STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2020

This report describes the laboratory and clinical characteristics of the 2,342 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2020. 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, Pia Thurø Hansen and Stine Frese-Madsen are thanked for technical assistance in the lab.

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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
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. 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 was used to analyze for trends for number of SAB, number of methicillin-resistant SAB and prevalence of *spa* types and clonal complex in relation to the total number of SAB cases (Stata 14.2, StataCorp, College Station, USA).

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).

1.3 Antimicrobial susceptibility data

The NRL-AMR extracted data on antimicrobial susceptibility from the Danish Microbiology Database (MiBa). The first *S. aureus* isolate per patient per year from blood was included. Resistance to penicillin, erythromycin, clindamycin, tetracycline, rifampicin, gentamycin, fusidic acid, sulfamethizol-trimethoprim, linezolid, mupirocin, vancomycin, and norfloxacin were retrieved.

2. Results

2.1 Cases and incidence

In 2020, 2,342 cases of SAB were recorded (Figure 1) of which 2,116 (90%) constituted primary and 226 subsequent episodes. The incidence rate of SAB was 40.2/100,000 inhabitants (Figure 2). The incidence rate has increased in average by 4% each year since 2011. Methicillin-resistant *S. aureus* (MRSA) was identified from 38 cases (1.6%) (Figure 3) and the incidence rate of SAB-MRSA was 0.65/100,000 inhabitants (Figure 4). In the last ten years there has been no significant increase of numbers of SAB MRSA in relation to annual numbers of SAB. There were more males than females (63% males vs. 37% females) among the cases of SAB in 2020. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

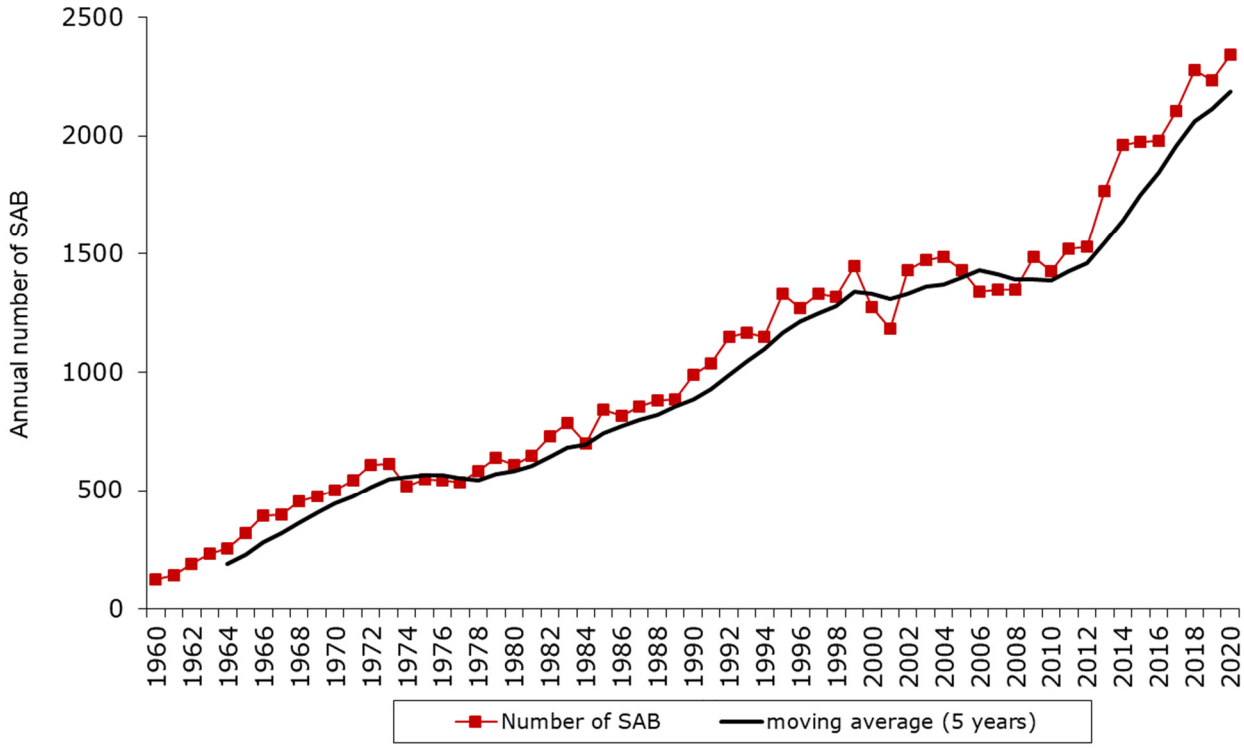

Figure 1. Number of SAB cases in Denmark 1960-2020.

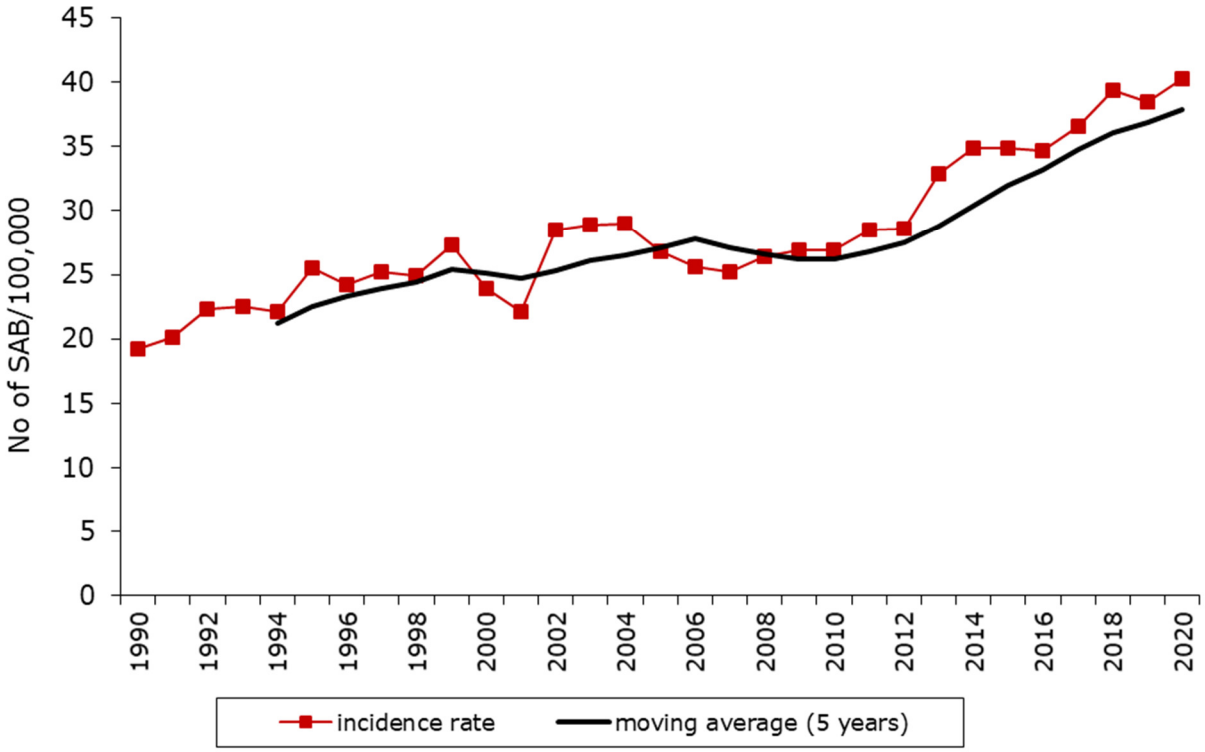

Figure 2. Incidence rate of SAB in Denmark per 100,000 inhabitants 1990-2020.

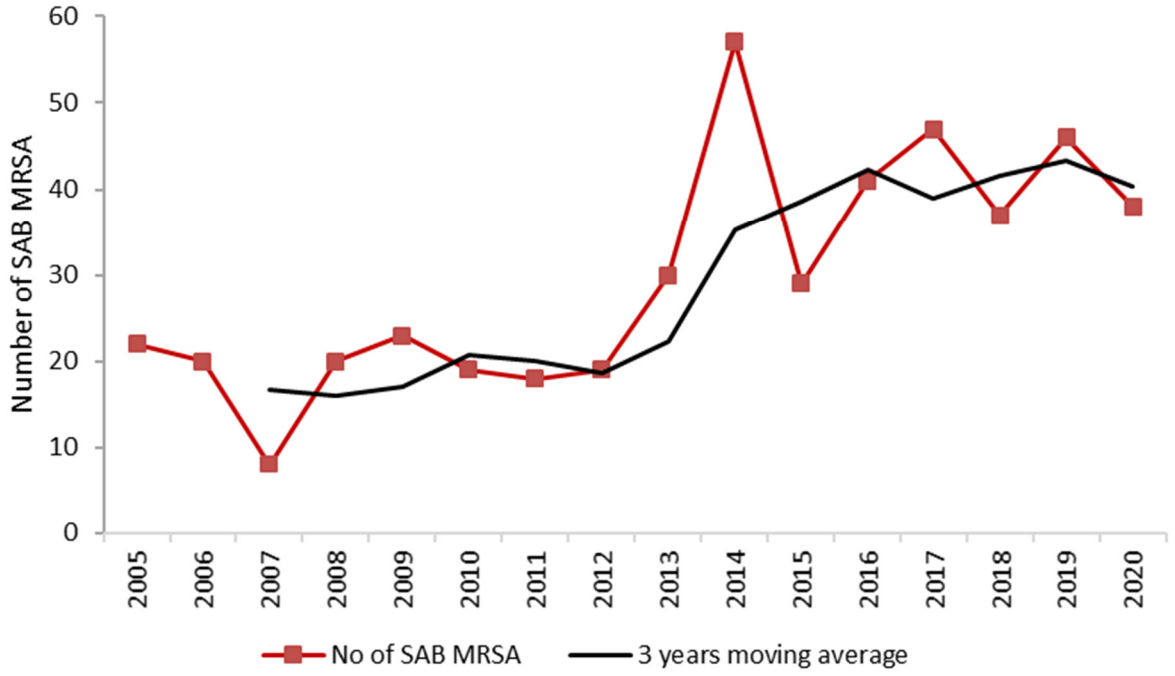

Figure 3. Number of SAB MRSA cases in Denmark 2005-2020

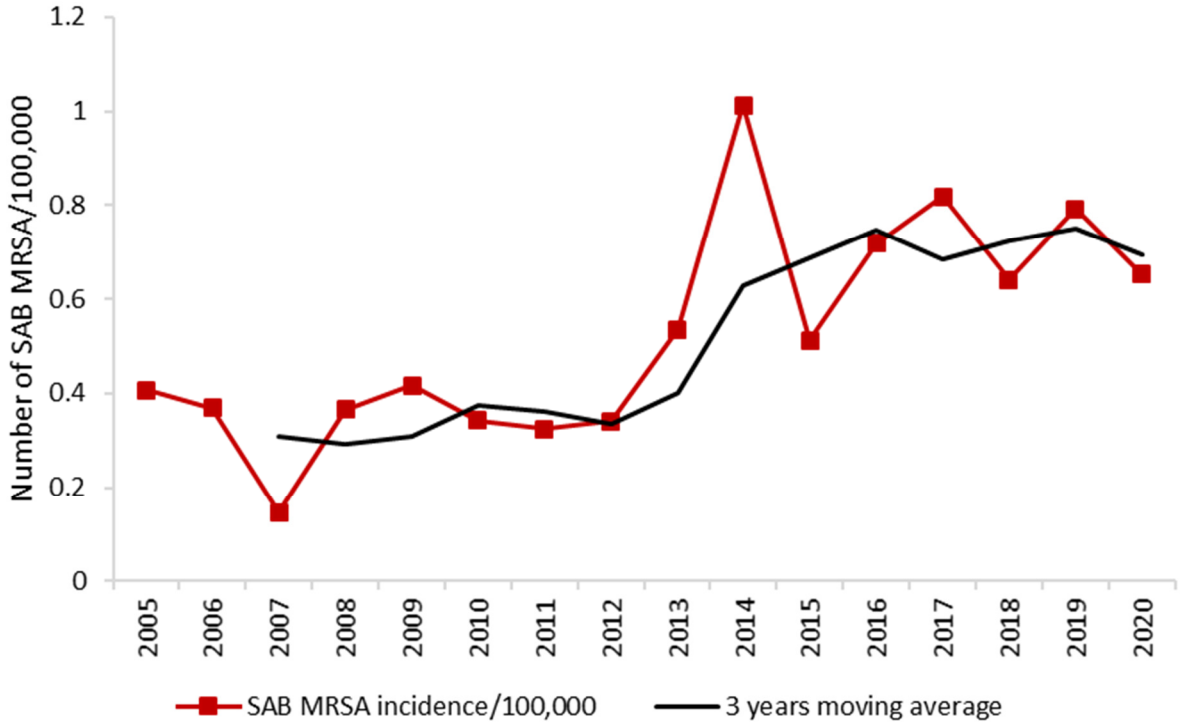

Figure 4. Incidence rate of SAB MRSA cases in Denmark per 100,000 inhabitants 2005-2020.

2.2 Age

More than 85% of the SAB patients in 2020 were older than 50 years and 26% were older than 80 years (Figure 5). The Danish population only included 4% older than 80 years in 2020 and the incidence of SAB among people above 80 years of age (265/100,000 inhabitants) was eight times higher than for the rest of the population (30.7/100,000 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%. A recent study showed that SAB rates between 2008 and 2015 in Denmark increased with 4.0% for person <80 years, with 8.4% for persons 80–89 years of age, and 13.0% for persons >90 years of age (Thorlacius-Ussing *et al*. 2019). Specific causes and mechanisms behind this increase among the elderly population are unresolved.

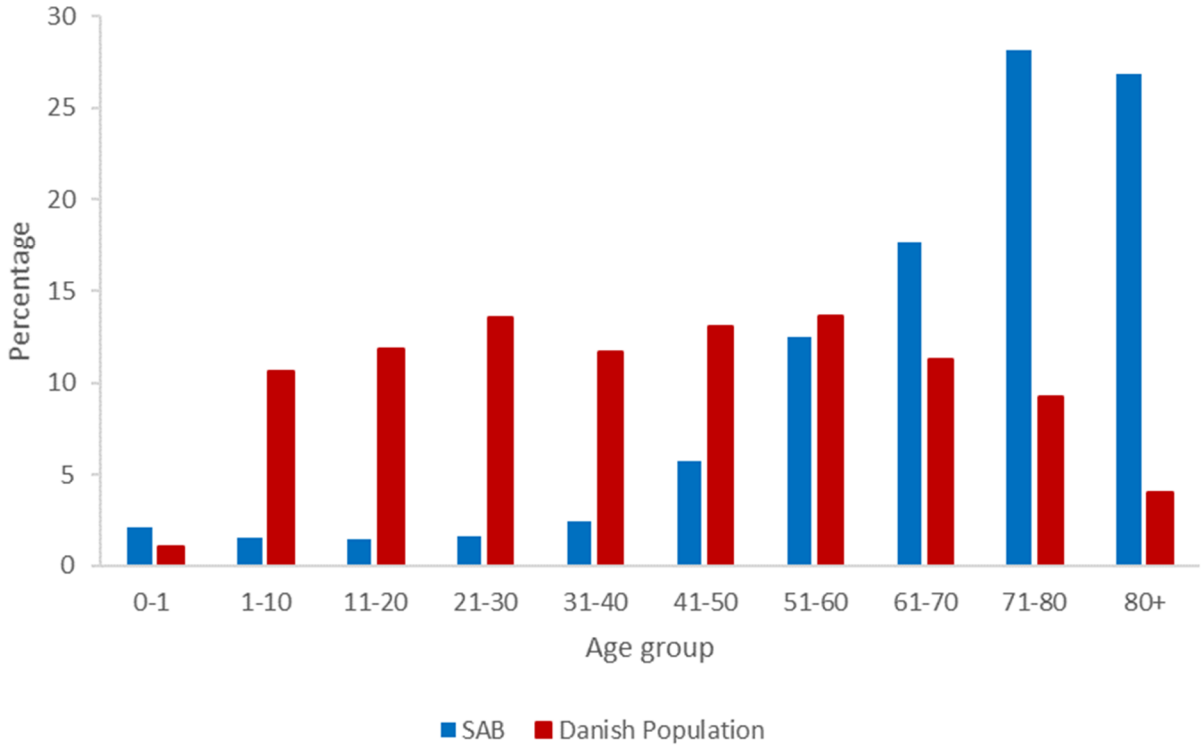

Figure 5. Age distribution of SAB patients and the Danish population in 2020 (%).

2.3 Case fatality

The 30-day all-cause case fatality was 20.7% in 2020 (Table 1). 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 (20.2% and 21.6%, respectively, p=0.42, Fisher's exact test). Case fatality was low between 1-40 years, increased from the age group of 51-60 years, and patients above 80 years had a case fatality rate of 39.1% (Table 1), almost twice as high as the average. The case fatality rate has been relatively constant for all age groups the last decade (Figure 8).

Table 1. Case fatality among Danish SAB patients in 2020 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	49	35	34	38	56	135	293	414	659	629	2342
No. case fatality	3	1	3	2	1	9	28	70	122	246	485
% case fatality	6.1	2.9	8.8	5.3	1.8	6.7	9.6	16.9	18.5	39.1	20.7

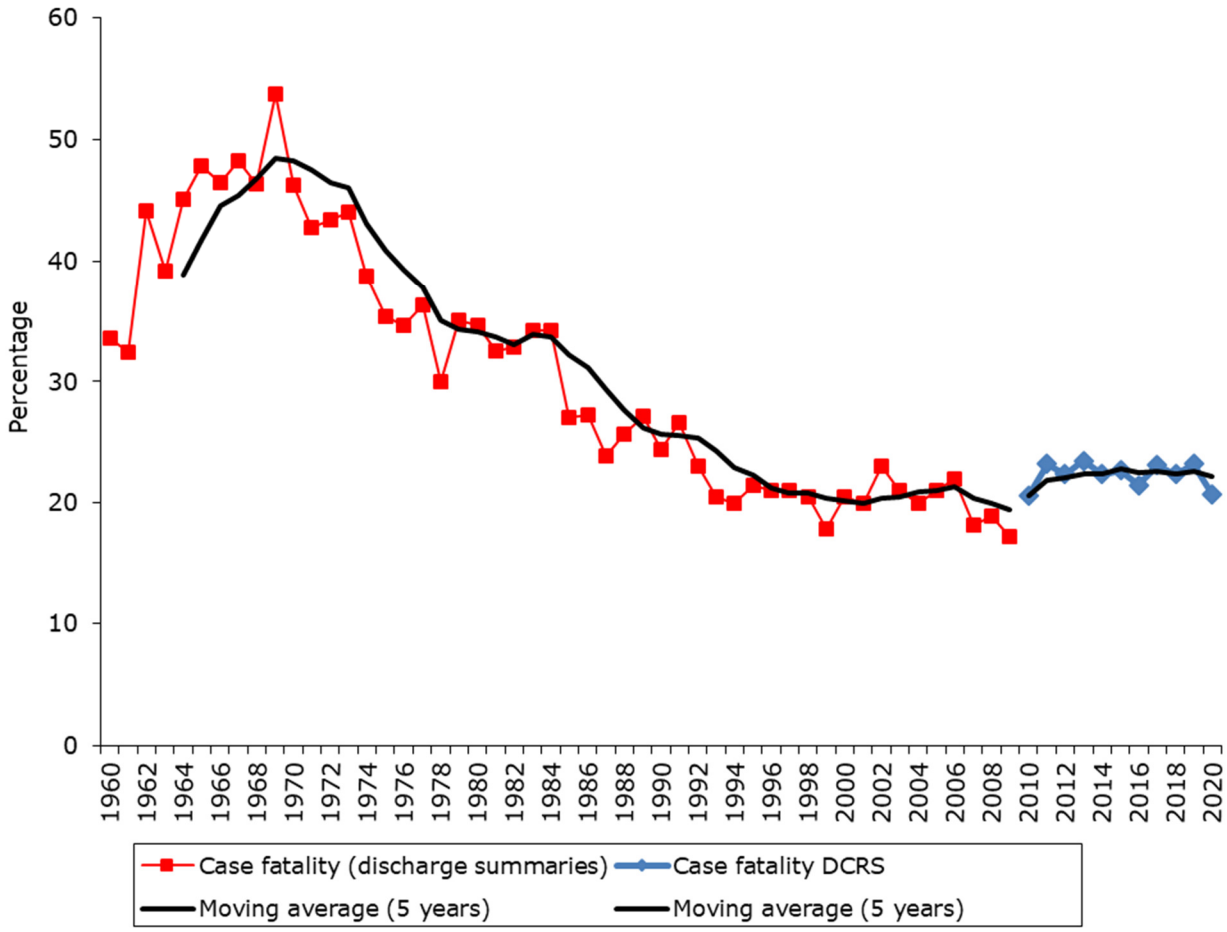

Figure 7. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2020.

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).

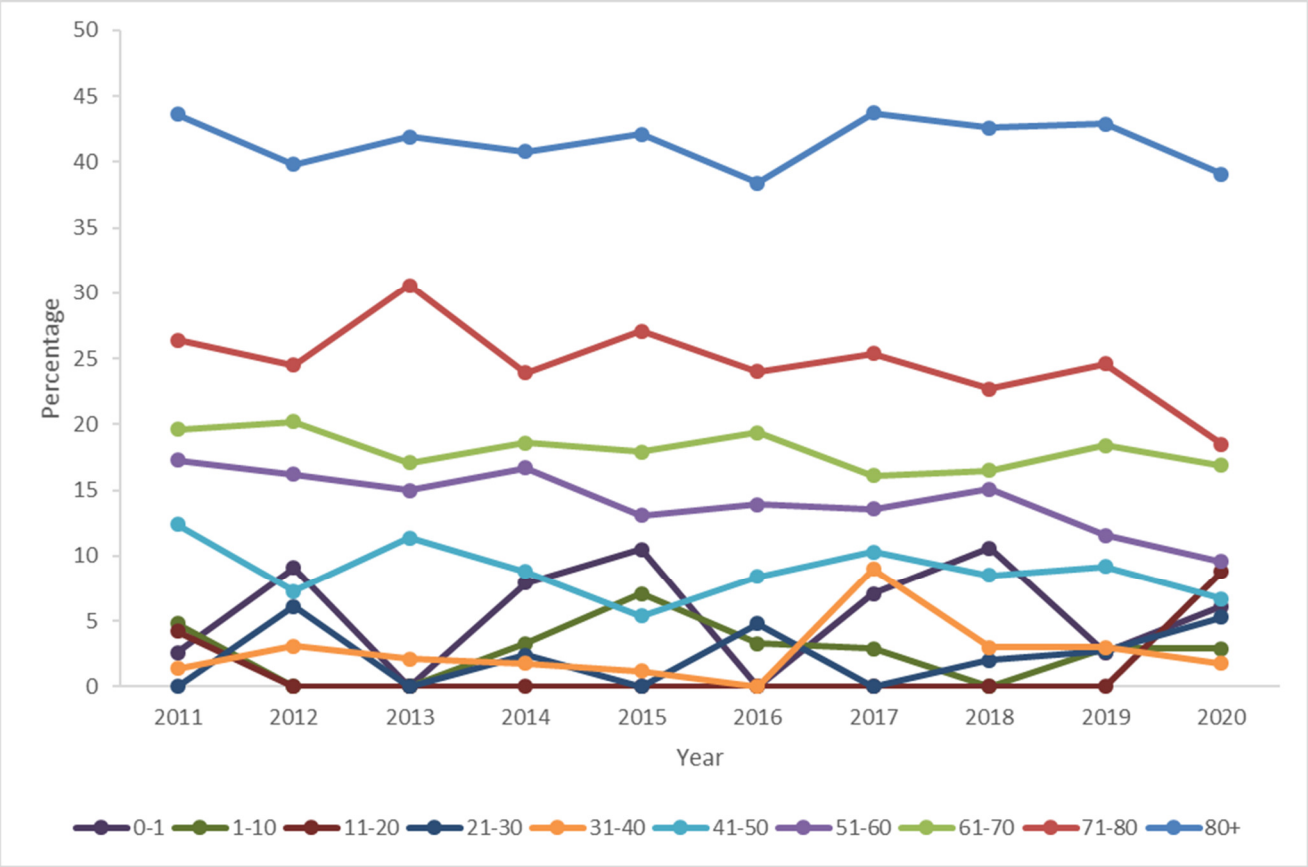

Figure 8. 30-day all-cause case fatality (%) of Danish SAB patients 2011-2020 by age-group.

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 485 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 2.6) nine (27.3%) died within thirty days from a positive blood culture which was not significantly different from *pvl* negative cases (20.6%, p=0.39, Fisher's exact test). Thirty-day case fatality among cases with MRSA was lower than for cases with MSSA (10.5% vs. 20.9%, however, the difference was not significant, p=0.16, Fisher's exact test).

2.4 Secondary infections

Within three months after SAB, the number of cases with a registered secondary infection was 599, corresponding to 25.6%. Endocarditis was the most prevalent secondary infection, followed by prosthetic infection, spondylitis, and arthritis (Table 2). Myositis, abdominal abscesses and tenosynovitis were all registered in less than 1%. No major changes in the prevalence of secondary infections in the period 2011 to 2020 have been observed (Figure 9); however, with the increasing numbers of cases, increasing numbers of secondary infections have been recorded, most notably for endocarditis and spondylitis (Figure 10).

Table 2. The most common secondary infections (%) among Danish SAB patients in 2020, recorded 3 months after admission.

Endocarditis	Spondylitis	Prosthetic infection	Arthritis	Osteomyelitis	Central nervous system
11.4	5.3	3.8	2.6	2.8	2.1

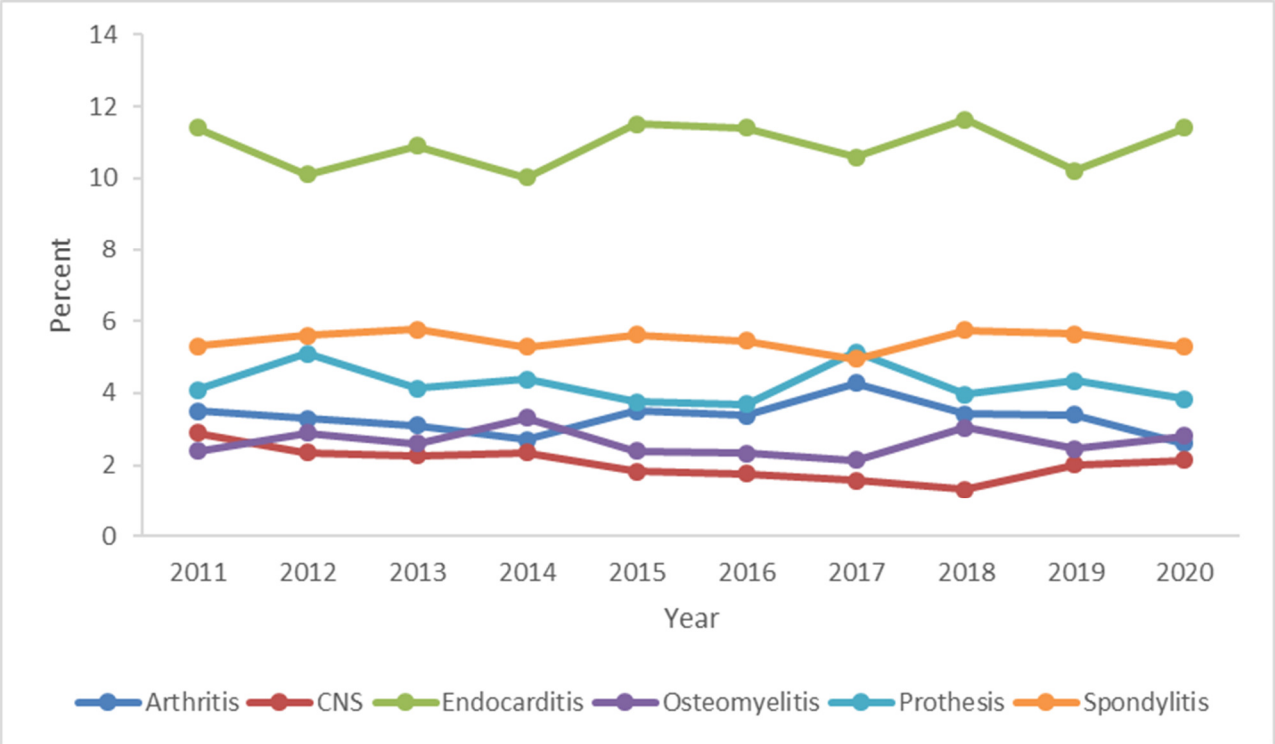

Figure 9. Prevalence of secondary infections (%) among Danish SAB patients 2011-2020, recorded three months after admission.

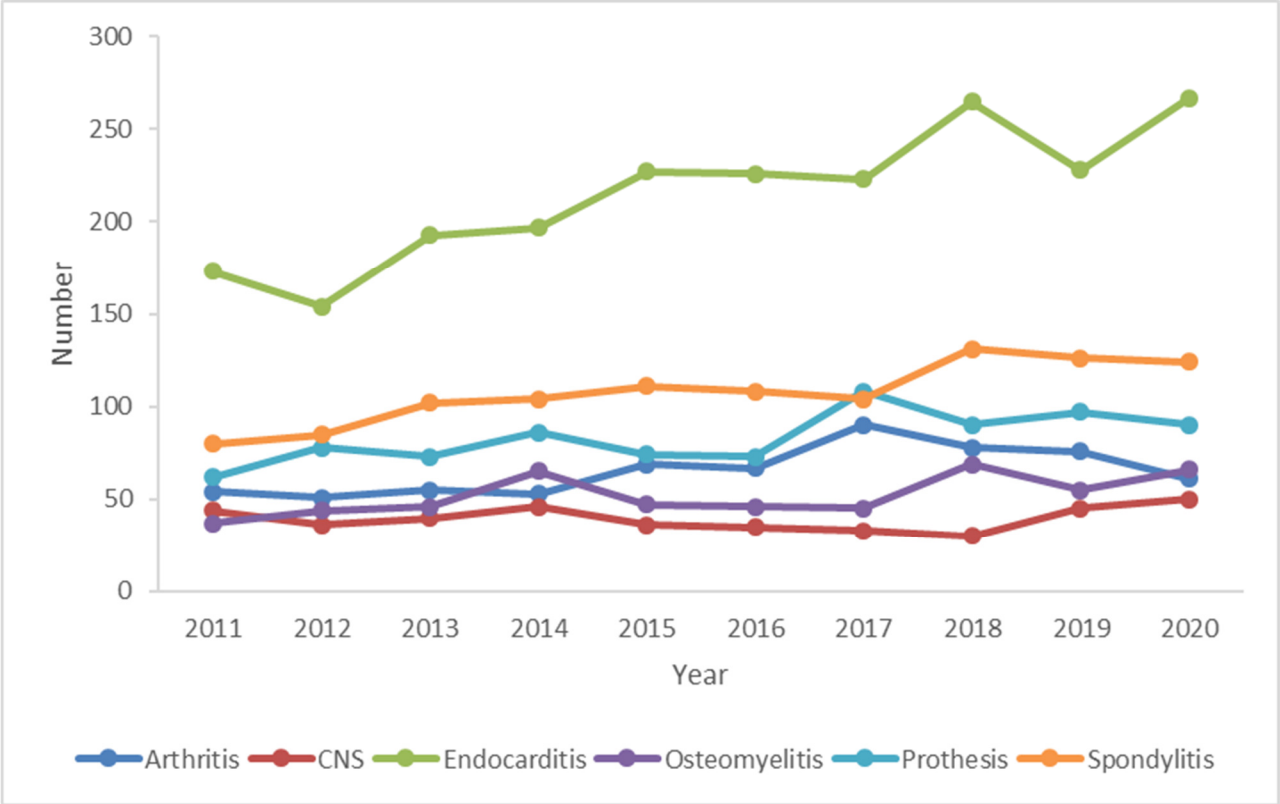

Figure 10. Number of secondary infections among Danish SAB patients 2011-2020, recorded three months after admission.

2.5 Comorbidities

SAB primarily affects people who are diagnosed with other diseases. In 2020, 796 cases (34%) had no comorbidities registered, while 894 cases (38%) had a comorbidity index score of 1-2, and 652 cases (28%) had a score of more than 2. Comorbidities were more often recorded among the older age groups (Table 3). Table 4 presents comorbidity based on the Charlson index. Malignancy (23.5%), diabetes without chronic complication (23.1%), and chronic pulmonary disease (17.3%) were the most frequently registered comorbidities among SAB patients in 2020. These three comorbidities has been among the most prevalent for the last ten years. Overall, the prevalence of comorbidities have been very stable for this period (Figure 11). 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 3. Prevalence (%) of comorbidity index score (CIS) per age group among Danish SAB cases 2020

	Age group									
CIS	**0-1**	**1-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**80+**
0	95	65	73	46	59	41	34	28	28	32
1-2	2	30	24	41	31	35	39	38	40	41
>2	2	5	3	14	10	24	27	35	32	27

Table 4. Prevalence of comorbidities among Danish SAB patients 2020

Comorbidity	%
AIDS/HIV	0
Any malignancy	23.5
Metastatic solid tumor	3.2
Diabetes without chronic complication	23.1
Diabetes with chronic complication	12.8
Dementia	4.7
Hemiplegia or paraplegia	1.1
Cerebrovascular disease	16.4
Myocardial infarction	6.9
Congestive heart failure	16.4
Chronic pulmonary disease	17.3
Peptic ulcer disease	5.8
Mild liver disease	7.2
Moderate or severe liver disease	3.0
Renal disease	14.4
Rheumatic disease	4.7
Peripheral vascular disease	13.3
Drug abuse	2.5

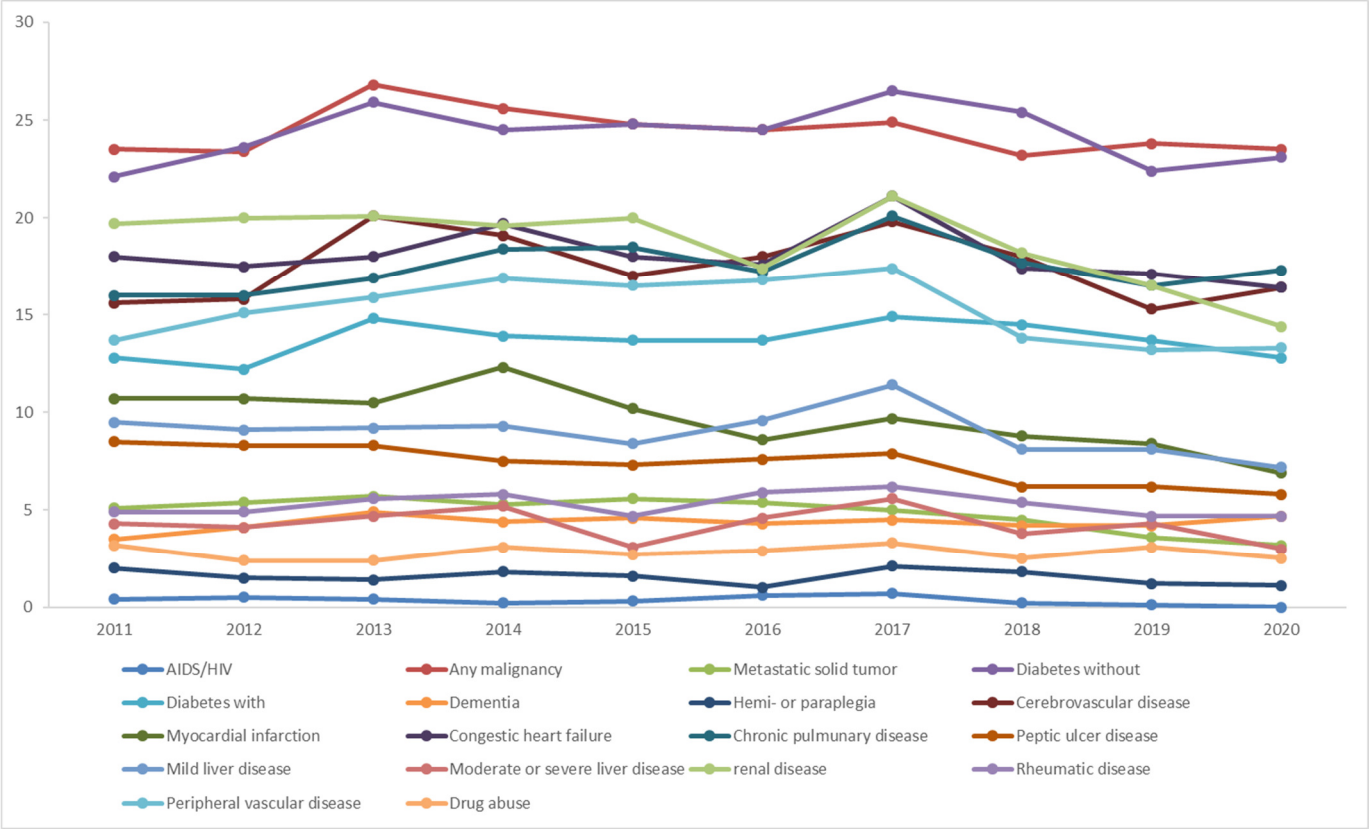

Figure 11. Prevalence (%) of comorbidities among Danish SAB patients 2011 - 2020

2.6 Typing

spa typing was successful for 2,331 isolates (99.5%). A total of 650 different *spa* types were identified, and ten *spa* types accounted for 32% of the isolates (Table 5). The same ten *spa* types have been the most prevalent since 2016 with some differences in ranking. *spa* type t230, which previously was the most common *spa* type among Danish SAB, and *spa* type t015, showed a significant decrease during the last 10 years, while *spa* type t701 increased in the same period (Table 5). A total of 437 *spa* types (67% of all *spa* types) were only found once. Putative assignment to MLST CC was possible for 2,070 isolates (88%). In the remaining cases, assignment was not possible due to an unresolved relationship with MLST typing. A total of 27 MLST CC groups were assigned. The three most prevalent CC groups constituted 39% of the SAB isolates in 2020 while the 10 most prevalent constituted 79% (Table 6). The most remarkable change of CC groups in the last decade was for CC398, which had an annual significant increase of 18% (Table 6). Thirty-three SAB isolates were *pvl* positive (1.4%), of which five were MRSA (two t008/CC8, two t034/CC398, and one t105 CC5). The *pvl* positive isolates were distributed among 25 different *spa* types and ten MLST CC groups; five 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 97 SAB cases (4.1%) in 2020 of which 11 were MRSA. Nine of the MRSA strains belonged to the livestock-associated clade of CC398. Two of the SAB CC398 MRSA patients had direct or indirect contact to livestock. None 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 86 SAB CC398 MSSA, 79 belonged to the human clade while 7 belonged to the live-stock associated clade. Case fatality rate among SAB CC398 MSSA was 20.9%.

Table 5. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2020 and the 10 year trend.

spa type	Number (%)	Trend
t084	118 (5.0)	ns
t127	111 (4.7)	ns
t091	92 (3.9)	ns
t002	92 (3.9)	ns
t230	76 (3.2)	0.93
t012	68 (2.9)	ns
t021	55 (2.3)	ns
t701	53 (2.3)	1.05
t015	46 (2.0)	0.94
t008	45 (1.9)	ns

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

Table 6. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2020 and the 10 year trend.

CC group	Number (%)	Trend
CC30	322 (13.7)	ns
CC45	307 (13.1)	0.95
CC15	285 (12.2)	ns
CC1	216 (9.2)	1.03
CC5	192 (8.2)	ns
CC8	165 (7.0)	ns
CC7	109 (4.7)	1.05
CC398	97 (4.1)	1.18
CC22	93 (4.9)	ns
CC97	62 (2.6)	1.04

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

Data retrieved from MiBa comprised 2,484 isolates. Susceptibility testing for different antimicrobials varied and only for penicillin all isolates were tested. Resistance to penicillin was 71.7% (71.9% in 2019) and resistance to fusidic acid increased to 14.2% (Table 7). Resistance to the remaining antimicrobials were all below 10%. Fully susceptible isolates comprised 21.6%. Figure 8 shows selected resistance prevalences from 1980 to 2020. Resistance to fusidic acid has increased from 0 to 15% while the proportion of fully susceptible isolates also increased from 12-13% to more than 20%.

Table 7. Resistance prevalence among SAB isolates retrieved from MiBa

Antimicrobial	Resistance (%)	Number of isolates tested
Penicillin	71.7	2,484
Erythromycin	7.2	2,277
Clindamycin	6.8	2,275
Fusidic acid	14.2	1,974
Tetracycline	2.9	877
Norfloxacin	6.1	295
Rifampicin	0.6	2,179
Linezolid	0*	2,104
TMP/SXT	0.8	600
Gentamicin	0.5	1,097
Mupirocin	0.2	1,251
Vancomycin	0	680

* Data from MiBa indicated five isolates resistant to linezolid. When testing was repeated in NRL-AMR, all isolates were sensitive, and thus 0% is reported. TMP/SXT=trimethoprim/sulfamethoxazole

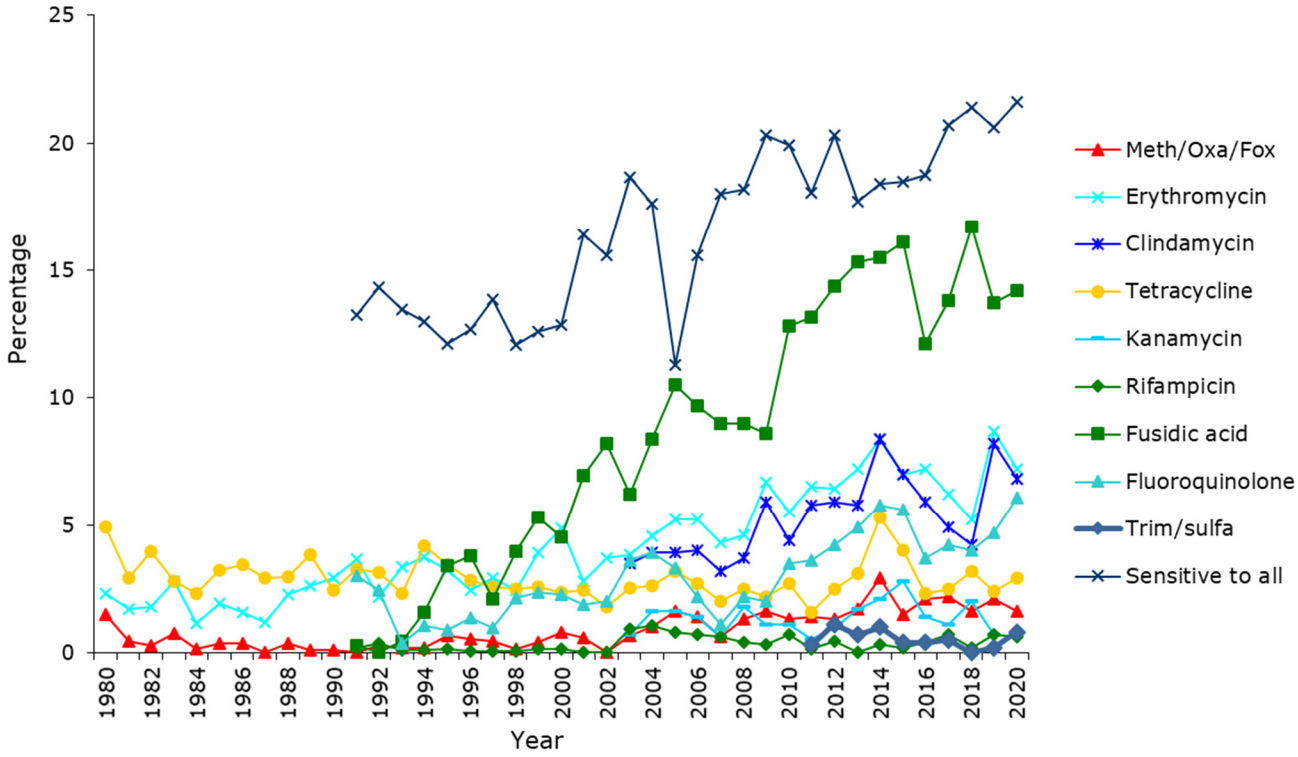

Figure 8. Prevalence of antimicrobial resistance in Danish SAB isolates (1980-2020).

3. Conclusions

The number of recorded SAB cases increased in 2020 and the long-term trends demonstrate increasing numbers and incidence. The prevalence of MRSA cases was 1.6%. The 30 day all-cause case fatality rate was 21% and this rate has been remarkably stable since the beginning of the 1990'ies. More than 20% of all SAB isolates were fully susceptible.

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. The number of secondary infections continues to increase; an effect that is believed to be caused by the changing demographics of cases of SAB, i.e. a growing proportion of the very old that appear more susceptible to SAB *per se* and to complications.

Overall, the epidemiology of SAB has varied very little over the past decade.

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