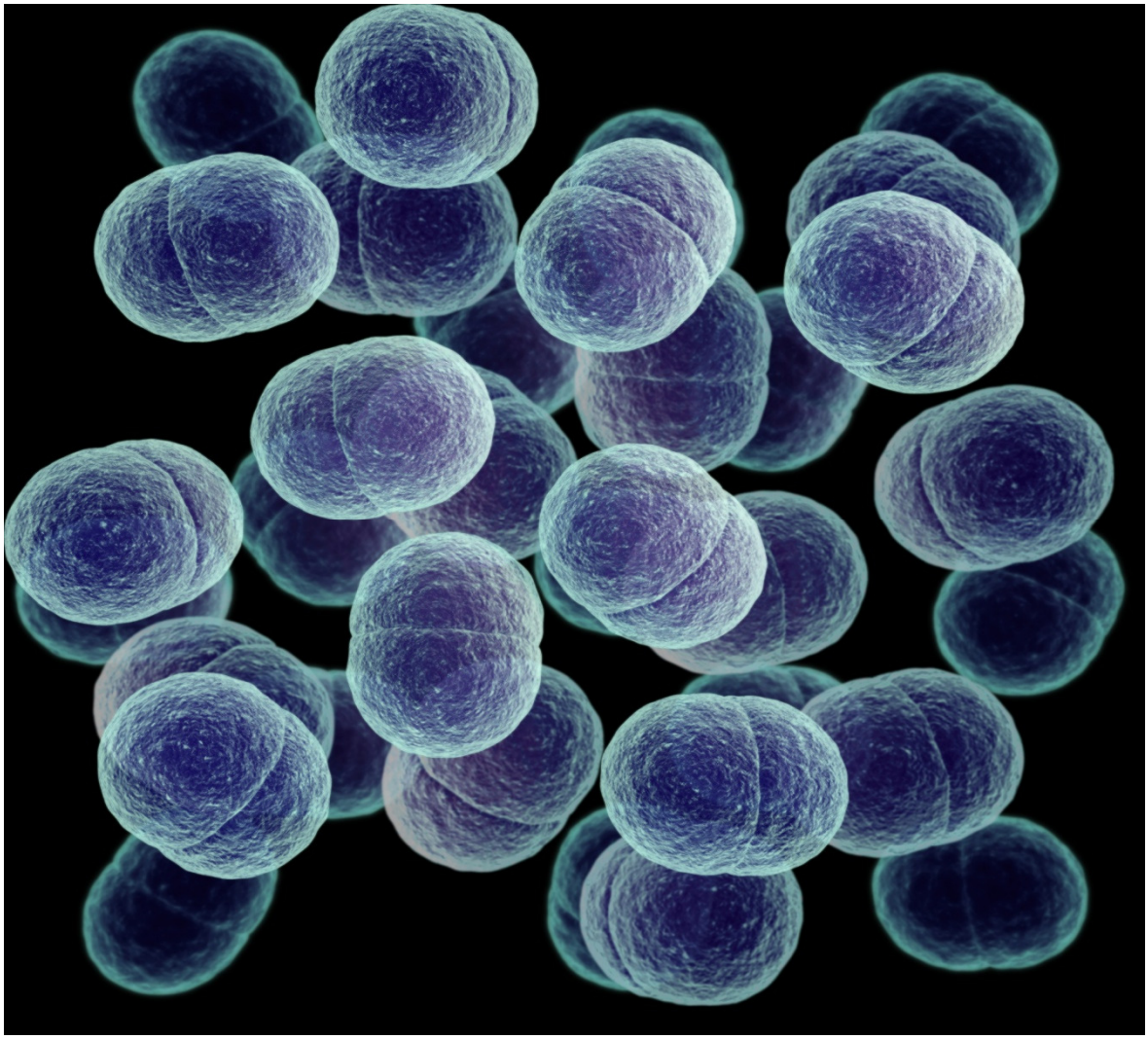
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2019



This report describes the laboratory and clinical characteristics of the 2,234 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2019. SAB has been surveyed by submission of blood culture isolates since 1957. The National Reference Laboratory for Antimicrobial Resistance (NRL-AMR) at Statens Serum Institut has undertaken strain characterization and collection of clinical and epidemiological information in collaboration with the Danish Departments of Clinical Microbiology (DCM).

ACKNOWLEDGEMENT

Isolates from SAB cases were received from all DCMs. We are grateful for their voluntary submission.

The location of the Danish Departments of Clinical Microbiology. The colors indicate the five regions which provide tax-paid health services to the Danish population.

Lone Ryste Hansen Kildevang, Alexandra Medina, Mikkel Christensen, Pia Thurø Hansen and Stine Frese-Madsen are thanked for technical assistance.

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Publisher: National Reference Laboratory for Antimicrobial Resistance (NRL-AMR)
Responsible institution: Statens Serum Institut
Design: Statens Serum Institut
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Content

LIST OF ABBREVIATIONS

CC: Clonal complex
CLSI: Clinical and Laboratory Standards Institute
DCM: Department of Clinical Microbiology
DCRS: Danish Civil Registration System
EUCAST: The European Committee on Antimicrobial Susceptibility Testing
ICD-10: International Classification of Diseases
lukF/S-pv: Genes encoding the Panton-Valentine leucocidin
mecA: The gene encoding for methicillin resistance
mecC: The gene coding for a variant *mecA* gene
MiBa: The Danish Microbiology Database
MLST: Multi Locus Sequence Typing
MSSA: Methicillin-susceptible *Staphylococcus aureus*
MRSA: Methicillin-resistant *Staphylococcus aureus*
NPR: The Danish National Patient Register ('Landspatientregistret')
PCR: Polymerase Chain Reaction
SAB: *Staphylococcus aureus* bacteraemia
spa: The gene encoding the staphylococcal protein A

1. Materials and Methods

1.1 *Staphylococcus aureus* bacteraemia (SAB) episodes

The Departments of Clinical Microbiology in Denmark referred one *S. aureus* isolate per bacteraemia episode to the NRL-AMR as part of the national SAB surveillance established in 1957. Subsequent isolates from the same patient were only included if the positive blood cultures were drawn at least one month apart (new episode).
Medical information on comorbidities and secondary foci (assessed three months after the onset of SAB) was extracted from The Danish National Patient Register (NPR, Lynge *et al*. 2011) for each patient with SAB. The register contains information for all occasions a citizen is in contact with the health care system in Denmark. It should be noted that a new version of the NPR was implemented during 2019 and the data structure was changed. However, it was possible to extract the same information that has been used since 2010. Comorbidities listed in the Charlson comorbidity index (1987) were extracted based on the ICD-10 codes by Quan *et al*. (2005); for intravenous drug use the definition of Elixhauser *et al*. (1998) was used. A comorbidity index score was calculated based on the revised weights by Quan *et al*. (2011). Thirty-day all cause case fatality was calculated based on data extracted from the Danish Civil Registration System (DCRS, Pedersen *et al*. (2006)). Demographic data was obtained from the homepage of Statistics Denmark (http://www.statistikbanken.dk/bef5).
Negative binomial regression analysis using Stata 14.2 (StataCorp, College Station, USA) was used to analyze for trends for number of SAB, number of methicillin-resistant SAB and prevalence of clonal complex in relation to the total number of SAB cases.

1.2 Typing

PCR detection of the *spa* gene confirmed the submitted isolates to be *S. aureus*. The PCR simultaneously detected the *spa*, *mec*A, *mec*C, and *luk*F/S-pv genes (*pvl*) (Stegger *et al*. 2012). The isolates were typed by sequencing of the *spa* gene. *spa* types and clonal complexes (CC) were annotated using Bionumerics 7.6 (Applied Maths, Sint-Martens-Latem, Belgium) and RidomStaphType 1.4 (Ridom GmbH, Würzburg, Germany). *spa* types were approximated to multilocus sequence typing (MLST) clonal complexes (CC), using the MLST homepage (https://pubmlst.org/saureus/). Whole genome sequencing was performed on all CC398 isolates and isolates were designated to the human or livestock-associated clade, based on their place in the phylogeny in Price *et al.* (2012).

1.3 Antimicrobial susceptibility testing

The NRL-AMR performed susceptibility testing of every second received isolate in the months February, March, June, July, August, September, and November (549 isolates, 25%) by MIC determination using a custom-made panel (DKSSP2, TREK Diagnostics). Table 1 presents the antimicrobials tested and the ranges included. Interpretation of antimicrobial resistance was based on The European Committee on Antimicrobial Susceptibility Testing (EUCAST) break-points. For kanamycin and norfloxacin the breakpoints of Clinical and Laboratory Standards Institute (CLSI) were used. *S. aureus* ATCC 29213 was included as quality control for each batch of resistance determination.

Table 1. Antimicrobials and ranges included in the susceptibility testing.

Antimicrobial	Range (mg/L)
Penicillin	0.06-0.12
Cefoxitin	screen: 4
Ceftaroline	0.5-2
Ceftobiprole	0.5-4
Erythromycin	1-4
Clindamycin, including induction	0.25-1 and D-test
Tetracycline	1-4
Rifampicin	0.25-1
Gentamicin	1-2
Kanamycin	Screen: 16
Fusidic acid	0.5-2
Sulfamethoxazole/Trimethoprim	2/38-8/152
Linezolid	2-8
Mupirocin	0.5-2 and screen: 256
Vancomycin	1-4
Daptomycin	0.5-2
Norfloxacin	4-8

In addition to the testing performed in the NRL-AMR, data from the Danish Microbiology Database (MiBa) was used. The first *S. aureus* isolate per patient per year from blood was included. Resistance to penicillin, cefoxitin, erythromycin, clindamycin, tetracycline, rifampicin, gentamycin, fusidic acid, sulfamethizol-trimethoprim, linezolid, mupirocin, vancomycin, and norfloxacin were retrieved. Prevalences of resistance were compared between the two data sets.

2. Results

2.1 Patient information

In 2019, 2,234 cases of SAB were recorded (Figure 1) of which 2,035 (91%) constituted primary and 199 subsequent episodes. The incidence rate of SAB was 38.5/100,000 inhabitants (Figure 2). Methicillin-resistant *S. aureus* (MRSA) was identified from 46 cases (2.1%) (Figure 3) and the incidence rate of MRSA-SAB was 0.79/100,000 inhabitants (Figure 4). The number of new cases has increased in average by 5% each year since 2010, also with the increasing population taken into account. From 2005 to 2019 there was an annual significant average increase of 4% SAB MRSA in relation to the total number of SAB. There was more males than females (63% males vs. 37% females) among the cases of SAB in 2019. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

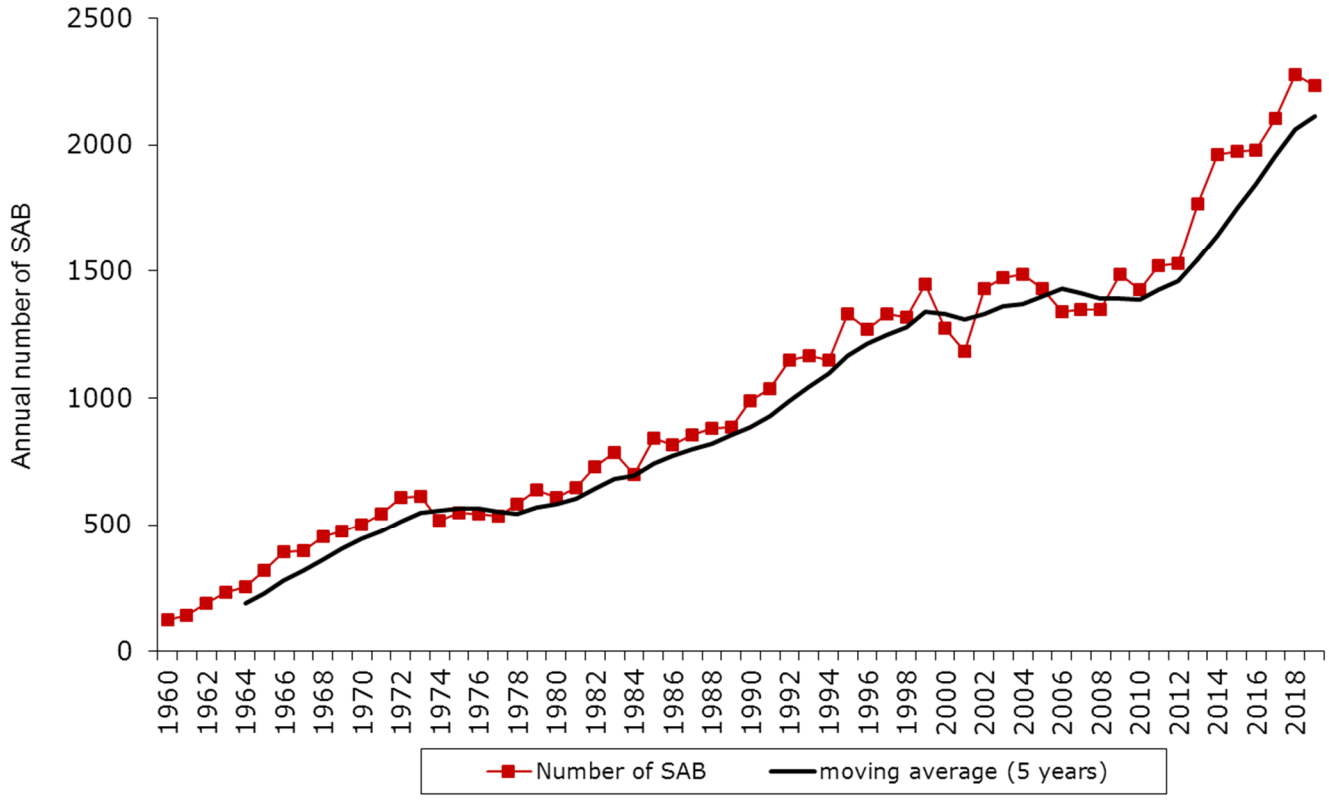

Figure 1. Number of SAB cases in Denmark 1960-2019.

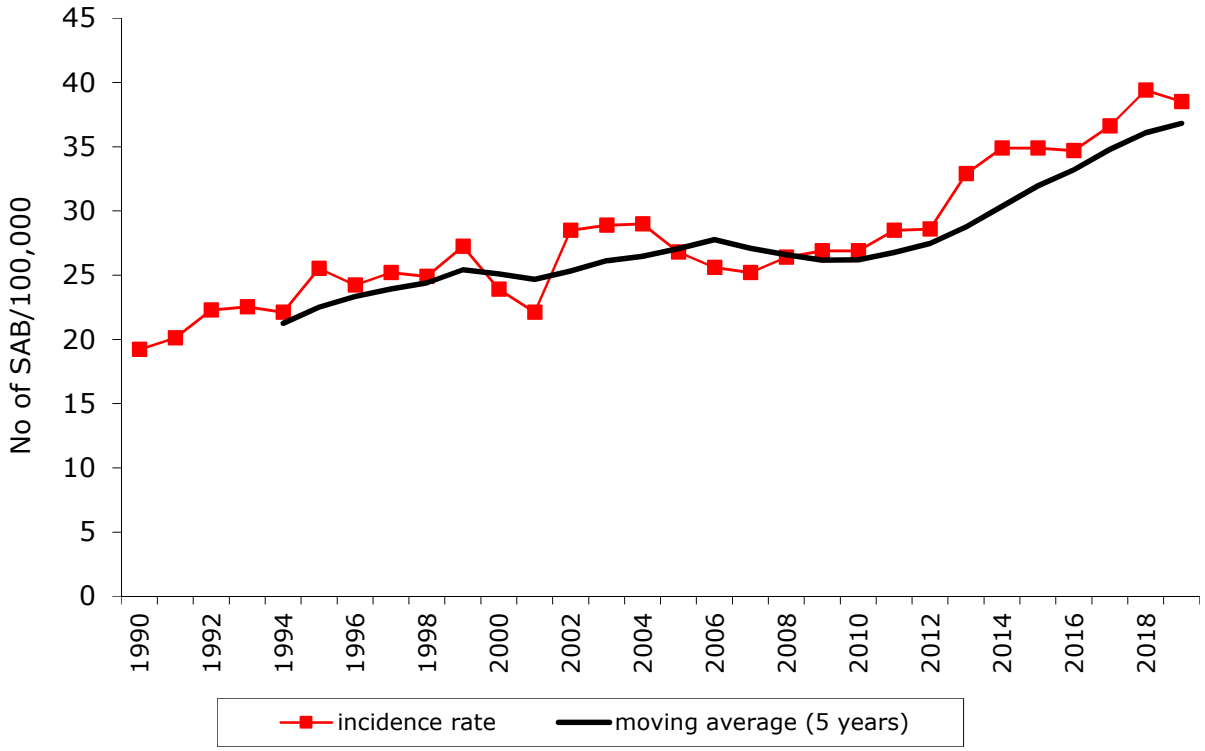

Figure 2. Incidence rate of SAB in Denmark per 100,000 inhabitants during 1990-2019.

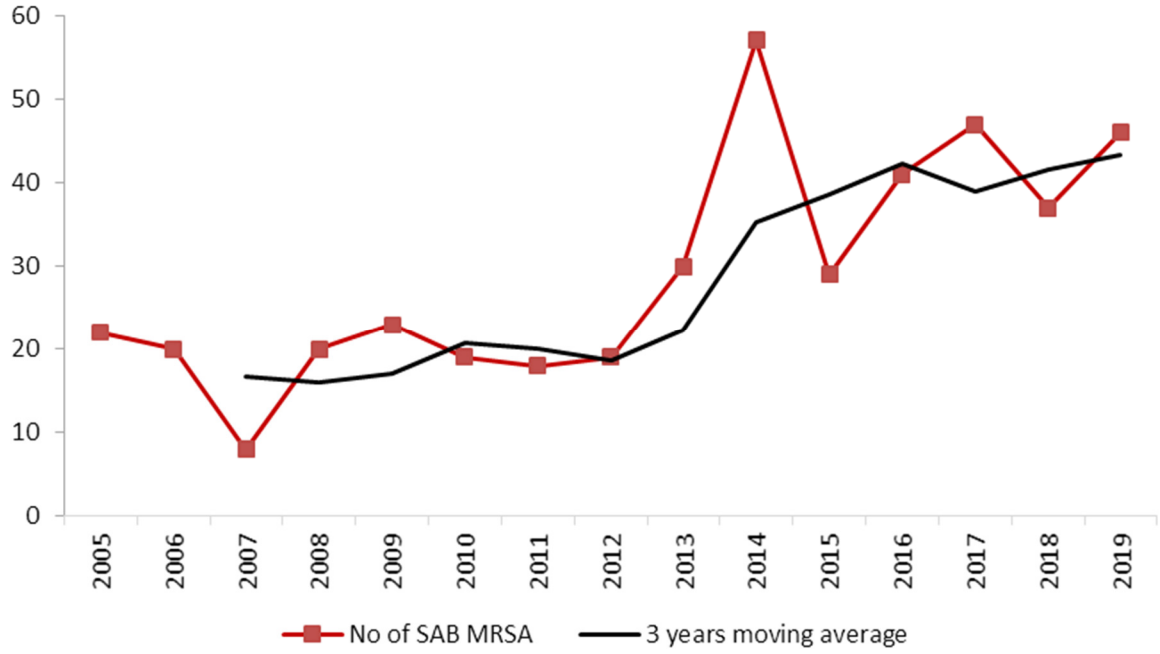

Figure 3. Number of MRSA SAB cases in Denmark 2005-2019.

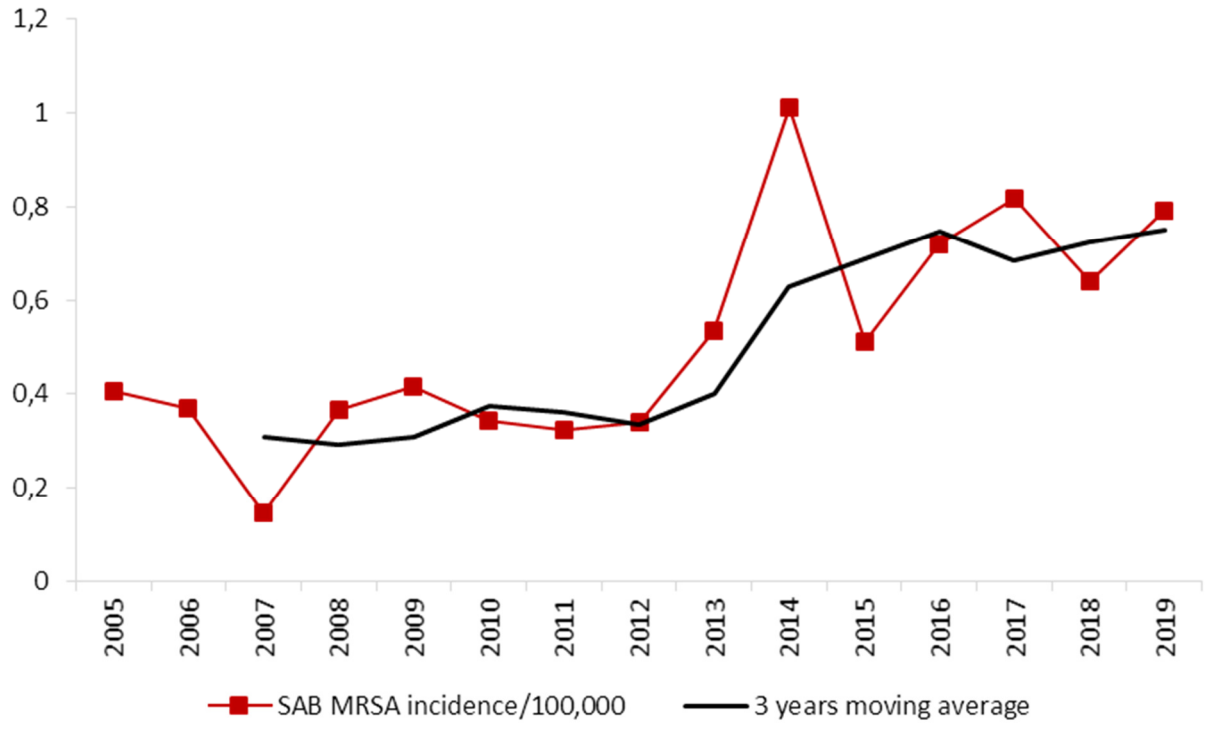

Figure 4. Incidence rate of MRSA SAB cases in Denmark per 100,000 inhabitants 2005-2019.

2.2 Age

More than 84% of the SAB patients in 2019 were older than 50 years and 24% were older than 80 years (Figure 5). The Danish population only included 4% older than 80 years in 2019 and the incidence of SAB among people above 80 years of age (239/100,000 inhabitants) was seven times higher than for the rest of the population (31/100,000 inhabitants). In the decades 1960-1969, 1970-1979 and 1980-1989 SAB patients older than 80 years only comprised below or around 10% of all patients, while in the last two decades, 2000-2009 and 2010-2019, this proportion was around or above 20% (Figure 6).

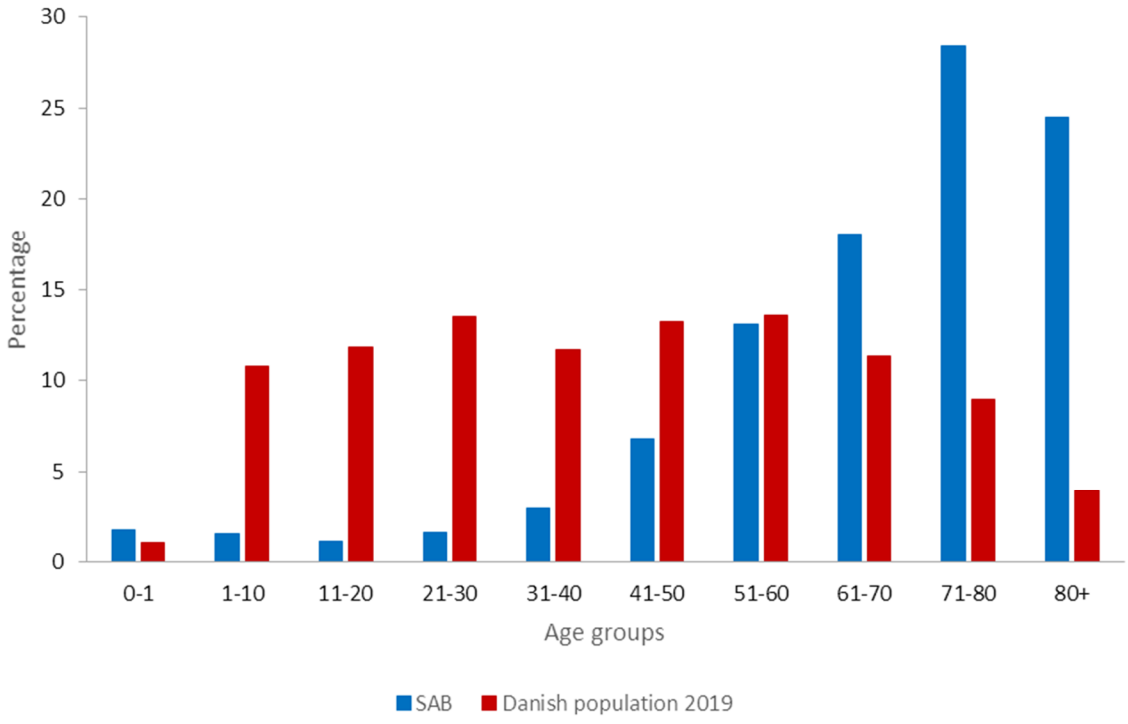

Figure 5. Age distribution of SAB patients and the Danish population in 2019 (%).

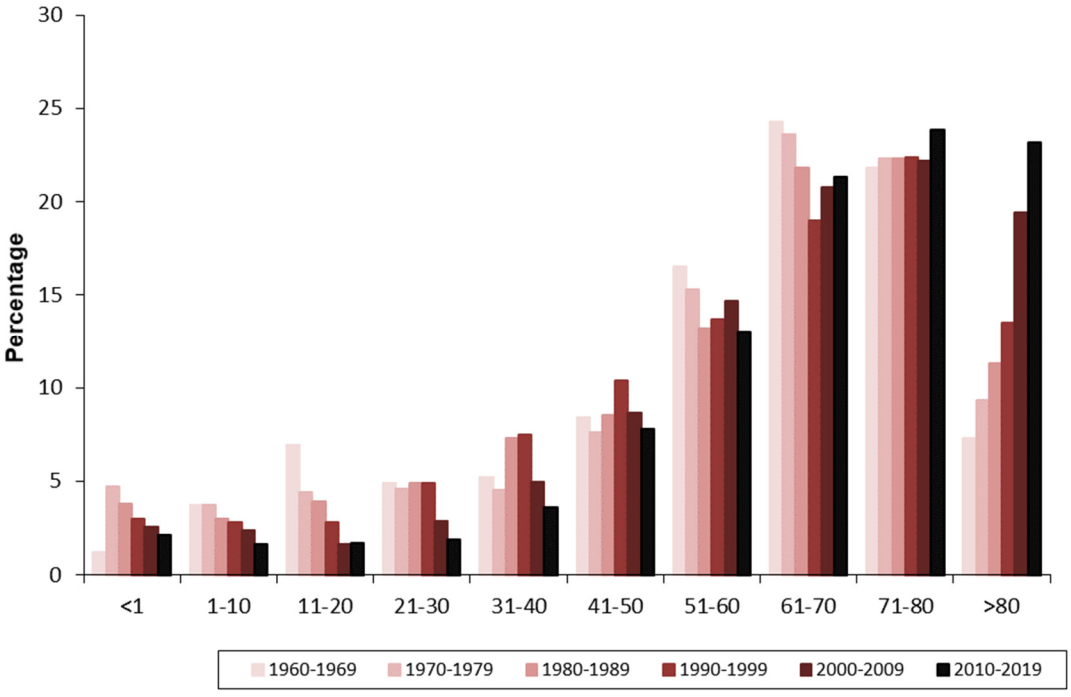

Figure 6. Age distribution of SAB patients in Denmark 1960 - 2019 (%).

2.3 Case fatality

The 30-day all-cause case fatality was 23.2% in 2019 (Table 2). The rate has been between 17-24% for the last 25 years (Figure 7). There was no difference in 30-day all-cause case fatality between men and women (22.3% and 24.7%, respectively, p=0.19, Fischer's exact test). Case fatality was low between 1-40 years, increased from the age group of 41-50 years, and patients above 80 years had a case fatality rate of 42.9% (Table 2), almost twice as high as the average. The case fatality rate for patients above 80 years has been relatively constant around 40% the last decade.

Table 2. Case fatality among Danish SAB patients in 2019 by age group and in total.

Age group (years)	0-1	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	80+	Total
No. SAB	39	35	26	37	67	152	293	403	634	548	2234
No. case fatality	1	1	0	1	2	14	34	74	156	235	518
% case fatality	2.6	2.9	0	2.7	3.0	9.2	11.6	18.4	24.6	42.9	23.2

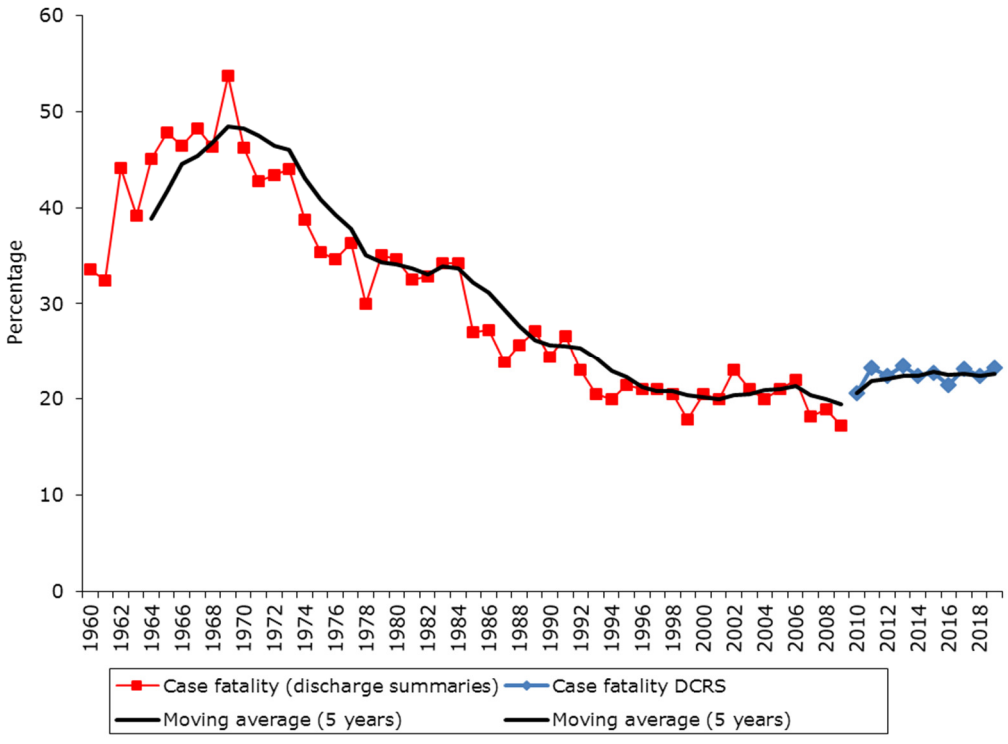

Figure 7. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2019.

Until 2009, data was extracted from discharge notes. From 2010 and onwards 30-day, all-cause case fatality was extracted from the Danish Civil Registration System (DCRS).

The outcome of SAB did not seem to depend on the specific type of *S. aureus* causing infection. The most prevalent *spa* types among the 518 isolates from cases dying within 30 days did not differ from the overall distribution of *spa* types. Of cases with *pvl* positive isolates (see section 2.7) only five (12%) died within thirty days from a positive blood culture. Thirty-day case fatality among MRSA-cases was also almost the same as the MSSA-case fatality (17.4% vs. 23.3%, p=0.48, Fischer's exact test).

2.4 Secondary infections

Within three months after SAB, the number of cases with a registered secondary infection was 553, corresponding to 24.8% (no data for one case). Endocarditis was the most prevalent secondary infection, followed by prosthetic infection, spondylitis, and arthritis (Table 3). Myositis, abdominal abscesses and tenosynovitis were all registered in less than 1%. No major changes of secondary infections in the period 2014 to 2019 have been observed (Table 3).

Table 3. The most common secondary infections (%) among Danish SAB patients in 2014-2019, recorded 3 months after admission.

Secondary infection	2014	2015	2016	2017	2018	2019
Endocarditis	10.0	11.5	11.4	10.6	11.6	10.2
Spondylitis	5.3	5.6	5.5	4.9	5.8	5.6
Prosthetic infection	4.4	3.8	3.7	5.1	4.0	4.3
Arthritis	2.7	3.5	3.4	4.3	3.4	3.4
Osteomyelitis	3.3	2.4	2.3	2.1	3.0	2.5
Central nervous system	2.3	1.8	1.8	1.6	1.3	2.0

2.5 Comorbidities

SAB primarily affects people who are diagnosed with other diseases. In 2019, 706 cases (32%) had no comorbidities registered, while 895 cases (40%) had a comorbidity index score of 1-2, and 632 cases (28%) had a score of more than 2 (one case with no data). Table 4 presents comorbidity based on the Charlson index. Malignancy (23.8%), diabetes without chronic complication (22.4%), and congestive heart failure (17.1%) were the most frequently registered comorbidities among SAB patients in 2019. These three comorbidities has been among the most prevalent for the last eight years. Overall, the prevalences of comorbidities have been very stable for this period. Comorbidities (and secondary infections) were extracted from discharge notes prior to 2010, and consisted of fewer and somewhat different categories, which makes comparisons longer back in time difficult.

Table 4. Number and percentage of comorbidities among Danish SAB patients 2019, with percentages for 2016 - 2018 for comparison.

Comorbidity	2019 Number	2016 %	2017 %	2018 %	2019 %
AIDS/HIV	3	0.6	0.7	0.2	0.1
Any malignancy	532	24.5	24.9	23.2	23.8
Metastatic solid tumor	80	5.4	5.0	4.5	3.6
Diabetes without chronic complication	500	24.5	26.5	25.4	22.4
Diabetes with chronic complication	307	13.7	14.9	14.5	13.7
Dementia	94	4.3	4.5	4.2	4.2
Hemiplegia or paraplegia	27	1.0	2.1	1.8	1.2
Cerebrovascular disease	342	18.0	19.8	18.0	15.3
Myocardial infarction	187	8.6	9.7	8.8	8.4
Congestive heart failure	381	17.6	21.1	17.4	17.1
Chronic pulmonary disease	369	17.2	20.1	17.7	16.5
Peptic ulcer disease	139	7.6	7.9	6.2	6.2
Mild liver disease	182	9.6	11.4	8.1	8.1
Moderate or severe liver disease	97	4.6	5.6	3.8	4.3
Renal disease	369	17.4	21.1	18.2	16.5
Rheumatic disease	105	5.9	6.2	5.4	4.7
Peripheral vascular disease	295	16.8	17.4	13.8	13.2
Drug abuse	69	2.9	3.3	2.5	3.1

2.6 Typing

spa typing was successful for 2,215 isolates (99.1%). A total of 630 different *spa* types were identified, and ten *spa* types accounted for 33% of the isolates (Table 5). The same ten *spa* types were the most prevalent in 2016, 2017 and 2018 but with some differences in ranking. A total of 426 *spa* types (68% of all *spa* types) were only found once. Putative assignment to MLST CC was possible for 1,898 isolates (85%). In the remaining cases, assignment was not possible due to low number of repeats in the *spa* type or an otherwise unresolved relationship with MLST typing. A total of 27 MLST CC groups were assigned. The three most prevalent CC

groups constituted 37% of the SAB isolates in 2019 while the 10 most prevalent constituted 76% (Table 6). Forty-one SAB isolates were *pvl* positive (1.8%), of which six were MRSA (two t008/CC8, two t021/CC30, one t019 CC30 and one t304/CC6). The *pvl* positive isolates were distributed among 22 different *spa* types and eleven MLST CC groups; six isolates had an unresolved relationship with MLST typing.

2.6.1 CC398

CC398 MRSA isolates have been associated with a reservoir in livestock. CC398 constituted 77 SAB cases (3.4%) in 2019 of which eight were MRSA. All eight MRSA stains belonged to the livestock-associated clade of CC398, and three of the SAB CC398 MRSA patients had direct or indirect contact to livestock. One of the SAB CC398 MRSA patients died within 30 days of diagnosis. Since 2007, 10 SAB patients with CC398 MRSA have died within 30 days. Among the 69 SAB CC398 MSSA, 63 belonged to the human clade while 6 belonged to the live-stock associated clade. Case fatality rate among SAB CC398 MSSA was 23.2% (23.8% for the human clade, 16.7% for the livestock-associated clade).

Table 5. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2019. Corresponding numbers and prevalences for the years 2015 to 2018 are shown for comparison.

spa type	2015	2016	2017	2018	2019
t127	96 (4.9)	81 (4.1)	128 (6.1)	137 (6.0)	117 (5.2)
t091	60 (3.0)	62 (3.1)	85 (4.0)	85 (4.0)	95 (4.3)
t084	89 (4.5)	83 (4.2)	75 (3.6)	105 (4.6)	91 (4.1)
t230	82 (4.2)	81 (4.1)	70 (3.3)	81 (3.6)	73 (3.3)
t002	84 (4.3)	87 (4.4)	81 (3.8)	101 (4.4)	72 (3.2)
t012	57 (2.9)	62 (3.1)	65 (3.1)	70 (3.1)	65 (2.9)
t021	46 (2.3)	39 (2.0)	52 (2.5)	61 (2.7)	65 (2.9)
t008	36 (1.8)	55 (2.8)	44 (2.1)	53 (2.3)	59 (2.6)
t701	45 (2.3)	37 (1.9)	51 (2.4)	40 (1.8)	56 (2.5)
t015	53 (2.7)	38 (1.9)	43 (2.0)	49 (2.2)	36 (1.6)

Table 6. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2019. Corresponding numbers and prevalences for the four previous years and the 10 year trend are shown.

Clonal complex	2015	2016	2017	2018	2019	10 year trend*
CC45	330 (16.7)	305 (15.4)	292 (13.9)	330 (14.5)	326 (14.6)	0.95
CC30	243 (12.3)	241 (12.2)	269 (12.8)	289 (12.7)	279 (12.5)	0.98
CC15	197 (10.0)	209 (10.6)	200 (9.5)	230 (10.1)	227 (10.2)	ns
CC1	169 (8.6)	149 (7.5)	208 (9.9)	224 (9.8)	209 (9.4)	1.04
CC5	169 (8.6)	171 (8.6)	176 (8.4)	193 (8.5)	166 (7.4)	ns
CC8	143 (7.2)	138 (7.0)	142 (6.7)	163 (7.2)	161 (7.2)	ns
CC7	69 (3.5)	72 (3.6)	94 (4.5)	86 (3.8)	100 (4.5)	1.05
CC22	58 (2.9)	64 (3.2)	80 (3.8)	89 (3.9)	86 (3.9)	ns
CC398	25 (1.3)	41 (2.1)	47 (2.2)	51 (2.4)	77 (3.4)	1.18
CC97	48 (2.4)	47 (2.4)	48 (2.3)	67 (2.9)	60 (2.7)	1.05

* trend is shown as significant in- or decrease per year of the particular clonal complex relative to the total number of SAB cases. Values below 1 denotes decrease, values above 1 denotes increase, ns denotes no significant trend.

2.7 Antimicrobial susceptibility testing

Table 7 shows the prevalence of resistance to the antimicrobials tested. The resistance profiles are shown in Figure 8. Figure 9 shows selected resistance prevalences from 1980 to 2019.

Resistance to penicillin was 71.9% (72.2% in 2018). Resistance to fusidic acid decreased to 13.7%. All tested SAB isolates were susceptible to ceftaroline, ceftobiprole, linezolid and vancomycin in 2019. The proportion of isolates susceptible to all antibiotics was 20.6%. The proportion of resistance to at least one antimicrobial in addition to penicillin was 22.6% and the proportion of resistance to at least two and three additional antimicrobials were 6.6% and 2.7%, respectively.

In MiBa, 2.231 *S. aureus* isolates from blood were found and the results of the antimicrobial resistance determinations performed at the regional DCMs were retrieved. These resistance

prevalences are also shown in Table 7. They are overall comparable to the results generated in the NRL-AMR with some minor differences; most notably resistance to norfloxacin where data from MiBa indicates a resistance prevalence 50% higher than NRL-AMR (7.2 vs. 4.7%). Antimicrobial resistance data in this report will in the future be based on the data obtained from MiBa.

Table 7. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2019 (n=549) generated at the National Reference Laboratory for Antimicrobial Resistance and retrieved from MiBa

	Resistance (%)		Minimal inhibitory concentration (MIC, mg/L)										
Antimicrobial	**NRL-AMR**	**MiBa**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
Penicillin	71.9	72.6		25.5	2.6	71.9							
Erythromycin	8.7	7.4						90.7	0.5	0	8.7		
Clindamycin	8.2§	7.6				99.1	0.2	0	0.7				
Fusidic acid	13.7	14.9					83.4	2.9	0.4	13.3			
Tetracycline	2.4	3.3						97.4	0.2	0.2	2.2		
Norfloxacin	4.7	7.2								95.3	0.9	3.8	
Rifampicin	0.7	0.5				99.1	0.2	0	0.7				
Linezolid	0	0.4							92.7	7.3	0		
Kanamycin	0.7	NA										99.3	0.7
TMP/SXT*	0.2	0.8							99.1	0.7	0	0.2	
Ceftaroline	0	NA					99.1	0.9	0				
Ceftobiprole	0	NA					97.8	2.2	0	0			
Daptomycin	2.6	NA					61.2	36.2	2.4	0.2			
Gentamicin	0.5	0.5						99.5	0.2	0.4			
Mupirocin	0.4	0.1					99.5	0.2	0.4				
Vancomycin	0	0						97.4	2.6	0			

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

§ *Inducible clindamycin resistance is included. * Trimethoprim/sulfamethoxazole; MIC expressed as the trimethoprim concentration. NA Not applicable*

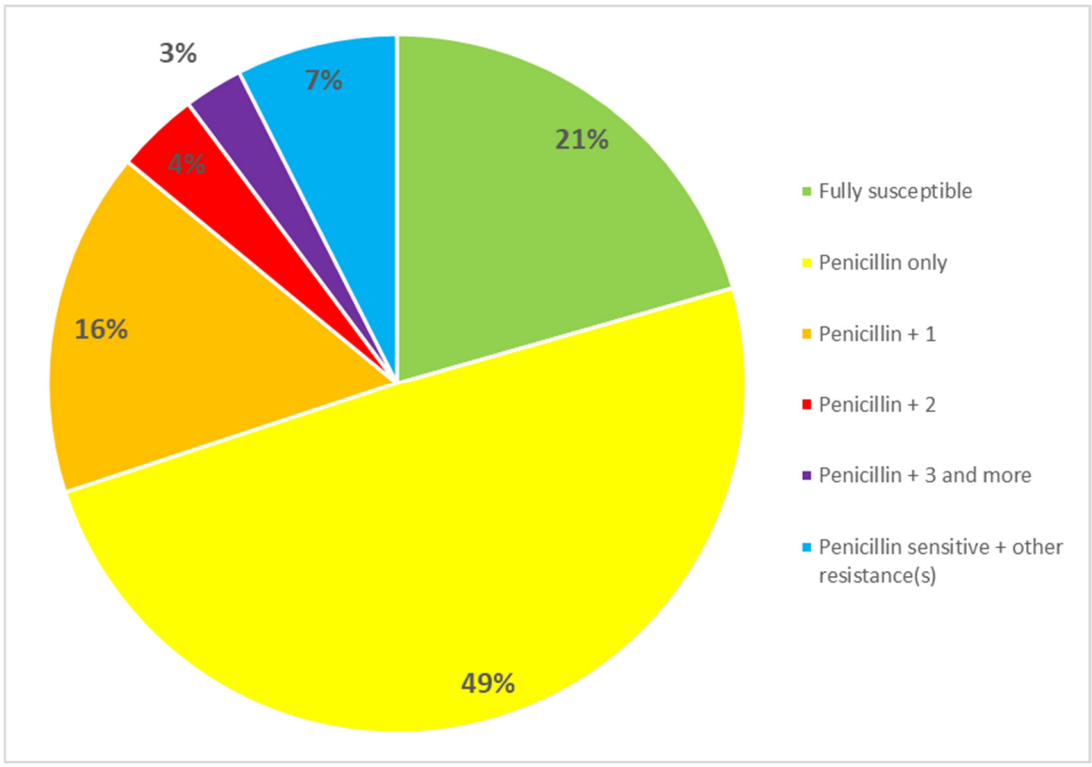

Figure 8. Resistance profiles of a subset of Danish SAB isolates 2019 (n=549) (%).

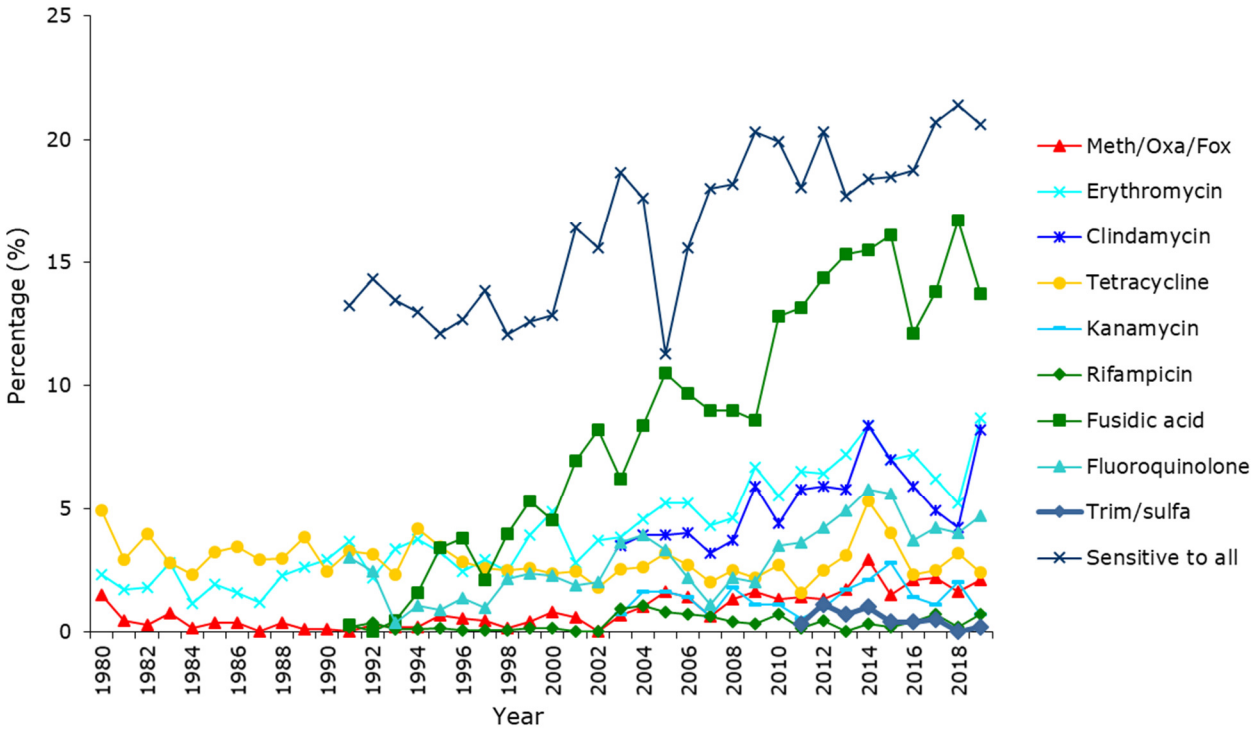

Figure 9. Prevalence of selected antimicrobial resistance in Danish SAB isolates (1980-2019).

3. Conclusions

The number of recorded SAB cases was at the same level as in 2018. However, the long-term trends demonstrate increasing numbers and incidence. The prevalence of MRSA cases was 2.1%. In three of the four last years, the prevalence has been above 2%, and the long term surveillance clearly indicates a slowly increasing MRSA prevalence in Danish SAB cases on in average 4% per year. The 30 day all-cause case fatality rate was 23% and this rate has been remarkably stable since the beginning of the 1990'ies. More than two-thirds of all blood isolates were either fully susceptible or resistant only to penicillin.

More than two-thirds of all patients had at least one comorbidity registered, and three months after onset of SAB, one-fourth of all cases had a registered secondary infection, reflecting that SAB primarily affects patients with a compromised immune status and has severe consequences.

Comparison of AST results obtained at the DCMs laboratories and in the NRL are with the exception of norfloxacin similar. Based on these results future reports will only include results from the DCM laboratories.

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