (non-
122 parametric test, two-tailed with Gaussian approximation) to compare between the MIC of
123 clinical and non-clinical isolates. Statistical significance was given to a p value < 0.05.
124
125 *DNA extraction and sequencing*
126 Overnight cultures derived from single colonies were pelleted and resuspended in lysis
127 buffer (Qiagen), transferred to 2 ml lysis matrix B tubes (MPBio) and subjected to bead
128 beating for 15 min at 30 Hz (Tissuelyser II, Qiagen) with RNAse A added. DNA was extracted
129 according to the QiaCube HT protocol with an additional 30 min incubation at 65 ^{o}C after
130 proteinase K addition and eluted into Tris-10mM HCL.
131
132 Libraries for sequencing were prepared using the Nextera XT DNA Library Prep Protocol and
133 sequenced on the Illumina MiSeq or NextSeq with a loading concentration of 1.8 picomolar.
134
135 *Genome analysis*

136 The raw reads were subject to FastQC quality control (8), adapters were trimmed using
137 Trimmomatic [v 0.39] (9) using the supplied NexteraXT adapter sequences. In some cases,
138 read normalisation was performed using BBNorm [v35.85] (10) to remove low coverage
139 contamination. The lowest coverage cutoff level parameter used was dependent on the
140 total coverage of the sequence since some sequencing runs had a high difference in
141 coverage level across the run. Finally, reads were concatenated if they originated from
142 Illumina NextSeq since this platform produces eight reads per sample, four forward and four
143 reverse. A total of 366 samples sequenced for this project passed QC and were suitable for
144 downstream analysis. These reads were used as described below. Sequences are available
145 from the European Nucleotide Archive, under project PRJEB31403.
146 To determine which antimicrobial resistance genes were present in each of our 364 NAS
147 genomes, reference gene sequences were downloaded from CARD v (11) and used as input
148 to ARIBA v2.13.2 (12) which generates local assemblies from sequence reads and reports
149 back which reference genes are identified, with a minimum percent identity cut off at 90%.
150 The tabulated results were evaluated for gene/mutation presence/absence relative to MIC
151 per antimicrobial. Twelve of the NCTC strains, for which sequence data were already
152 available, were analysed by ABRIcate v0.9.7 (13) using CARD v2.0.0 (11) as the reference
153 database with a minimum DNA coverage of 90%. Protein level conservation was assessed
154 using BLAST v2.10.1 against the NCBI AMR database. Hits were recorded for greater than
155 40% identity at the protein level over 80% of the query and subject sequence.
156
157 **Results & Discussion**

158 Our NAS collection comprised over 30 species of *Staphyloccocus*, including 10 or more
159 isolates of *S. epidermidis*, *S. capitis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. simulans*
160 and *S. warneri* (Table 1). Isolates were collected over a four-year period from 2013 to 2016.
161 The range of antimicrobials tested were selected based upon clinical relevance and
162 availability (Table S1). Observed MIC distributions per antimicrobial are shown in Figure 1.
163

164 *Cefoxitin screening does not correlate with* mecA *presence in clinically relevant NAS*

165 Cefoxitin is used to screen for methicillin resistance in *S. aureus* as it induces *mecA*
166 expression. However, while methicillin resistant *S. aureus* (MRSA) have a high public profile,
167 much less is known about methicillin resistant NAS (MRNAS). EUCAST guidelines state that
168 for MRSA "cefoxitin is a very sensitive and specific marker of *mecA/mecC*-mediated
169 methicillin resistance including in heterogeneous expressing strains and is the agent of
170 choice" (14). In this collection, we found 174/394 (44%) displayed reduced susceptibility to
171 cefoxitin with MICs $\geq$ 4 µg/ml (Table S1). The vast majority of these isolates were from
172 clinical samples (Figure 1) but analysis at the nucleotide level (Table S2 and S3) indicated
173 that only 80/168 (47.6 %) with an MIC $\geq$ 4µg/ml harboured a known *mecA.* MecA is
174 extremely well-conserved and searching at the protein level yielded the same results (Table
175 S4). Other *mec* elements were also identified (e.g. *mecC*, *mecI* and *mecR1*) but only ever in
176 addition to *mecA*. No *mecA* was detected in twelve species with high cefoxitin MICs and for
177 *S. haemolyticus, S. warneri, S. sciuri* and *S. simulans* the percentage of the population that
178 exhibited MIC $\geq$ 4µg/ml with no *mecA* was found between 58 and 88.9% (Figure 2).
179 Conversely, presence of *mecA* did not result in a high cefoxitin MIC in multiple isolates of *S.*
180 *hominis*, *S. haemolyticus* and *S. epidermidis*. Our results therefore do not support cefoxitin

181 as a universally good indicator of *mecA* presence in *S. epidermidis* (7), and suggest that it
182 performs poorly in the less common, but still clinically relevant NAS.
183
184 *Clinical resistance only partly explained by known mechanisms*
185 When exposed to gentamicin and tetracycline, isolates could broadly be divided into two
186 populations, displaying susceptible or reduced susceptibility phenotypes (Figure 1 GEN and
187 TET). In isolates with an MIC ≥1 µg/ml gentamicin, 49/130 harboured *aac(6')-le-aph(2")-la*
188 (Table 2) which is associated with gentamicin resistance in *Enterococcus* (15, 16) but has
189 also been observed in *Staphylococcus* (17, 18). A total of 12 isolates had a match for
190 *aph(3')IIIa*, but only five of them were associated with reduced susceptibility.
191 Six isolates that contained *aac(6')-le-aph(2")-la* displayed susceptible MICs, making them
192 the equivalent of major errors (MEs) in public health terms, as the isolates were
193 genotypically resistant but phenotypically susceptible (19). Accordingly, the 75/130 isolates
194 with reduced susceptibility (≥1 µg/ml) that harboured no *aac(6')-le-aph(2")-la* represented
195 the equivalent of very major errors (VMEs) as they were genotypically susceptible but
196 phenotypically resistant (19). This is highly suggestive of novel mechanisms of resistance and
197 was a feature of other antimicrobials tested (Figure S2).
198 To identify whether efflux pumps might play a role these phenotypes, we assessed the
199 ARIBA output for the staphylococcal-specific *norABC, mgrA, mepR* and *qac* genes (20). In the
200 sequenced NAS collection 168/378 (44.4 %) contained *norA*, however of these less than two
201 thirds had reduced susceptibility to gentamicin.
202 According to the Comprehensive Antimicrobial Resistance Database [CARD] (11), *tetK* is by
203 far the most common tetracycline resistance mechanism in *S. aureus* and *S. epidermidis* (10-
204 20%), followed by *tetL* (<1%) and *tetM* (<1%). This was borne out in our NAS collection,

205 where 48/148 (32.4 %) isolates with MICs ≥ 2 µg/ml of tetracycline contained *tetK*, as
206 compared to 5/223 (2.2 %) with MICs below 2 µg/ml. One animal isolate with an MIC of 16
207 µg/ml carried *tetL* and one clinical isolate with an MIC of 64 µg/ml carried *tetM*; neither had
208 any other tetracycline resistance genes. Again, this demonstrated that 98/148 isolates
209 displayed a reduced susceptibility phenotype that did not associate with a known resistance
210 determinant, indicative of uncharacterised resistance mechanisms.
211
212 The distribution of erythromycin phenotypes was more complex. With this antimicrobial, we
213 observed both susceptible isolates and those with reduced susceptibility, but the latter
214 appeared to consist of two populations, one with MICs between 2 and 256 µg/ml and one
215 with MICs ≥512 µg/ml (Figure 1 ERY). We had sequence data available from 135 of the 2-256
216 µg/ml population and 66 of the ≥512 µg/ml population, and identified the presence of a
217 resistance gene (*ermA, ermC, msrA)* in 65 % (88/135) of the 2-256 µg/ml population and
218 52/66 (79 %) of the ≥512 µg/ml population (Table 2). Our results indicated that the presence
219 of *ermC* rather than *msrA* was the major cause of MICs exceeding 256 µg/ml. In the total
220 sequenced NAS collection *qac* was observed 100 times, with 64 isolates having a reduced
221 susceptibility to erythromycin (2-256 µg/ml population). Seven isolates had no other known
222 mechanisms. *qac* was identified 36 with an MIC of ≥512 µg/ml and in 23 of these cases *ermC*
223 was also present, only twice was *qac* found with no other known erythromycin resistance
224 mechanisms. A total of 101 isolates with an MIC ≥ 2 µg/ did not contain *qac.*
225
226 For daptomycin, approximately half the collection displayed reduced susceptibility (MIC ≥ 1
227 µg/ml, Figure 1 DAP and Table S1). A small subset, comprising seven isolates from clinical
228 samples only, displayed MICs ≥ 4 µg/ml; such high MICs to daptomycin have not been

229 previously reported, according to the European Committee on Antimicrobial Susceptibility
230 Testing (EUCAST) and The British Society for Antimicrobial Chemotherapy (BSAC)
231 surveillance data. These MICs were repeated a second time and confirmed. This is
232 concerning given that daptomycin is a current therapeutic choice for treating soft tissue
233 infections caused by NAS (21). Our ARIBA analysis (Table S2) indicated only a single *S.*
234 *epidermidis* isolate with an MIC of 1 µg/ml contained genes implicated in daptomycin
235 resistance: *gshF* and *liaFRS*. The remaining 169 isolates with an MIC ≥1 µg/ml did not
236 harbour any of these genes. Several mutations or genes are associated with daptomycin
237 resistance in *S. aureus* (including *mprF*, and SNPs in *rpoC*) but none of these were identified
238 in the NAS collection (6, 22). SNPs in *walK* have also been associated with daptomycin
239 resistance in *S. aureus* (23); this gene was identified by protein BLAST as present across the
240 NAS collection but with several differences at the protein level which raises doubts about
241 whether the SNPs observed in *S. aureus* can be directly extrapolated to NAS. We therefore
242 conclude that there is a potentially novel daptomycin resistance mechanism present in
243 these strains, which we are evaluating further.
244
245 *Resistance to vancomycin found in clinical samples*
246 Vancomycin is a treatment option in prosthetic joint infection, and 94 % of isolates had an
247 MIC below 4 µg/ml (Figure 1 VAN). However, of the 24 isolates with reduced susceptibility,
248 22 (92 %) came from clinical samples and only 2/24 were found in healthy volunteers. This is
249 indicative of a wider trend, where isolates associated with clinical samples had significantly
250 higher MICs ($p < 0.005$) than non-clinical isolates for cefoxitin, erythromycin, gentamicin,
251 tetracycline, daptomycin and vancomycin (Figure S1). Given the importance of NAS in
252 nosocomial infections, this is a worrying prospect both in terms of what is presenting in the

253 clinic and also the possibility of AMR gene transfer into organisms more capable of causing
254 infection, including *S. aureus*. In addition, no known mechanisms of resistance were
255 identified for vancomycin, rifampicin or teicoplanin (Figure S2 and Table S3).
256
257 *Over half of the NAS collection displayed susceptibility to multiple antimicrobials*
258 Out of the all the isolates tested, 49 % (187/394) had reduced susceptibility to three or more
259 antimicrobials. Twenty-four isolates had reduced susceptibility to six antimicrobials, and
260 three isolates had reduced susceptibility to seven antimicrobials; of these 23/24 and 3/3
261 were isolated from clinical samples (Table S1). The implications of these are difficult to treat
262 infections and potentially a large reservoir of staphylococcal resistance genes within the
263 patient under antimicrobial treatment.
264
265 *Animal isolates have similar MIC distributions to human isolates*
266 It is generally acknowledged that the presence of reduced susceptibility in microorganisms
267 isolated from animals can impact upon public health if those organisms also cause infection
268 in humans (24, 25). In our collection there were 40 NAS isolated from animals (including 7
269 NCTC strains), of which we obtained genome sequences from 29. Although in much fewer
270 numbers than the human isolates in the collection, the animal isolates displayed very similar
271 MIC distributions and harboured corresponding genetic mechanisms. This does not rule out
272 the possibility that animals could be a reservoir of AMR for staphylococci.
273
274 **Conclusion**
275 Genome analysis of isolates displaying MICs to cefoxitin of $\geq$ 4 µg/ml indicated that
276 approximately half harboured the *mecA* element. The absence of *mecA* from the other half

277 suggests that other mechanisms are likely present. This was apparent across many of the
278 antimicrobials tested as between 0 and 70 % of phenotypic resistance in clinical isolates
279 could be attributed to known resistance mechanisms. The remaining 30-100 % suggests that
280 there are potentially numerous unknown mechanisms underpinning NAS resistance, which
281 warrant further investigation.
282

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293 **Transparency declarations**

294 None to declare.

295

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376

377

378

379

380 **Figure legends**

381 Figure 1. Antibiotic susceptibility of NAS collection

382 MIC distributions for 400 NAS isolates grown in the presence of antimicrobials. FOX
383 cefoxitin, GEN gentamicin, TET tetracycline, ERY erythromycin, DAP daptomycin, VAN
384 vancomycin, RIF rifampicin and TEC teicoplanin.

385

386 Figure 2. Presence of *mecA* in relation to high cefoxitin MIC.

387 Per staphylococcal species, bars display the number of isolates found to have a cefoxitin
388 MIC of ≥4 µg/ml [red] or <4 µg/ml [peach] (4 isolates had no MIC data [blue], in relation to
389 the presence [stripe] and absence [dots] of *mecA*. Sum of the MIC bar is the total number of
390 isolates sequenced for that species.
391
392
393

394 Table 1

395 Frequency of non-*aureus* staphylococcal species in the study collection

Staphylococcus auricularis	1
Staphylococcus capitis	20
Staphylococcus caprae	2
Staphylococcus carnosus	2
Staphylococcus chromogenes	3
Staphylococcus cohnii	1
Staphylococcus condimenti	1
Staphylococcus devriesei	1
Staphylococcus epidermidis	191
Staphylococcus equorum	1
Staphylococcus haemolyticus	43
Staphylococcus hominis	45
Staphylococcus jettensis	1
Staphylococcus lugdunensis	7
Staphylococcus massiliensis	1
Staphylococcus microti	1
Staphylococcus muscae	1
Staphylococcus nepalensis	1
Staphylococcus pasteuri	4
Staphylococcus petrasii	1
Staphylococcus pettenkferi	1
Staphylococcus piscifermentans	1
Staphylococcus rostri	1
Staphylococcus saprophyticus	22
Staphylococcus sciuri	5
Staphylococcus simiae	1
Staphylococcus simulans	10
Staphylococcus sp [1]	1
Staphylococcus stepanovicii	1
Staphylococcus succinus	1
Staphylococcus vitulinus	3
Staphylococcus warneri	18
Staphylococcus xylosus	1

396

397 *Species designated by MALDI-TOF (Bruker)

398

399 Table 2 Genetic mechanisms of resistance identified with ARIBA and ABRIcate using CARD
400 database compared to the MIC data (of sequenced isolates only) using 90 % match ID
401 (partial and interrupted sequences are not included, see table S2)
402

Antimicrobial	*Mechanism (Accession No.)*	*No. isolates above breakpoint MIC (≥2 µg/ml)*		*No. isolates below breakpoint*
TET	*tetK* (NC_013452)	48/148 (32.4%)		8/223 (3.6 %)
	tetL (M11036.0)	1/148 (0.7 %)		0/223
	tetM (AM180355)	1/148 (0.7 %)		0/223
	All	**50/148 (33.8 %)**		**8/232 (3.6 %)**
		No. isolates above breakpoint MIC (≥1 µg/ml)		
GEN	*aac(6')-Ie-aph(2")-Ia* (NC_005024)	49/130 (37.7 %)		6/ 246 (2.4 %)
	aph(3)IIIa (CP004067) **All***	13/ 130 (10 %) **59 [g1]/130 (22.3%)**		7/ 246 (2.8 %) **12[g2] /246 (4.9 %)**
		MIC ≥512 µg/ml	*MIC ≥2 - 256 µg/ml*	
ERY	*msrA* (NC_022598.1)	6/ 66 (9 %)	75/135 (55.5 %)	9/171(5.2 %)
	ermC (M12730)	42/ 66 (63.6 %)	13/135 (9.6 %)	9/171 (5.2 %)
	ermA (NC_009632)	6/ 66 (9%)	1/135 (0.7 %)	0/171
	emeA (AB091338) **All***	1/ 66 (1.5 %) **52[e1] /66 (78.7 %)**	0/135 **88[e2]/135 (65.2 %)**	0/171 **18/171 (10.5 %)**

403
404 *Some isolates harboured multiple resistance genes. TET tetracycline, GEN gentamicin, ERY
405 erythromycin; g1: 3 - isolates contained multiple resistance genes; g2: 1 isolate contained
406 multiple resistance genes; e1: 3 isolates contained multiple resistance genes; e2; 1 isolate
407 contained multiple resistance genes
408

409 Supplementary data

410 Table S1. MIC characterisation of the NAS collection

411 MIC data for all isolates tested; all MIC are shown in µg/ml; FOX cefoxitin, GEN gentamicin,
412 TET tetracycline, ERY erythromycin, DAP daptomycin, VAN vancomycin, RIF rifampicin and
413 TEC teicoplanin.

414

415 Table S2. ARIBA characterisation of all sequenced NAS isolates, grouped by relevant
416 antimicrobial.

417

418 Table S3. Summary of ARIBA/ABRIcate match data for all sequenced isolates.

419

420 Table S4. Comparison of gene presence (using ARIBA) and protein BLAST presence. Data
421 includes all genes identified in the CARD database.

422

423 Figure S1. MICs in clinical and non-clinical isolates

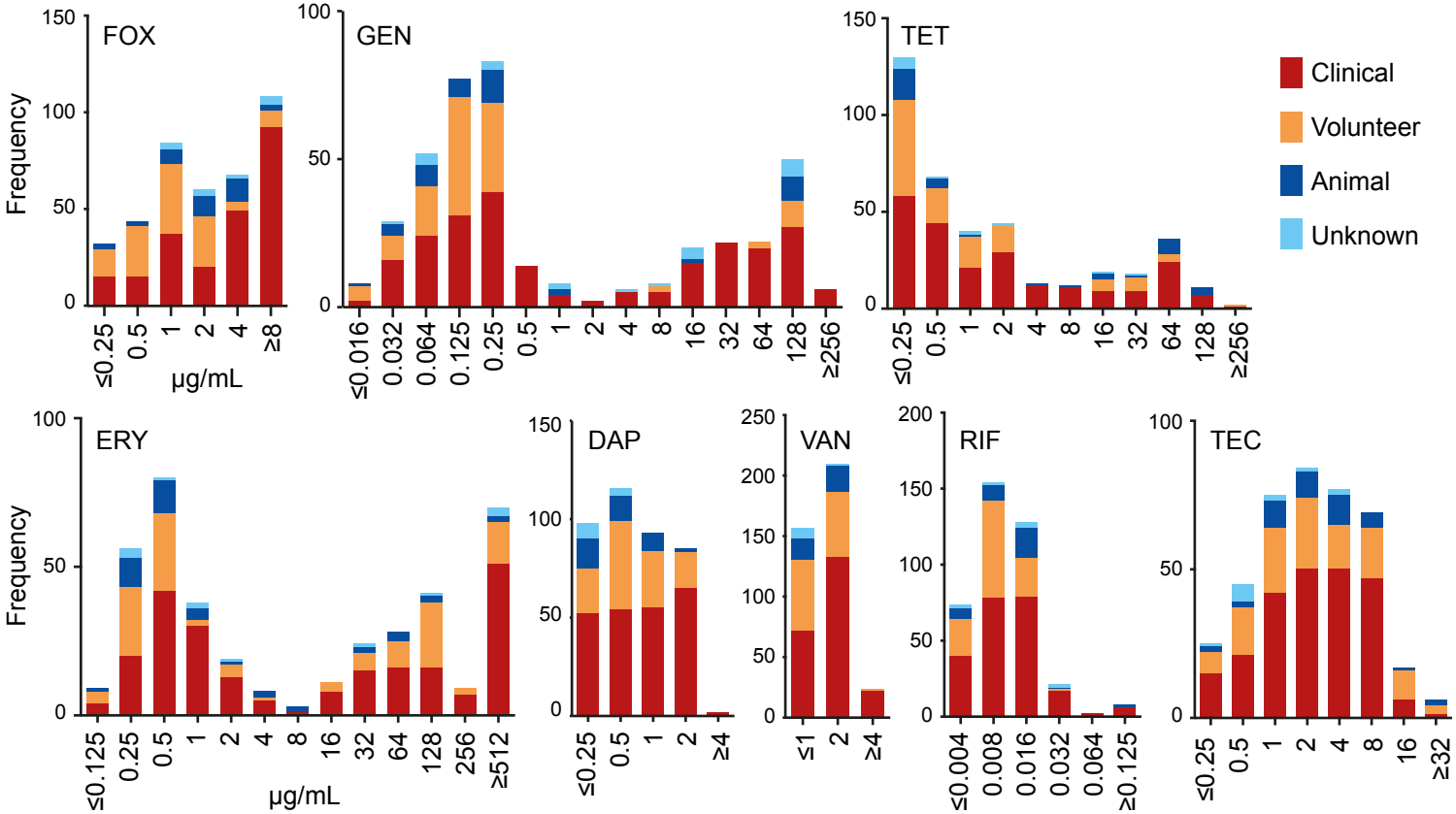
424 Box and whisker plot of MIC distribution per antimicrobial for clinical (orange) and non-
425 clinical (yellow) isolates. Thick black bar indicates the median MIC which resided within the
426 Interquartile Range. The extreme lines are represented as dotted lines and indicate the data
427 outside the upper (75%) and lower (25%) quartiles, open circles represent potential outliers.
428 Levels of significance from Mann-Whitney U test denoted by **** ($p<0.0001$), ***
429 ($p<0.005$), ** ($p=0.001$) and * ($p=0.01$).

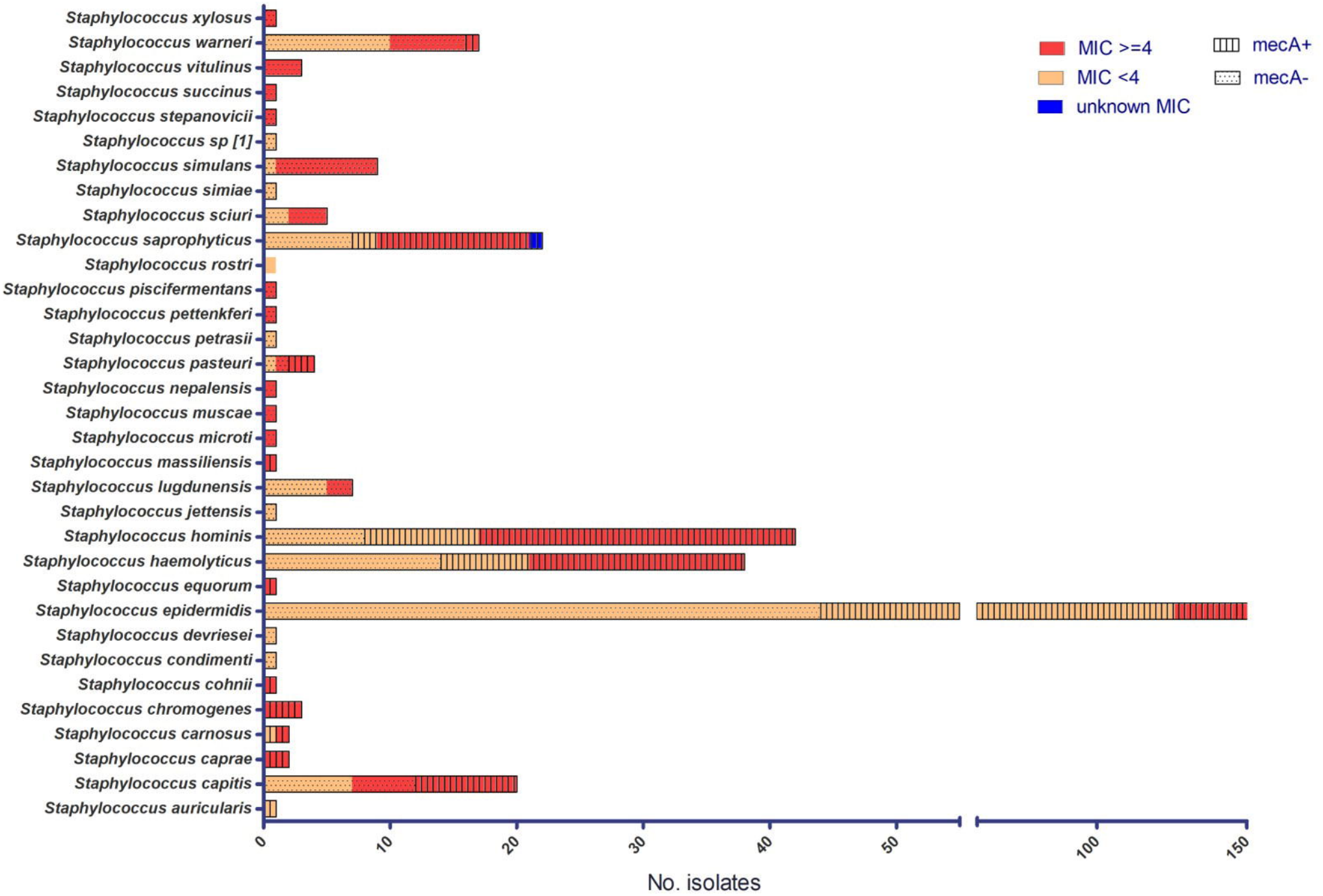
430

431 Figure S2. Mechanisms of resistance

432 Percentage of known genetic mechanisms identified in sequenced NAS isolates with
433 reduced susceptibility. Number of isolates with reduced susceptibility per antimicrobial

434 given in parentheses. Known mechanisms found per antimicrobial are shown in italics. FOX
435 cefoxitin, GEN gentamicin, TET tetracycline, ERY erythromycin, DAP daptomycin, VAN
436 vancomycin, RIF rifampicin and TEC teicoplanin.
437


FOX
GEN
TET
ERY
DAP
VAN
RIF
TEC
Frequency
μg/mL
Clinical
Volunteer
Animal
Unknown
≤0.25
0.5
1
2
4
≥8
≤0.016
0.032
0.064
0.125
0.25
8
16
32
64
128
≥256
≤0.125
256
≥512
≥4
≤1
≤0.004
0.008
0.016
0.032
0.064
≥0.125
≥32


Staphylococcus xylosus
Staphylococcus warneri
Staphylococcus vitulinus
Staphylococcus succinus
Staphylococcus stepanovicii
Staphylococcus sp [1]
Staphylococcus simulans
Staphylococcus simiae
Staphylococcus sciuri
Staphylococcus saprophyticus
Staphylococcus rostri
Staphylococcus piscifermentans
Staphylococcus pettenkferi
Staphylococcus petrasii
Staphylococcus pasteuri
Staphylococcus nepalensis
Staphylococcus muscae
Staphylococcus microti
Staphylococcus massiliensis
Staphylococcus lugdunensis
Staphylococcus jettensis
Staphylococcus hominis
Staphylococcus haemolyticus
Staphylococcus equorum
Staphylococcus epidermidis
Staphylococcus devriesei
Staphylococcus condimenti
Staphylococcus cohnii
Staphylococcus chromogenes
Staphylococcus carnosus
Staphylococcus caprae
Staphylococcus capitis
Staphylococcus auricularis
MIC >=4
MIC <4
unknown MIC
mecA+
mecA-
0
10
20
30
40
50
100
150
No. isolates