STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2021

This report describes the laboratory and clinical characteristics of the 2,512 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2021. 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, Leona Berg Takagi Andersen, Magnus Lindberg Jørgensen and Stine Frese-Madsen are thanked for technical assistance in the lab.

Authors: Andreas Petersen, Thomas Benfield and Anders Rhod Larsen
Front cover photography: Alexandra Medina and Lone Ryste Hansen Kildevang
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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) for each patient with SAB. The register contains information for all occasions a resident is in contact with the health care system in Denmark (Lynge *et al*. 2011). 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 were annotated using Bionumerics 8.1 (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 moxifloxacin were retrieved.

2. Results

2.1 Cases and incidence

In 2021, 2,512 cases of SAB were recorded (Figure 1) of which 2,270 (90%) constituted primary and 242 subsequent episodes. The incidence rate of SAB was 43.0/100,000 inhabitants (Figure 2). The incidence rate has increased in average by 4% each year since 2012. Methicillin-resistant *S. aureus* (MRSA) was identified from 40 cases (1.6%) (Figure 3) and the incidence rate of SAB-MRSA was 0.62/100,000 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 (62% males vs. 38% females) among the cases of SAB in 2021. This proportion has been relatively constant with males comprising 60%-64% of cases during the last 20 years.

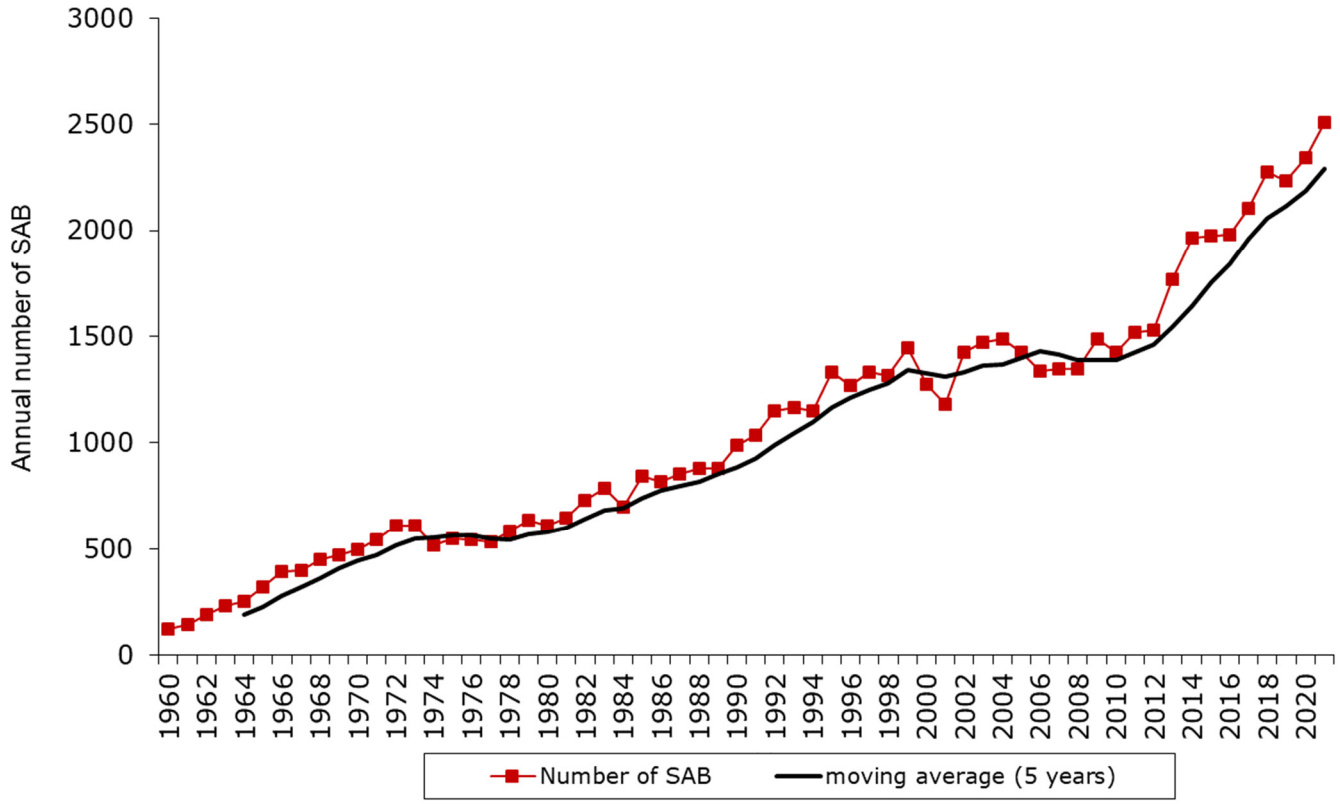

Figure 1. Number of SAB cases in Denmark 1960-2021.

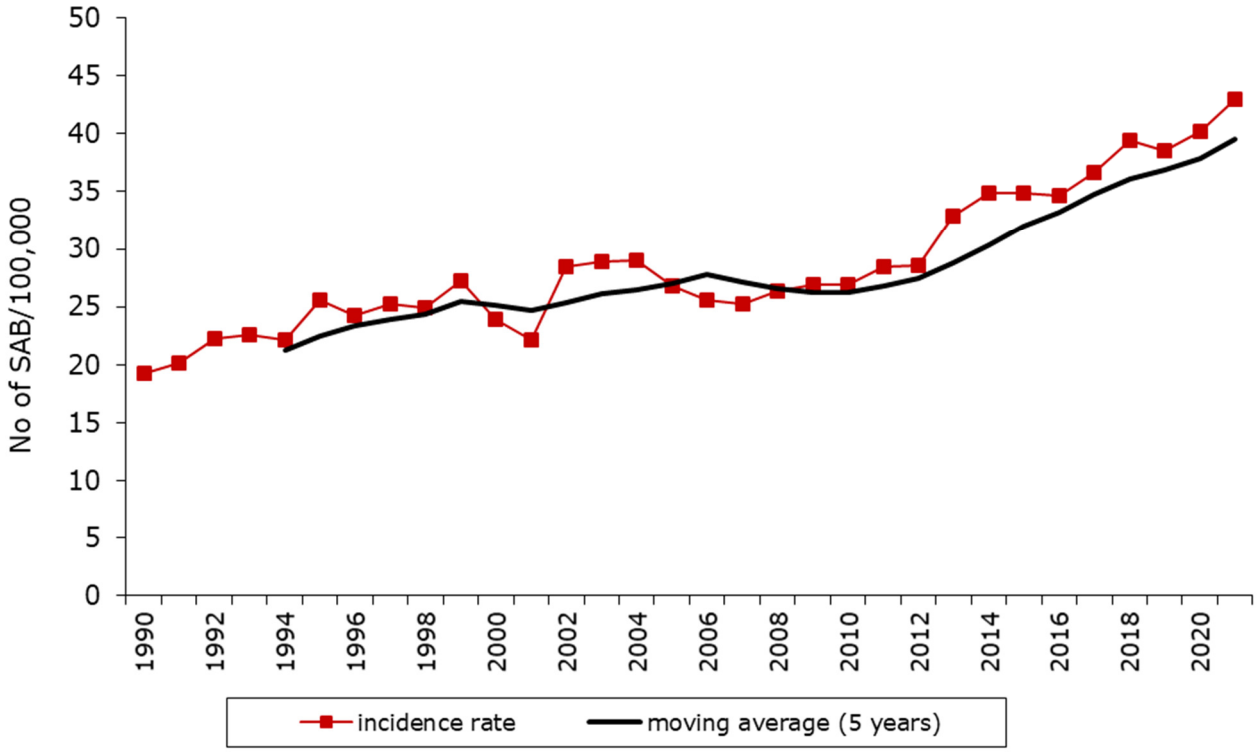

Figure 2. Incidence rate of SAB in Denmark per 100,000 inhabitants 1990-2021.

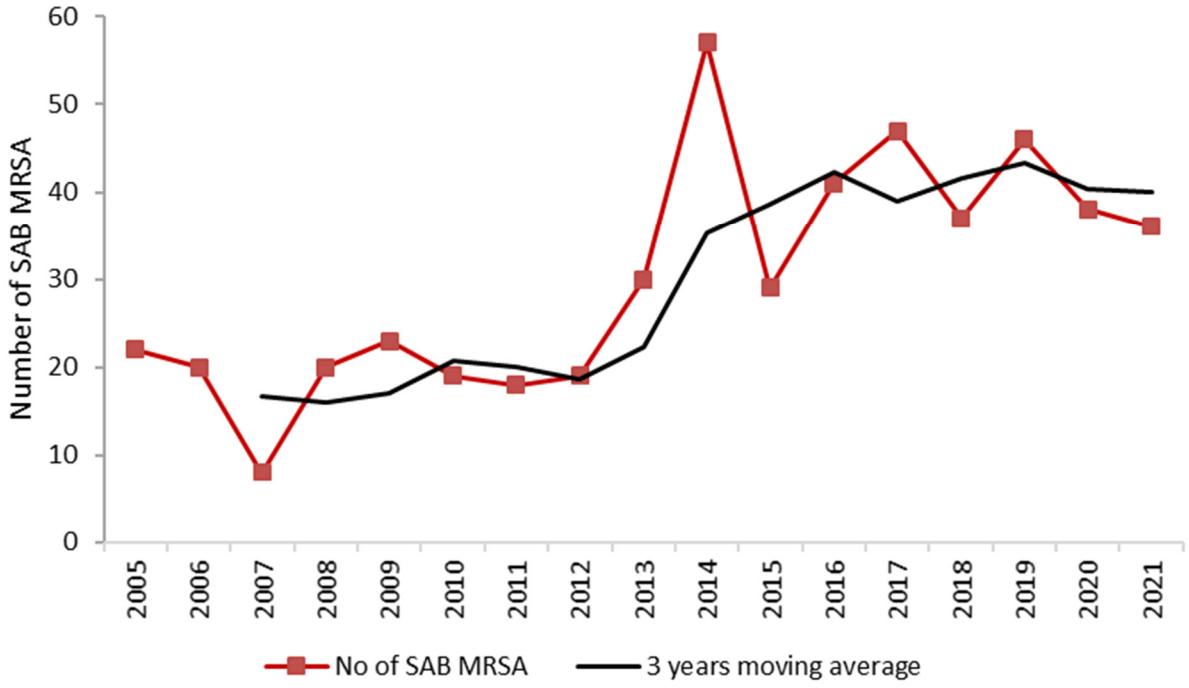

Figure 3. Number of SAB MRSA cases in Denmark 2005-2021

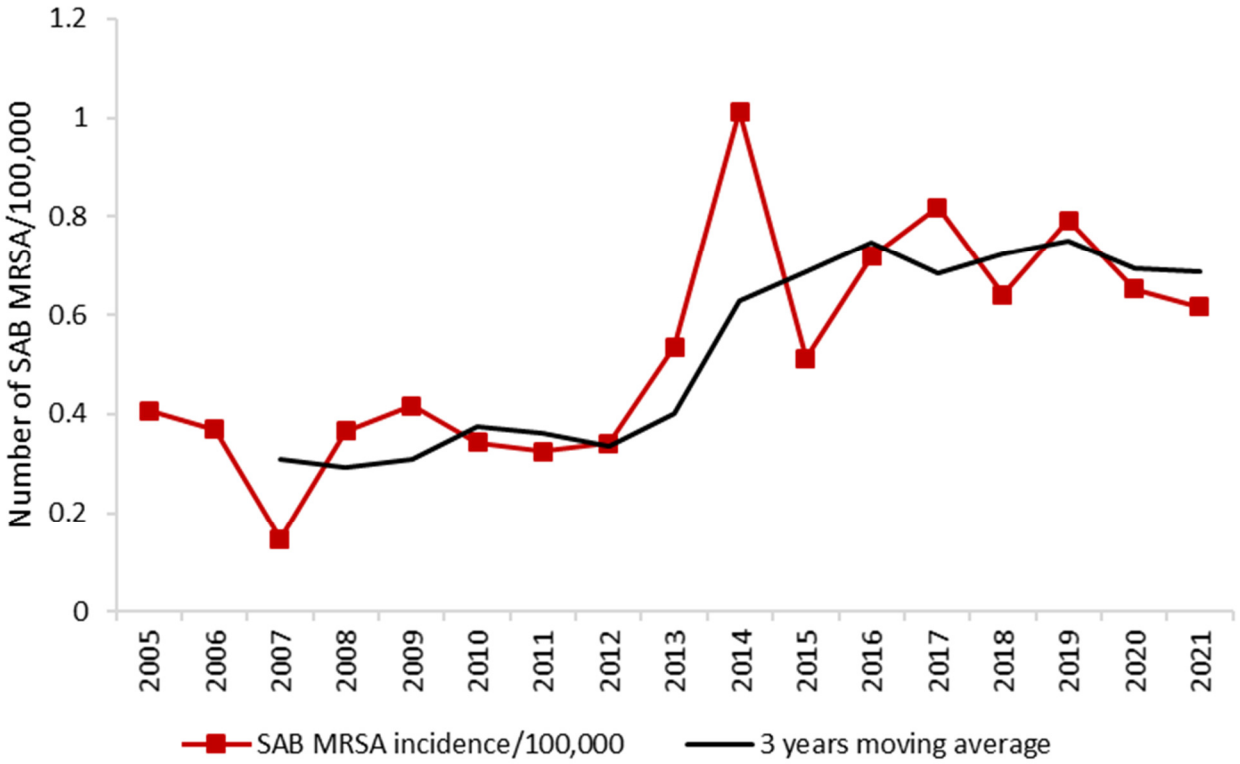

Figure 4. Incidence rate of SAB MRSA cases in Denmark per 100,000 inhabitants 2005-2021.

2.2 Age

Eighty-five percent of the SAB patients in 2021 were older than 50 years and 27% were older than 80 years (Figure 5). The Danish population only included 4% older than 80 years in 2021 and the incidence of SAB among people above 80 years of age (275/100,000 inhabitants) was eight times higher than for the rest of the population (32.9/100,000 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.

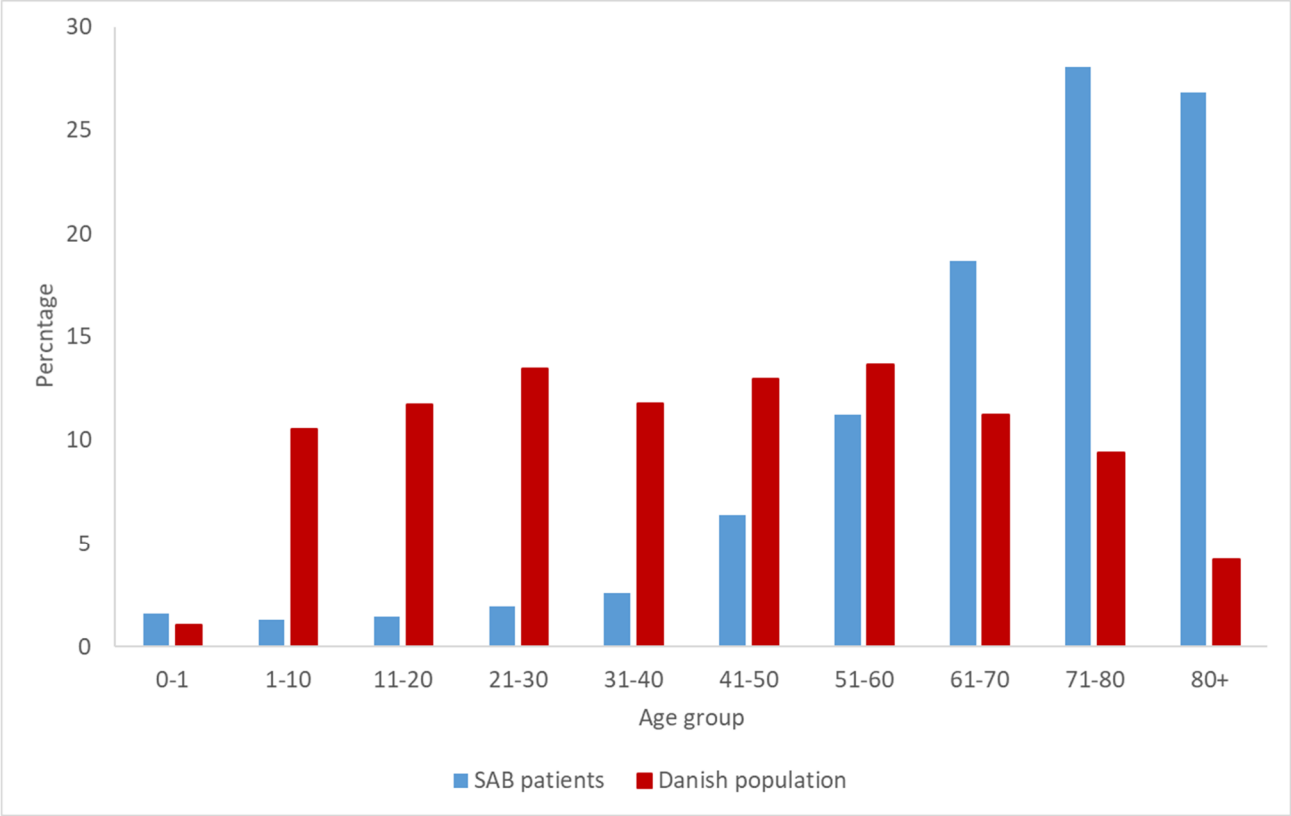

Figure 5. Age distribution of SAB patients and the Danish population in 2021 (%).

2.3 Case fatality

The 30-day all-cause case fatality was 22.2% in 2021 (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 (23.1% and 21.9%, respectively, p=0.52, Fisher's exact test). Case fatality was low in patients between 1-40 years, increased from the age group of 51-60 years, and patients above 80 years had a case fatality rate of 37.9% (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 2021 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	40	33	37	49	65	160	282	469	704	673	2512
No. case fatality	2	1	3	0	4	15	40	69	169	255	558
% case fatality	5.0	3.0	8.1	0	6.2	9.4	14.2	14.7	24.0	37.9	22.2

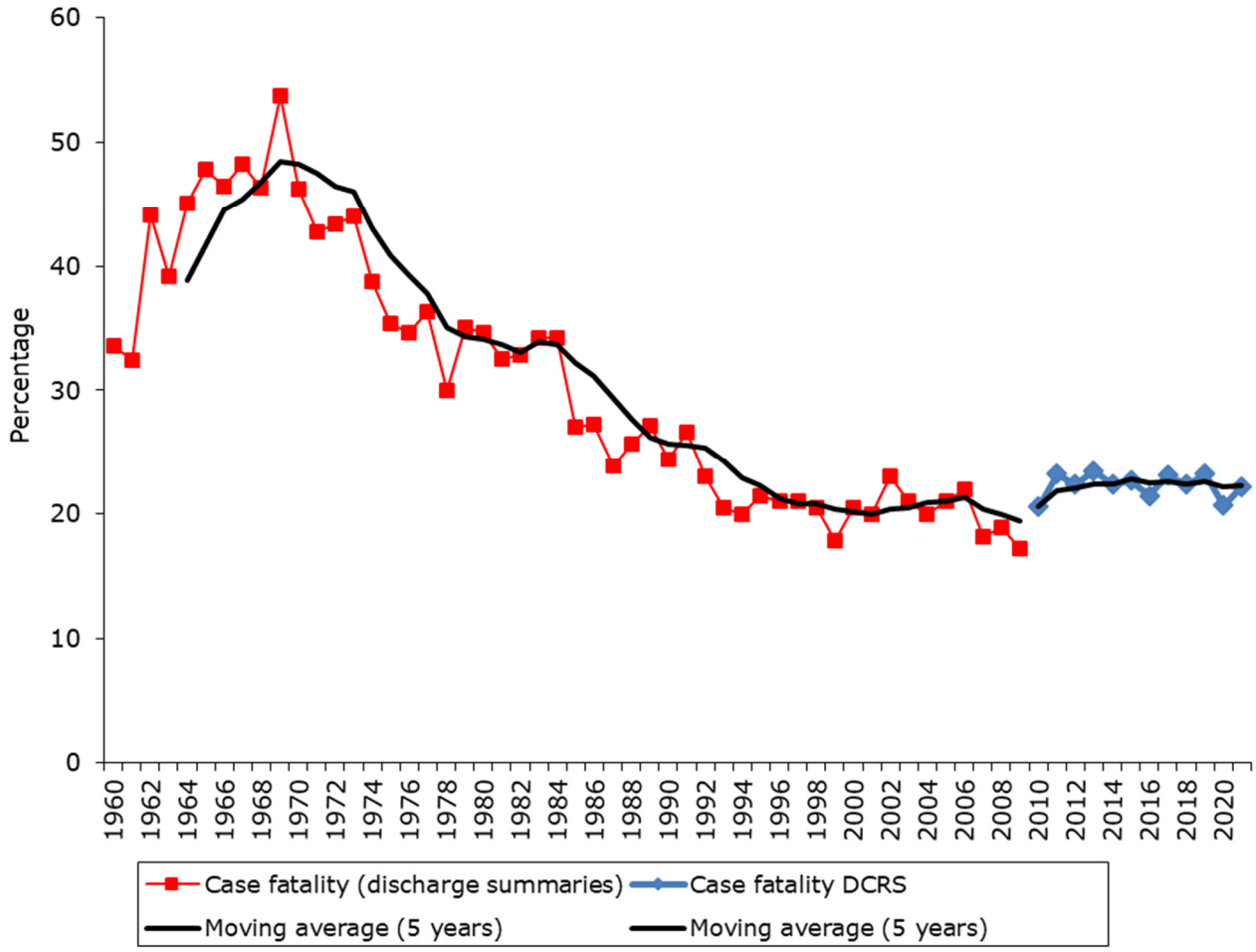

Figure 7. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2021.

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).

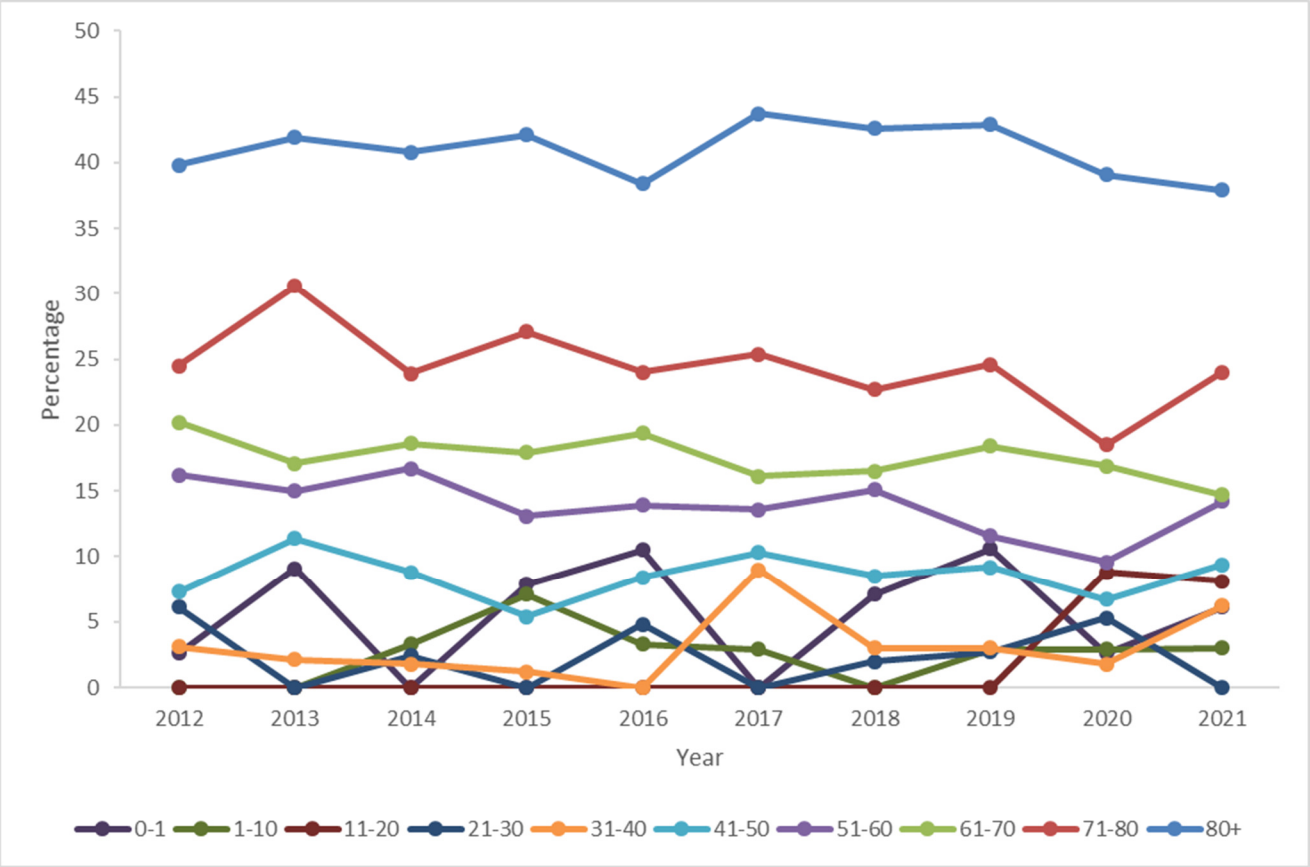

Figure 8. 30-day all-cause case fatality (%) of Danish SAB patients 2012-2021 by age-group.

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 558 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) four (16.7%) died within thirty days from a positive blood culture which was not significantly different from *pvl* negative cases (22.3%, p=0.63, Fisher's exact test). Thirty-day case fatality among cases with MRSA was the same as for cases with MSSA (20% vs. 22.3%, p=0.85, Fisher's exact test).

2.4 Secondary infections

Within three months after SAB, the number of cases with a registered secondary infection was 590, corresponding to 23.5%. This prevalence has been stable for the last 10 years, comprising 24-26% of SAB patients (Figure 9). 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 2012 to 2021 have been observed (Figure 9); however, with the increasing numbers of cases, increasing numbers of secondary infections have been recorded, most notably for endocarditis (Figure 10).

Table 2. The most common secondary infections (%) among Danish SAB patients in 2021, recorded 3 months after admission.

Endocarditis	Spondylitis	Prosthetic infection	Arthritis	Osteomyelitis	Central nervous system
10.7	4.9	4.7	3.6	2.0	1.2

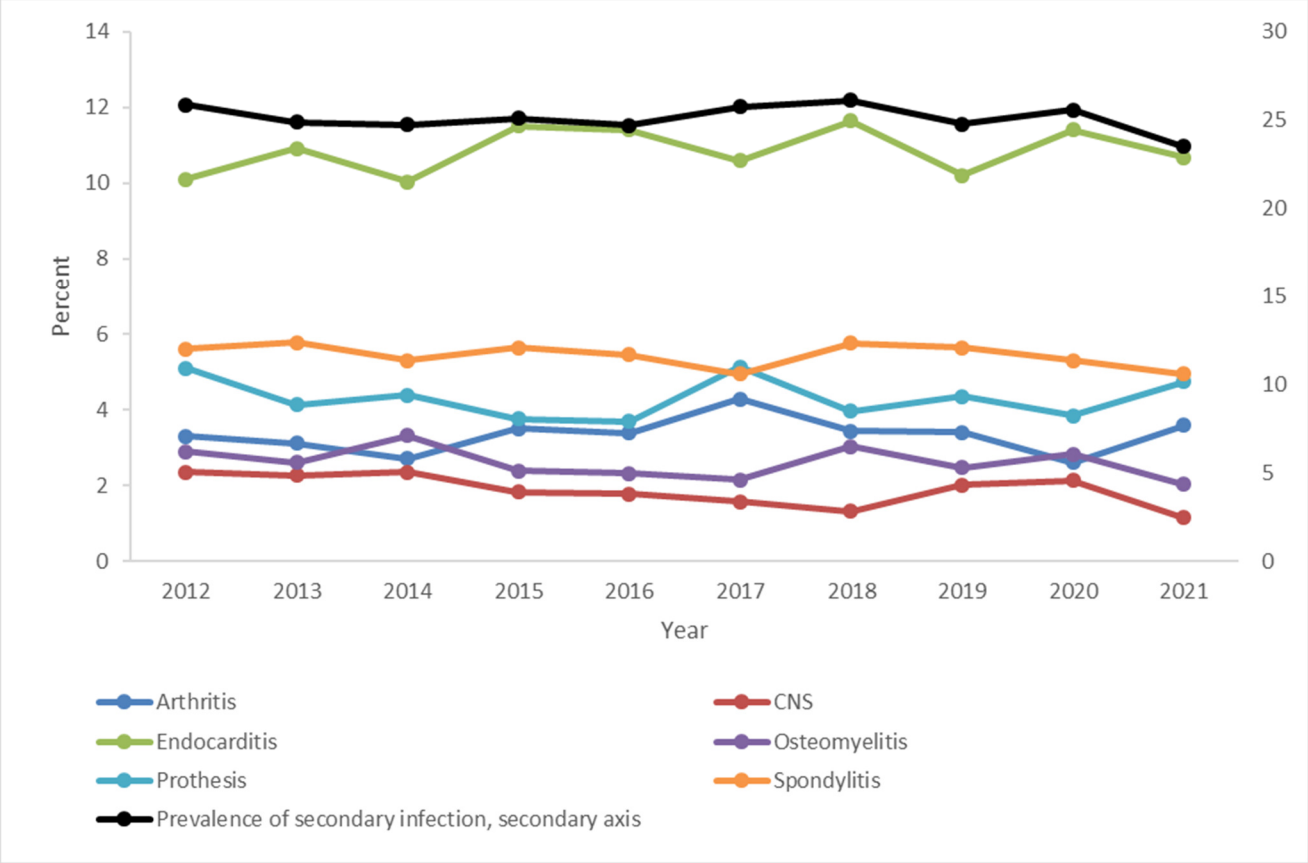

Figure 9. Prevalence of specific secondary infections and overall prevalence of any secondary infection (%) among Danish SAB patients 2012-2021, recorded three months after admission.

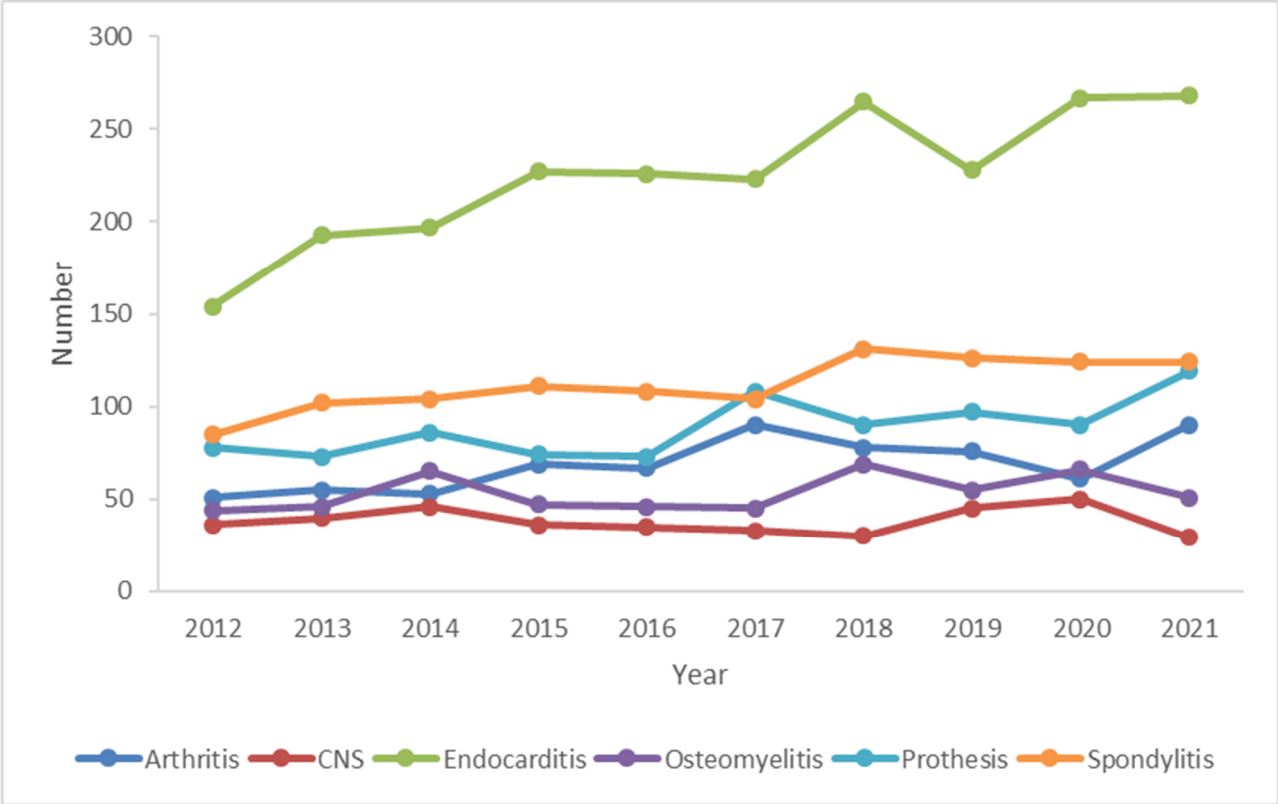

Figure 10. Number of secondary infections among Danish SAB patients 2012-2021, recorded three months after admission.

2.5 Comorbidities

SAB primarily affects people who are diagnosed with other diseases. In 2021, 876 cases (35%) had no comorbidities registered or a comorbidity score of 0, while 932 cases (37%) had a comorbidity index score of 1-2, and 703 cases (28%) had a score of more than 2. This distribution has been constant during the last decade. Comorbidities were more often recorded among the older age groups (Table 3). Table 4 presents prevalence of 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 2021. 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 2021

	Age group									
CIS	**0-1**	**1-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**80+**
0	100	52	70	69	43	32	37	34	26	35
1-2	0	42	22	24	37	33	38	36	40	39
>2	0	6	8	6	20	36	25	30	35	26

Table 4. Prevalence (%) of comorbidities among Danish SAB patients 2021

Comorbidity	Total
AIDS/HIV	0.1
Any malignancy	22.3
Metastatic solid tumor	3.5
Diabetes without chronic complication	21.4
Diabetes with chronic complication	11.6
Dementia	4.1
Hemiplegia or paraplegia	1.0
Cerebrovascular disease	15.1
Myocardial infarction	7.2
Congestive heart failure	15.1
Chronic pulmonary disease	17.4
Peptic ulcer disease	5.0
Mild liver disease	9.9
Moderate or severe liver disease	3.8
Renal disease	15.0
Rheumatic disease	4.7
Peripheral vascular disease	11.4
Drug abuse	2.8

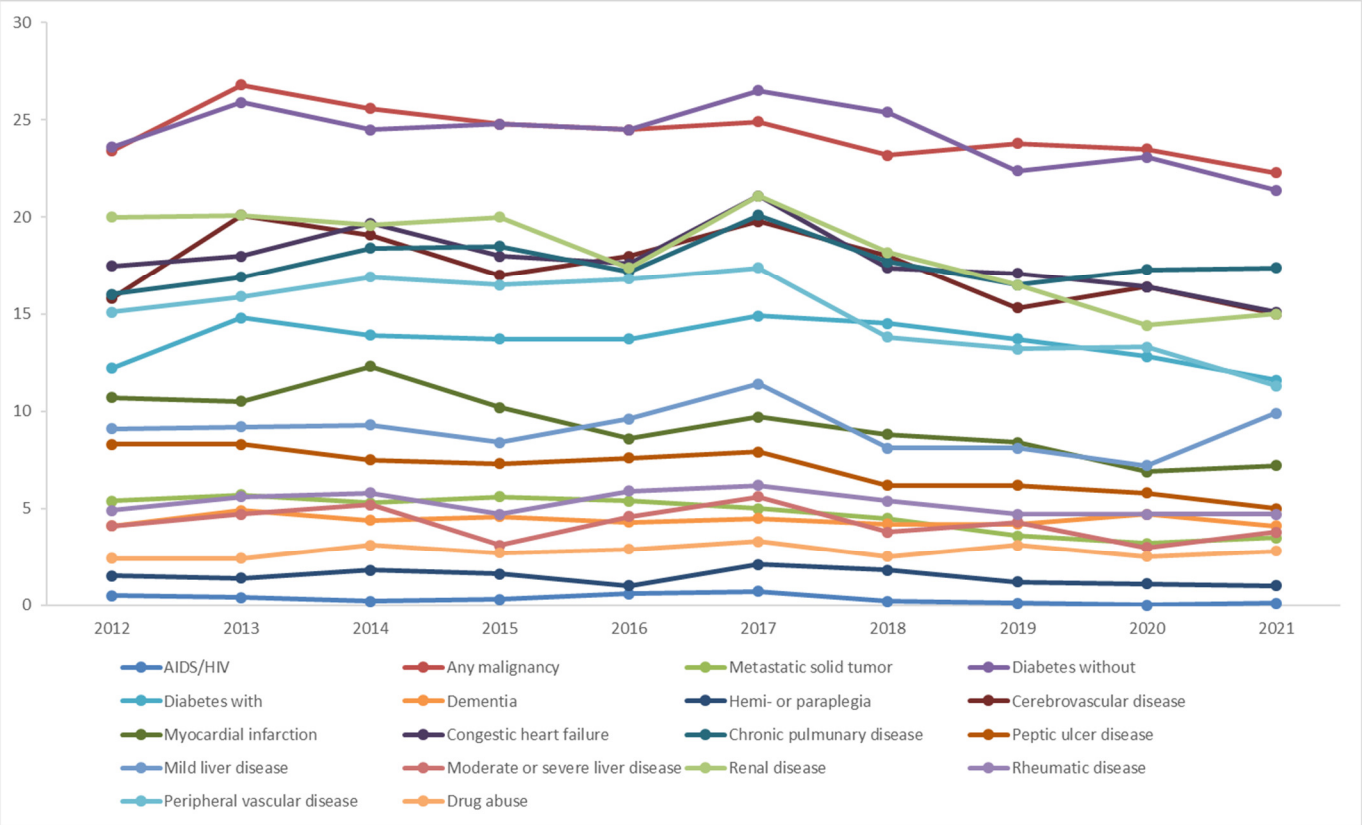

Figure 11. Prevalence (%) of comorbidities among Danish SAB patients 2012 - 2021

2.6 Typing

spa typing was successful for 2,495 isolates (99.3%). A total of 712 different *spa* types were identified, and ten *spa* types accounted for 31% 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 t091 increased in the same period (Table 5). A total of 482 *spa* types (68% of all *spa* types) were only found once. Assignment to MLST CC was possible for 2,131 isolates (85%). In the remaining cases, assignment was not possible due to an unresolved relationship with MLST typing. A total of 27 MLST CC were assigned. The three most prevalent CC constituted 36% of the SAB isolates in 2021 while the 10 most prevalent constituted 76% (Table 6). The most remarkable change of CC in the last decade was for CC398, which had an annual significant increase of 16% (Table 6). Twenty-four SAB isolates were *pvl* positive (0.96%), of which three were MRSA (t005/CC22, t008/CC8, and t021/CC30). The *pvl* positive isolates were distributed among 17 different *spa* types and nine 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 98 SAB cases (3.9%) in 2021 of which 6 were MRSA. One of the SAB CC398 MRSA patients had indirect contact to livestock. Two of the SAB CC398 MRSA patients died within 30 days of diagnosis. Since 2007, 12 SAB patients with CC398 MRSA have died within 30 days. Case fatality rate among SAB CC398 MSSA was 19.6%.

Table 5. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2021 and the 10 year trend.

spa type	Number (%)	Trend
t002	112 (4.5)	ns
t091	108 (4.3)	1.06
t127	103 (4.1)	ns
t084	96 (3.8)	ns
t230	92 (3.7)	0.94
t012	63 (2.5)	ns
t008	58 (2.3)	ns
t021	55 (2.2)	ns
t701	54 (2.1)	ns
t015	40 (1.6)	0.93

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 2021 and the 10 year trend.

CC group	Number (%)	Trend
CC45	340 (13.5)	0.96
CC30	290 (11.5)	0.98
CC15	266 (10.6)	ns
CC5	265 (10.5)	ns
CC1	195 (7.8)	ns
CC8	169 (6.7)	ns
CC7	123 (4.9)	1.05
CC398	98 (3.9)	1.16
CC22	94 (3.7)	ns
CC97	63 (2.5)	NA

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 2,659 isolates. Susceptibility testing for different antimicrobials varied and only for penicillin all isolates were tested. Resistance to penicillin was 69.0% (71.7% in 2020) and resistance to fusidic acid decreased to 12.8% (14.2% in 2020; Table 7). Resistance to the remaining antimicrobials were all below 10%. Fully susceptible isolates increased to 24.7%. Figure 8 shows selected resistance prevalences from 1980 to 2021. Resistance to fusidic acid has increased from 0 to 15% while the proportion of fully susceptible isolates also increased from 12-13% to almost 25%.

Table 7. Resistance prevalence among Danish SAB isolates 2021, retrieved from MiBa

Antimicrobial	Resistance (%)	Number of isolates tested
Penicillin	69.0	2,659
Erythromycin	7.2	2,418
Clindamycin	6.9	2,405
Fusidic acid	12.8	2,082
Tetracycline	2.2	891
Moxifloxacin	3.7	2,193
Rifampicin	0.6	2,326
Linezolid	0*	2,232
TMP/SXT	0.2	621
Gentamicin	0.2	1,074
Mupirocin	0.5	1,322
Vancomycin	0	751

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

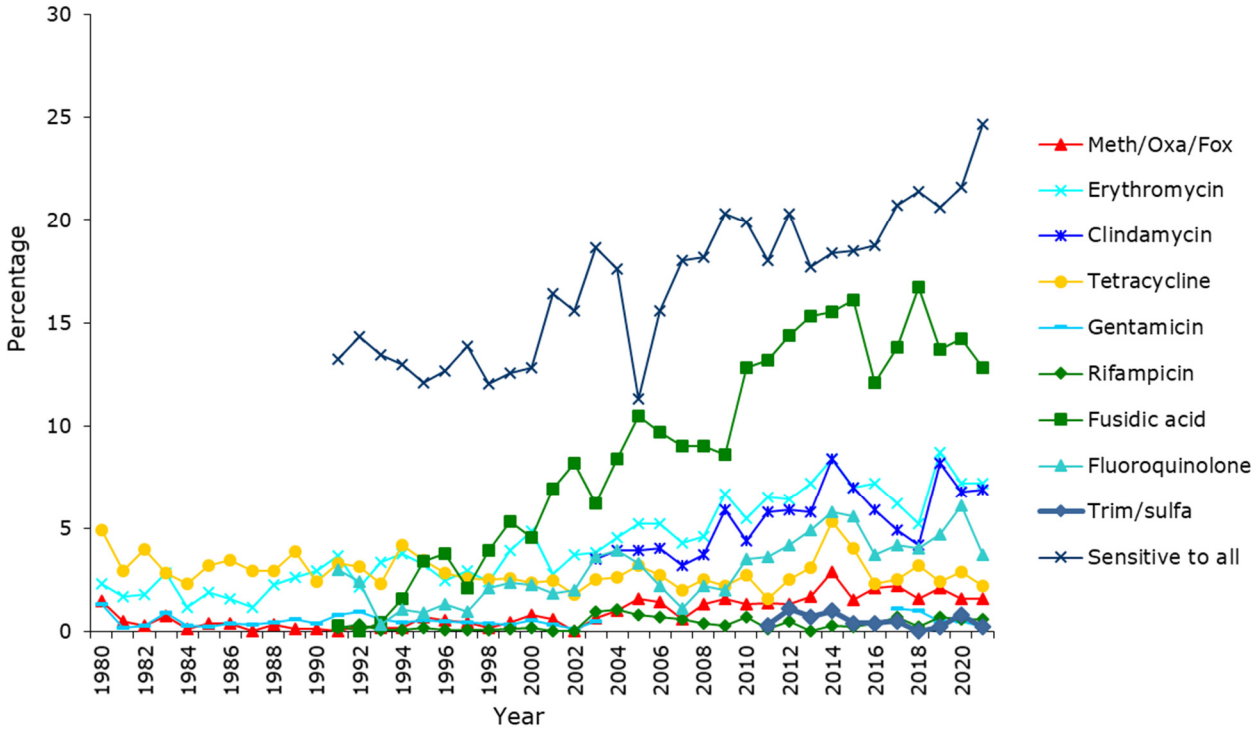

Figure 8. Prevalence of antimicrobial resistance in Danish SAB isolates (1980-2021).

3. Conclusions

The number of recorded SAB cases increased in 2021 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 22% and this rate has been remarkably stable since the beginning of the 1990'ies. Almost 25% of all SAB isolates were fully susceptible to the tested antimicrobials.

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.

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Statens Serum Institut
Artillerivej 5
2300 København S
Danmark

T 3268 3268
F 3268 3868
@ serum@ssi.dk
W ssi.dk

CVR nr. 46 83 74 28
EAN nr. 5798000362192