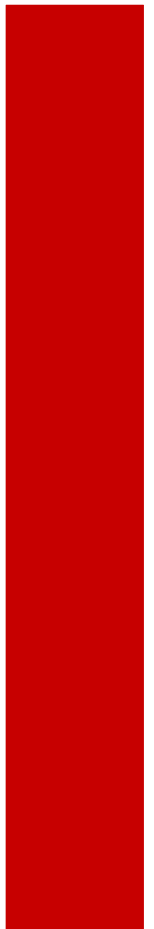




# *Staphylococcus aureus* bacteraemia

Cases in Denmark 2022





This report describes the laboratory and clinical characteristics of the 2,578 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2022. 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, Ditte Marie Brix, Magnus Lindberg Jørgensen and Stine Frese-Madsen are thanked for technical assistance in the lab.

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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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## LIST OF ABBREVIATIONS

CC: Clonal complex	<i>mecC</i> : The gene coding for a variant <i>mecA</i> gene
CLSI: Clinical and Laboratory Standards Institute	MiBa: The Danish Microbiology Database
DCM: Department of Clinical Microbiology	MLST: Multi Locus Sequence Typing
DCRS: Danish Civil Registration System	MSSA: Methicillin-susceptible <i>Staphylococcus aureus</i>
EUCAST: The European Committee on Antimicrobial Susceptibility Testing	MRSA: Methicillin-resistant <i>Staphylococcus aureus</i>
ICD-10: International Classification of Diseases	NPR: The Danish National Patient Register
<i>lukF/S</i> -pv: Genes encoding the Panton-Valentine leucocidin	PCR: Polymerase Chain Reaction
<i>mecA</i> : The gene encoding for methicillin resistance	SAB: <i>Staphylococcus aureus</i> bacteraemia
	<i>spa</i> : 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 voluntary 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 (Lyngé *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 <https://www.statistikbanken.dk/20021>.

Negative binomial regression analysis was used to analyse for trends for incidence 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*, *mecA*, *mecC*, and *lukF/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

The Danish population constituted 5,873,420 citizens by January 1st 2022. A total of 2,578 cases of SAB were recorded (Figure 1) of which 2,310 (90%) patients had their first episode. The incidence rate of SAB was 43.9/100,000 inhabitants (Figure 2). The incidence rate has increased in average by 3% each year since 2012. Methicillin-resistant *S. aureus* (MRSA) was identified from 50 cases (1.9%) (Figure 3) and the incidence rate of SAB-MRSA was 0.85/100,000 inhabitants (Figure 4). In the last ten years there has been no significant changes of numbers of SAB MRSA in relation to annual numbers of SAB. There were more males than females (64% males vs. 36% females) among the cases of SAB in 2022. This proportion has been relatively constant with males comprising 60%-64% of cases during the last 20 years.

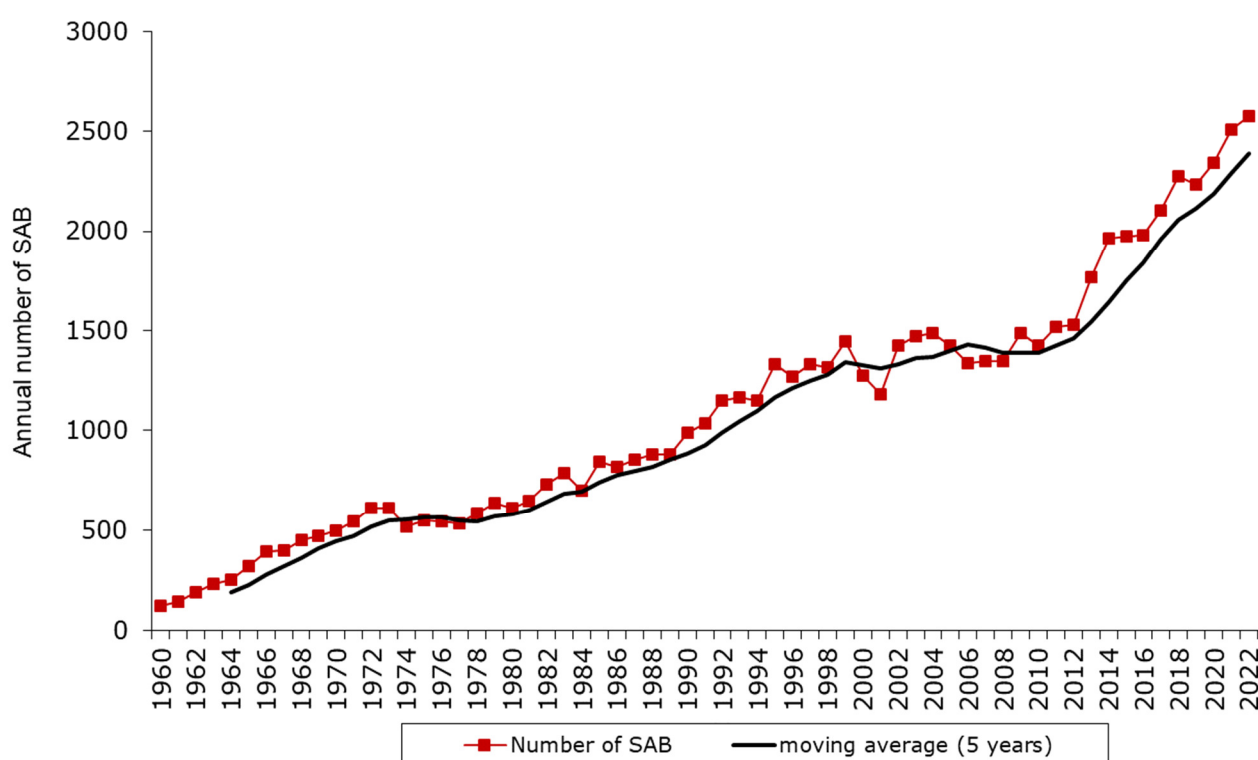


Figure 1. Number of SAB cases in Denmark 1960-2022.

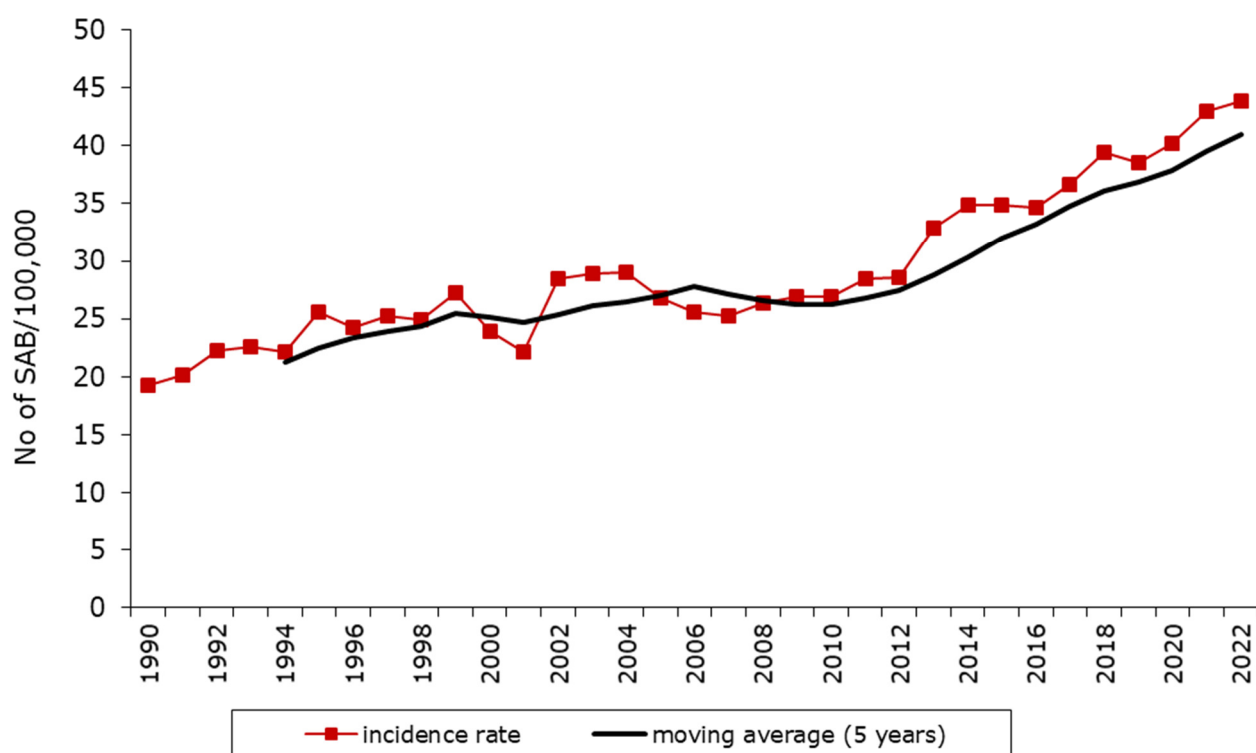


Figure 2. Incidence rate of SAB in Denmark per 100,000 inhabitants 1990-2022.

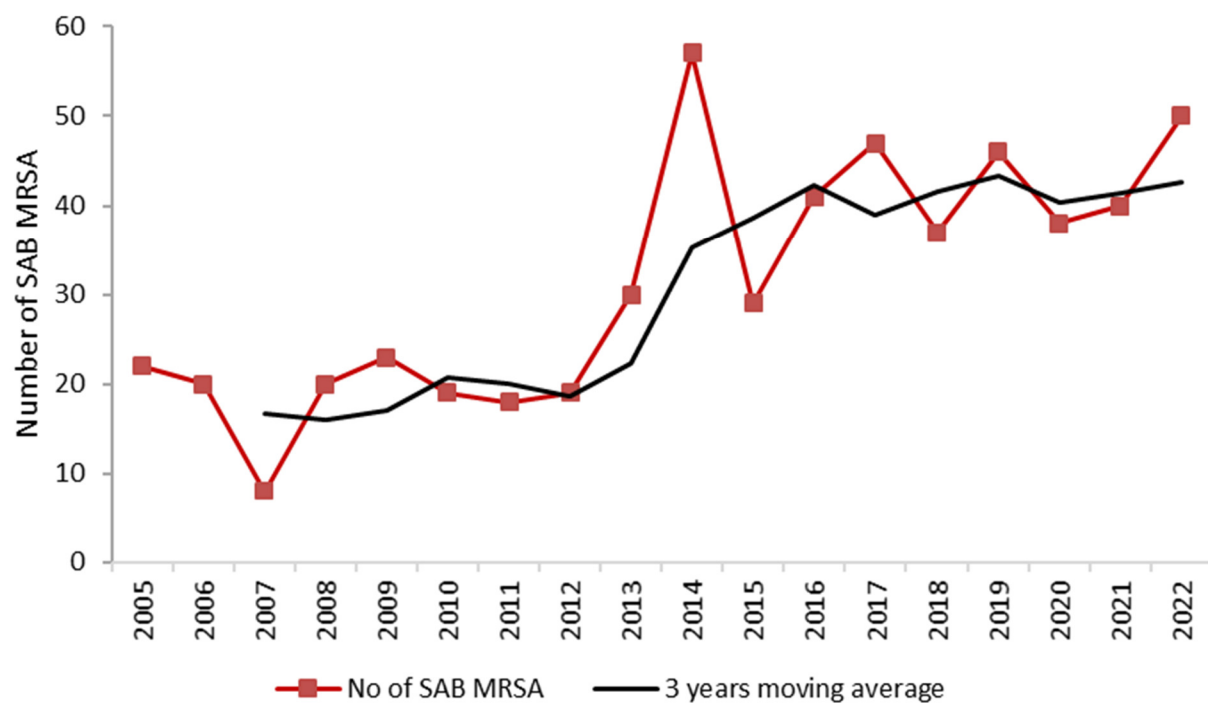
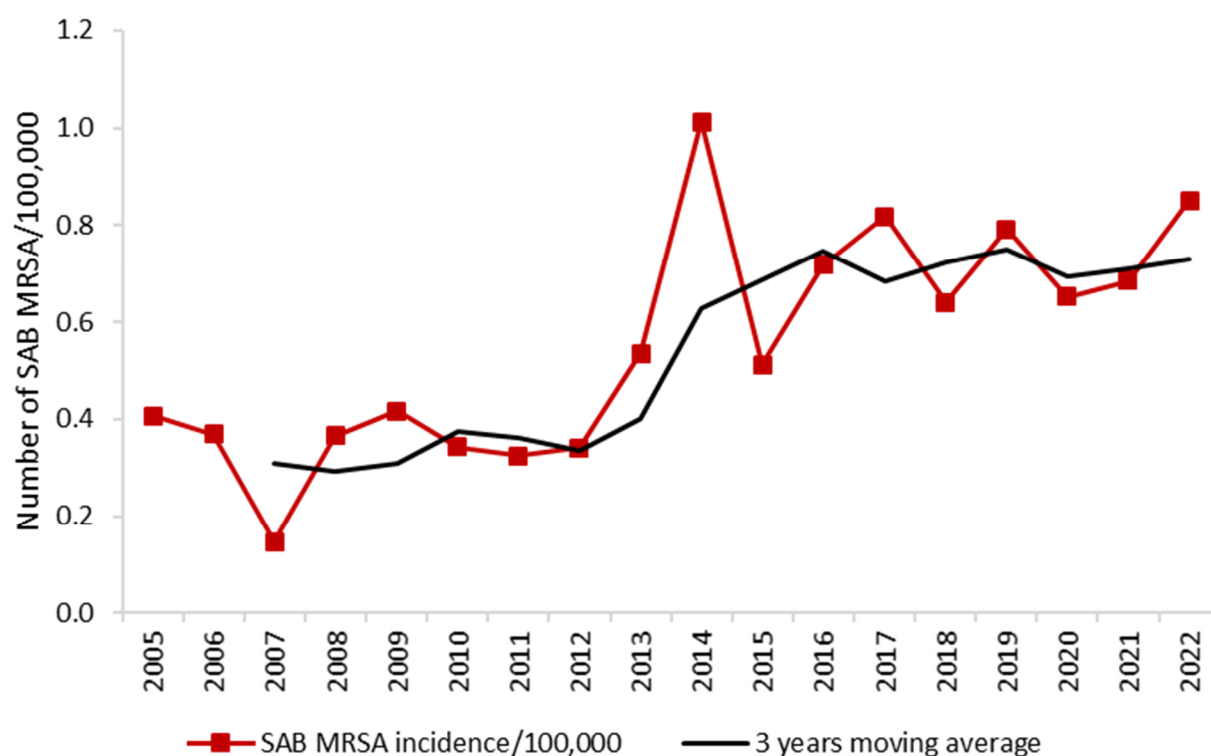


Figure 3. Number of SAB MRSA cases in Denmark 2005-2022



**Figure 4. Incidence rate of SAB MRSA cases in Denmark per 100,000 inhabitants 2005-2022.**

## 2.2 Age

Eighty-five percent of the SAB patients in 2022 were older than 50 years and 28% were older than 80 years (Figure 5). The Danish population only included 4% older than 80 years in 2022 and the incidence of SAB among people above 80 years of age (286/100,000 inhabitants) was eight times higher than for the rest of the population (33.0/100,000 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%. Specific causes and mechanisms behind this increase among the elderly population are unresolved.

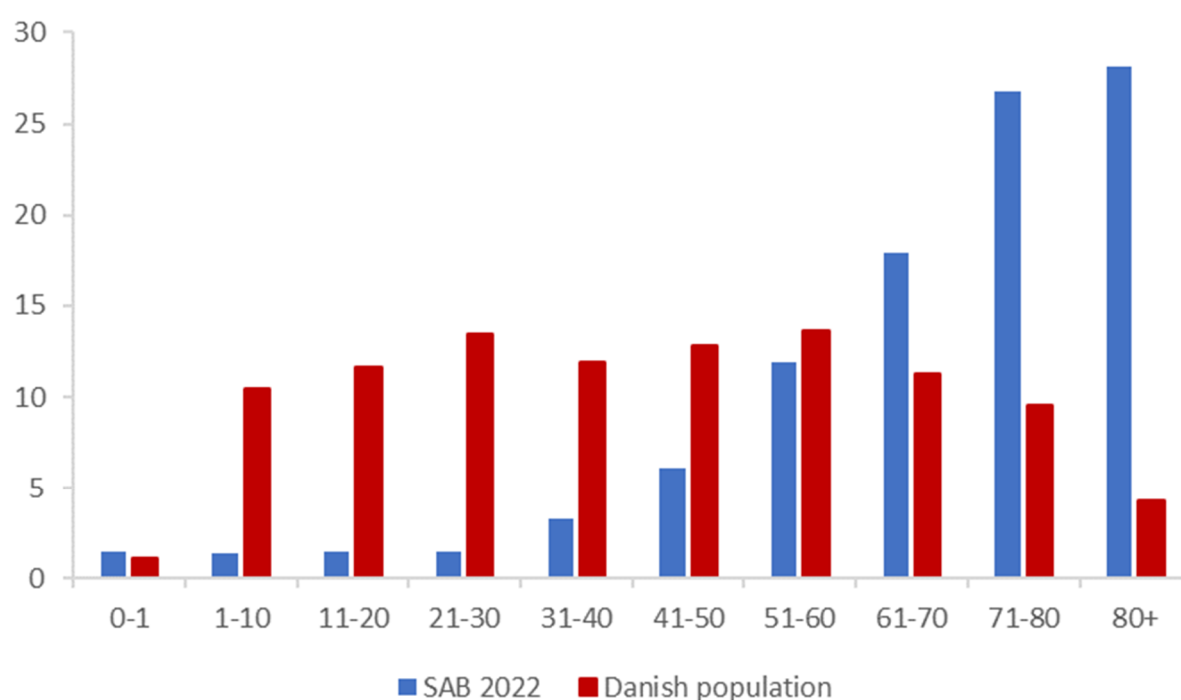


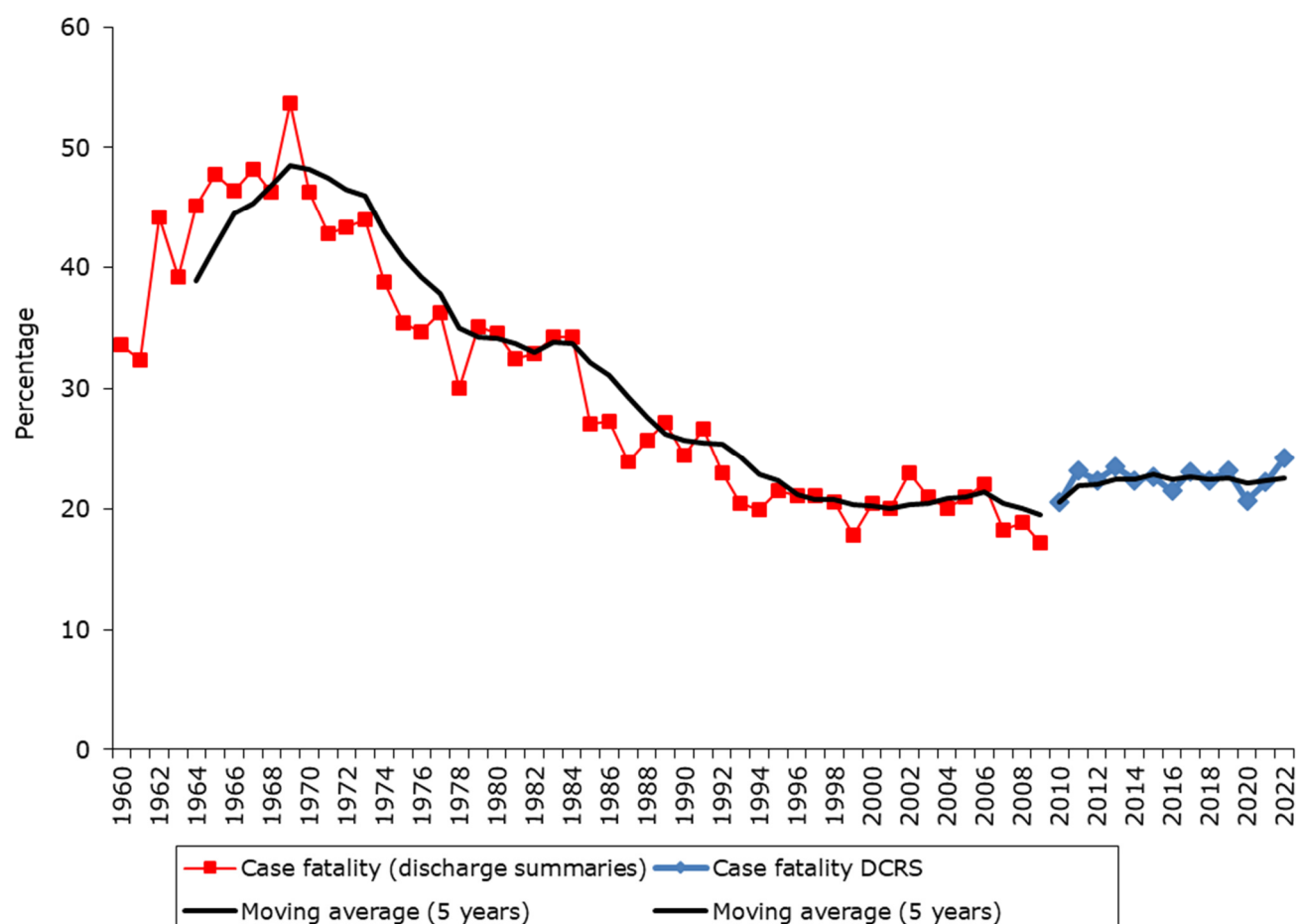
Figure 5. Age distribution of SAB patients and the Danish population in 2022 (%).

### 2.3 Case fatality

The 30-day all-cause case fatality was 24.3% in 2022 (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 (23.8% and 25.2%, respectively,  $p=0.44$ , 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 41.7% (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).

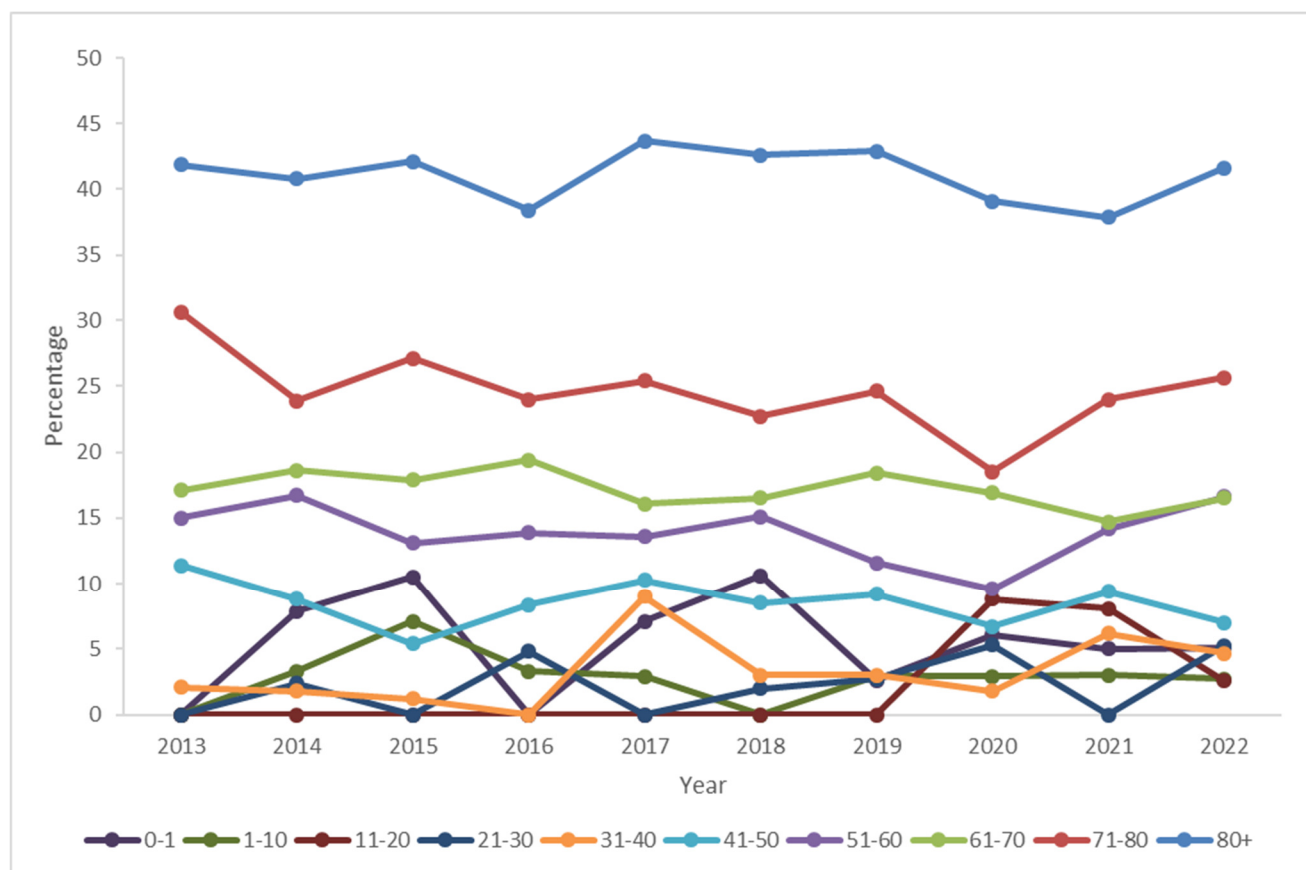
Table 1. Case fatality among Danish SAB patients in 2022 by age group and in total.

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	37	39	38	86	157	307	460	690	725	2578
No. case fatality	2	1	1	2	4	11	51	76	177	302	627
% case fatality	5.1	2.7	2.6	5.3	4.7	7.0	16.6	16.5	25.7	41.7	24.3



**Figure 7. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2022.**

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 2013-2022 by age-group.**

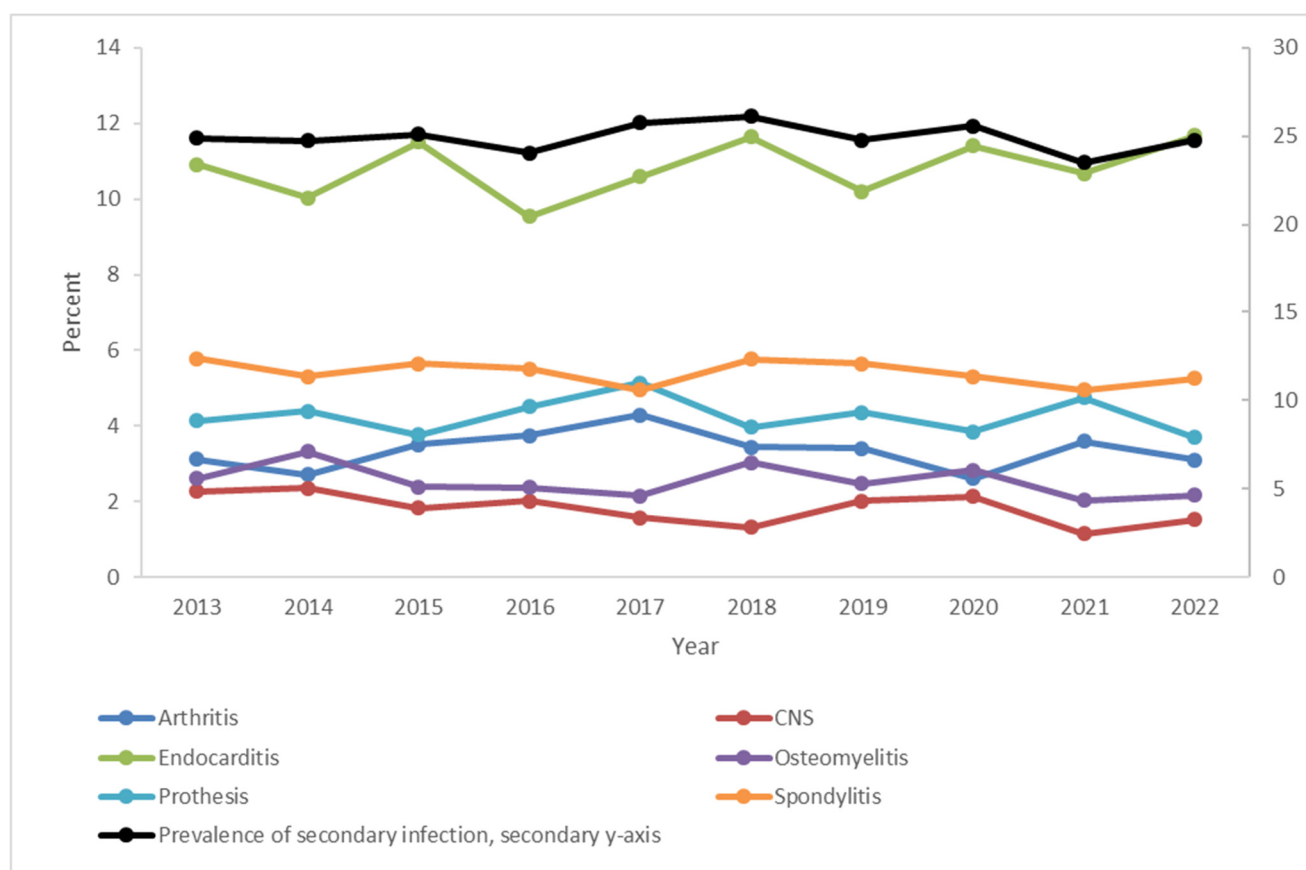
The outcome of SAB did not seem to depend on the specific type of *S. aureus* causing infection. The most prevalent *spa* types among the 627 isolates from cases dying within 30 days did not differ from the overall distribution of *spa* types. Two patients with a *pvl* positive isolate (see section 2.6) died within thirty days from a positive blood culture. The proportion (9.5%) was lower, but not significantly different from *pvl* negative cases (24.4%,  $p=0.13$ , Fisher's exact test). Thirty-day case fatality among cases with MRSA was the same as for cases with MSSA (18% vs. 24.4%,  $p=0.40$ , Fisher's exact test). The case fatality rate for MRSA was 17.6% and for MSSA 22.4%,  $p=0.10$ , Chi-square, for the years 2018-2022.

## 2.4 Secondary infections

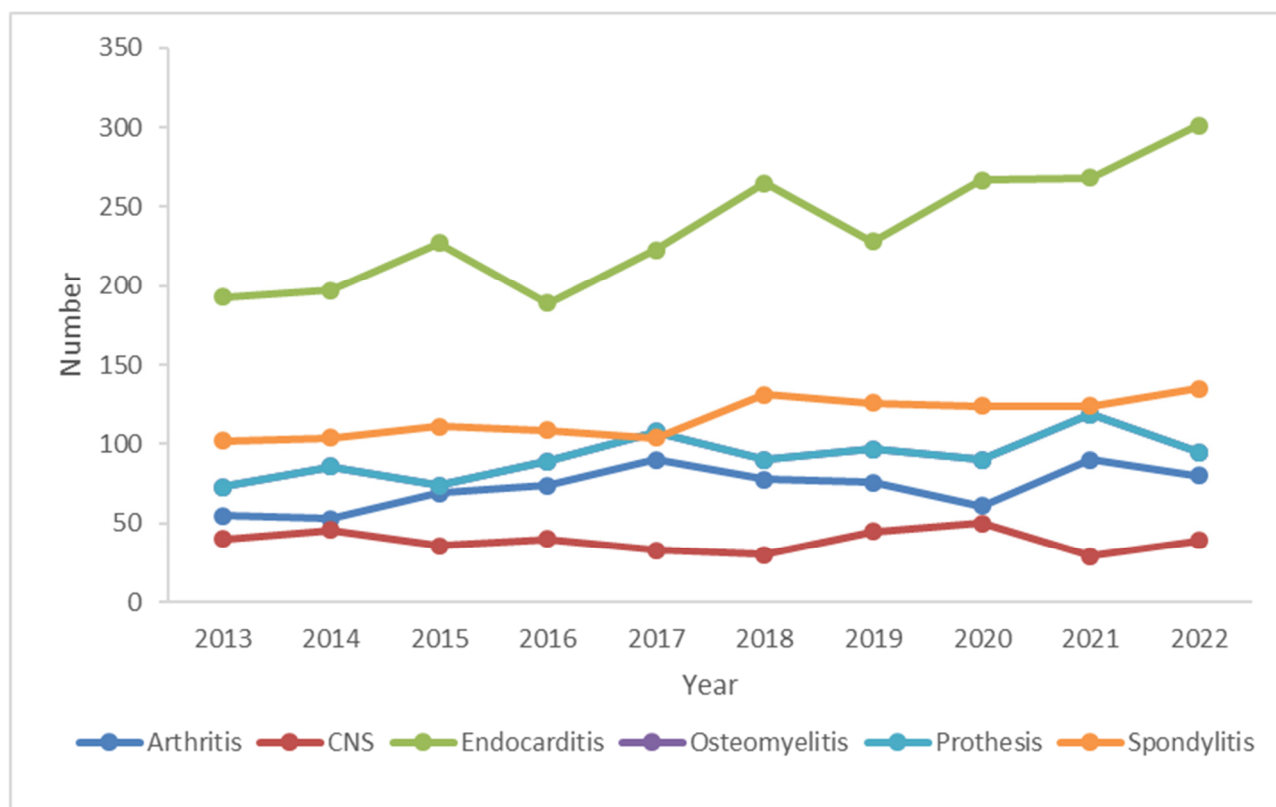
The number of cases with registered secondary infections within three months after SAB was 639, corresponding to 24.8%. This prevalence has been stable for the last 10 years, comprising 24-26% of SAB patients (Figure 9). Endocarditis was the most prevalent secondary infection, followed by spondylitis, prosthetic infection, 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 2013 to 2022 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 2022, recorded until three months after admission.**

Endocarditis	Spondylitis	Prosthetic infection	Arthritis	Osteomyelitis	Central nervous system
11.7	5.2	3.7	3.1	2.2	1.5



**Figure 9. Prevalence of specific secondary infections and overall prevalence of any secondary infection (%) among Danish SAB patients 2013-2022, recorded until three months after admission.**



**Figure 10. Number of secondary infections among Danish SAB cases 2013-2022, recorded until three months after admission.**

## 2.5 Comorbidities

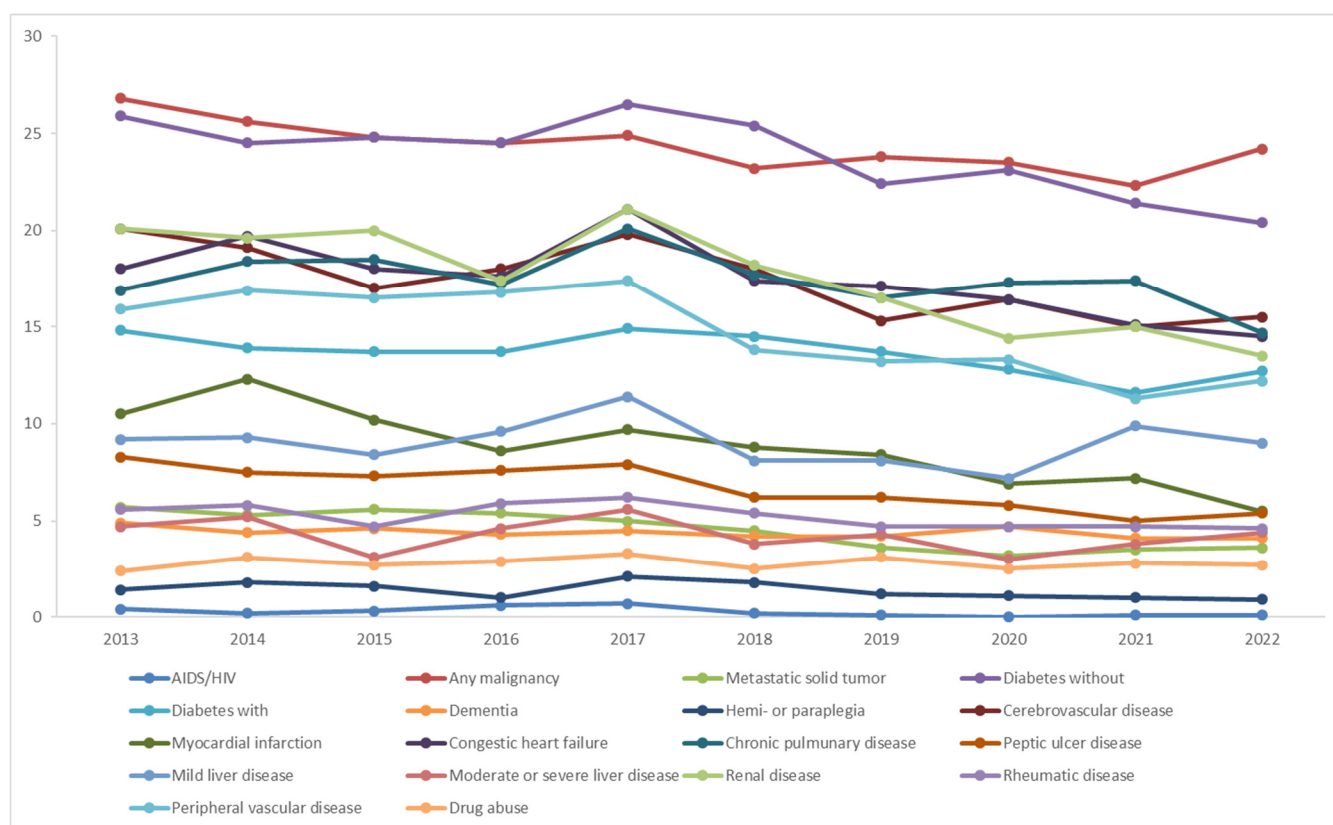
SAB primarily affects people who are diagnosed with other diseases. In 2022, 871 cases (34%) had no comorbidities registered or a comorbidity index score (CIS) of 0, while 1,020 cases (40%) had a CIS of 1-2, and 687 cases (27%) had a score of more than 2. This distribution has been constant during the 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 (24.2%), diabetes without chronic complication (20.4%), and cerebrovascular disease (15.5%) were the most frequently registered comorbidities among SAB patients in 2022. 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 2022**

CIS	Age group									
	0-1	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	80+
0	90	62	74	61	38	29	36	31	27	33
1-2	8	27	23	29	47	41	37	37	42	43
>2	3	11	3	11	15	29	27	32	31	24

**Table 4. Prevalence (%) of comorbidities among Danish SAB cases 2022**

Comorbidity	Total
AIDS/HIV	0.1
Any malignancy	24.2
Metastatic solid tumor	3.6
Diabetes without chronic complication	20.4
Diabetes with chronic complication	12.7
Dementia	4.1
Hemiplegia or paraplegia	0.9
Cerebrovascular disease	15.5
Myocardial infarction	5.5
Congestive heart failure	14.5
Chronic pulmonary disease	14.7
Peptic ulcer disease	5.4
Mild liver disease	9.0
Moderate or severe liver disease	4.4
Renal disease	13.5
Rheumatic disease	4.6
Peripheral vascular disease	12.2
Drug abuse	2.7



**Figure 11. Prevalence (%) of comorbidities among Danish SAB patients 2013 - 2022**

## 2.6 Typing

*spa* typing was successful for 2,565 isolates (99.5%). A total of 727 different *spa* types were identified, and ten *spa* types accounted for 31% of the isolates (Table 5). *spa* type t1451 which only was found among three SAB patients in 2013, has increased by in average 32% each year since and is now among the ten most common *spa* types among Danish SAB patients in 2022 (Table 5). The remaining top ten *spa* types have been among 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 499 *spa* types (69% of all *spa* types) were only found once. Assignment to MLST CC was possible for 2,212 isolates (86%). 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 38% of the SAB isolates in 2022 while the 10 most prevalent constituted 77% (Table 6). The most remarkable change of CC in the last decade was for CC398, which had an annual significant increase of 15% (Table 6). The successful *spa* type t1451 belongs to CC398. Twenty-one SAB isolates were *pvl* positive (0.8%), of which six were MRSA (t002/CC5, t005/CC22, t019/CC30, t034/CC398, t044/CC80 and t1028/CC80). The *pvl* positive isolates were distributed among 15 different *spa* types and 10 MLST CC groups; two isolates had an unresolved relationship with MLST CC typing.

### 2.6.1 CC398

CC398 MRSA isolates have been associated with a reservoir in livestock. CC398 constituted 118 SAB cases (4.6%) in 2022 of which 8 were MRSA. Three of the SAB CC398 MRSA patients had direct contact to livestock. Four of the SAB CC398 MRSA patients died within 30 days of diagnosis. Since 2007, 16 SAB patients with CC398 MRSA have died within 30 days. Case fatality rate among SAB CC398 MSSA in 2022 was 21.8%.

**Table 5. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2022 and the 10 year trend.**

<i>spa</i> type	Number (%)	Trend
t127	120 (4.6)	ns
t084	115 (4.5)	ns
t091	101 (3.9)	1.05
t002	96 (3.7)	ns
t230	78 (3.0)	0.94
t008	73 (2.8)	ns
t012	69 (2.2)	ns
t701	55 (2.1)	ns
t015	51 (2.0)	0.95
t1451	48 (1.9)	1.32

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 2022 and the 10 year trend.**

CC group	Number (%)	Trend
CC45	369 (14.3)	0.97
CC15	307 (11.9)	ns
CC30	293 (11.4)	0.98
CC5	228 (8.8)	ns
CC1	216 (8.4)	ns
CC8	191 (7.4)	ns
CC398	118 (4.6)	1.15
CC7	111 (4.3)	1.04
CC22	92 (3.6)	ns
CC97	63 (2.4)	ns

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.

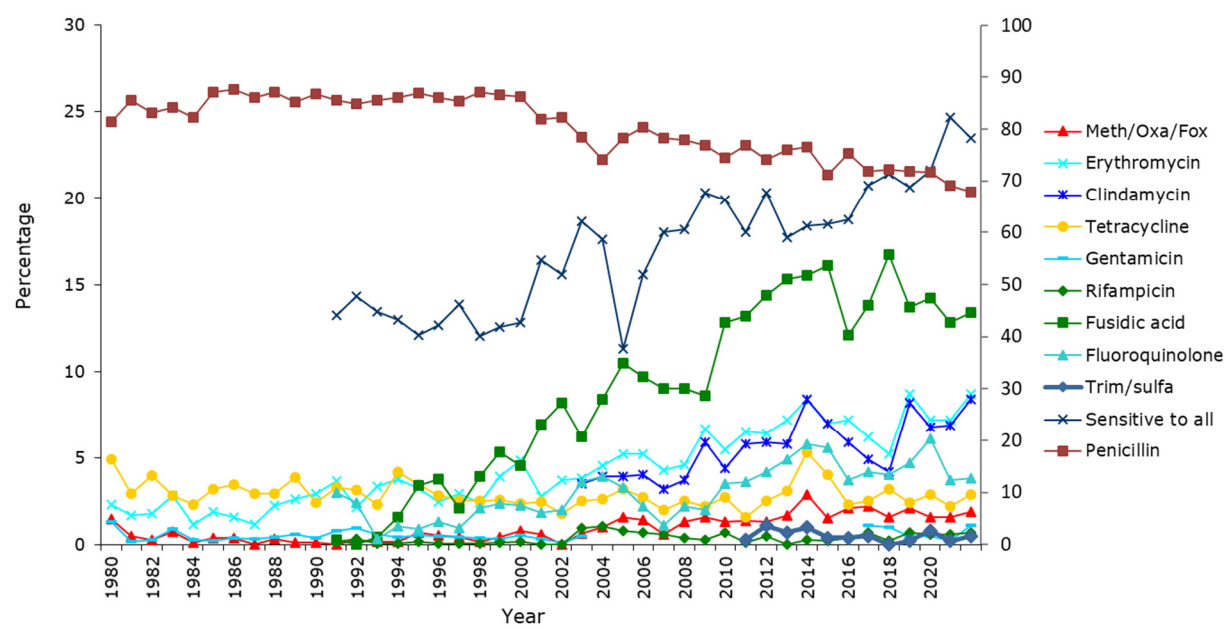
## 2.7 Antimicrobial susceptibility testing

Data retrieved from MiBa comprised 2,570 isolates. Susceptibility testing for different antimicrobials varied between DCMs and only for penicillin almost all isolates were tested. Resistance to penicillin was 67.6% (69.0% in 2021) and resistance to fusidic acid increased to 13.4% (12.8% in 2021; Table 7). Resistance to the remaining antimicrobials were all below 10%. Fully susceptible isolates decreased to 23.5% (24.7% in 2021). Figure 8 shows selected resistance prevalences from 1980 to 2022. 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 2022, retrieved from MiBa**

Antimicrobial	Resistance (%)	Number of isolates tested
Penicillin	67.6	2,562
Erythromycin	8.7	2,442
Clindamycin	8.4	2,431
Fusidic acid	13.4	2,020
Tetracycline	2.9	801
Moxifloxacin	3.8	2,042
Rifampicin	0.7	2,140
Linezolid	0*	2,052
TMP/SXT	0.5	638
Gentamicin	1.1	888
Mupirocin	0.2	1,197
Vancomycin	0	859

\* 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 (1980-2022). Prevalence of penicillin resistance is shown on the right y-axis.**

### 3. Conclusions

The number of recorded SAB cases increased in 2022 and the long-term trends demonstrate increasing numbers and incidence. The prevalence of MRSA cases was 1.9%. The 30 day all-cause case fatality rate was 24% and this rate has not decreased 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.

The diversity of *S. aureus* causing bacteraemia is increasing, with 69% of *spa* types representing isolates from single SAB cases. Thus few successful lineages accounts for the majority of SAB cases, with top ten representing 30% of all cases. *spa* type t1451 (CC398) has increased the last decade and is now found among the top 10 *spa* types causing SAB in Danish patients.

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