bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

1 **Title:** Trends and characteristics of multidrug resistant MRSA in

2 Norway 2008-2020

3

4 **Torunn Gresdal Rønning[1,2], Hege Enger[1], Jan Egil Afset[2,3], Christina Gabrielsen Ås [1,2]**

5 1. The Norwegian MRSA Reference laboratory, Department of Medical Microbiology, Clinic of

6 Laboratory Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

7 [2]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology,

8 Trondheim, Norway

9 [3]Department of Medical Microbiology, Clinic of Laboratory Medicine, St. Olavs Hospital, Trondheim

10 University Hospital, Trondheim, Norway

11

12 **Correspondence:**

13 Christina Gabrielsen Ås

14 christina.gabrielsen@stolav.no

15

16

17 **Short title**: MDR-MRSA in Norway 2008-2020

18

19 Keywords: ***Staphylococcus aureus*, MRSA, multidrug resistance, AMR, epidemiology, Norway**

20

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

21 # Abstract

22 Infections caused by multidrug-resistant (MDR) bacteria are recognized as a critical One Health
23 concern which poses a significant threat to public health, leading to increased morbidity and
24 mortality across both high- and low-income countries. In this study, we investigated the
25 epidemiology and molecular mechanisms of multidrug-resistant methicillin-resistant *Staphylococcus*
26 *aureus* (MDR-MRSA) strains identified in Norway from 2008 to 2020, in order to gain a better
27 understanding of the evolution and dissemination of multidrug resistance in *S. aureus*.

28 A total of 452 MDR-MRSA strains isolated from 429 individuals were analyzed from a dataset of
29 23,412 MRSA strains. Methods included epidemiological characterization, antimicrobial susceptibility
30 testing (AST) and genetic analysis of a selection of strains using nanopore sequencing to identify
31 antimicrobial resistance (AMR) genes and mutations, as well as their location on plasmids, SCC*mec*
32 and other mobile genetic elements (MGEs).

33 The study revealed an overall increasing trend in MDR-MRSA strains, with healthcare-associated
34 strains being more prevalent among MDR-MRSA compared to the overall MRSA population.
35 Significant heterogeneity in *spa*-types and clonal complexes exhibiting multidrug resistance was
36 observed, with high resistance rates against multiple antibiotic groups, particularly erythromycin,
37 ciprofloxacin/norfloxacin, tetracycline, gentamicin, and clindamycin in addition to cefoxitin. The
38 predominant MDR-MRSA clones included t1476/CC8, t127/CC1, t189/CC188 and t030, t037/CC239. A
39 broad range of AMR genes and mutations were detected, linked to a wide variety of MGEs,
40 highlighting the complex mechanisms of resistance development and dissemination within the MRSA
41 population.

42 This study highlights the rising challenge posed by MDR-MRSA strains, and reveals the multifactorial
43 nature of AMR in *S. aureus*, thus emphasizing the importance of continued surveillance, antibiotic

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

44 stewardship and infection control measures, as well as global cooperation, in order to combat the
45 spread of these multidrug-resistant pathogens.

46 Author Summary

47 In our study, we explored the landscape of multidrug-resistant methicillin-resistant *Staphylococcus*
48 *aureus* (MDR-MRSA) in Norway from 2008 to 2020. This research is possible because it draws on a
49 robust national surveillance system that has been active for over a decade, aimed at preventing the
50 establishment of these dangerous pathogens in our healthcare facilities. While the overall incidence
51 of MDR-MRSA was relatively low, we noticed an upward trend in the number of these resistant
52 strains over time. This pattern, along with shifts in the molecular profiles of the strains, suggests that
53 certain MDR-MRSA clones have become well-established and are spreading globally.

54 One of the most important findings was that the majority of MDR-MRSA strains were acquired
55 abroad. This indicates that international travel and migration are significant contributors to the
56 spread of these resistant strains, particularly from regions like Asia and Africa. This underscores the
57 necessity for global collaboration in surveillance and antibiotic stewardship to combat the threat
58 posed by these pathogens.

59 Additionally, we found that a high proportion of MDR-MRSA strains were associated with healthcare
60 settings, primarily isolated from patients during hospital admissions. This is concerning, as it suggests
61 that the most resistant strains are often found in hospitals, where vulnerable patients are at risk. The
62 high antibiotic exposures in these environments likely contributes to the selection and spread of
63 these resistant clones.

64 Interestingly, we discovered that many of the MDR-MRSA strains were detected in asymptomatic
65 carriers rather than in clinical infections. This could be due to the strains being acquired abroad and
66 subsequently identified through routine screening in healthcare settings. The overall potential

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

67 implications for public health are however significant, especially since the resistance profiles of these
68 strains can severely limit treatment options.

69 By utilizing advanced nanopore sequencing technology, we were able to delve deeper into the
70 genetic elements responsible for antibiotic resistance, highlighting the extensive heterogeneity of
71 resistance mechanisms among the MDR-MRSA strains. We found that resistance genes are primarily
72 located on plasmids and other mobile genetic elements, which enhances their potential for spread
73 among different strains. This complexity of resistance mechanisms and the adaptive strategies
74 employed by MRSA highlight the ongoing battle against antibiotic resistance.

75 In conclusion, our study sheds light on the evolving landscape of MDR-MRSA, emphasizing the need
76 for continued vigilance and coordinated efforts to mitigate the spread of these resistant strains.

77

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

78 # Introduction

79 *Staphylococcus aureus* colonizes the skin and mucosal surfaces of about 30 % of the human
80 population [1]. This bacterium is however also an important human pathogen, causing a wide range
81 of infections ranging from mild skin and soft-tissue infections to severe and invasive disease, such as
82 endocarditis, osteomyelitis, bloodstream infection and sepsis [2].

83 *S. aureus* is furthermore a bacterial pathogen which has the capasity to incorporate a wide variety of
84 mobile genetic elements (MGEs) making it able to adapt to different hosts and environments [3].
85 These MGEs, which include plasmids, transposons, bacteriophages and staphylococcal cassette
86 chromosome (SCC) elements, can facilitate the horizontal transfer of genes that encode important
87 virulence factors as well as antibiotic resistance determinants providing resistance against almost all
88 the clinically relevant groups of antibiotics.

89 Plasmids play a pivotal role in horizontal gene transfer, significantly contributing to the dissemination
90 of antimicrobial resistance (AMR) among bacteria [4]. Well-known examples in *S. aureus* include the
91 widely disseminated *blaZ*-encoding plasmids that provide resistance to penicillins [5]. Furthermore,
92 the aquistion of the staphylococcal cassette chromosome *mec* (SCC*mec*) carrying the *mecA* (or *mecC*)
93 gene provides resistance to all beta-lactam antibiotics defining *S. aureus* as methicillin-resistant
94 (MRSA) [6]. SCC*mec* can furthermore contain additional antibiotic resistance- and virulence genes
95 contributing to the adaptability and pathogenicity of MRSA strains [7, 8]. Bacteriophages, or phages,
96 are prevalent in the genome of most bacteria, often introducing additional genes that enhance
97 virulence and antibiotic resistance [9]. In human-adapted *S. aureus* strains, Sa3int phages are
98 particularly significant as they carry genes that help bacteria evade the immune system, thus
99 increasing their virulence [9]. These examples illustrate the importance of horizontal gene transfer
100 and MGEs in the dissemination of AMR and the evolution of bacterial pathogenicity in *S. aureus*.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

101 Infections caused by multidrug-resistant (MDR) bacteria, including MRSA, are recognized as a critical
102 One Health concern which poses a significant threat to public health [10]. These infections result in
103 increased mortality and morbidity across both high- and low-income countries [11]. To better
104 understand the mechanisms driving the spread of multidrug resistance in MRSA, this study aimed to
105 examine the epidemiology and molecular mechanisms of multidrug-resistant MRSA strains identified
106 in Norway in the period 2008 to 2020.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

107 # Results

108 ## Epidemiological characteristics of multidrug-resistant MRSA in Norway 2008-2020

109 A subset of 452 MDR-MRSA strains isolated from 429 persons were included in the study, from a
110 total of 23,412 MRSA strains (1.9 %) (Table 1) in the study period from 2008 through 2020. Although
111 the number of MDR-MRSA strains per year was low (ranging from 28 to 73) and with some
112 fluctuations, we observed an overall increasing trend (Fig 1), except for the COVID-19 pandemic year
113 2020. This coincided with an overall increase in the total number of MRSA strains in Norway in the
114 same period.

115

116 **Fig 1. Yearly number of MDR-MRSA strains in Norway in 2008-2020.**

117 Strains are classified as Healthcare-Associated (HA) or Community-Associated (CA) as indicated by
118 the coloured key and scale on the left axis. Total number of MRSA strains per year indicated by black
119 line, with scale on the right axis.

120

121 In total, 275 (60.8 %) of the MDR-MRSA strains were classified as carriage strains and 129 (28.5 %)
122 were classified as infection strains. Of the infection strains, the majority were associated with
123 wounds (74.4 %), abcesses (14.7 %) or pus (7.8 %). In total, only three strains were from invasive
124 infections (0.7 %). No information on sampling site was available for 48 (10.6 %) of the strains. In
125 total, 158 strains (35.0 %) were classified as healthcare-associated (HA), and 135 strains (29.9 %)
126 were from patients admitted to hospital. The remaining 294 strains (65.0 %) were classified as
127 community-associated (CA). Only two of the MDR-MRSA strains (0.4 %) were registered as related to
128 outbreaks.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

129 According to the registered place of acquisition for the MDR-MRSA strains, 13.1 % were aquired in
130 Norway, and 38.1 % were aquired abroad, while no information about place of acquisition was
131 available for 48.9 % of the strains. Of the strains that were acquired abroad, the majority (20.1 %)
132 were aquired in Asia, followed by Africa (8.4 %) and Europe (excluding Norway, 5.8 %).

133 The overall sex distribution of the MDR-MRSA strains was even, with 235 (52.0 %) from females, and
134 217 (48.0 %) from males. The mean age of persons was 40.6 years, with a median age of 38 years.
135 More than one strain was isolated from 18 individuals (4.2 %). The strains were isolated at varying
136 time intervals, ranging from one to six years between each collection. All strains exhibited consistent
137 *spa*-types (or in one case clonal complex) between isolate one and isolates two or three from the
138 same individual.

139 All MDR-MRSA strains in this study showed phenotypic resistance to cefoxitin and contained the
140 *mecA*-gene, while 20.4 % (n=92) of strains contained the virulence factor and epidemiological marker
141 Panton-Valentine leucocidin (PVL).

142

143 **Table 1. Epidemiological and molecular characteristics of the strains included in the study,**
144 **compared to data from Rønning *et al.* [12] (2024).**

Study	**Current study**		**Rønning *et al.* 2024**	
Study period	2008-2020		2008-2017	
Inclusion criteria	MDR-MRSA strains		All MRSA strains	
	N	**%**	**N**	**%**
Strains	452	100.0 %	15200	100.0 %
Persons	429	100.0 %	14386	100.0 %
Female	235	52.0 %	7173	49.9 %
Male	217	48.0 %	7211	50.1 %
Mean age	40.6	-	36.0	-
Median age	38.0	-	31.0	-
Carriage	275	60.8 %	7780	51.2 %
Infection	129	28.5 %	5407	35.6 %
Unknown	48	10.6 %	1516	10.0 %
Invasive infections	3	0.7 %	122	0.8 %
Healthcare-associated	158	35.0 %	4566	30.0 %
Admitted to hospital	135	29.9 %	3004	19.8 %
Nursing home	6	1.3 %	629	4.1 %
Healthcare personell	17	3.8 %	933	6.1 %
Community-associated	294	65.0 %	10634	70.0 %
Outbreak-related	2	0.4 %	299	2.0 %
Single strain	411	95.8 %	13689	95.2 %

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Multiple strains	18	4.2 %	1394	9.2 %
PVL positive	92	20.4 %	5163	34.0 %
PVL negative	360	79.6 %	8483	55.8 %
Place of acquisition				
Not registered	221	48.9 %	7123	47 %
Norway	59	13.1 %	4199	28 %
Abroad	172	38.1 %	3878	26 %
Africa	38	8.4 %	430	3 %
Asia	91	20.1 %	1872	3 %
Europe (excl. Norway)	26	5.8 %	896	6 %
North-America	6	1.3 %	119	0.8 %
Oceania	1	0.2 %	26	0.2 %

145

146 **Successful MDR-MRSA clones and phenotypic antimicrobial susceptibility profiles**

147 Of the 452 MDR-MRSA strains included in the study, 361 (79.9 %) showed antibiotic resistance
148 towards five different antibiotic groups, while 70 (15.5 %) demonstrated resistance against six
149 antibiotic groups. Furthermore, 17 strains (3.8 %) displayed resistance against seven antibiotic
150 groups, and four strains (0.9 %) showed antibiotic resistance against eight antibiotic groups (Fig 2).

151

152 **Fig 2. Core genome phylogeny of whole genome sequenced MDR-MRSA strains (n=101).**
153 The nodes within the tree are assigned distinct colors based on clonal complex and SCC*mec* type.
154 Phenotypic resistance profiles based on AST are visually represented through coloured boxes (box
155 with fill indicates resistant, while outlined box indicates intermediate resistance).

156

157 For strains showing antibiotic resistance against five or six antibiotic groups, we observed a very
158 heterogeneous collection of *spa*-types. Strains resistant to 5 antibiotic groups belonged to more than
159 50 distinct *spa*-types of 22 different CCs, while strains resistant to six antibiotic groups belonged to
160 22 *spa*-types of 9 different CCs. Conversely, in strains showing resistance against seven or eight
161 antibiotic groups, a very limited number of *spa*-types were observed. These included *spa*-types t008,
162 t030, t034, t037, t064, t1476 and t451, belonging to clonal complexes 8, 239 and 398.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

163 Overall, phenotypic antibiotic susceptibility testing revealed almost universal resistance towards
164 erythromycin (93.1 %) and ciprofloxacin/norfloxacin (92.9 %) in addition to cefoxitine (100.0 %) (Fig
165 3). High levels of resistance were also observed to tetracycline (83.9 %), gentamicin (81.7 %), and
166 clindamycin (69.3 % total). 175/452 (38.7 %) of the strains showed constitutive resistance against
167 clindamycin, while 140 (31.0 %) showed inducible resistance against clindamycin. Moderate to low
168 levels of resistance were observed for fusidic acid (27.8 %), trimethoprim-sulfamethoxazole (19.4 %),
169 rifampicin (13.2 %) and mupirocin (10.0 %). No isolates were resistant towards linezolid (0.0 %) or
170 vancomycin (0.0 %).

171

172 **Fig 3. Phenotypic susceptibility of all MDR-MRSA strains categorized as resistant, intermediate or**
173 **susceptible towards tested antibiotics.**

174

175 Of the most successful MDR-MRSA *spa*-types over the course of the study period were t127/CC1,
176 t189/CC188, t030, t037/CC239, and t1476/CC8 (Fig 4). Collectively, these *spa*-types accounted for
177 45.1 % (204/452) of the strains included in this study.

178 MDR-MRSA t1476/CC8 (n=63, 13.9 %) was the most successful clone in the study period (Fig 4). The
179 number of strains which belonged to this genotype increased considerably from 2008 to 2019 (from
180 0 strains in 2008 to 29 strains in 2019). In this group a majority of cases were from females (65.1 %),
181 and most of the strains were from carriage (76.2 %). Based on country of acquistion, 38.1 % of the
182 strains were associated with countries in Sub-Saharan Africa, while 42.9 % had no record of
183 aqcuisition (Fig 5). All the strains were resistant against beta-lactams, aminoglycosides,
184 fluoroquinolones and tetracyclines. The majority were additionally resistant against macrolides
185 (n=62, 98.4 %) (Table 2).

186 **Fig 4. Yearly relative distribution of MDR-MRSA *spa*-types (>5 per year) in the period 2009-2020.**

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

187 The years 2010 and 2011 are excluded due to missing data. Major *spa*-types are highlighted.

188

189 **Fig 5. Map showing the country of aquisition for the major MDR-MRSA clones.**

190 The major clones include t1476 (green), t127 (blue), t189 (yellow) and t030/t037 (red), and the

191 number of strains is indicated by the size of the circles as shown by the key.

192

193 **Table 2. Proportion of strains resistant to the tested antibiotics, for the four most prevalent MDR-**

194 **MRSA clones.** Color gradient from red to green indicates high to low percentage of resistant strains

195 accordingly. ICR, inducible clindamycin resistance; TMP-SMX, trimethoprim-sulfamethoxazole.

		Cefoxitin	Erytromycin	Clindamycin	ICR	Fusidic acid	Gentamicin	TMP-SMX	Tetracycline	Norfloxacin/ ciprofloxacin	Rifampicin	Mupirocin	Linezolid	Vancomycin
spa-type	No. of isolates	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
t1476	63	100.0	96.7	9.3	79.4	0.0	1.6	100.0	100.0	100.0	1.6	1.6	0.0	0.0
t030, t037	56	100.0	87.5	50.9	19.6	14.3	50.0	98.2	92.9	92.9	66.1	1.8	0.0	0.0
t127	43	100.0	95.3	4.7	20.9	97.7	2.3	74.4	51.2	76.7	0.0	0.0	0.0	0.0
t189	42	100.0	100.0	100.0	0.0	2.4	9.5	88.1	100.0	100.0	0.0	0.0	0.0	0.0

196

197 MDR-MRSA t127/CC1 was the second most frequent *spa*-type in this study, accounting for 43 out of

198 452 strains (9.5 %). All of these strains were resistant against five antibiotic groups (Table 2), the

199 most common profile being resistance to beta-lactams, MLS, tetracyclines, fusidanes and

200 fluoroquinolones (n=20, 46.5 %). The sex distribution was 48.8 % female and 51.1 % male, and the

201 proportion of carriage (51.1 %) was similar to infections (48.8 %). For most of the cases from whom

202 MDR-MRSA t127/CC1 strains were isolated, there was no record of place of aquisition (79.0 %).

203 Known countries of aquisition however included European as well as Asian and African countries (Fig

204 5).

205 MDR-MRSA t189/CC188 was the third most frequent *spa*-type in this study, accounting for 42 out of

206 452 strains (9.3 %). These strains showed resistance against five antibiotic groups, the most common

207 profile included resistance to beta-lactams, MLS, aminoglycosides, tetracyclines and

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

208 fluoroquinolones (n=37, 88.1 %) (Table 2). The proportion of female cases was high in this group
209 (69.0 %), and carriage (57.1 %) was more frequent than infection (40.5 %). For 35.8 % of strains, the
210 place of acquistion were Southeast Asian countries (Fig 5).

211 MDR-MRSA t037/CC239 and t030/CC239 were the fourth (n=36, 8.0 %) and fifth (n=20, 4.4 %) most
212 frequent *spa*-types in this study. These were most frequent at the start of the study period, while the
213 number of strains declined in more recent years. These two *spa*-types belong to the same clonal
214 complex (CC239) and share similar phenotypic antibiotic resistance patterns. A majority of strains in
215 the two groups were resistant towards tetracyclines (98.2 %), aminoglycosides (92.9 %), MLS (87.5
216 %), fluoroquinolones (92.9 %), TM/S (50.0 %) and ansamycins (66.1 %) (Table 2). Notably, two strains
217 (3.6 %), both t037, were resistant against all the antibiotics tested except for linezolid and
218 vancomycin. In contrast to the other successful clones, there were more men (64.3 %) than women
219 (42.9 %) in this group, and a majority of strains were from carriage (62.5 %). 42.9 % of the strains
220 were registrered as acquired in Asian countries (Northern, Western, Southern, and South-eastern
221 subregions) (Fig 5).

222

223 **Genotypic resistance determinants associated with different groups of antibiotics**

224 For the sequenced MDR-MRSA strains (n=101), the presence of AMR-associated genes and mutations
225 were predicted using the AMRFinder Plus tool and database. In total, 39 different AMR genes and 31
226 different AMR mutations were identified, with a median of 10 (range 7-15) different genes and 3
227 (range 1-10) mutations per strain. In accordance with phenotypic restistance data, the most common
228 AMR determinants detected provided resistance against beta-lactams, MLS, fluoroqinolones,
229 tetracyclines and aminoglycosides (Fig 2, Table 3). The specific resistance determinants associated
230 with each group of antibiotics are more closely described in the following subsections.

Table 3: AMR traits detected in MDR-MRSA strains in Norway, and association with clonal complex and detected MGEs. AMR phenotype is given as percentage of strains with the specific AMR trait where AST is interpreted as resistant (R), susceptible with increased exposure (I) or susceptible (S) towards the specific antibiotic(s) tested in each group: GEN, gentamicin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; ICR, Inducible clinidamycin resistance; FUS, fusidic acid, NOR, norfloxacin; CIP, ciprofloxacin; RIF, rifampicin; FOX, cefoxitin; TMP-SMX, Trimethoprim-sulfamethoxazole; MUP, mupirocin; LIN, linezolid.

Antibiotic group	Gene/ variant	Mutation/ MGE	AMR Phenotype						Clonal complexes												MGE(s)				
			R ERY	S ERY	R CLI	S CLI	R ICR	S ICR	1	5	8	9	30	59	72	80	188	239	398	672	SCCmec	IS/Tn	Plasmid	Plasmid name (mobsuite)	Phage
MLS	*erm(A)*	MGE	31.9 %	0.0 %	46.3 %	18.3 %	12.5 %	37.7 %		5								239			SCCmec II, III	Tn554			
	erm(B)	MGE	14.9 %	0.0 %	34.1 %	0.0 %	0.0 %	20.3 %						59			188					Tn551, Tn917	non-mobilizable, mobilizable plasmid	AB973	
	erm(C)	MGE	36.2 %	0.0 %	17.1 %	45.0 %	81.3 %	11.6 %	1	5	8	9			72	80		239	398				non-mobilizable plasmid	AA411, AA770, AC627	
	mph(C)	MGE	18.1 %	0.0 %	0.0 %	28.3 %	6.3 %	21.7 %	1	5	8		30							672	SCCmec IV		conjugative, non-mobilizable and mobilizable plasmid	AB628, AA411, AA840	
	msr(A)	MGE	18.1 %	0.0 %	0.0 %	28.3 %	6.3 %	21.7 %	1	5	8		30							672	SCCmec IV		conjugative, non-mobilizable and mobilizable plasmid	AB628, AA411, AA840	
	vga(A)	MGE	1.1 %	0.0 %	2.4 %	0.0 %	0.0 %	1.4 %											398				mobilizable plasmid	AA003	
	lsa(E)	MGE	1.1 %	28.6 %	7.3 %	0.0 %	0.0 %	4.3 %				9							398				non-mobilizable plasmid	AA409	
	lnu(A)	MGE	9.6 %	14.3 %	4.9 %	13.3 %	3.1 %	13.0 %	1	5	8		30				188						conjugative, non-mobilizable and mobilizable plasmid	AA083, AB631, AB840, AB924, AB973, AC333, AA411, AB631	
	lnu(B)	MGE	1.1 %	28.6 %	7.3 %	0.0 %	0.0 %	4.3 %				9							398				non-mobilizable plasmid	AA409	
	lmrS	Core	100.0 %	85.7 %	100.0 %	98.3 %	100.0 %	98.6 %	1	5	8	9	30	59	72	80	188	239	398	672					
			R TET	S TET																					
Tetracyclines	*tet(K)*	MGE	56.8 %	0.0 %					1	5	8		30	59	72	80	188	239	398		SCCmec III and IV		non-mobilizable, mobilizable plasmid	AB924, AB973, AC333, AA411, AA770, AA841, AA850	
	tet(M)	MGE	48.9 %	0.0 %						5	8	9						239	398		SCCmec IX	Tn6224			
	tet(L)	MGE	3.4 %	0.0 %					1										398				non-mobilizable, mobilizable plasmid	AA411, AA764	
	tet(38)	Core	100.0 %	100.0 %					1	5	8	9	30	59	72	80	188	239	398	672					
			R FUS	S FUS																					
Fucidanes	*fusB*	MGE	4.2 %	0.0 %												80							mobilizable plasmid	AA770	
	fusC	MGE	45.8 %	0.0 %					1				30							672	SCCmec I, IV and V				
	FusA L461K	Mutation	37.5 %	0.0 %						5	8							239							
			R FOX	S FOX																					
Beta-lactams	*mecA*	MGE	100.0 %	0.0 %					1	5	8	9	30	59	72	80	188	239	398	672	SCC*mec*				
	blaZ	MGE	71.3 %	0.0 %					1	5	8		30	59	72	80	188	239	398	672		Tn552	non-mobilizable, mobilizable plasmid	AB628, AB924, AB973, AA409, AA411, AA770, AA840, AA841, AA848, AA850, AA069, AA764	
	blaPC1	MGE	30.7 %	0.0 %							8		30					239			SCCmec IV	Tn552	mobilizable plasmid	AA069, AA850	
			R GEN	S GEN																					
Aminoglycosides	*aac(6')-Ie/ aph(2'')-Ia*	MGE	96.6 %	25.0 %					1	5	8	9	30	59	72		188	239	398	672	SCCmec IV and V		conjugative, non-mobilizable and mobilizable plasmid	AB110, AB083, AB924, AB973, AA409, AA840, AA850	
	ant(9)-Ia	MGE	33.7 %	16.7 %						5								239	398		SCCmec II, III	Tn554			
	ant(6)-Ia	MGE	36.0 %	25.0 %					1	5	8	9	30	59		80		239	398	672	SCCmec IV		non-mobilizable, mobilizable plasmid	AB628, AA411, AA840, AA850, AA409	
	aph(3')-IIIa	MGE	36.0 %	25.0 %					1	5	8		30			80		239		672	SCCmec IV		non-mobilizable, mobilizable plasmid	AB628, AA411, AA840, AA850, AA764	

	aadD1	MGE	9.0 %	41.7 %					1	5	8		30				188		398		SCCmec IV		conjugative and mobilizable plasmid	AA083, AB924, AA973, AC333, AA411	
	str	MGE	2.2 %	0.0 %													188						mobilizable plasmid	AA010	
	spw	MGE	1.1 %	0.0 %								9											non-mobilizable plasmid	AA409	
	aac(6')-Ie	MGE	1.1 %	0.0 %													188						mobilizable plasmid	AB973	
			R TMP-SMX	**S TMP-SMX**																					
Folate pathway inhibitors	*dfrK*	MGE	3.1 %	0.0 %															398			Tn559			
	dfrG	MGE	37.5 %	8.7 %					1	5	8	9	30		72			239		672					phiSa2wa, ECel-2020q
	dfrS1	MGE	15.6 %	5.8 %							8		30					239	398		SCCmec IV		mobilizable plasmid	AA850	
	dfrE	MGE	3.1 %	7.2 %													188						mobilizable plasmid	AB973, AA843	
	FolP F17L	Mutation	37.5 %	4.3 %							8							239							
	FolP E208K	Mutation	18.8 %	0.0 %							8														
	FolA L21V	Mutation	6.3 %	0.0 %														239							
	FolA F99Y	Mutation	9.4 %	0.0 %														239							
			R RIF	**S RIF**																					
Ansamycins	RpoB H481N	Mutation	86.2 %	0.0 %							8							239							
	RpoB L466S	Mutation	27.6 %	0.0 %														239							
	RpoB A473T	Mutation	17.2 %	0.0 %														239							
	RpoB A477T	Mutation	17.2 %	0.0 %														239							
	RpoB A477D	Mutation	3.4 %	0.0 %														239							
	RpoB I527M	Mutation	13.8 %	0.0 %							8														
	RpoB I527L	Mutation	3.4 %	0.0 %														239							
	RpoB S529L	Mutation	6.9 %	0.0 %														239							
			R CIP/ NOR	**S CIP/ NOR**																					
Fluoro-quinolones	GyrA S84L	Mutation	97.9 %	0.0 %					1	5	8	9			72	80	188	239	398	672					
	GyrA S85P	Mutation	1.1 %	0.0 %														239							
	GyrA G106D	Mutation	8.5 %	0.0 %														239							
	GyrA E88G	Mutation	7.4 %	0.0 %						5															
	ParC S80Y	Mutation	36.2 %	0.0 %					1	5	8							239							
	ParC S80F	Mutation	62.8 %	0.0 %					1	5	8				72	80	188	239	398	672					
	ParC E84K	Mutation	8.5 %	0.0 %						5								239							
	ParC E84G	Mutation	1.1 %	0.0 %														239							
	ParE D432V	Mutation	1.1 %	0.0 %														239							
	ParE P451S	Mutation	2.1 %	0.0 %														239							
	ParE P585S	Mutation	2.1 %	0.0 %						5															
			R MUP	**S MUP**																					
Carboxylic acids	*mupA*	MGE	100.0 %	0.0 %						5	8		30					239					Conjugative plasmid	AA083	
	IleS V588F	Mutation	20.0 %	1.0 %							8							239							
			R LIN	**S LIN**																					
Oxazolidinones	23S C2220T (single copy)	Mutation	0.0 %	1.0 %														239							
	23S C2534T (single copy)	Mutation	0.0 %	2.0 %						5															

235

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

236 ***Beta-lactam resistance***

237 All MDR-MRSA strains included in this study (n=452) harboured the *mecA*-gene. In addition, we
238 detected penicillin resistance genes in 98.0 % of the sequenced strains (Table S1). Of these, 71.3 %
239 harboured the *blaZ*-gene, and 26.7 % contained *blaPC1*. 4 % of strains had *mecA*, *blaZ* and *blaPC1*
240 genes, while two strains (2 %) solely harboured the *mecA*-gene.

241 ***Macrolide and lincosamide resistance***

242 Almost all (95.0 %) of the sequenced MDR-MRSA strains were phenotypically resistant against
243 erythromycin and clindamycin (Table S1). This was however linked to different genes and gene
244 combinations. The most common gene was *erm(C)*, detected in 35.4 % of MLS-resistant strains (Table
245 3, Fig 6), followed by *erm(A)*, detected in 31.3 % of MLS-resistant strains. The different genetic
246 profiles were not associated with distinct MLS phenotypic profiles, but rather included erythromycin
247 resistance with either constitutive or inducible resistance or susceptibility to clindamycin. The *erm(B)*
248 gene was found in 14 strains (Table 3, Table S1), and all of these had a phenotypic profile of
249 erythromycin resistance and constitutive clindamycin resistance.

250

251 **Fig 6. Upset plot showing all combinations AMR genes and mutations (set) associated with the**
252 **major antibiotic groups in sequenced MDR-MRSA strains.**

253 The major antibiotic groups include aminoglycosides, tetracyclines, macrolide, lincosamide and
254 streptogramin (MLS), fucidanes, fluoroquinolones and ansamycins. Strains (intersection) are colored
255 according AST, interpreted as resistant (R), susceptible with increased exposure (I) or susceptible (S)
256 towards the specific antibiotic(s) tested in each group: GEN, gentamicin; TET, tetracycline; ERY,
257 erythromycin; CLI, clindamycin; FUS, fusidic acid, NOR, norfloxacin; CIP, ciprofloxacin; RIF, rifampicin.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

258 The combination of *mph(C)* and *mrs(A)* was present in the erythromycin resistant and inducible
259 clindamycin resistant-profile, and the only erythromycin resistant profile (Fig 6). The rarest genes
260 associated with MLS resistance were *vga(A)* and the combination of *lsa(E)* and *lnu(B),* with the
261 phenotypic profile erythromycin susceptible and constitutive clindamycin resistance. The gene *lmrS*
262 was present in both erythromycin resistant and susceptible strains, and did not appear to provide
263 phenotypic resistance to erythromycin at levels sufficient for detection by the methods used in this
264 study.

265 ***Ciprofloxacin/norfloxacin resistance***

266 Of the sequenced MDR-MRSA strains, 94 (93.1 %) exhibited phenotypic resistance to ciprofloxacin or
267 norfloxacin. The most frequent mutations found associated with quinolone resistance were GyrA
268 S84L (91 strains, 90.1 %), ParC S80F (58 strains, 57.4 %) and ParC S80Y (34 strains, 33.7 %) (Table 3,
269 Table S1). Combinations of quinolone-conferring mutations were found in 92 strains (91.1 %). The
270 most frequent combinations of mutations were GyrA S84L and ParC S80F (found in 46 strains, 45.5
271 %) (Fig 6) and GyrA S84L and ParC S80Y (found in 26 strains, 25.7 %). Mutations in the chromosomal
272 *gyrA, parC* and *parE* genes were found in all quinolone-resistant strains, among multiple *spa*-types of
273 different clonal complexes. This thus appears to be a quite common adaptation in the general MDR-
274 MRSA population to acquire quinolone resistance.

275 ***Tetracycline resistance***

276 88 (87.1 %) of sequenced MDR-MRSA strains exhibited phenotypic resistance to tetracycline, having
277 either the *tet(M)* (42.6 %), *tet(K)* (49.5 %) or in a few cases the *tet(L)* (3.0 %) genes in different
278 combinations (Table 3, Table S1, Fig 6). In two strains we did not detect any gene(s) likely causing
279 tetracycline resistance. Previously described chromosomal mutations associated with tetracycline
280 resistance in MepA (N369Y), RpsJ (Y58D) or 16S rDNA, were not identified in any of the strains. The
281 *tet(38)*-gene was detected in all the sequenced strains in this study, but did not appear to cause

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

282 phenotypic tetracycline resistance at levels sufficient for detection by the methods used in this study
283 (Table 3).

284 ***Gentamicin resistance***

285 Of the sequenced MDR-MRSA strains, 89 (88.1 %) exhibited phenotypic resistance to gentamicin. The
286 most common gene encoding aminoglycoside resistance were *aac(6')-Ie/aph(2'')-Ia*, found in 86 of
287 the 89 (96.9 %) (Table 3, Table S1). 35 strains (34.7 %) had the *aph(3')-IIIa*-gene encoding
288 amikacin/kanamycin resistance, 35 strains (34.7 %) had *ant(6)-Ia*-gene encoding streptomycin
289 resistance and 32 (31.7 %) had *ant(9)-Ia*-gene encoding spectinomycin resistance. 13 strains (12.9 %)
290 had *aadD1*-gene encoding kanamycin/tobramycin resistance. Single strains had the *aac(6')-Ie*-gene
291 encoding amikacin/kanamycin/tobramycin-resistance and the aminoglycoside *spw*-gene, and three
292 strains had the *str*-gene encoding streptomycin. The most frequently found combination of
293 aminoglycoside-genes was *aac(6')-Ie/aph(2'')-Ia* together with the *ant(9)-Ia*-gene, found in 18 strains
294 (17.8 %) (Fig 6).

295 ***Trimethoprim-sulfamethoxazole resistance***

296 Overall, 34 (33.7 %) of sequenced MDR-MRSA strains exhibited phenotypic resistance to TMP-SMX.
297 As phenotypic testing is performed with the TMP-SMX combination drug, and resistance to both
298 components separately is required for resistance, the detection and interpretation of genes and
299 mutations contributing to TMP-SMX resistance can be complex. While we observed full concordance
300 between phenotypic susceptibility and lack of previously described genes/mutations encoding TMP-
301 SMX resistance, there was only a 23/34 (67.6 %) concordance between phenotypic resistance and
302 having a combination of genes/mutations previously reported to confer phenotypic TMP-SMX
303 resistance (Table 3, Table S1). In the concordant cases cases, the *drfG, dfrS1, dfrK* or *dfrE* genes
304 encoding trimetoprim resistance were detected, either in a single, two or three copies, toghether
305 with the F17L and E208K-mutations in FolP providing sulfametoxazole resistance (Table S1).

306 ***Rifampicin resistance***

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

307 Overall, 26 (25.7 %) of sequenced MDR-MRSA strains exhibited phenotypic resistance to rifampicin.
308 Chromosomal mutations in *rpoB* conferring rifampicin resistance described by Guerillot *et al.* were
309 found in 26 of the sequenced strains [13]. The most common mutations were RpoB H481N alone or
310 in combination (Table 3, Fig 6, Table S1). The chromosomal *rpoB*-mutations conferring rifampicin
311 resistance seemed to be clonal, and were found only in strains belonging to CC8 and CC239, which
312 included the most resistant strains in this study with resistance towards seven or eight antibiotic
313 groups. The H481N mutation has furthermore been reported to promote the emergence of a
314 subpopulation of small colony variants with reduced susceptibility to vancomycin and daptomycin.
315 Although not investigated here, this raises an additional concern regarding the use of rifampicin
316 treatment and the possible effect on emergence of MDR-MRSA strains.

317 ***Fusidic acid resistance***

318 Of the sequenced MDR-MRSA strains, 25 (24.8 %) exhibited phenotypic resistance to fusidic acid. The
319 most common gene that was associated with fusidic acid resistance was *fusC*, found in 11 strains
320 (Table 3, Table S1). The *fusB*-gene was present in one strain (t044, CC80). The *fusA* L461K mutation
321 was found in nine strains, were *spa*-type t037 was the most frequent *spa*-type. For three strains, we
322 did not detect any genetic determinants likely causing phenotypic fusidic acid resistance. These
323 strains however had disk diffusion inhibition zones close to the susceptibility breakpoint (S>24 mm).
324 There was 100 % concordance for phenotypic susceptibility to fusidic acid and having no genetic
325 determinants detected.

326 ***Mupirocin resistance***

327 Of the sequenced MDR-MRSA strains, five (5.0 %) exhibited phenotypic resistance to mupirocin
328 (Table 3, Table S1). All of these strains contained the *mupA*-gene. One strain had two copies of the
329 gene, and one strain furthermore had the chromosomal *IleS* V588F mutation in addition to the
330 *mupA*-gene. The V588F IleS-mutation was however also detected in one mupirocin susceptible strain.

331 ***Linezolid resistance***

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

332 No phenotypic resistance to linezolid was observed in this study, and no genes associated with
333 linezolid resistance (*cfr, optrA, poxtA*) were detected in the sequenced MDR-MRSA strains. Linezolid
334 resistance may also be caused by mutations in copies of the 23S rDNA genes (7). We identified two
335 mutations associated with linezolid resistance in three MDR-MRSA strains; C2192T (n=1) and C2534T
336 (n=2) [14, 15] (Table 3). However, neither of the strains demonstrated phenotypic resistance when
337 exposed to linezolid, as all had mutations in only a single copy of the gene.

338

339 **Mobile genetic elements associated with antibiotic resistance determinants**

340 For the sequenced MDR-MRSA strains (n=101), the presence of AMR genes within specific types of
341 MGEs were investigated. The specific resistance determinants associated with each type of MGE are
342 more closely described in the following subsections.

343 ***Plasmids***

344 In the present study, a total of 191 plasmids were identified, in 83.2 % of the sequenced MDR-MRSA
345 strains. The number of plasmids per strain varied from 1 to 6, with a median of 2 plasmids per strain,
346 and a median size of 10,318 bp. The mobilizable group of plasmids included both the largest and
347 some of the smallest plasmids, ranging in size from 1,299-98,879 bp (Fig S1). A majority of the
348 plasmids were previously described in *S. aureus* (86.4 %) or other Staphylococci (10.5 %). However,
349 plasmids previously described in *Escherichia coli* (2.1 %), *Lactobacillus pentosus* (0.5 %) and
350 *Salmonella enterica* 0.5 %) were also detected.

351 The plasmids were clustered into 29 sub communities using Pling, while two of the nodes were
352 excluded due to likely being partial plasmids or transposons. Among these sub communities, 15
353 comprised of multiple plasmids while 17 were singletons. The largest sub community included 117
354 plasmids, indicating that a majority of the plasmids in this study were related. The largest sub
355 community included plasmids from strains of 25 different *spa*-types and 11 different sequence types.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

356 These plasmids encoded various combinations of 21 different AMR-genes, covering resistance
357 against beta-lactams, MLS, aminoglycosides, trimethoprim, tetracyclines, fosfomycins and fusidanes.
358 The second largest sub community included 11 plasmids, from strains with five different *spa*-types,
359 and four different sequence types. These plasmids contained combinations of five different AMR-
360 genes, covering resistance against beta-lactams, aminoglycosides, lincosamide and mupirocin.

361 The majority of plasmids were convergent plasmids (48.4 %), carrying combinations of AMR-, stress
362 and virulence-genes (e.g. AA411) (Table S2). Some of the detected plasmids were however strictly
363 AMR- (32.3 %), stress- (12.9 %) or virulence-plasmids (6.5 %), carrying genes of only one specific
364 category (e.g. AC627, AA851 and AA379, respectively). In total, 112 plasmids (58.6 %) carried one or
365 several AMR genes, with a median of 4 AMR genes per plasmid (ranging from 1 to 14). The AMR
366 genes most commonly found on plasmids were *blaZ* (n=45), *aac(6')-Ie/aph(2'')-Ia* (n=30), *erm(C)*
367 (n=26) and *tet(K)* (n=24). The *blaZ* gene was found on 15 different plasmids, the *aac(6')-Ie/aph(2'')-Ia*
368 on nine different plasmids, the *erm(C)* gene on three different plasmids and the *tet(K)* gene on eight
369 different plasmids (Fig 7). Other genes were only found on specific plasmids; e.g. *mup(A)* was only
370 found in the conjugative plasmid AA083. The most common AMR plasmids identified in the MDR-
371 MRSA strains were the non-mobilizable plasmid AC627 (n=20), and the mobilizable plasmids AA411,
372 AB973 and AC333 (n=13). Conjugative AMR plasmids included the AA083 (n=4) and the AB110 (n=3)
373 plasmids.

374

375 **Fig 7. Plasmid type, predicted mobility and AMR genes found within plasmids in sequenced MDR-**
376 **MRSA strains from Norway.**

377

378 The small non-mobilizable plasmid AC627 (Fig 7, Table S2), which only holds the *erm(C)*-gene, was
379 found in multiple strains belonging to CC1 (t127), CC239 (t037 and t632), CC398 (t011), CC72 (t3092)

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

380 and CC8 (t064, t1476, t1952 and t451). For seven strains where we initially detected no genetic cause
381 of macrolide resistance, it was upon further inspection detected that this plasmid was present, but
382 had not been assembled correctly. Previous studies have also reported challenges in the detection of
383 small plasmids using long read assemblers [16]. Consequently, this is an important aspect to consider
384 for analysis of small plasmids when employing this methodological approach.

385 Most of the detected plasmids (76.0 %) were found in a single clonal complex (Fig 7), and thus
386 appeared to be relatively stably maintained within a specific genetic background while not being
387 disseminated to other clones to any large extent. Consequently, these plasmids likely have
388 diminished capacity for disseminating AMR traits across the more widely distributed MRSA clones.
389 For instance, the medium sized mobilizable plamid AB973, holding the AMR genes *erm(B), blaZ,*
390 *blaR1, blaI, dfrE, lnu(A), tet(K), aac(6')-Ie/aph(2'')-Ia* and *aadD1,* was only found in strains belonging
391 to CC188 (Fig 7, Table S2). A few groups of AMR-plasmids (18.8 %) however appeared to be spreading
392 more successfully to diverse MRSA backgrounds. The medium sized conjugative plasmid AA083,
393 holding *mup(A), lnu(A), qacC, aac(6')-Ie/aph(2'')-Ia* and *aadD1*, was found in CC239 (t037), CC30
394 (t665) as well as CC5 (t067 and t9408). Furthermore, the small plasmids AC333 and AC627, the small
395 to medium-sized plasmids AA411, AB924 and AA840, were found in multiple clonal backgrounds, and
396 thus demonstrate the highest potential for horizontal transfer between different MRSA clones.

397 ***Staphylococcal chromosome cassette mec (SCCmec)***

398 SCC*mec* was detected in all but one (99.0 %) of the sequenced MDR-MRSA strains. The lengths of
399 SCC*mec* chromosome cassettes were quite uniform, with a median length of 45,587 bp. The smallest
400 and largest SCC*mec* elements detected were both SCC*mec* type IV, of length 24,060 bp and 83,838
401 bp, respectively. The most prevalent SCC*mec* types identified in the sequenced strains were type IV
402 (n=47, 46.5 %) followed by type III (n=25, 24.8 %). Both SCC*mec* types contained a wide variety of
403 AMR genes (Fig 8). Type IV was typically detected in strains belonging to CC8 and CC188. The most
404 commonly detected AMR genes in this SCC*mec*, in addition to *mecA*, were *aac(6')-Ie/aph(2'')-Ia*, *drfS1*

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

405 (n=17), and *tet(K)* (n=16). SCC*mec* type III was only detected in strains belonging to CC239. The most
406 predominant AMR genes found within this type, aside from *mecA,* were *ant(9)-Ia* and *erm(A)* (n=14).
407

408 **Fig 8. AMR genes identified in SCC*mec* elements in sequenced MDR-MRSA strains from Norway.**
409 NT, non-typeable.
410

411 ***Prophages***

412 A total of 256 prophages harboring virulence and/or AMR-genes were identified in 86 (85.1 %) of the
413 sequenced MDR-MRSA strains. Strains which harboured prophages had on average three prophages
414 per genome (range 1-7) with a mean length of 25,151 bp. Only three strains (3.0 %) had prophages
415 encoding an AMR gene (Fig 9), specifically the *dfrG* gene providing trimetoprim resistance. This gene
416 was detected, in single or multiple copies, in the *Staphylococcus* phages ECel-2020q and phiSa2wa-
417 st72 accordingly. The prophages identified were however associated with several known virulence
418 factors. This included the well-known PVL toxin (encoded by *lukF-PV* and *lukS-PV*), δ-hemolysin (*hld)*
419 and the enterotoxin-encoding genes *sea, sec2, sek, sel, sep* and *seq.* Prophages encoding the human
420 immune evasion cluster were also detected, encoding the staphylokinase gene *(sak)* and the
421 staphylococcal complement inhibitor (*scn)*. The prophages harbouring this cluster were phage
422 23MRA, ECel-2020g, P630, phiNM3, phiSAa119, SA1014ru, Sa2wa, SA345ru, SA7, SA780ru, SAP090B
423 and tp310-3, which were present in 79 (78.2 %) of the sequenced MDR-MRSA strains.
424

425 **Fig 9. AMR and virulence genes in identified prophages in sequenced MDR-MRSA strains from**
426 **Norway.**
427

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

428 ***Composite transposons***

429 A total of 207 composite transposons containing AMR-genes were identified in 76 (75.2 %) of the
430 sequenced MDR-MRSA strains. Within these strains, we detected a median of one transposon per
431 genome, with a mean length of 6,032 bp. All of these transposons contained AMR gene(s). The
432 Tn*5405*, Tn*551* and Tn*552* were found in both the chromosome and on plasmids (Fig 10). The Tn*554*,
433 Tn*558*, Tn*559*, Tn*6224* and Tn*917* were only found in the chromosomes of the analysed strains.

434

435 **Fig 10. AMR genes identified in composite transposons in sequenced MDR-MRSA strains from**
436 **Norway.** Genomic location indicated by chromosome (Chr) or plasmid.

437

438 The AMR genes predominantly found within composite transposons were the aminoglycoside
439 resistance gene *ant(9)-Ia* and the macrolide resistance gene *erm(A)* (n=54) (Fig 10). Additionally, the
440 beta-lactam resistance genes *blaPC1* (n=32) and *blaZ* (n=17) were found in transposons, as well as
441 the MLS gene *erm(B)* (n=17). Genes found more rarely in transposons were *tet(M)* (n=2) and *dfr(K)*
442 (n=1).

443 Tn*554*-transposons (carrying the *ant(9)-Ia*- and *erm(A)* AMR genes), were present in one to four
444 copies per strain. The Tn*551* and the Tn*552*- transposons was detected in a single or two copies when
445 present in the strains. The Tn*664*-, Tn*5405*-, Tn*558*-, Tn*559* and Tn*917*-transposons, were present in a
446 single copy per strain.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

447 # Discussion

448 This study included all MDR-MRSA strains (n=452) detected in Norway during the study period 2008-
449 2020, from a total of 23,412 MRSA strains. This project was made possible by the continued
450 Norwegian MRSA surveillance effort that has been ongoing since 2008, with the aim of keeping
451 multidrug-resistant pathogens from becoming endemic in healthcare institutions in Norway.
452 Although the overall and yearly proportion of MDR-MRSA was relatively low, we observed an
453 increase in the number of MDR-MRSA strains over time, as well as major changes in molecular
454 epidemiology, during the study period. Specifically, we observed temporal shifts and predominace of
455 certain genotypes (*spa*-types t1476, t127, t188 and t030/t037), indicating that these were
456 successfully established and disseminating MDR-MRSA clones.

457 Although the information on place of acquisition for the MDR-MRSA strains is inherently limited by
458 some uncertainty, it is interesting to note that only 13.1 % of strains were registered as aquired in
459 Norway, while a majority were registered as aquired abroad. Compared to the overall MRSA
460 population [12], this suggests import to be responsible for a comparatively larger proportion of MDR-
461 MRSA strains, and similarly that Asia and Africa are the most frequent routes of transmission. This is
462 also supported by the fact that we detect specific clones that have previously been reported as
463 endemic or prevalent in these specific regions and countries [17, 18]. These findings highlight the
464 importance of global cooperation, surveillance, antibiotic stewardship and infection control efforts
465 that can limit both the emergence and global dissemination of these important pathogens.

466 A high proportion of the MDR-MRSA strains were healthcare-associated, mostly isolated from
467 patients during hospital admission. The proportion of healthcare-associated strains was markedly
468 higher among the MDR-MRSA strain collection than that reported in our surveillance study of the
469 whole Norwegian MRSA population [12]. This suggests that the most highly resistant MRSA clones
470 are more often hospital-associated than less resistant MRSA clones, although they have become
471 more common in the community setting as well. This may among other factors be due to the high

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

472 selection pressures and competition between MRSA clones provided by high antibiotic exposure [19].
473 MRSA are also endemic in many hospital environments wordwide [20], thus providing reservoirs for
474 efficient spread into a vulnerable populations, especially in resource-limited settings with inadequate
475 infection control [21]. A limitation to this study is the lack of temporal data on hospital and nursing
476 home admissions, and thus that we had to rely on a broad definition of healthcare-associated MRSA.
477 With this in mind, it is possible that the number of HA- and specifically hospital-associated cases are
478 overestimated. However, the same definition of HA-MRSA was used in the surveillance study of the
479 whole Norwegian MRSA population, in support of the relative differences observed in this study.

480 Interestingly, there was a larger proportion of MDR-MRSA strains from carriage than from clinical
481 infections, compared to the corresponding numbers for the overall MRSA population in Norway [12].
482 This finding may reflect that a majority of these strains have been acquired abroad, and are thus (in
483 Norway) mainly detected from asymptomatic carriers, e.g. due to screening upon contact with the
484 healthcare system and differing screening practices. While it is possible that some MDR-MRSA strains
485 have reduced virulence due to e.g. the physiological costs of maintaining resistance mechanisms, this
486 can vary significantly between different strains and settings [22-24]. The prevalence of PVL-positive
487 strains was 20.4 %, which was much lower than for the whole Norwegian MRSA strain population
488 [12]. This also likely reflects the low number of infection compared to carriage strains, as PVL positive
489 strains have typically been linked to clinical infections, whereas PVL-negative strains are more
490 commonly associated with asymptomatic carriage [25].

491 In the MDR-MRSA strain collection, we observed almost universal resistance to antibiotics such as
492 erythromycin and ciprofloxacin/norfloxacin in addition to cefoxitine. High levels of resistance were
493 also observed for tetracycline, gentamicin, and clindamycin. Thus, in this group of strains, the choice
494 of antibiotic treatment for potential infections is alarmingly and severely restricted. Resistance to
495 mupirocin is however low, meaning that this is still an option for eradication/decolonization of a
496 majority of these strains, while vancomycin and linezolid remain as treament options for all strains.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

497 We observed a very large heteogeneity of *spa*-types and clonal complexes that were resistant
498 towards five or six groups of antibiotics, indicating that multi-drug resistance is indeed a major
499 challenge within the general MRSA population, not only in a few specific clones. The limited number
500 of genotypes that were resistant to seven or eight groups of antibiotics however included well-
501 known clones like MRSA CC239, that have been evolving antimicrobial resistance in high selection
502 pressure environments for many decades, given their continous presence and global spread since the
503 large hospital outbreaks of the 1970s [26, 27]. This underscores the critical importance of preventing
504 the introduction of MRSA into hospitals and other healthcare facilities, especially in low-prevalence
505 countries, and furthermore the importance of antibiotic stewardship and infection control in
506 mitigating the risk of developing highly resistant strains within these environments.

507 Overall, we observed significant concordance between phenotypic (AST) and genotypic antimicrobial
508 resistance profiles. In almost all cases, previously described AMR genes or mutations were detected,
509 that could explain the observed phenotypes. Additionally, we noticed high clonality of resistance
510 profiles, which corresponds well with the apparent clonality of specific MGEs carrying these AMR
511 genes. Consequently, one can reasonably anticipate an antimicrobial profile based on genotyping
512 results, although we do not suggest that antimicrobial susceptibility testing should be bypassed. On
513 the other hand, certain genes and mutations which do not appear to confer phenotypic resistance in
514 *S. aureus*, at least not to an extent which provides resistance according to clinical breakpoints,
515 continue to be reported as resistance genes in the major AMR databases. Furthermore, especially for
516 some groups of antibiotics, the genetics underlying the resistance phenotypes are still not well
517 enough understood and/or are diffucult to interpret. This underscores the necessity for further
518 investigation into the mechanisms underlying bacterial resistance as well as the importance of
519 continuous curation and updating of AMR databases.

520 Nanopore sequencing technology was used to investigate the specific MGEs associated with AMR
521 genes and mutations. Long-read sequencing techologies such as Oxford Nanopore Techologies (8)

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

522 have facilitated a much more comprehensive investigation of MGEs, which can often be problematic
523 to resolve by short-read sequencing (9). Consequently, our investigation revealed that AMR genes in
524 MDR-MRSA strains are predominantly located within plasmids, as well as within staphylococcal
525 cassette chromosome *mec* (SCC*mec*) elements and transposons, with a limited presence on
526 prophages. The most common AMR genes were associated with wide range of MGEs, including
527 different SCCmec types and multiple transposons and plasmids. Some MGEs were clonal, while
528 others were widely distributed, indicating high potential for spread within the MRSA population. A
529 majority of the plasmids were furthermore closely related, although differing in AMR traits, thus
530 indiciating high plasticity. The most prevalent AMR phenotypes were furthermore caused by multiple
531 AMR genes and/or mutations. As an example, we observed eight different genes or gene
532 combinations in strains with MLS resistance. This large variation is likely a consequence of high
533 selection pressure and thus multiple adaptations of MRSA clones over a long period of time, which
534 now serves as an arsenal of genes available for providing resistance against different MLS antibiotics.
535 This high diversity both in AMR genes and in acquisition mechanisms thus provide a significant
536 advantage for dissemination of antibiotic resistance within the MRSA population. These findings
537 highlight the complexity of resistance gene dissemination and the adaptive strategies employed by *S.*
538 *aureus* in response to antimicrobial pressures.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

539 # Materials and Methods

540 ## Study design and population

541 This study is based on MRSA strains submitted to the Norwegian MRSA reference laboratory from
542 2008-2020. Inclusion was based on phenotypic resistance to five or more of the following
543 antibiotic/resistance groups, with the antibiotic tested provided in parenteses; beta-lactams
544 (cefoxitin), macrolide/lincosamide/streptogramin B (MLS) (erythromycin and clindamycin),
545 aminoglycosides (gentamicin), tetracyclines (tetracycline), fucidanes (fusidic acid), fluoroquinolones
546 (ciprofloxacin or norfloxacin), folate pathway inhibitors (trimethoprim-sulfamethoxazole, TMP-SMX),
547 ansamycins (rifampicin), oxazolidinones (linezolid) and glycopeptides (vancomycin). Although all
548 MRSA by current definitions [28] are regarded as multidrug-resistant (MDR), the strains included in
549 this study are either not phenotypically resistant to, or have not been tested against, sufficient
550 antibiotic groups to be designated as extensively drug-resistant (XDR). For lack of a better
551 terminology, we thus refer to this strain collection as MDR-MRSA throughout this manuscript.

552 ## Clinical and epidemiological data

553 Clinical and epidemiological data on all cases was collected from the Norwegian Surveillance System
554 for Communicable Diseases (MSIS) and request forms from the referring laboratory or treating
555 physician available from the laboratory information system (LIMS) at the national reference
556 laboratory. The Information from MSIS included age, sex, admission to hospital or nursing home,
557 place of acquisition and if it was part of a known outbreak (data from the Norwegian outbreak rapid
558 alert system Vesuv) [9]. The data obtained from the LIMS included sample date, sampling site/type of
559 sample and laboratory results. All MDR-MRSA cases were categorized as carriage, infection, invasive
560 infection or unknown based on sampling site/type of sample. Age groups were defined according to
561 Diaz et al. [10]. Due to lack of temporal data for hospitalized patients and nursing home stays, a case
562 was classified as healthcare-associated (HA) if it was diagnosed during a hospital or nursing home

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

563 stay, or if MDR-MRSA was detected in healthcare workersMLS (HCWs). Conversely, all other cases
564 were classified as community-acquired (CA).

565 **Genotyping and antimicrobial susceptibility testing**

566 Culturing, DNA extraction, *spa*-typing and assignment of *spa*-types to clonal complexes (CC) was
567 performed as described previously [12]. The strains included in this study had previously undergone
568 phenotypic antimicrobial susceptibility testing (AST) towards the following antibiotics: cefoxitin,
569 erythromycin, clindamycin, fusidic acid, trimetoprim-sulfamethoxalate, tetracycline, gentamicin,
570 ciprofloxacin/norfloxacin, rifampicin, mupirocin, linezolid and vancomycin. Susceptibility testing was
571 performed as previously described [29] on all strains using the EUCAST (European Committee on
572 Antimicrobial Susceptibility Testing) disk diffusion method and categorized as either susceptible,
573 intermediate, or resistant according to the current EUCAST breakpoints at the time of testing. For
574 clindamycin, inducible resistance was recorded as described in the EUCAST expert rules [30]. For
575 vancomycin, the gradient strip test from BioMeriéux (2008-2013) or Liofilchem (2014-2020) was
576 used.

577 **Whole genome sequencing**

578 A selection of strains (n=101) were subjected to whole genome sequencing (WGS) by nanopore
579 methodology, hereafter referred to as sequencing. These included all strains resistant to seven or
580 more antibiotic groups (n=21), and a randomized selection among the remaining strains (n=80).
581 Bacterial cells were first treated with proteinase K (2 mg/mL) and lysostaphin (0.1 mg/mL) for 15 min
582 with shaking at 37 °C, before heating for 15 min at 65 °C. Genomic DNA was then isolated using the
583 EZ1 DNA tissue kit with an EZ1 Advanced XL instrument (Qiagen). Sequencing libraries were prepared
584 and multiplexed using the Rapid Barcoding Sequencing kit (SQK-RBK004), according to the
585 RBK_9054_v2_revJ_14Aug2019 protocol. Sequencing libraries were loaded onto a R9.4.1 SpotON
586 flow cell (FLO-MIN106D) and sequenced on a MinION Mk1B sequencer (Oxford Nanopore
587 Technologies).

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

588 **Bioinformatic analyses**

589 Dorado [31] v.0.4.2 was used for basecalling (SUP v3.6 model) and demultiplexing. Assembly was
590 performed using Flye v.2.9.2 [32, 33]. Racon and Medaka (SUP v5.0.7 model) were used for polishing.
591 Additionally, Homopolish [34] was used to remove possible systematic errors from the nanopore
592 sequencing. The sequences are available from GenBank under BioProject ID PRJNA1186082.
593 Annotation was performed with Prokka v.1.14.6 [35]. Definition of the core genome and phylogeny
594 were performed using Roary v3.13.0 with default settings [36] and FastTree with the GTR model [37].

595 Plasmid classification was performed by MOB-suite v.3.1.8 [38], with the typing and clustering
596 modules. Mashtree [39] was then used to construct a plasmid phylogeny. Plasmids were furthermore
597 merged into communities/subcommunities using the Pling bioinformatic tool [40].

598 Putative prophages were detected using Phastest [41] including only intact phages, and identified
599 using nBLAST against reference *S. aureus* phages in the GenomeNet Virus-Host DataBase (VHDB)
600 [42]. Transposons and IS-elements were detected using the MobileElementFinder [43]. *SCCmec*
601 chromosome cassettes were typed using SCCmecFinder [44]. Whole *SCCmec* elements were
602 extracted by *in silico* PCR with SeqKit [45], using modified primers from Ito *et al* [46].

603 For whole genomes as well as for specific MGEs, AMR genes and point mutations were detected
604 using AMRFinderPlus v3.10.30 with organism-specific settings for *S. aureus*, and cut-off of 90 % on
605 protein identity and 50 % on coverage [47].

606 **Visualization**

607 Upset plots were created using R studio v4.3.3 with the ggplot2 [48], ComplexUpset [49, 50] and
608 cowplot [51] packages. Visualization of the core genome tree with phenotypic and genotypic traits
609 was performed with iTol v.5 [52]. The world map was created using GeoPandas [53] and Matplotlib
610 [54].

611 **Ethical statement**

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

612 The project was approved, with exemption from informed consent of participants, by the Norwegian
613 Regional Committees for Medical and Health Research Ethics (REC) South-East with reference
614 number 352380. A Data Protection Impact Assessment was performed and approved by the
615 responsible institution, the Clinic of Laboratory Medicine at St. Olavs Hospital, Trondheim University
616 Hospital.

617 **Acknowledgements**

618 The authors would like to acknowledge the contribution of all submitting medical microbiology
619 laboratories to the continued MRSA surveillance effort in Norway, as well as the laboratory
620 personnel who have contributed to the analysis of these strains at the Norwegian MRSA reference
621 laboratory at St. Olavs Hospital.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

622 # References

623 1. Howden BP, Giulieri SG, Wong Fok Lung T, Baines SL, Sharkey LK, Lee JYH, et al.
624 Staphylococcus aureus host interactions and adaptation. Nat Rev Microbiol. 2023;21(6):380-95.
625 2. Boucher HW, Corey GR. Epidemiology of methicillin-resistant Staphylococcus aureus. Clin
626 Infect Dis. 2008;46 Suppl 5:S344-9.
627 3. Richardson EJ, Bacigalupe R, Harrison EM, Weinert LA, Lycett S, Vrieling M, et al. Gene
628 exchange drives the ecological success of a multi-host bacterial pathogen. Nat Ecol Evol.
629 2018;2(9):1468-78.
630 4. Zhao W, Zeng W, Pang B, Luo M, Peng Y, Xu J, et al. Oxford nanopore long-read sequencing
631 enables the generation of complete bacterial and plasmid genomes without short-read sequencing.
632 Frontiers in Microbiology. 2023;14.
633 5. Mores CR, Montelongo C, Putonti C, Wolfe AJ, Abouelfetouh A. Investigation of Plasmids
634 Among Clinical Staphylococcus aureus and Staphylococcus haemolyticus Isolates From Egypt. Front
635 Microbiol. 2021;12:659116.
636 6. Ito T, Kuwahara-Arai K, Katayama Y, Uehara Y, Han X, Kondo Y, et al. Staphylococcal Cassette
637 Chromosome mec (SCCmec) analysis of MRSA. Methods Mol Biol. 2014;1085:131-48.
638 7. Katayama Y, Ito T, Hiramatsu K. Genetic organization of the chromosome region surrounding
639 mecA in clinical staphylococcal strains: role of IS431-mediated mecI deletion in expression of
640 resistance in mecA-carrying, low-level methicillin-resistant Staphylococcus haemolyticus. Antimicrob
641 Agents Chemother. 2001;45(7):1955-63.
642 8. Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire
643 mec DNA of pre-methicillin-resistant Staphylococcus aureus N315. Antimicrob Agents Chemother.
644 1999;43(6):1449-58.
645 9. Leinweber H, Sieber RN, Larsen J, Stegger M, Ingmer H. Staphylococcal Phages Adapt to New
646 Hosts by Extensive Attachment Site Variability. mBio. 2021;12(6):e0225921.
647 10. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, et al. Antibiotic
648 Resistance: One Health One World Outlook. Front Cell Infect Microbiol. 2021;11:771510.
649 11. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden
650 of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet. 2022;399(10325):629-
651 55.
652 12. Ronning TG, Enger H, Afset JE, As CG. Insights from a decade of surveillance: Molecular
653 epidemiology of methicillin-resistant Staphylococcus aureus in Norway from 2008 to 2017. PLoS One.
654 2024;19(3):e0297333.
655 13. Guerillot R, Goncalves da Silva A, Monk I, Giulieri S, Tomita T, Alison E, et al. Convergent
656 Evolution Driven by Rifampin Exacerbates the Global Burden of Drug-Resistant Staphylococcus
657 aureus. mSphere. 2018;3(1).
658 14. Howe RA, Wootton M, Noel AR, Bowker KE, Walsh TR, MacGowan AP. Activity of AZD2563, a
659 novel oxazolidinone, against Staphylococcus aureus strains with reduced susceptibility to vancomycin
660 or linezolid. Antimicrob Agents Chemother. 2003;47(11):3651-2.
661 15. Liakopoulos A, Spiliopoulou I, Damani A, Kanellopoulou M, Schoina S, Papafragas E, et al.
662 Dissemination of two international linezolid-resistant Staphylococcus epidermidis clones in Greek
663 hospitals. J Antimicrob Chemother. 2010;65(5):1070-1.
664 16. Johnson J, Soehnlen M, Blankenship HM. Long read genome assemblers struggle with small
665 plasmids. Microb Genom. 2023;9(5).
666 17. Lawal OU, Ayobami O, Abouelfetouh A, Mourabit N, Kaba M, Egyir B, et al. A 6-Year Update
667 on the Diversity of Methicillin-Resistant Staphylococcus aureus Clones in Africa: A Systematic Review.
668 Front Microbiol. 2022;13:860436.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

669 18. Mohamad Farook NA, Argimon S, Abdul Samat MN, Salleh SA, Sulaiman S, Tan TL, et al.
670 Diversity and Dissemination of Methicillin-Resistant Staphylococcus aureus (MRSA) Genotypes in
671 Southeast Asia. Trop Med Infect Dis. 2022;7(12).
672 19. de Vos AS, de Vlas SJ, Lindsay JA, Kretzschmar MEE, Knight GM. Understanding MRSA clonal
673 competition within a UK hospital; the possible importance of density dependence. Epidemics.
674 2021;37:100511.
675 20. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant
676 Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods.
677 International Journal of Antimicrobial Agents. 2012;39(4):273-82.
678 21. Christopher S, Verghis RM, Antonisamy B, Sowmyanarayanan TV, Brahmadathan KN, Kang G,
679 et al. Transmission dynamics of methicillin-resistant Staphylococcus aureus in a medical intensive
680 care unit in India. PLoS One. 2011;6(7):e20604.
681 22. Rudkin JK, Edwards AM, Bowden MG, Brown EL, Pozzi C, Waters EM, et al. Methicillin
682 Resistance Reduces the Virulence of Healthcare-Associated Methicillin-Resistant Staphylococcus
683 aureus by Interfering With the agr Quorum Sensing System. The Journal of Infectious Diseases.
684 2012;205(5):798-806.
685 23. Taglialegna A, Varela MC, Rosato RR, Rosato AE. VraSR and Virulence Trait Modulation during
686 Daptomycin Resistance in Methicillin-Resistant <i>Staphylococcus aureus</i> Infection. mSphere.
687 2019;4(1):10.1128/msphere.00557-18.
688 24. Rao Y, Peng H, Shang W, Hu Z, Yang Y, Tan L, et al. A vancomycin resistance-associated
689 WalK(S221P) mutation attenuates the virulence of vancomycin-intermediate Staphylococcus aureus.
690 J Adv Res. 2022;40:167-78.
691 25. Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant Staphylococcus aureus:
692 the role of Panton–Valentine leukocidin. Laboratory Investigation. 2007;87(1):3-9.
693 26. Shanson DC. Antibiotic-resistant Staphylococcus aureus. Journal of Hospital Infection.
694 1981;2:11-36.
695 27. Monecke S, Slickers P, Gawlik D, Müller E, Reissig A, Ruppelt-Lorz A, et al. Molecular Typing of
696 ST239-MRSA-III From Diverse Geographic Locations and the Evolution of the SCCmec III Element
697 During Its Intercontinental Spread. Frontiers in Microbiology. 2018;9.
698 28. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-
699 resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal
700 for interim standard definitions for acquired resistance. Clinical Microbiology and Infection.
701 2012;18(3):268-81.
702 29. Enger H, Larssen KW, Damås ES, Aamot HV, Blomfeldt A, Elstrøm P, et al. A tale of two STs:
703 molecular and clinical epidemiology of MRSA t304 in Norway 2008-2016. Eur J Clin Microbiol Infect
704 Dis. 2022;41(2):209-18.
705 30. EUCAST. The European Committee on Antimicrobial Susceptibility Testing Expert Rules v 3.2
706 http://www.eucast.org/clinical_breakpoints/. 2023.
707 31. Samarakoon H, Ferguson JM, Gamaarachchi H, Deveson IW. Accelerated nanopore
708 basecalling with SLOW5 data format. Bioinformatics. 2023;39(6).
709 32. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat
710 graphs. Nat Biotechnol. 2019;37(5):540-6.
711 33. Lin Y, Yuan J, Kolmogorov M, Shen MW, Chaisson M, Pevzner PA. Assembly of long error-
712 prone reads using de Bruijn graphs. Proc Natl Acad Sci U S A. 2016;113(52):E8396-E405.
713 34. Huang YT, Liu PY, Shih PW. Homopolish: a method for the removal of systematic errors in
714 nanopore sequencing by homologous polishing. Genome Biol. 2021;22(1):95.
715 35. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068-
716 9.
717 36. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, et al. Roary: rapid large-scale
718 prokaryote pan genome analysis. Bioinformatics. 2015;31(22):3691-3.
719 37. Price MN, Dehal PS, Arkin AP. FastTree: Computing Large Minimum Evolution Trees with
720 Profiles instead of a Distance Matrix. Molecular Biology and Evolution. 2009;26(7):1641-50.

bioRxiv preprint doi: https://doi.org/10.1101/2025.01.16.633344; this version posted January 17, 2025. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

38. Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb Genom. 2018;4(8).
39. Katz LS, Griswold T, Morrison SS, Caravas JA, Zhang S, den Bakker HC, et al. Mashtree: a rapid comparison of whole genome sequence files. J Open Source Softw. 2019;4(44).
40. Frolova D, Lima L, Roberts L, Bohnenkämper L, Wittler R, Stoye J, et al. Applying rearrangement distances to enable plasmid epidemiology with pling. bioRxiv. 2024:2024.06.12.598623.
41. Wishart DS, Han S, Saha S, Oler E, Peters H, Grant Jason R, et al. PHASTEST: faster than PHASTER, better than PHAST. Nucleic Acids Research. 2023;51(W1):W443-W50.
42. Mihara T, Nishimura Y, Shimizu Y, Nishiyama H, Yoshikawa G, Uehara H, et al. Linking Virus Genomes with Host Taxonomy. Viruses. 2016;8(3):66.
43. Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, Petersen TN. Detection of mobile genetic elements associated with antibiotic resistance in Salmonella enterica using a newly developed web tool: MobileElementFinder. Journal of Antimicrobial Chemotherapy. 2020;76(1):101-9.
44. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesøe RL, et al. SCC<i>mec</i>Finder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome <i>mec</i> in Staphylococcus aureus Using Whole-Genome Sequence Data. mSphere. 2018;3(1):10.1128/msphere.00612-17.
45. Shen W, Le S, Li Y, Hu F. SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. PLOS ONE. 2016;11(10):e0163962.
46. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2001;45(5):1323-36.
47. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, et al. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep. 2021;11(1):12728.
48. Wickham H. ggplot2: Elegant Graphics for Data Analysis: Springer-Verlag New York; 2016.
49. Krassowski M. ComplexUpset. https://doiorg/105281/zenodo3700590. 2020.
50. Lex A, Gehlenborg N, Strobelt H. UpSet: Visualization of Intersecting Sets,. IEEE Transactions on Visualization and Computer Graphics. 2014;20(12):1983–92.
51. Wilke CO. cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. R package version 1.1.3. 2024.
52. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Research. 2021;49(W1):W293-W6.
53. Jordahl K, Bossche JVd, Fleischmann M, Wasserman J, McBride J, Tratner JG, et al. geopandas/geopandas: v0.8.1. Zenodo. 2020.
54. Hunter JD. Matplotlib: A 2D Graphics Environment. Computing in Science & Engineering. 2007;vol. 9, no. 3, pp. 90-95, May-June 2007.

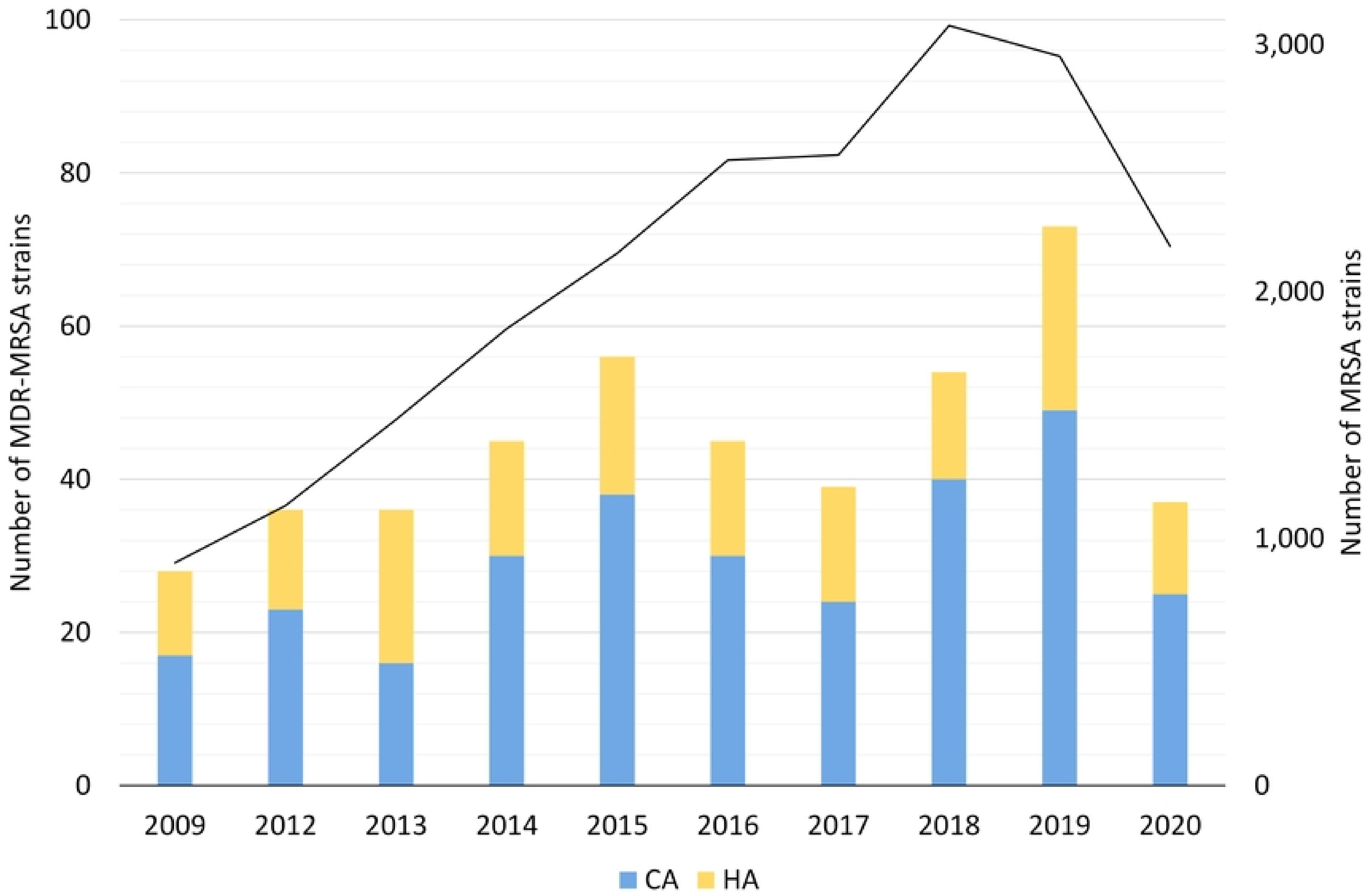


Figure 1

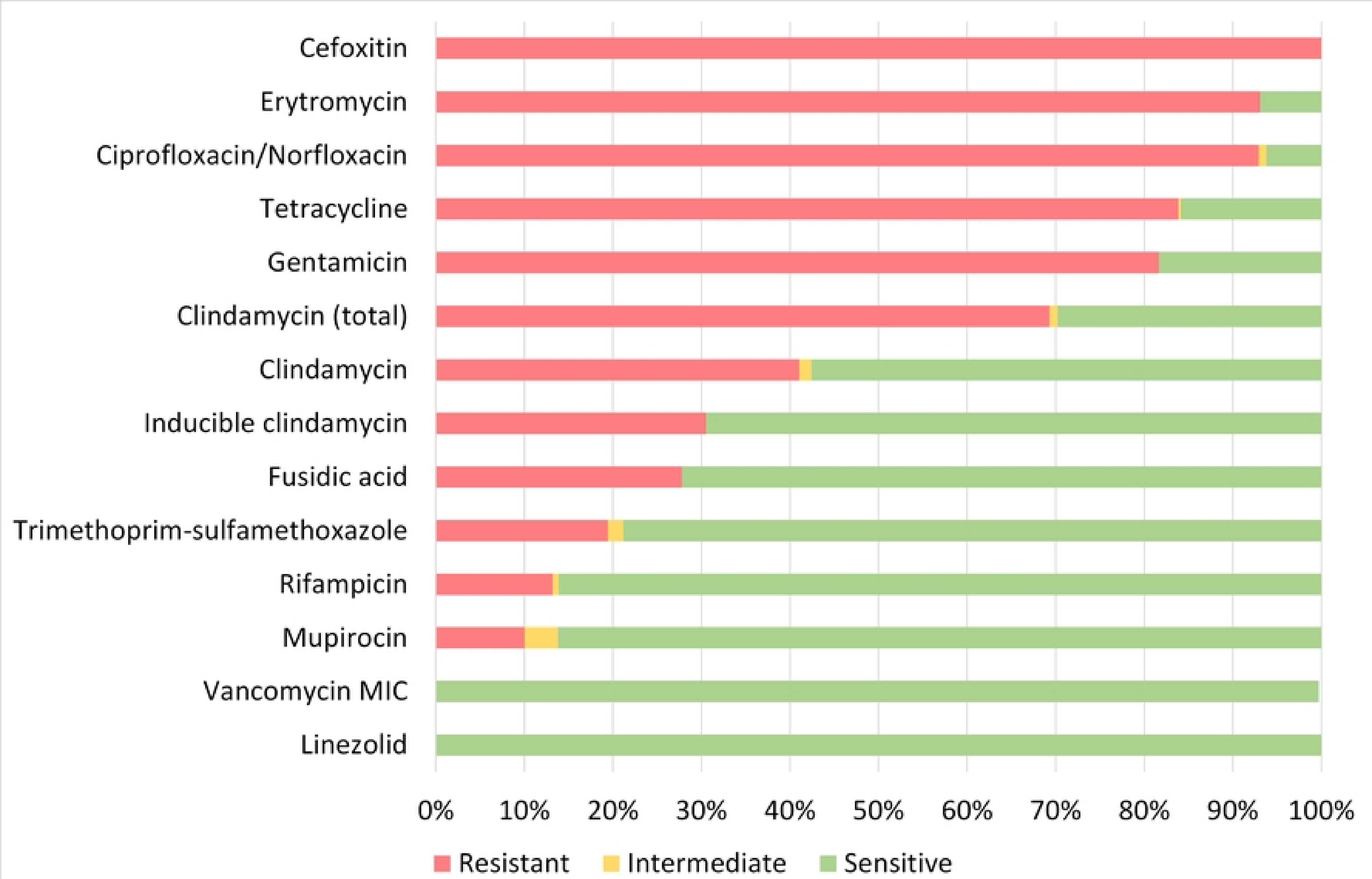


Figure 3

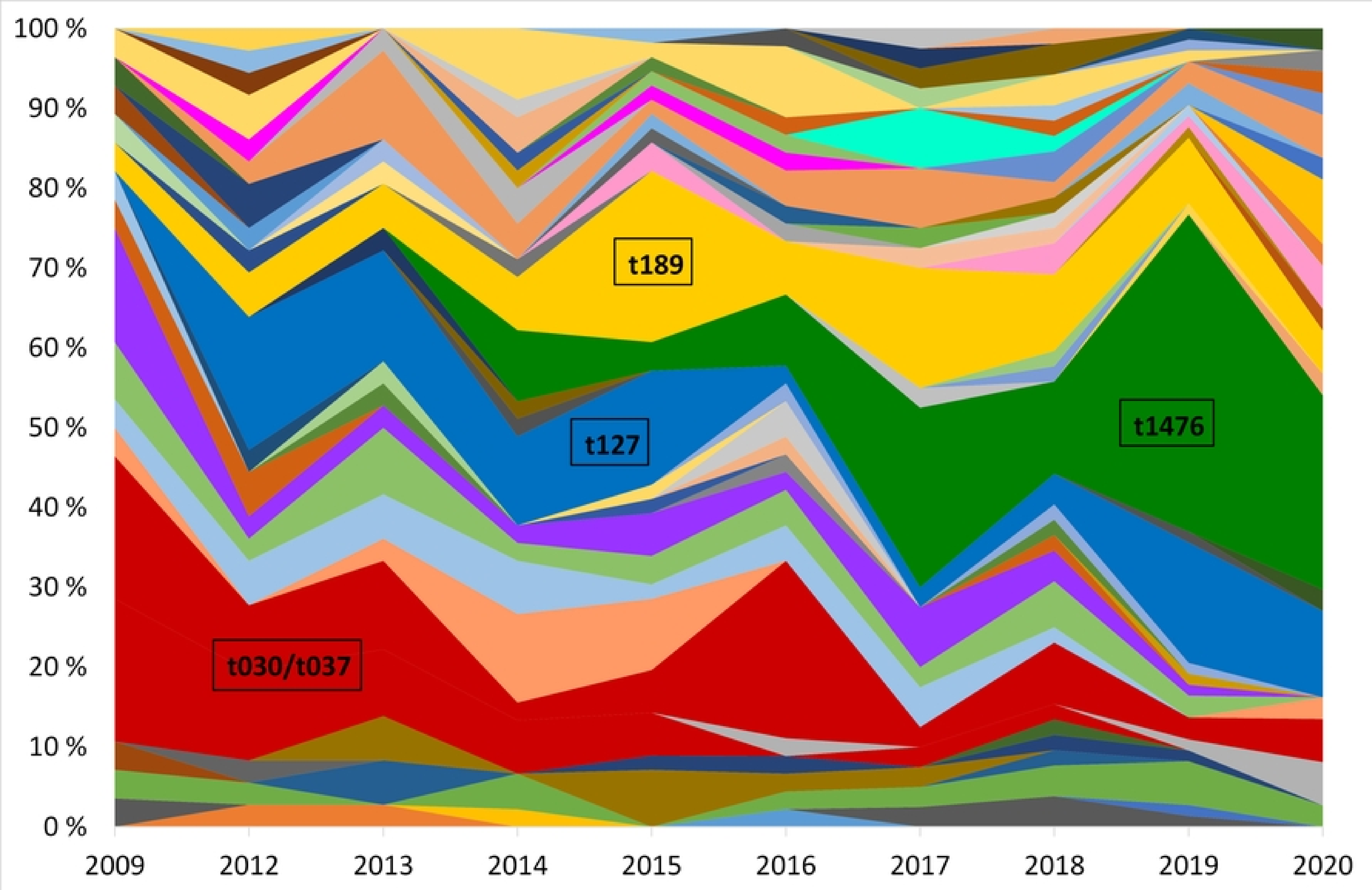


Figure 4

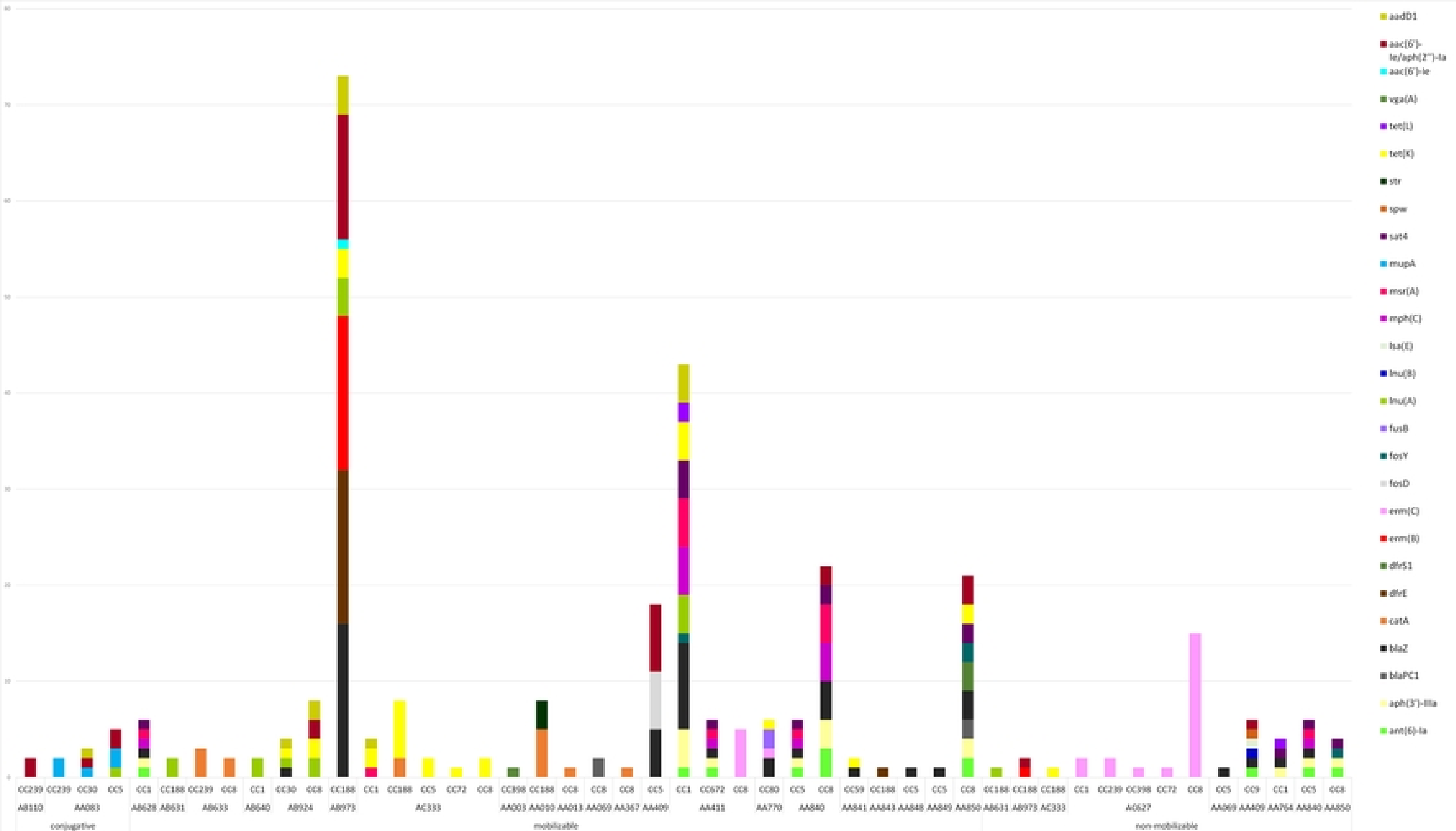


Figure 7

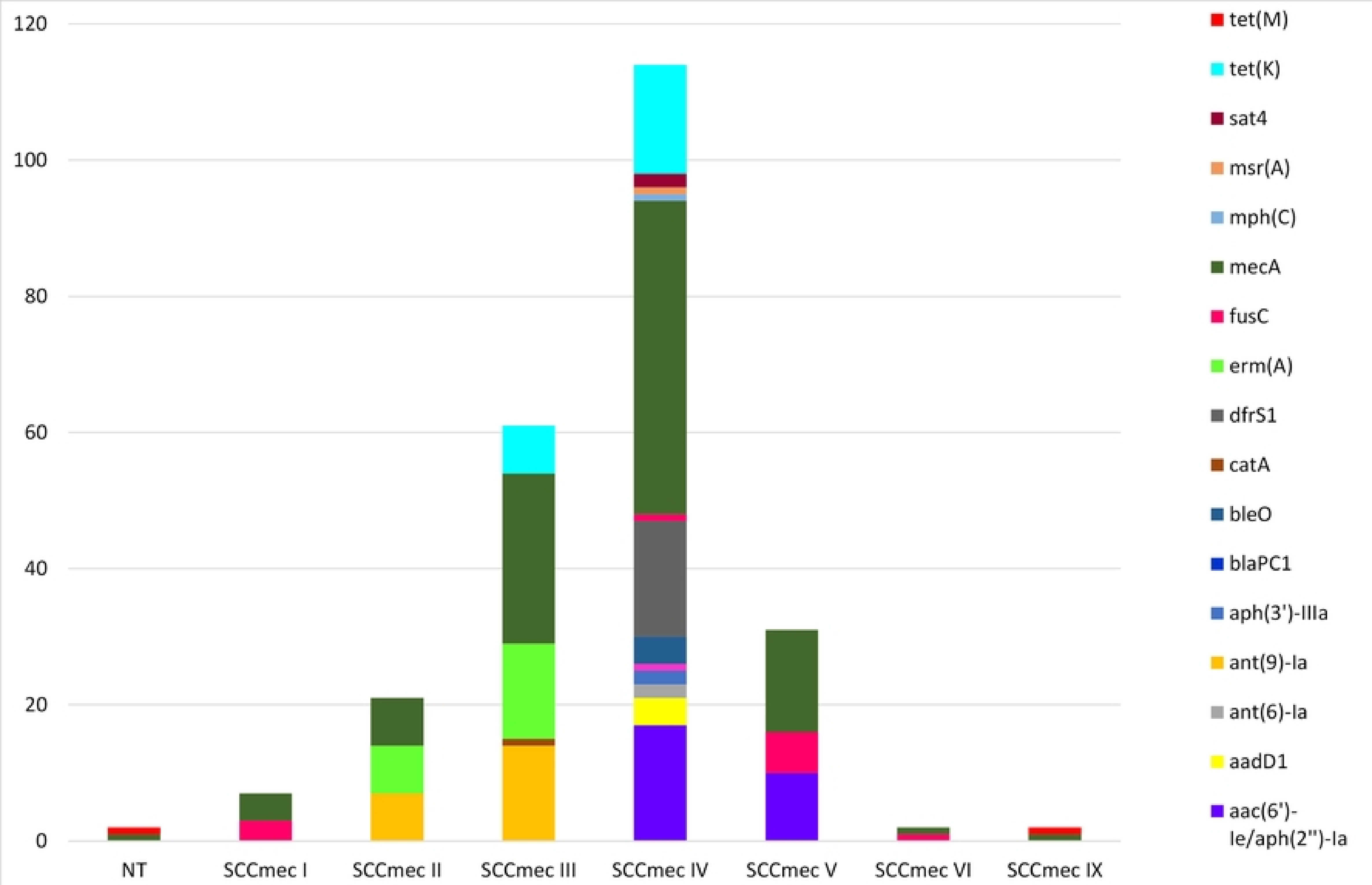


Figure 8

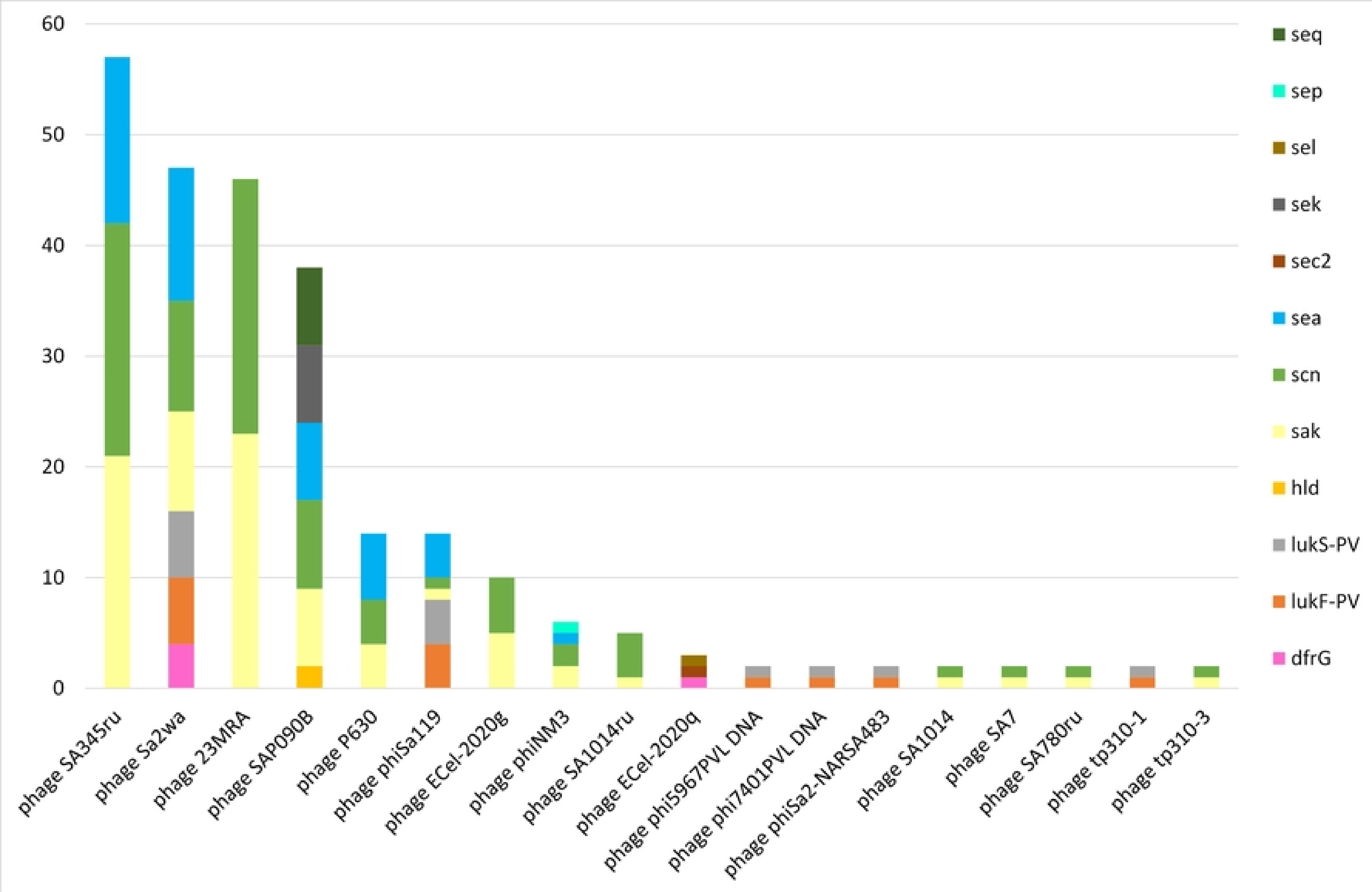


Figure 9

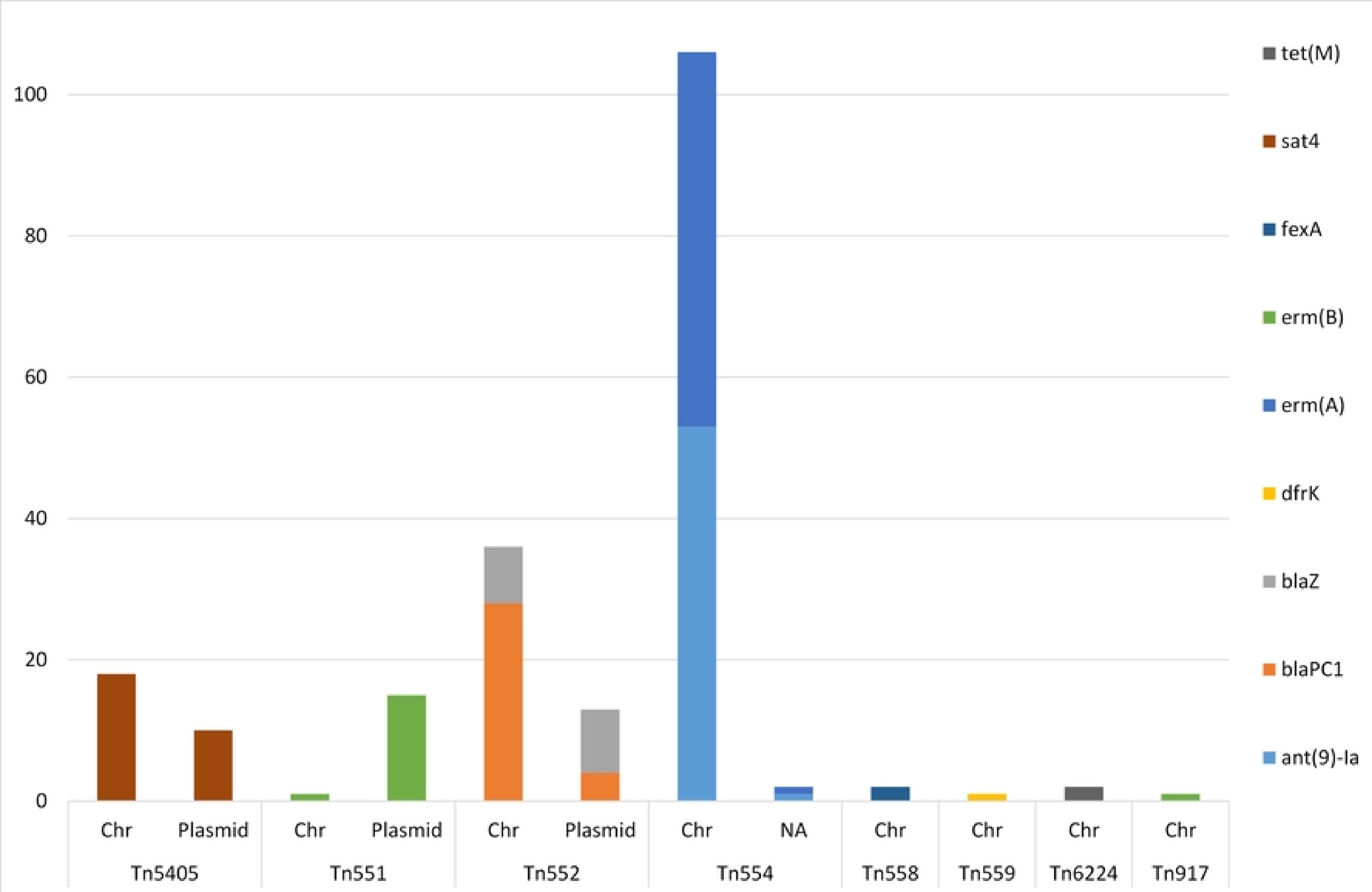


Figure 10

Tree scale: 0.01

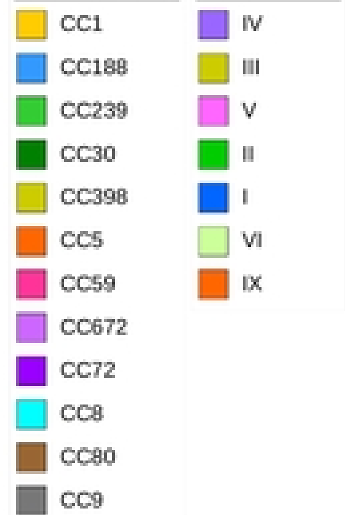


Figure 2

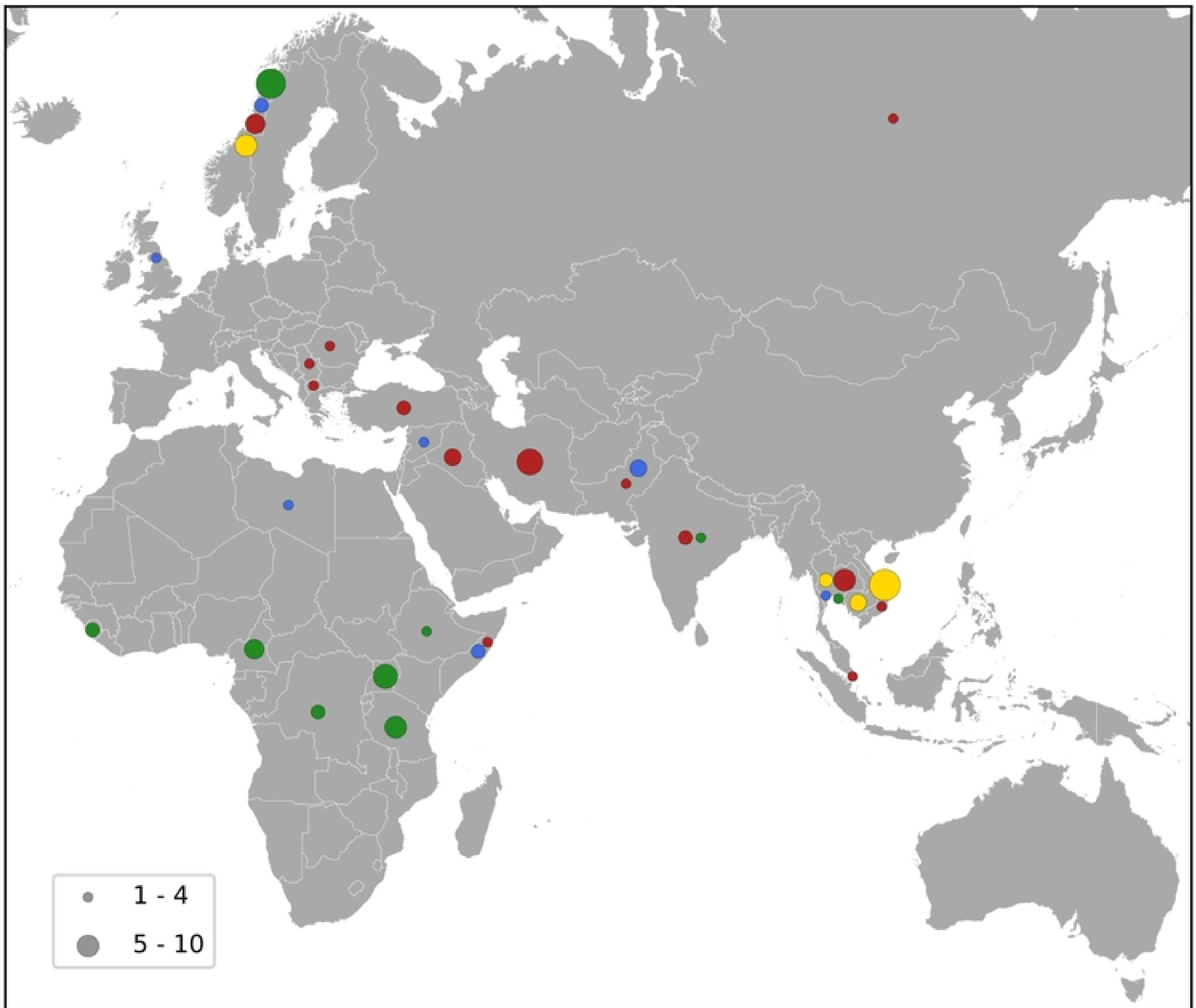
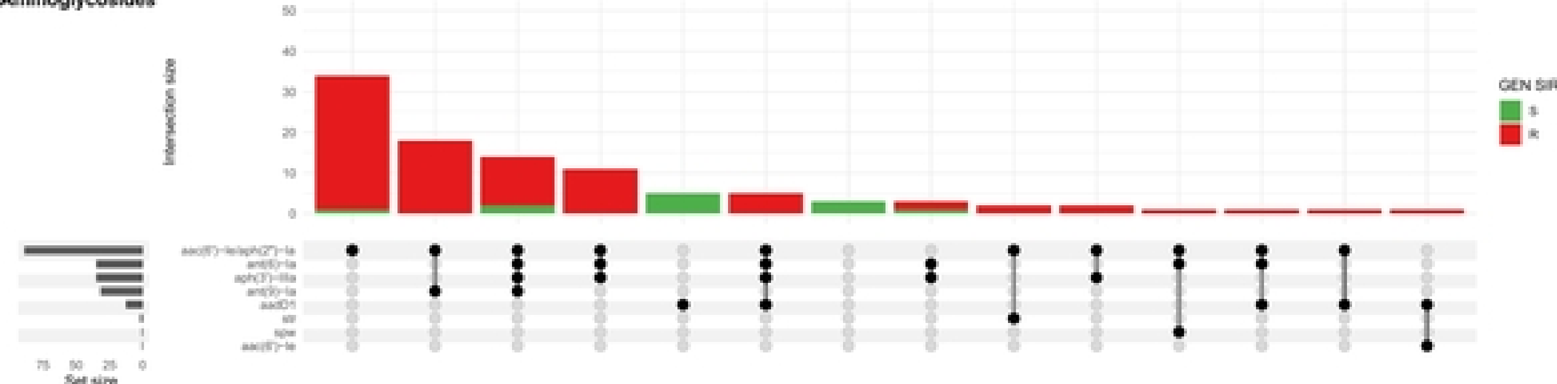
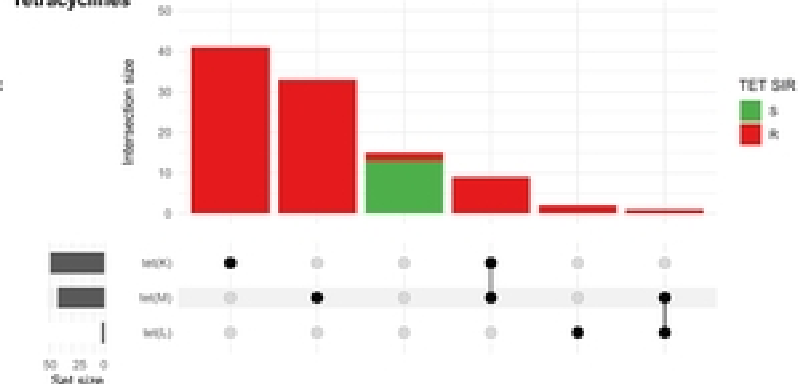
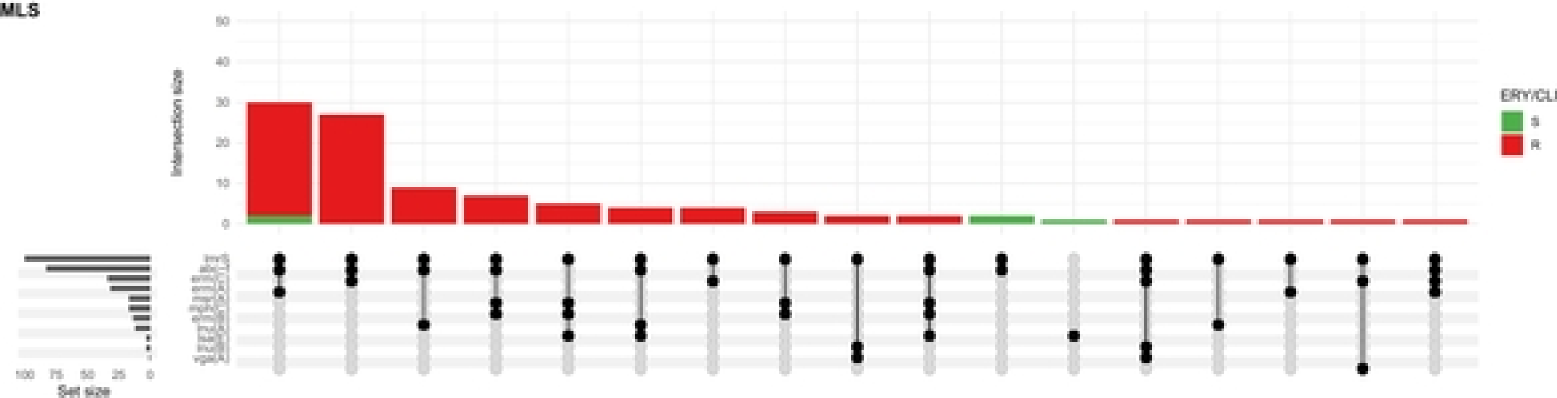
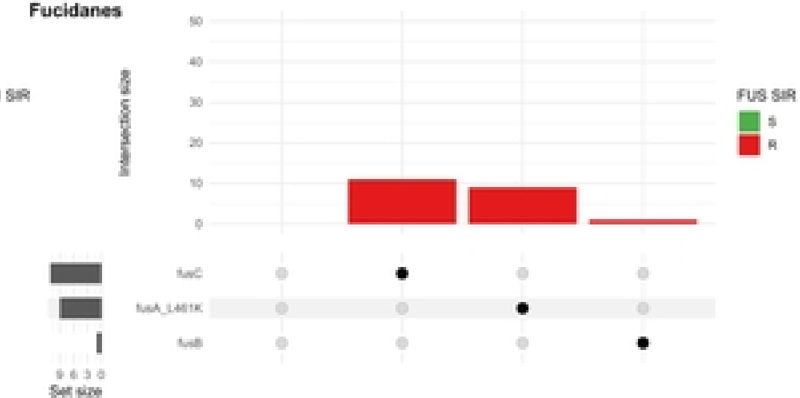


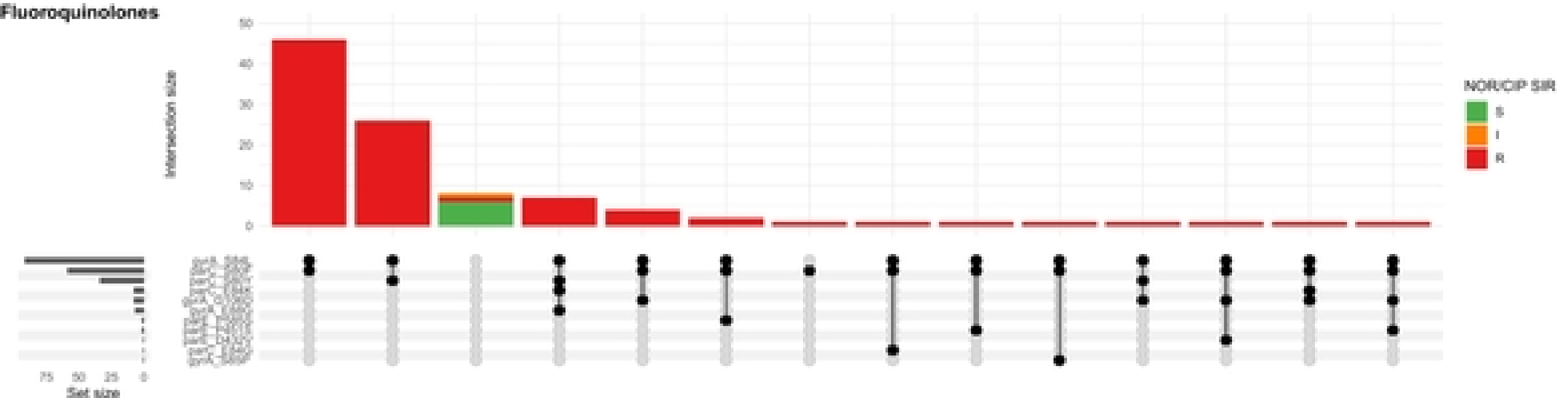
Figure 5











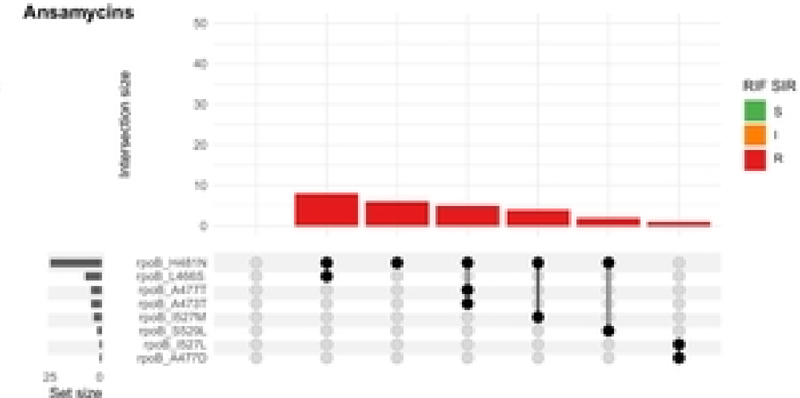


Figure 6