STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2023

This report describes the laboratory and clinical characteristics of the 2,571 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2023. 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.

Lone Ryste Hansen Kildevang, Ditte Marie Brix and Stine Frese-Madsen are thanked for technical assistance in the laboratory.

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.

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Contents

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
***luk*F/S-pv:**	Genes encoding the Panton-Valentine leucocidin
***mec*A:**	The gene encoding for methicillin resistance
***mec*C:**	The gene coding for a variant *mec*A gene
MiBa:	The Danish Microbiology Database
MLST:	Multi Locus Sequence Typing
MSSA:	Methicillin-susceptible *Staphylococcus aureus*
MRSA:	Methicillin-resistant *Staphylococcus aureu*
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 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 (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 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*, *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 Ridom SeqSphere+ 10.0.0 (Ridom GmbH, Münster, 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 https://mibaen.ssi.dk/miba). The first *S. aureus* isolate per patient per year from blood was included. Results from antimicrobial susceptibility testing 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 2023, a total of 2,571 cases of SAB were recorded (Figure 1) of which 2,315 patients (90%) had their first episode. This is almost the exact same number of cases as in 2022 (2,578). The number of cases has in general increased every year, most notably in the last decade. The number of cases in 2023 was thus 30% higher compared to 2014. The Danish population constituted 5,932,654 citizens by January 1st 2023 and the incidence rate of SAB was 43.3/100,000 inhabitants (Figure 2). The incidence rate has increased in average by 3% each year in the last decade. Methicillin-resistant *S. aureus* (MRSA) was identified from 39 cases (1.5%) (Figure 3) and the incidence rate of SAB-MRSA was 0.66/100,000 inhabitants (Figure 4). There were more males than females (64% males vs. 36% females) among the cases of SAB in 2023. This proportion has been relatively constant with males comprising 60%-64% of cases during the last 20 years.

The voluntary submission of isolates consisted of 97.5% of positive cases in MiBa.

Figure 1. Number of SAB cases in Denmark 1960-2023

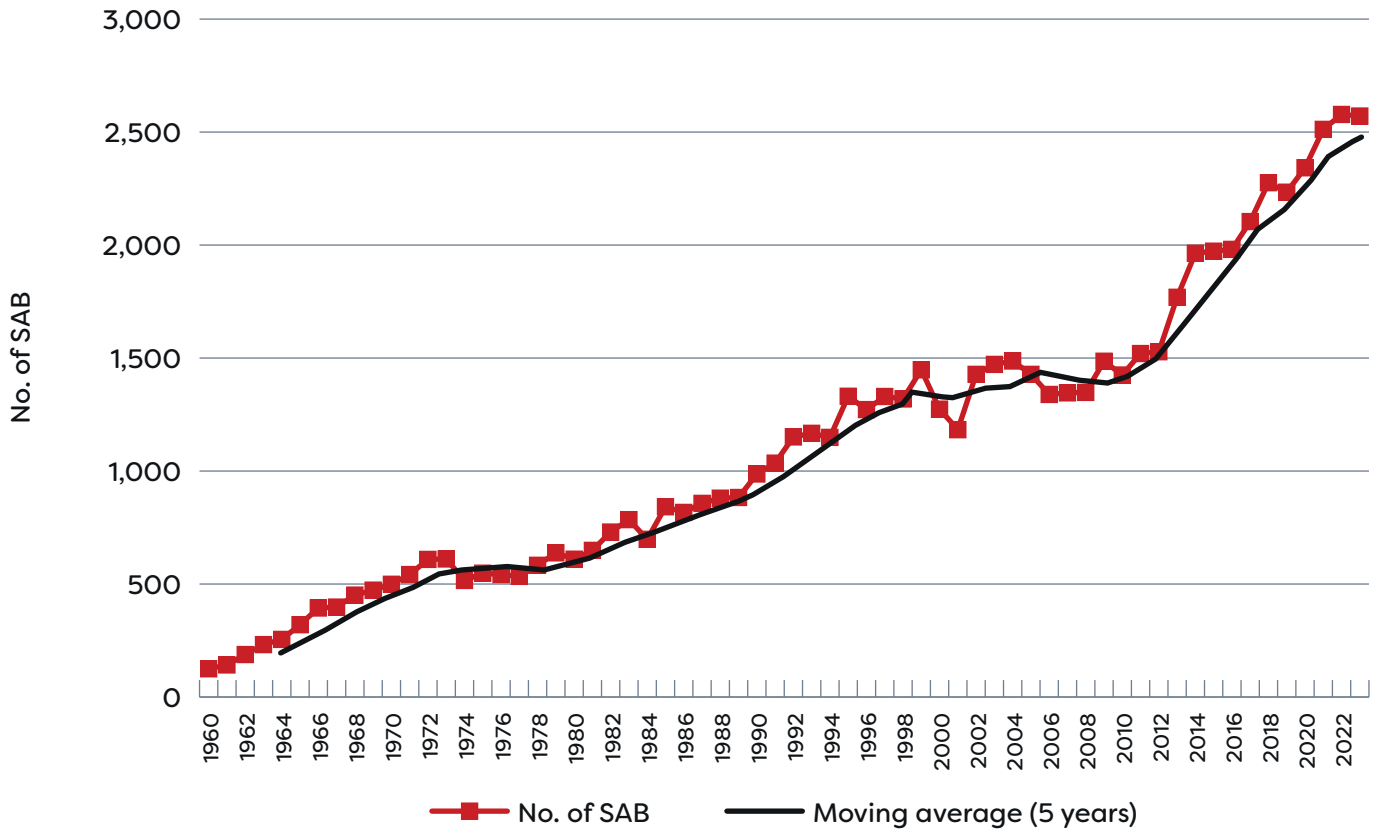


Figure 2. Incidence rate of SAB in Denmark per 100,000 inhabitants 1990-2023

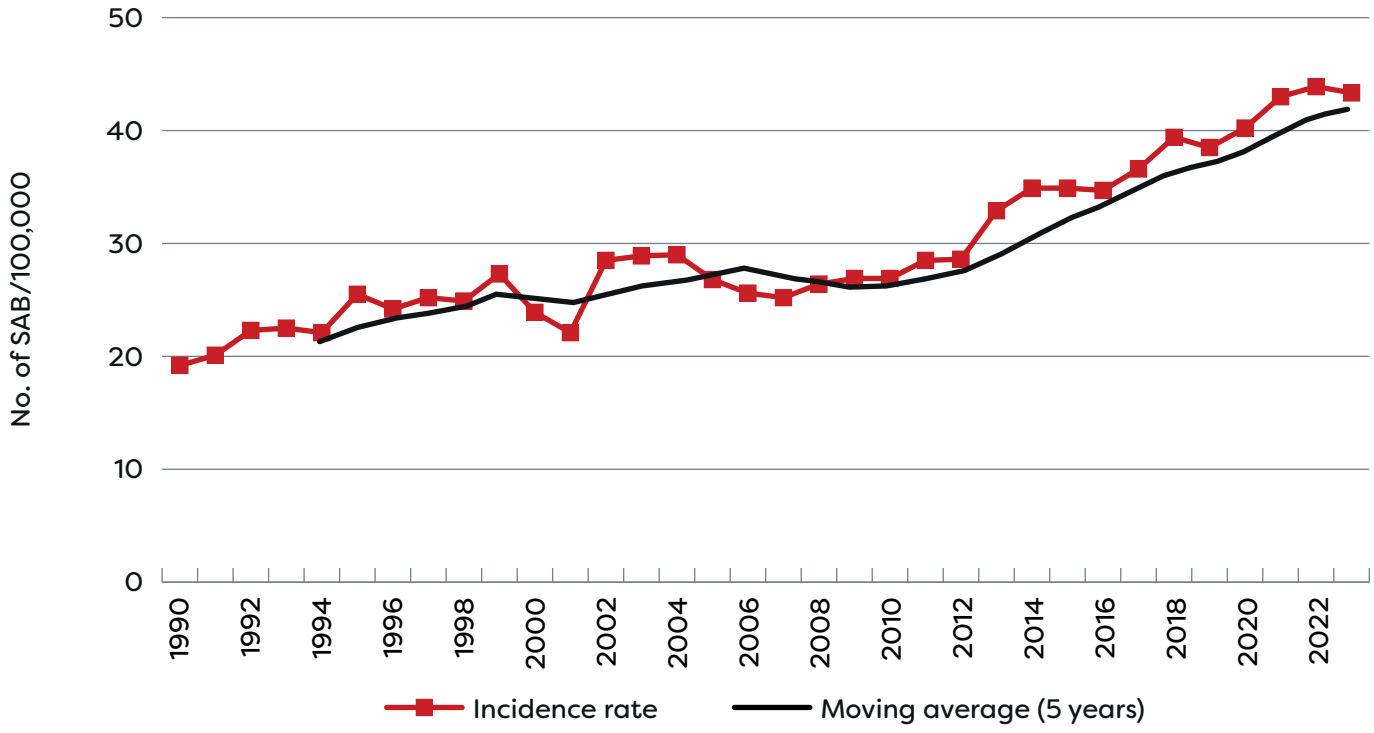


Figure 3. Number of SAB MRSA cases in Denmark 2005-2023

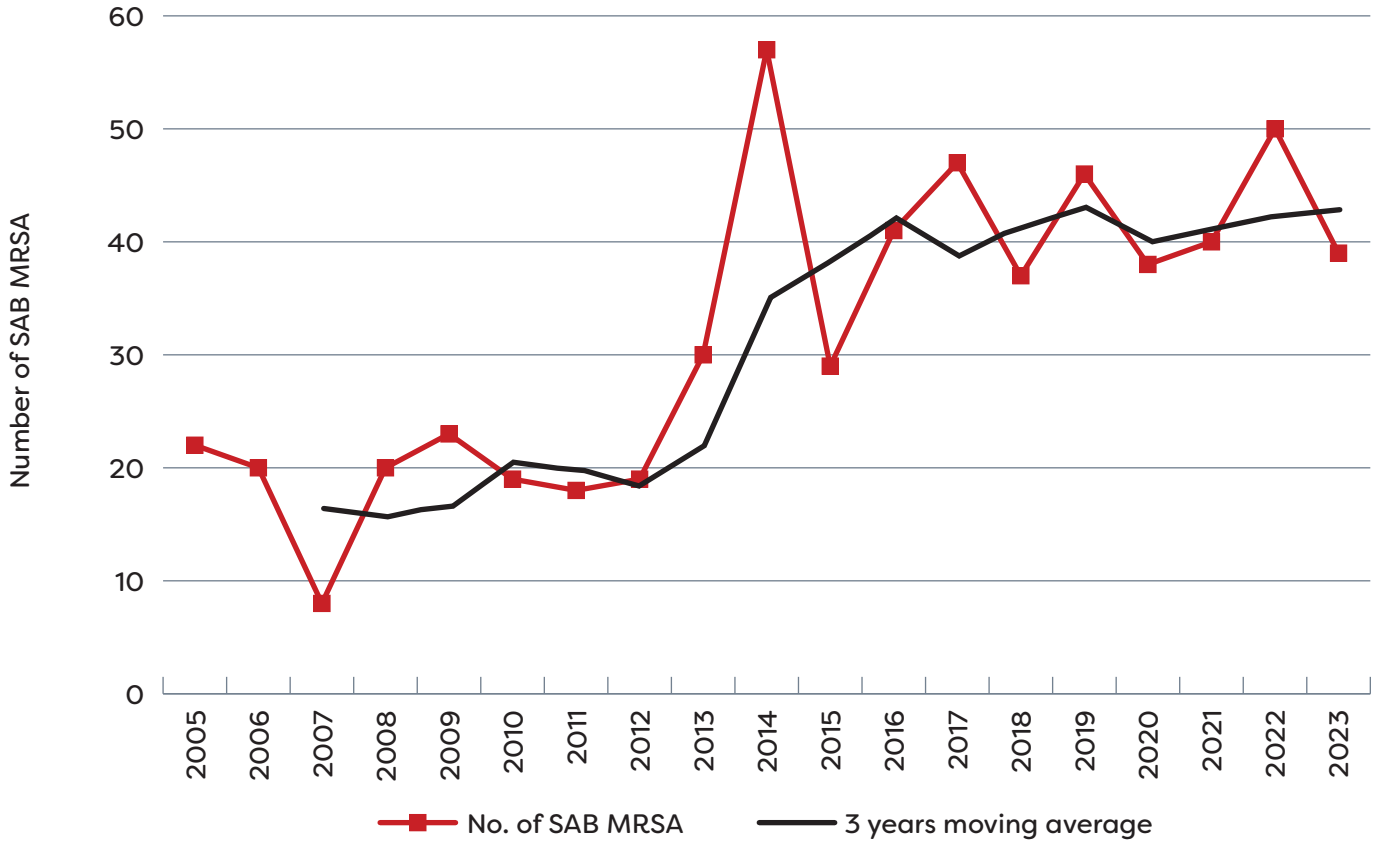
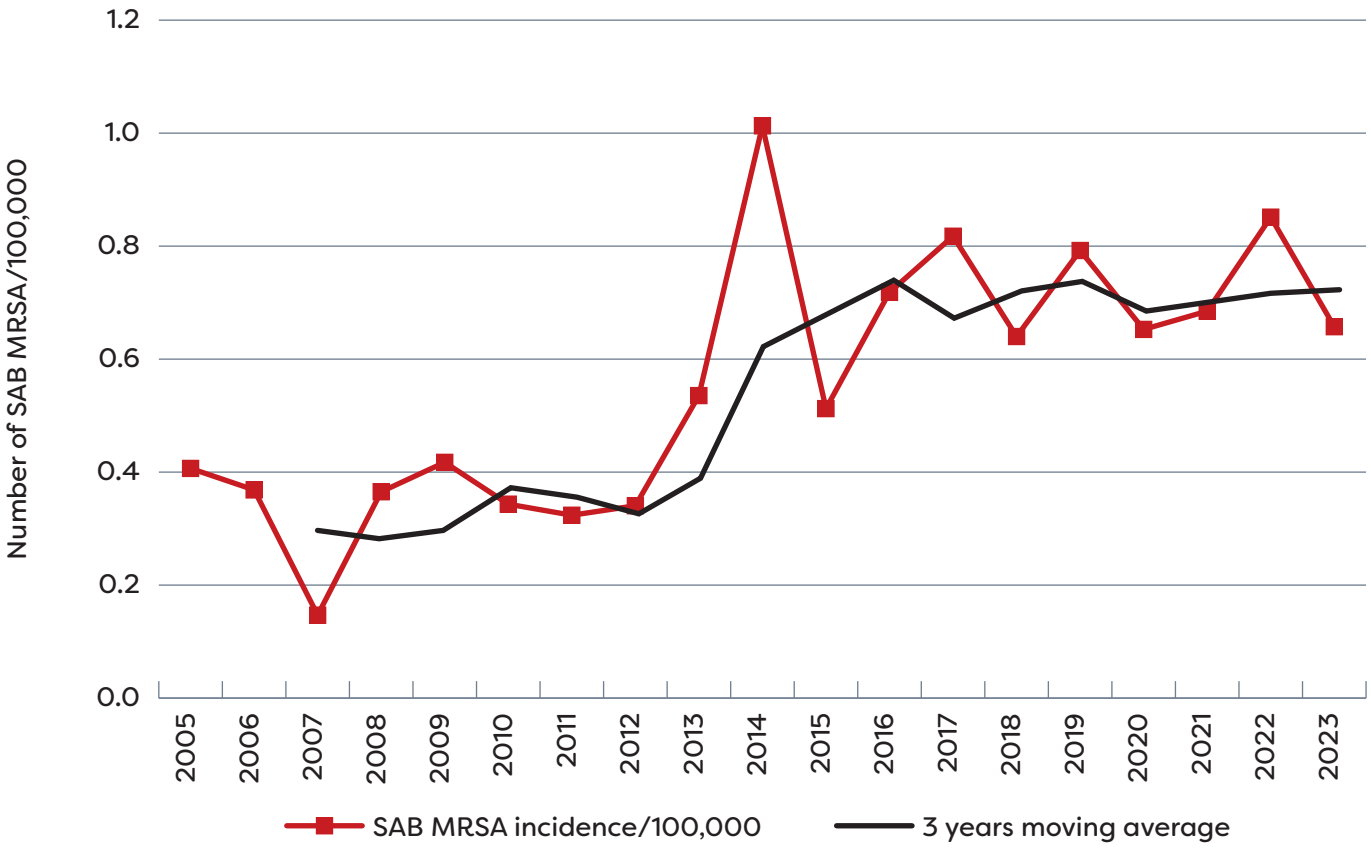


Figure 4. Incidence rate of SAB MRSA cases in Denmark per 100,000 inhabitants 2005-2023



2.2 Age distribution

Eighty-four percent of the SAB patients in 2023 were older than 50 years and 29% were older than 80 years (Figure 5). The Danish population only included 4% older than 80 years in 2023 and the incidence of SAB among people above 80 years of age (282/100,000 inhabitants) was eight times higher than for the rest of the population (32.2/100,000 inhabitants). In the last decade the incidence for age groups 71-80 and 80+ increased significantly with 2% and 3% per year, respectively (Figure 6). A significant increase in incidence was also seen in the age group 41-50 (2%). The remaining age groups saw no significant changes in the incidence.

Figure 5. Age distribution of SAB patients and the Danish population in 2023 (%)

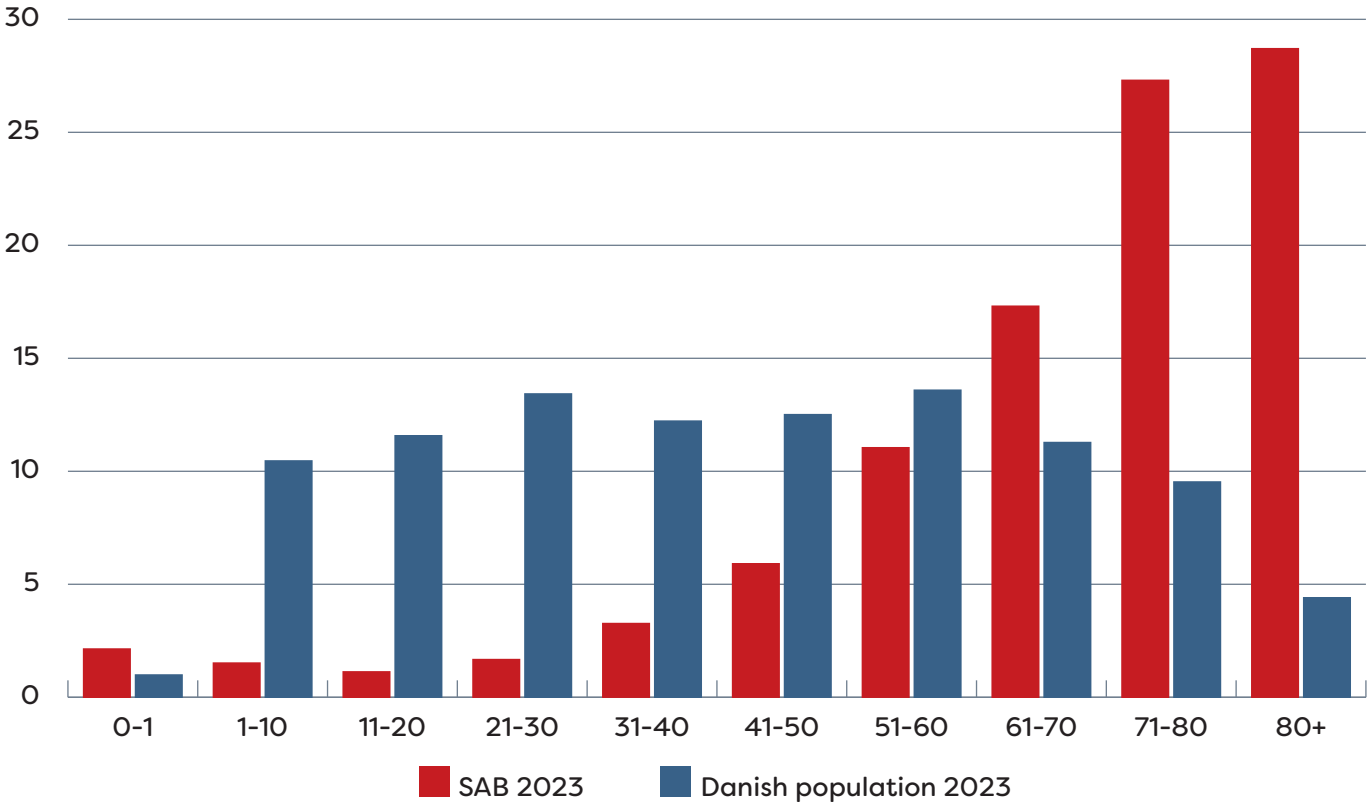
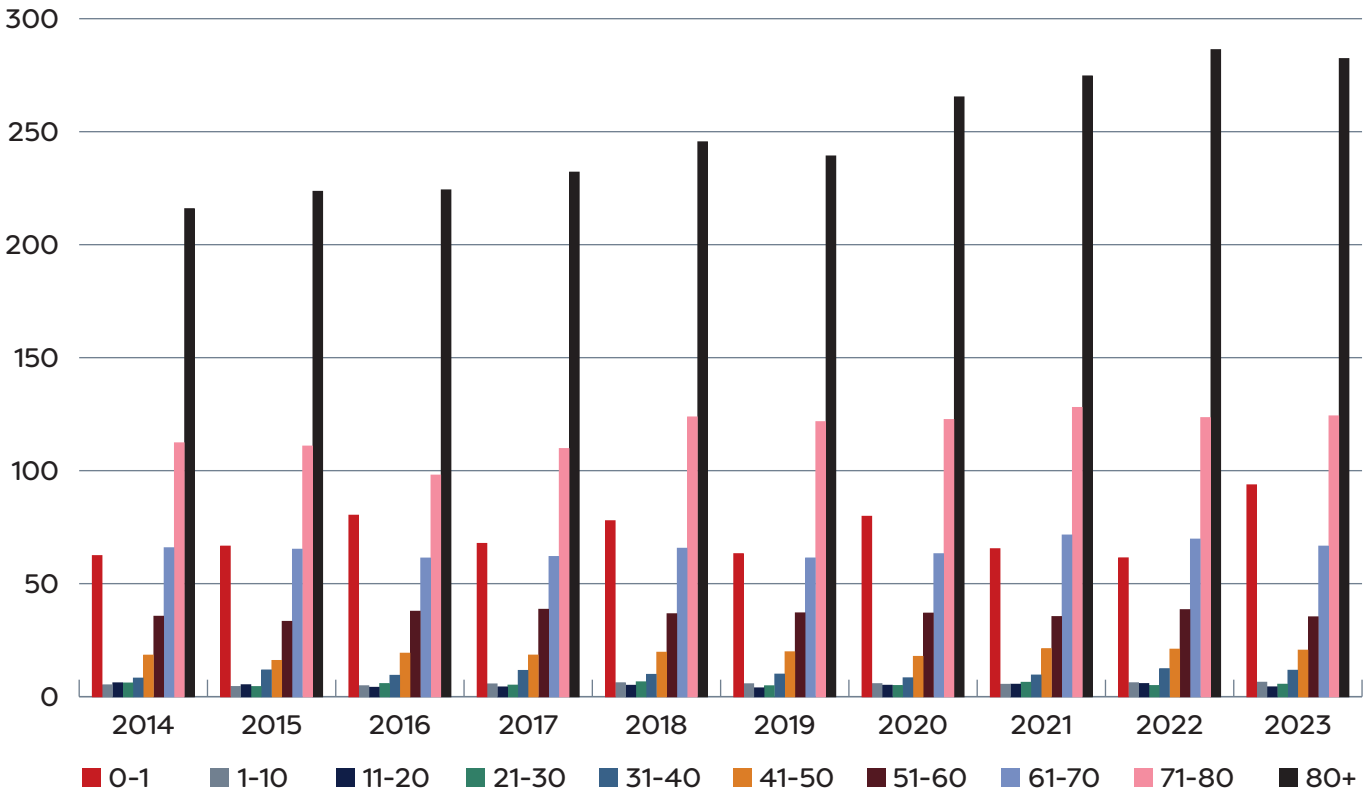


Figure 6. Incidence of SAB per age group among Danish cases 2014-2023



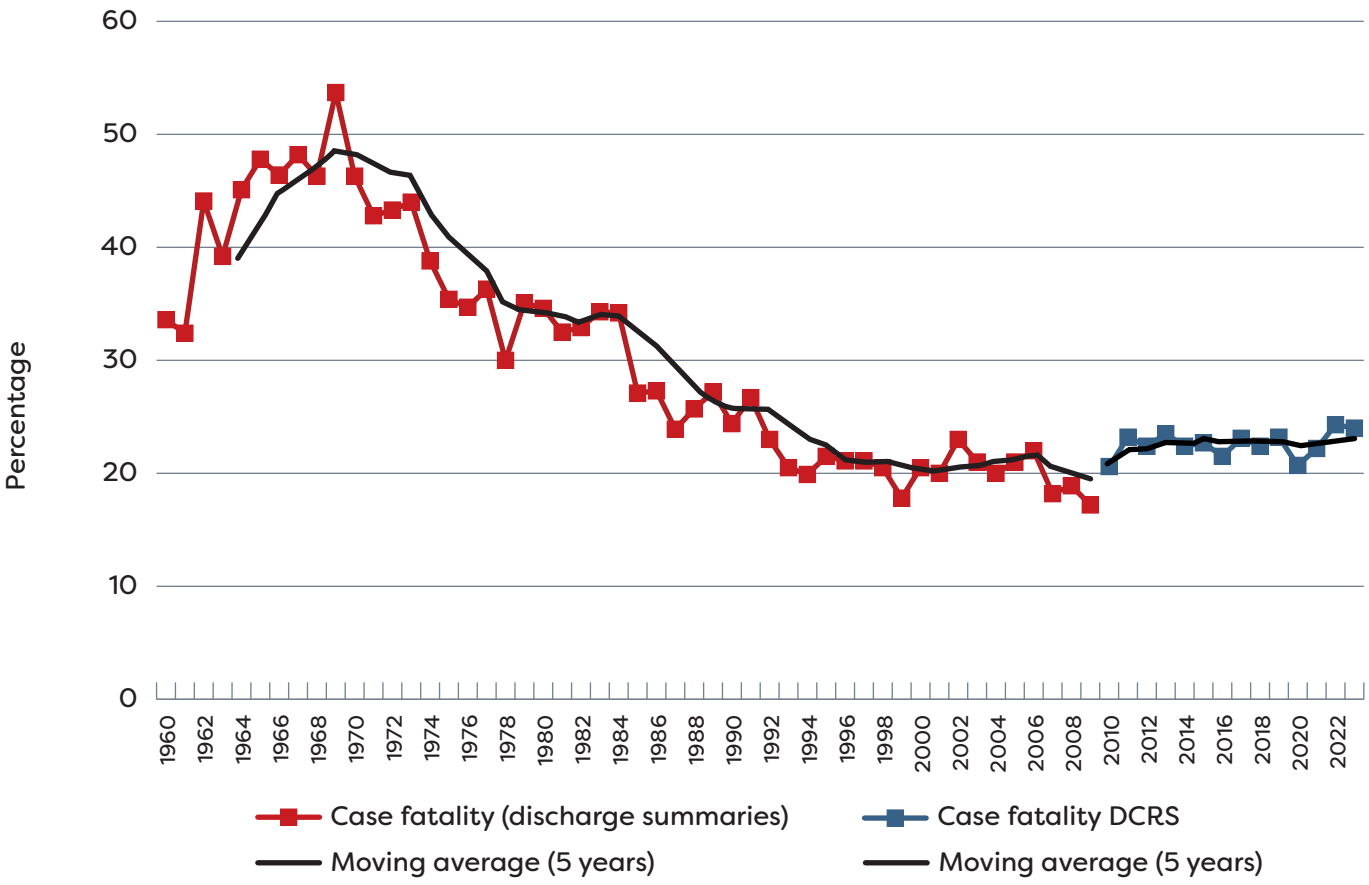
2.3 Case fatality

The 30-day all-cause case fatality was 24% in 2023 (Table 1). The rate has been between 21-24% in the last decade (Figure 7), but was more than 40% in the beginning of the 1970'ies and above 30% until the mid-80'ies. There was no difference in 30-day all-cause case fatality between men and women (23.8% and 24.6%, respectively, p=0.70, 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 40% (Table 1). 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 2023 by age group and in total

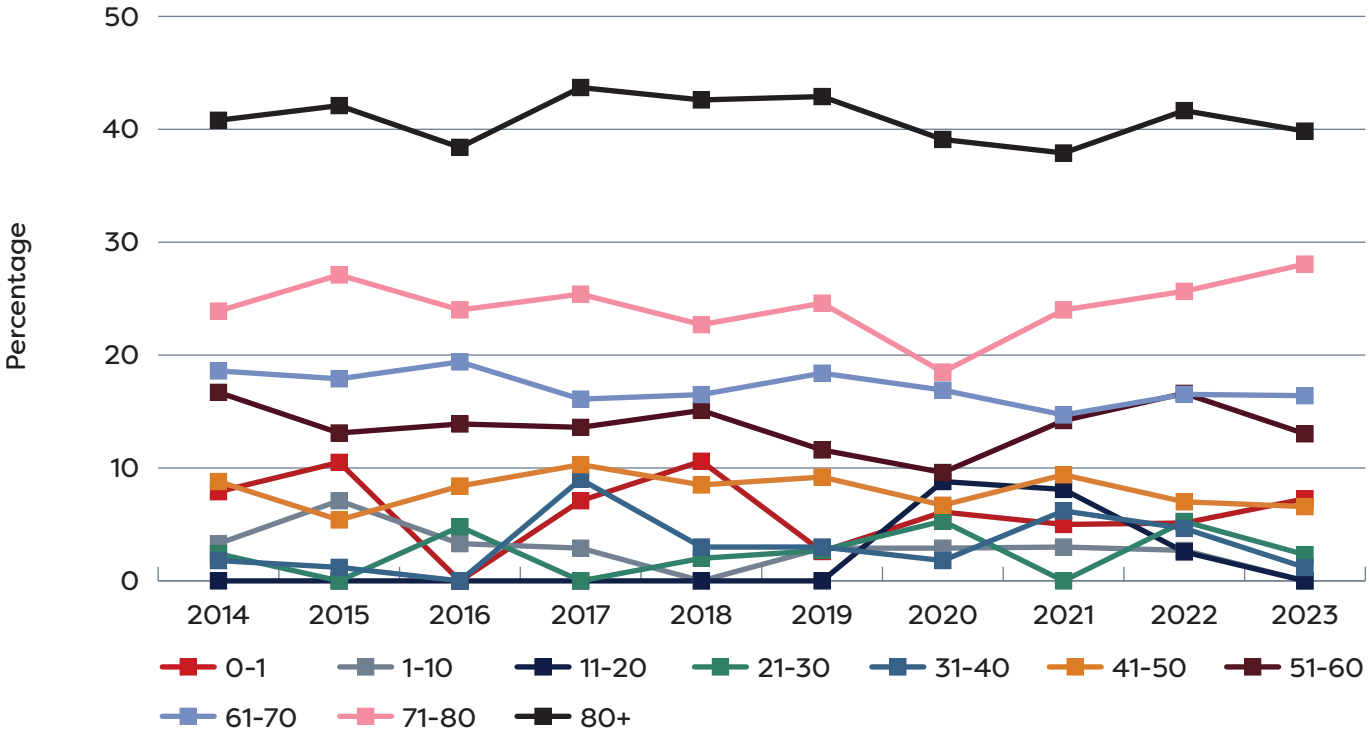
Age group (years)	0-1	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	80+	Total
No. SAB	55	39	29	43	84	152	284	445	702	738	2,571
No. case fatality	4	0	0	1	1	10	37	73	197	294	617
% case fatality	7	0	0	2	1	7	13	16	28	40	24

Figure 7. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2023



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 2014-2023 by age-group



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. Thus, the outcome of SAB did not seem to depend on the specific type of *S. aureus* causing infection. Six patients with a *pvl* positive isolate (see section 2.6) died within thirty days from a positive blood culture. The proportion (15.8%) was lower, but not significantly different from *pvl* negative cases (24.1%, p=0.34, Fisher's exact test). Thirty-day case fatality among cases with MRSA was not different for cases with MSSA (28.2% vs. 23.4%, p=0.57, Fisher's exact test).

2.4 Secondary infections

The prevalence of secondary infections in SAB patients has been stable for the last 10 years (Figure 9). In 2023, 23% of the cases was registered with secondary infections within three months after SAB, corresponding to 600 patients. 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%. With the increasing numbers of cases, increasing numbers of secondary infections have been recorded, most notably for endocarditis, even though in 2023 the numbers and prevalence were lower for endocarditis than in the previous decade (Figure 10).

Table 2. The most common secondary infections (%) among Danish SAB patients in 2023, recorded until three months after admission

Endocarditis	Spondylitis	Prosthetic infection	Arthritis	Osteomyelitis	Central nervous system
9.5	5.2	4.0	3.2	2.5	1.3

Figure 9. Prevalence of specific secondary infections (%) among Danish SAB patients 2014-2023, recorded until three months after admission

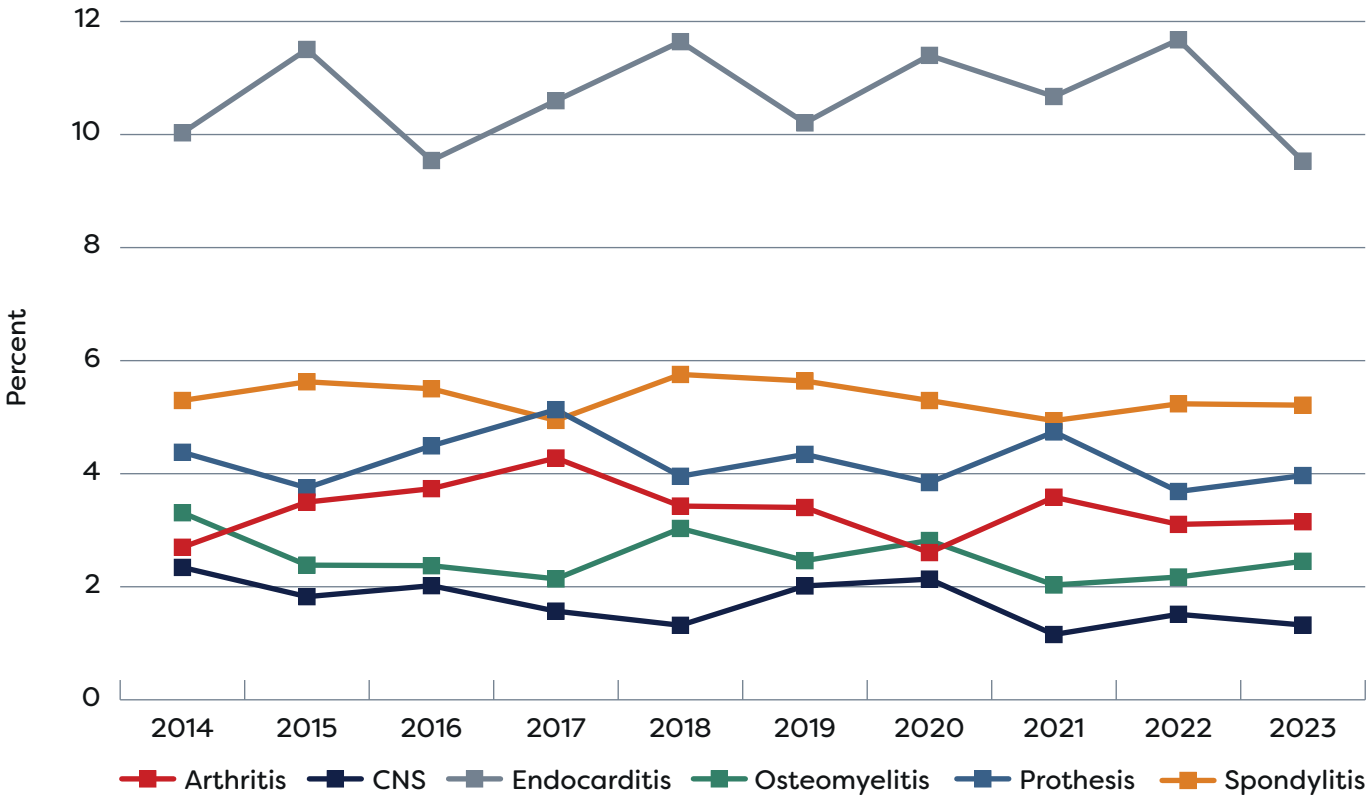
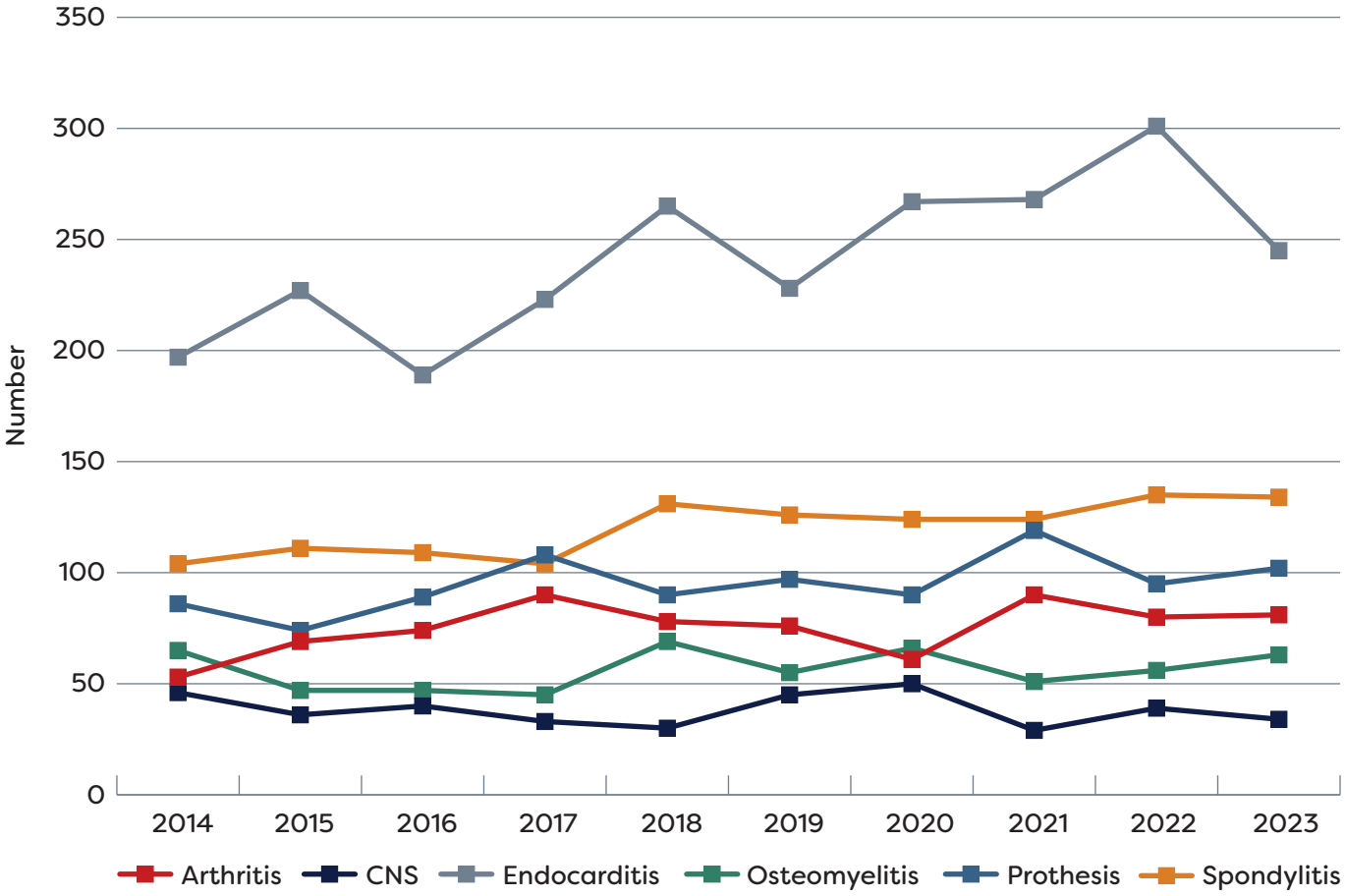


Figure 10. Number of secondary infections among Danish SAB cases 2014-2023, recorded until three months after admission



2.5 Comorbidities

SAB is often associated with comorbidity. In 2023, 915 cases (36%) had no comorbidities registered or a comorbidity index score (CIS) of 0, while 1,000 cases (39%) had a CIS of 1-2, and 656 cases (26%) had a score of more than 2. This distribution has been constant during the last decade. Comorbidities were more often seen in older patients (Table 3). Malignancy (23%), diabetes without chronic complication (20%), and cerebrovascular disease (16%) were the most frequently registered comorbidities among SAB patients in 2023 (Table 4). These three comorbidities have been among the most prevalent for the last ten years. Overall, the prevalence of comorbidities has been very stable for this period (Figure 11).

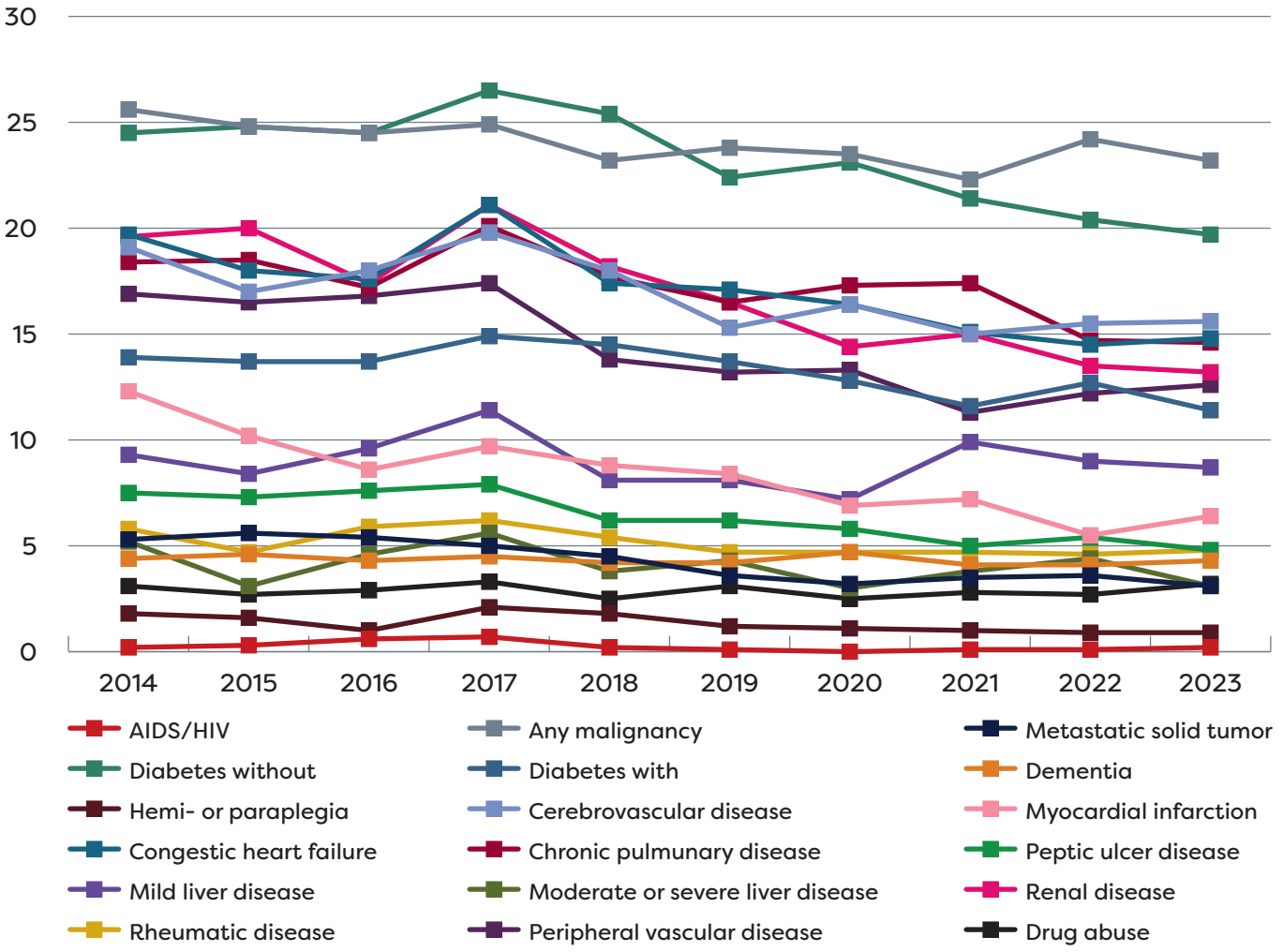
Table 3. Prevalence (%) of comorbidity index score (CIS) per age group among Danish SAB cases 2023

	Age group									
CIS	**0-1**	**1-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**80+**
0	86	64	83	51	44	36	34	33	31	33
1-2	9	33	14	37	41	41	43	36	39	42
>2	5	3	3	12	15	23	23	31	30	25

Table 4. Prevalence (%) of comorbidities among Danish SAB cases 2023

Comorbidity	Total
AIDS/HIV	<1
Any malignancy	23
Metastatic solid tumor	3
Diabetes without chronic complication	20
Diabetes with chronic complication	11
Dementia	4
Hemiplegia or paraplegia	<1
Cerebrovascular disease	16
Myocardial infarction	6
Congestive heart failure	15
Chronic pulmonary disease	15
Peptic ulcer disease	5
Mild liver disease	9
Moderate or severe liver disease	3
Renal disease	13
Rheumatic disease	5
Peripheral vascular disease	13
Drug abuse	3

Figure 11. Prevalence (%) of comorbidities among Danish SAB patients 2014-2023



2.6 Typing

spa typing proved to be a reliable typing method for SAB as 2,562 isolates (99.6%) could be typed. A total of 764 different *spa* types were identified, and the ten most common *spa* types accounted for 31% of the isolates (Table 5) while 539 *spa* types only were found in one case. The diversity of *spa* types has been constant in the last decade as the number of different *spa* types closely follow the numbers of SAB cases. *spa* type t1451 which only was found among four SAB patients in 2014, has increased by in average 29% each year since and has in the last two years been among the ten most common *spa* types among Danish SAB patients. The remaining top ten *spa* types have been among the most prevalent since 2016 with some differences in ranking. Only *spa* type t002 showed a small, but significant decrease during the last 10 years (Table 5). Assignment to MLST CC was possible for 2,244 isolates (87%). In the remaining cases, assignment was not possible due to an unresolved relationship with MLST typing. A total of 30 MLST CC were assigned. The three most prevalent CC constituted 37% of the SAB isolates in 2023 while the 10 most prevalent constituted 77%. The most remarkable change of CC in the last decade was for CC398, which had an annual significant increase of 15%. The successful *spa* type t1451 belongs to CC398. Thirty-eight SAB isolates were *pvl* positive (1.5%), of which eight were MRSA (t002/CC5, t019/CC30, t021/CC30, t024/CC8, t034/CC398, t304/CC6, t314/CC121 and t903/CC97). The *pvl* positive isolates were distributed among 24 different *spa* types and 13 MLST CC groups; four isolates had an unresolved relationship with MLST CC typing.

2.6.1 Livestock associated CC398

LA-CC398 MRSA isolates have a reservoir in livestock. CC398 constituted 144 SAB cases (5.6%) in 2023 of which 9 were LA-MRSA. Four of the LA-MRSA patients had direct contact to livestock. Two of the LA-MRSA patients died within 30 days of diagnosis (22.2%). Since 2007, 18 SAB patients with LA-MRSA have died within 30 days. Case fatality rate among SAB CC398 MSSA in 2023 was 25.4%.

Table 5. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2023 and the 10-year trend

spa type/CC group	Number (%)	Trend
t127/CC1	125 (4.9)	ns
t084/CC15	116 (4.5)	ns
t091/CC7	87 (3.4)	ns
t230/CC45	80 (3.1)	ns
t002/CC5	76 (3.0)	0.97
t021/CC30	69 (2.7)	ns
t012/CC30	68 (2.6)	ns
t008/CC8	65 (2.5)	ns
t1451/CC398	61 (2.4)	1.29
t701/CC8	44 (1.7)	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.

2.7 Antimicrobial susceptibility testing

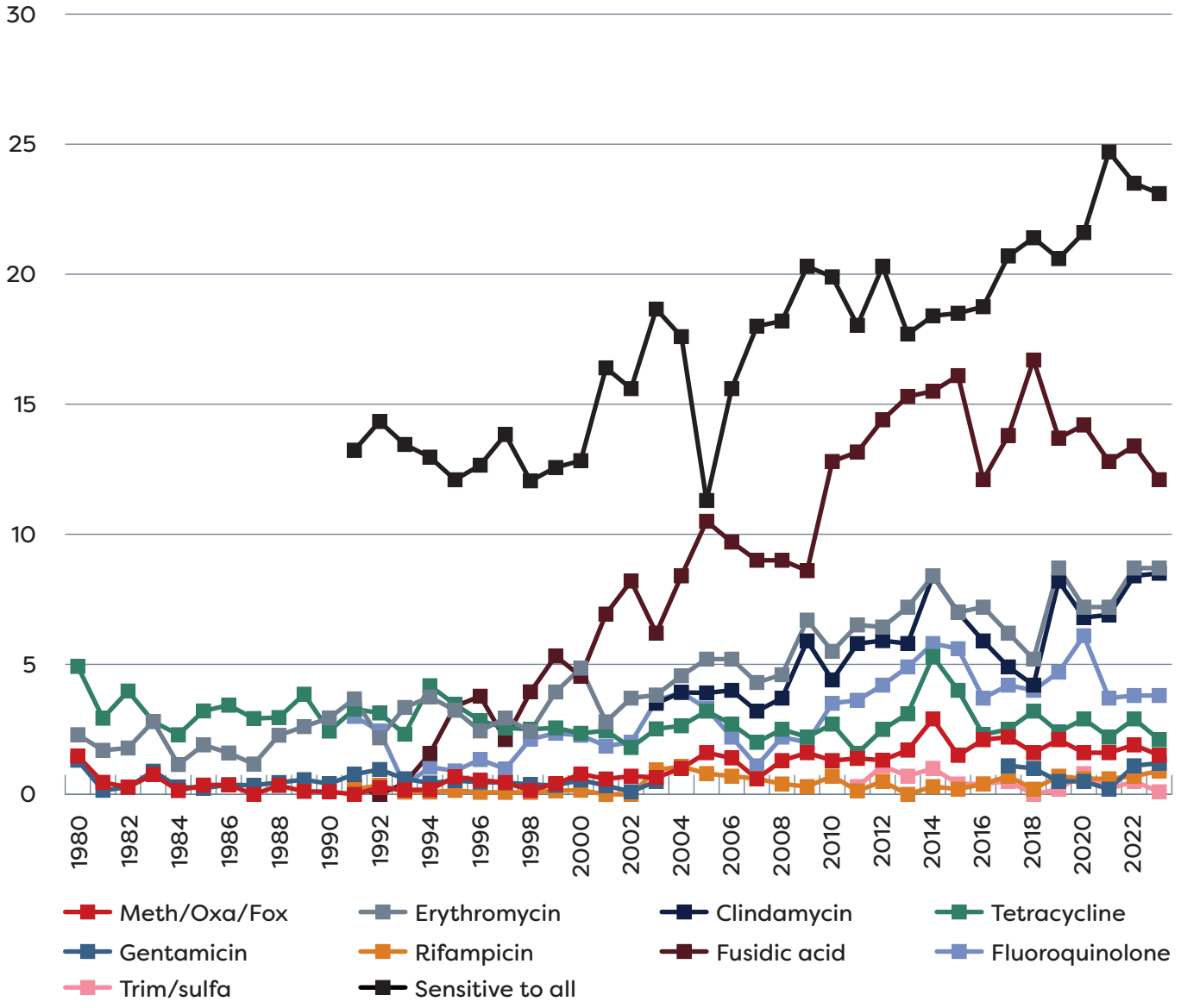
Data extracted from MiBa comprised 2,539 isolates. The DCMs included different antimicrobials in their susceptibility testing and only for penicillin almost all isolates were tested. Resistance to penicillin remained under 70% (67.9%; 67.6% in 2022) and resistance to fusidic acid decreased to 12.1% (13.4% in 2022; Table 6). Resistance to the remaining antimicrobials were all below 10%. The percentage of fully susceptible isolates was almost the same in 2023 as in 2022 (23.1% and 23.5%, respectively). Figure 12 shows selected resistance prevalences from 1980 to 2023. Resistance to fusidic acid has increased from 0 to 15%, but in the last decade a minor decrease has been recorded. The proportion of fully susceptible isolates also increased from 12-13% in the 1990'ies to 23-24% in recent years (Figure 12).

Table 6. Resistance prevalence among Danish SAB isolates 2023, retrieved from MiBa

Antimicrobial	Resistance (%)	Number of isolates tested
Penicillin	67.9	2,524
Erythromycin	8.7	2,413
Clindamycin	8.5	2,402
Fusidic acid	12.1	2,017
Tetracycline	2.1	839
Moxifloxacin	3.8	2,022
Rifampicin	0.9	2,138
Linezolid	0*	2,060
TMP/SXT	0.1	709
Gentamicin	1.2	914
Mupirocin	0.2	1,253
Vancomycin	0	1,040

* Data from MiBa indicated four 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 12. Prevalence of antimicrobial resistance in Danish SAB isolates (1980-2023)



3. Conclusions

The number of recorded SAB cases was almost identical to the number of cases in 2022. The prevalence of MRSA cases was 1.5% and 23% of the isolates were susceptible to all of the tested antimicrobials The 30 day all-cause case fatality rate was 24% and this rate has not changed since the beginning of the 1990'ies.

Two-thirds of all patients had at least one comorbidity registered, and three months after onset of SAB, almost 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 71% of *spa* types representing isolates only found in single SAB cases. Thus few successful lineages accounts for the majority of SAB cases, with top ten representing 31% of all cases. *spa* type t1451 (CC398) continues to increase and is for the second year in a row among the top 10 *spa* types causing SAB in Danish patients.

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