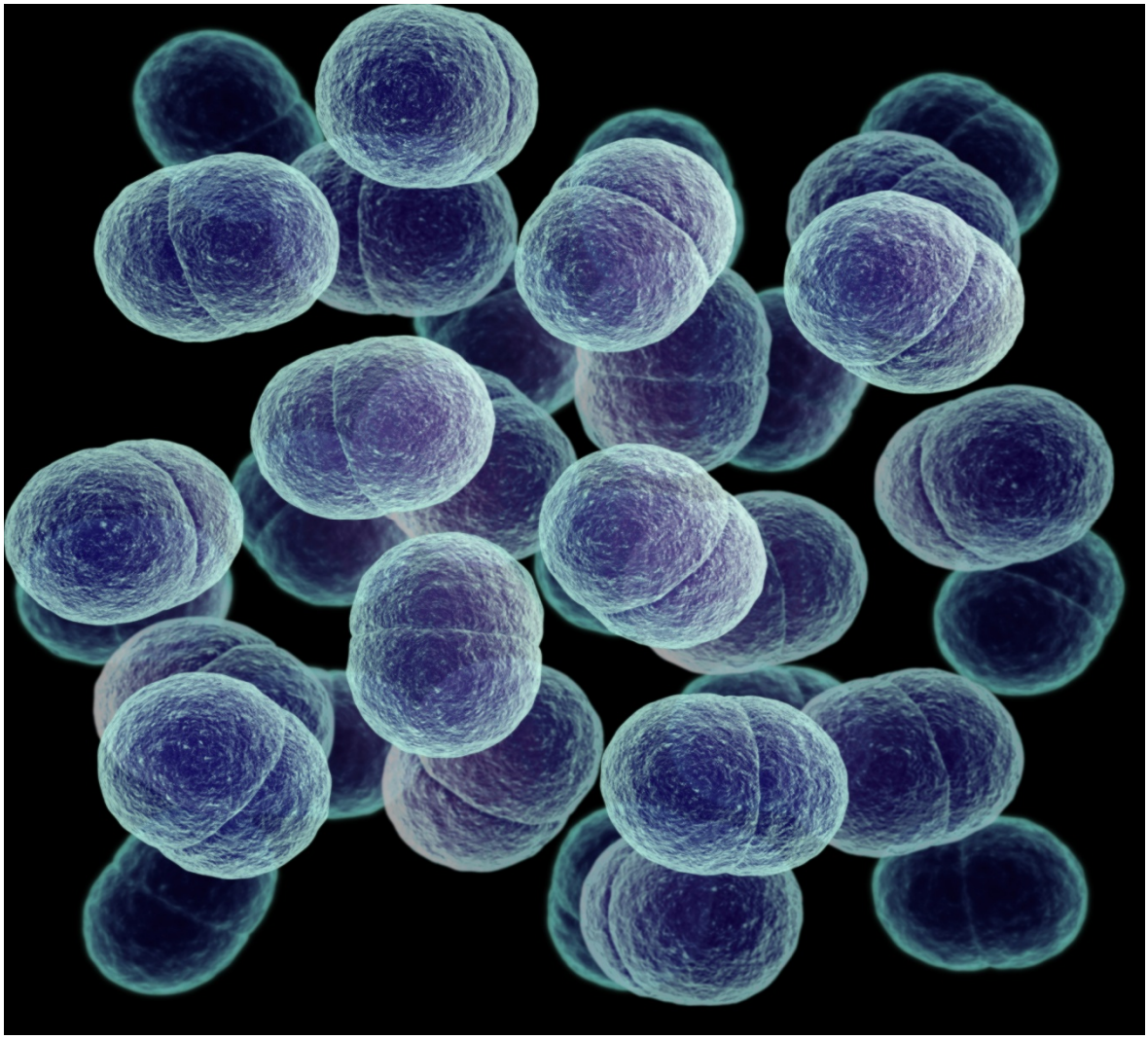
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2018



This report describes the laboratory and clinical characteristics of the 2,276 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2018. SAB has been surveyed by submission of blood culture isolates since 1957. The Staphylococcus Laboratory 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.

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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
HA: Hospital acquired
ICD-10: International Classification of Diseases
LA: livestock-associated
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
MLST: Multi locus sequence typing
MRSA: Methicillin-resistant *Staphylococcus aureus*
MSSA: Methicillin-susceptible *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 Staphylococcus Laboratory as part of an ongoing collaboration 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 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. The following data were extracted: onset of infection in relation to hospital admission, comorbidities and secondary foci (assessed during admission and 3 months after the onset of SAB). Onset of infection was classified as hospital acquired (HA) if *S. aureus* was found by blood culture more than two days after admission. 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). ICD-10 codes used to identify secondary infections are shown in Appendix 1. 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).

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 6.6 (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 and eBURST (https://pubmlst.org/saureus/).

1.3 Antimicrobial susceptibility testing

The National Reference Laboratory performed susceptibility testing of every second received isolate in the months January, March, April, September, October and November (504 isolates, 22%) 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) breakpoints. 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

2. Results

2.1 Patient information

In 2018, 2,276 cases of SAB were recorded (Figure 1); of which 2,043 (90%) constituted primary and 233 subsequent episodes. Methicillin-resistant *S. aureus* (MRSA) was identified from 37 cases (1.6%). The incidence rate of SAB was 39.4/100,000 inhabitants (Figure 2) and of MRSA-SAB 0.64/100,000 inhabitants. The number of new cases has increased by 5% each year since 2010, also when taken the increasing population into account. There was more males than females (64% males vs. 36% females) among the cases of SAB in 2018. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

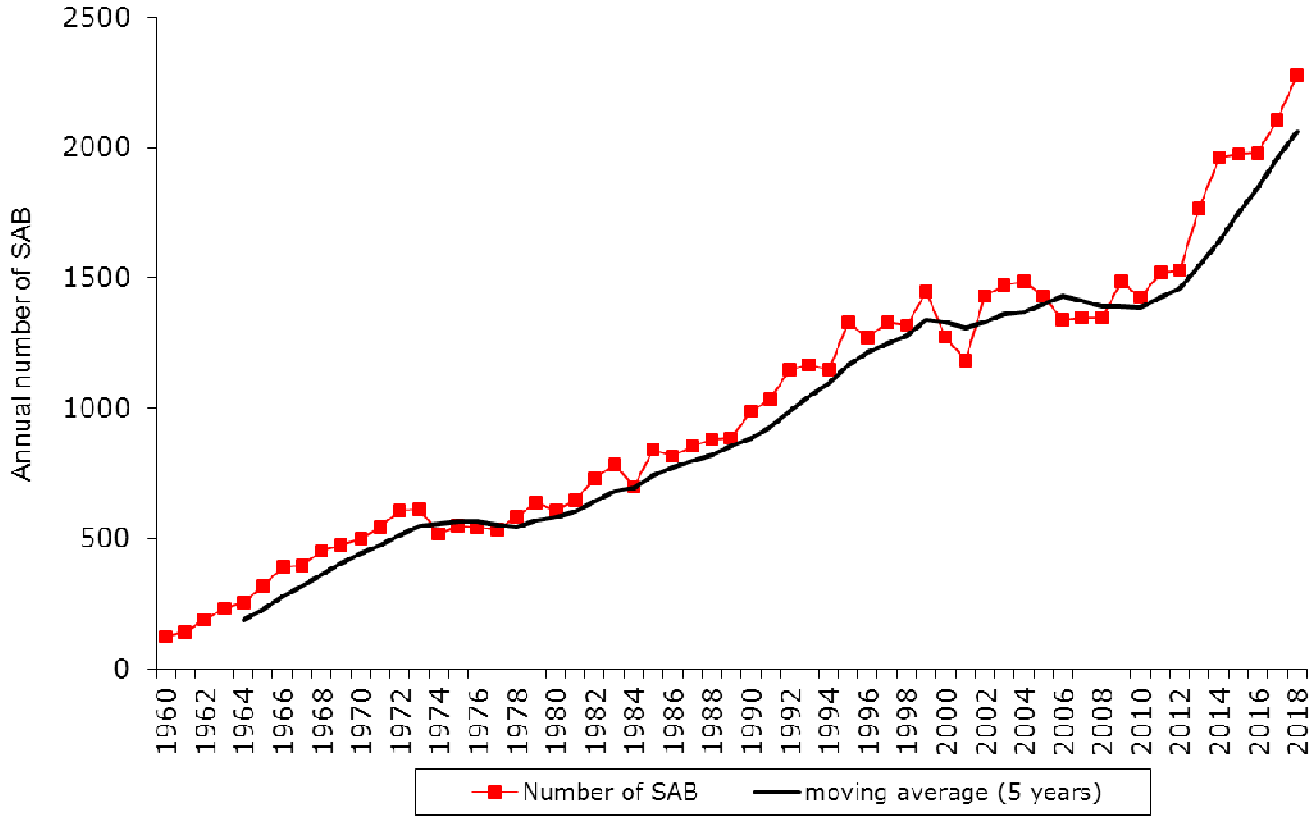

Figure 1. Number of SAB cases 1960-2018.

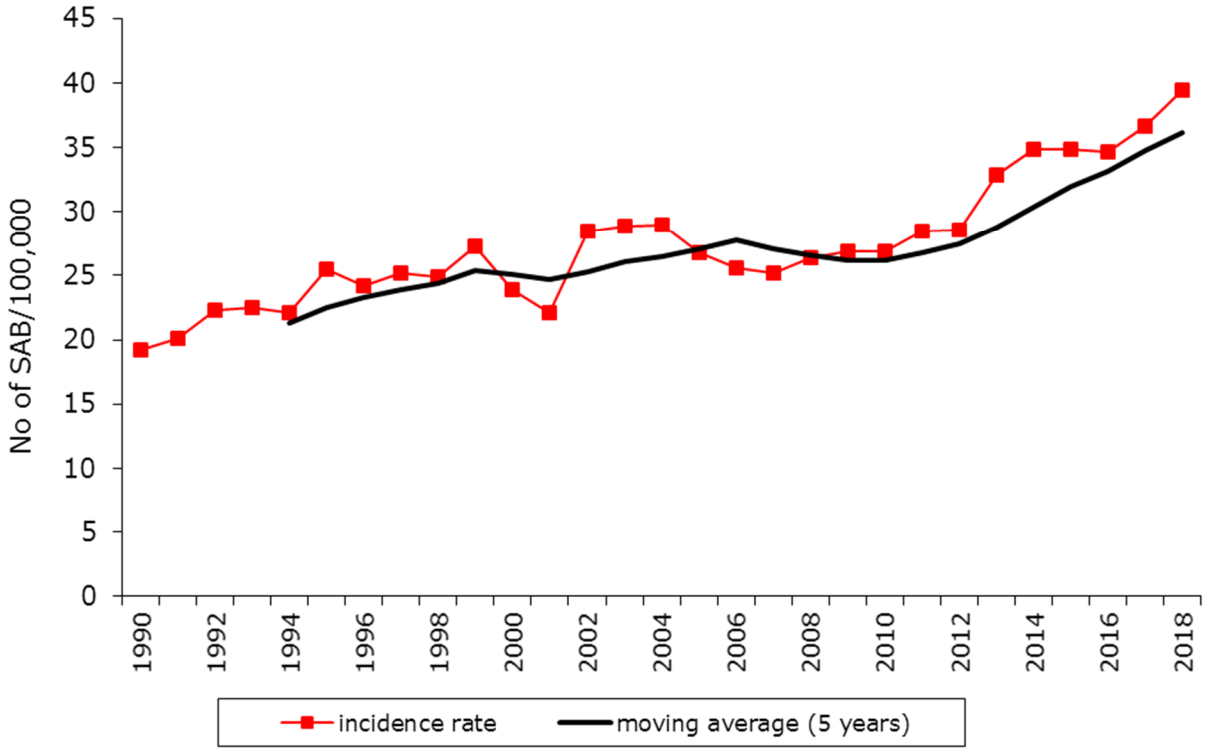

Figure 2. Incidence rate of SAB per 100,000 inhabitants during 1990-2018.

2.2 Age

More than 80% of the SAB patients in 2018 were older than 50 years and 24% were older than 80 years (Figure 3). The Danish population only included 4% older than 80 years in 2018 and the incidence of SAB among people above 80 years of age (245.4/100,000 inhabitants) was seven times higher than for the rest of the population (31.1/100,000 inhabitants). The age distribution of SAB cases shows a trend towards the two oldest age groups. In 2010 41% of the SAB cases were above 70 years of age while in 2018 they constituted 51%.

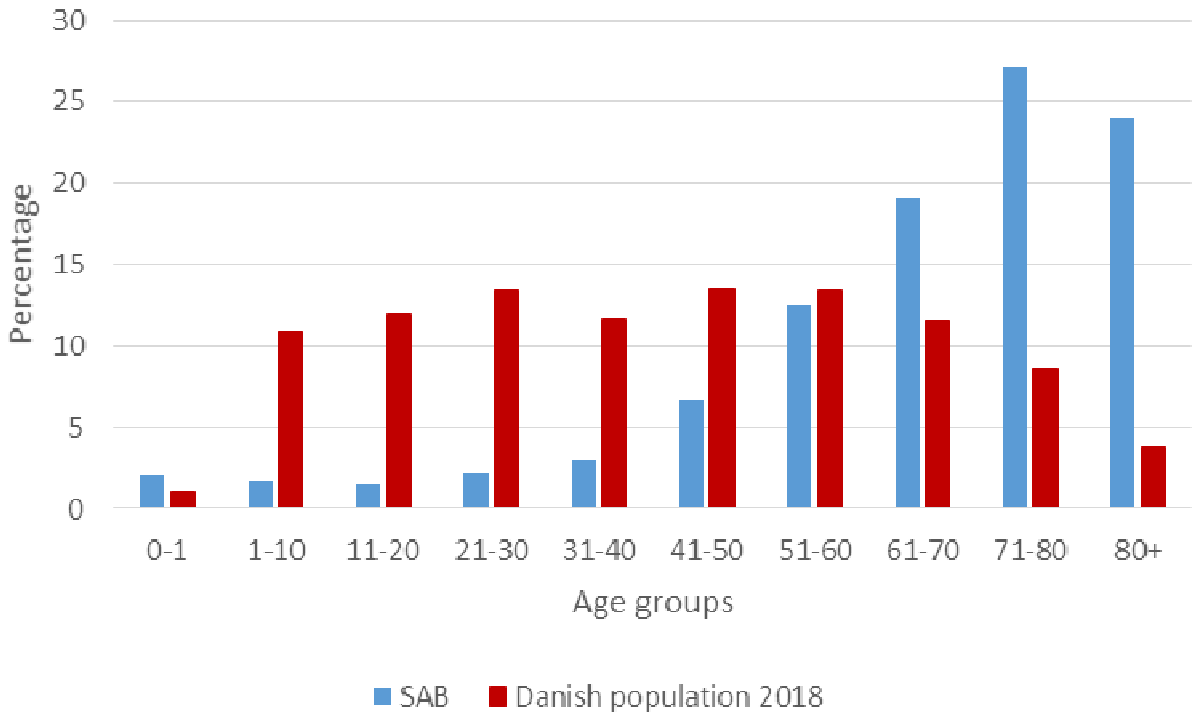

Figure 3. Age distribution of *S. aureus* bacteraemia patients and the Danish population in 2018 (%).

2.3 Case fatality

The 30-day all-cause case fatality was 22.4% in 2018 (Table 2). The rate has been between 17-24% for the last 25 years (Figure 4). Case fatality was low between 1-30 years, increased from the age group of 31-40 years, and patients above 80 years had a case fatality rate of 42.6% (Table 2), almost twice as high as the average. There was no difference in 30-day all-cause case fatality between men and women (22.4% and 22.9%, respectively).

Table 2. Case fatality among Danish SAB patients in 2018 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	47	38	34	50	66	153	285	436	618	549	2276
No. case fatality	5	0	0	1	2	13	43	72	140	234	510
% case fatality	10.6	0	0	2	3.0	8.5	15.1	16.5	22.7	42.6	22.4

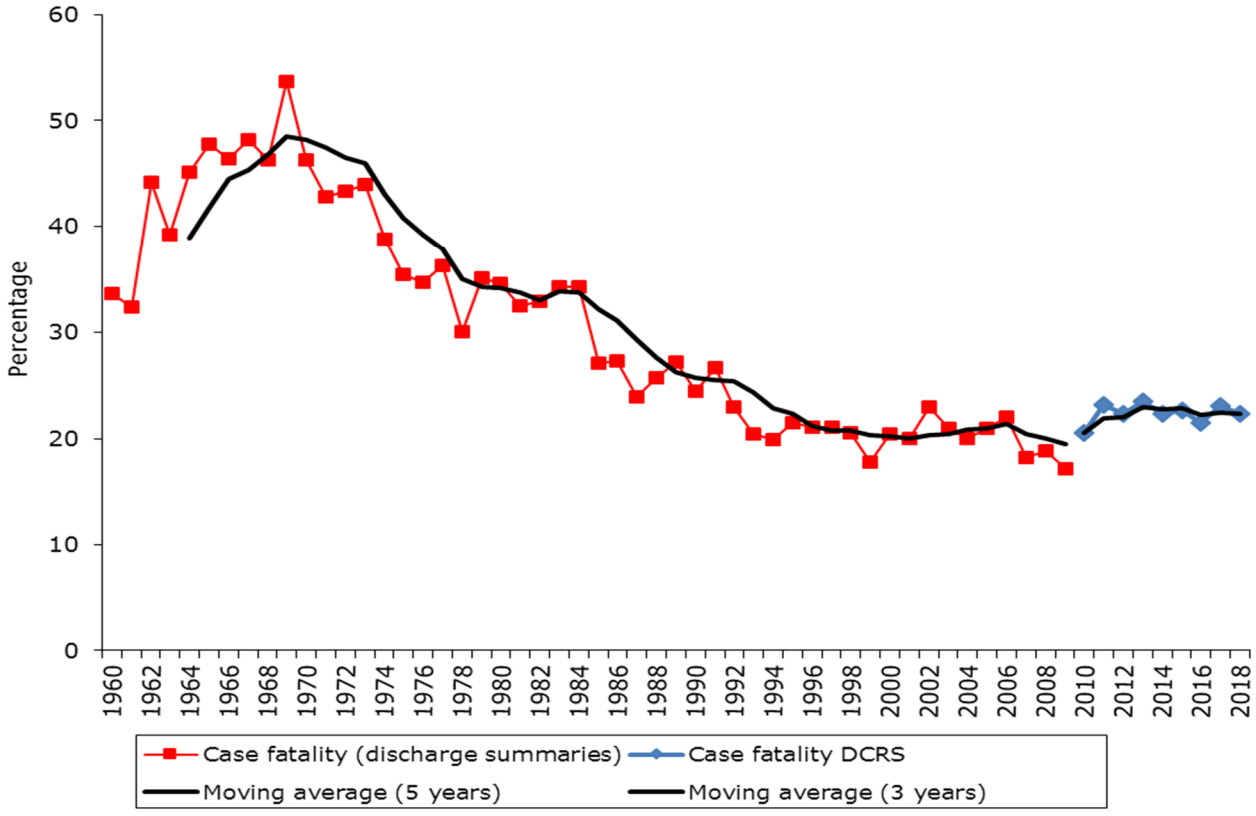

Figure 4. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2018. Until 2009, data was extracted from discharge notes. From 2010 and onwards 30-day, all-cause case fatality was extracted from the Danish Civil Registration System (DCRS).

The outcome of SAB did not seem to depend on the specific type of *S. aureus* causing infection. Thirty-day case fatality among MRSA-cases was also almost the same as the MSSA-case fatality (18.9% vs. 22.5%, p=0.70, Fischer's exact test). The most prevalent *spa* types among the 510 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 two (7%) died within thirty days from a positive blood culture.

2.4 Acquisition

A total of 542 cases (23.8%) had an onset of infection two or more days after admission to a hospital (HA). The corresponding percentage was 25.8% in 2017 and has been steadily decreasing since 2002 (from 41%). Assignment of acquisition to health-care related cases with a community onset (HACO) and community acquired was not possible with data from NPR. The HACO category constituted an increasing number throughout the period and an increasing part of the cases up until 2009.

2.5 Secondary infections

254 cases (11.2%) had a secondary infection registered during admission and within three months, the number was 594 cases, corresponding to 26.1%. 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 2013 to 2018 have been observed; however CNS shows a decreasing trend (Table 4).

Table 3. Secondary infections (%) among Danish SAB patients in 2018, recorded during admission and 3 months after.

	Endocarditis	Spondylitis	Prosthetic infection	Arthritis	Osteomyelitis	CNS
During admission	3.7	1.9	2.4	1.5	1.1	0.5
3 months follow-up	11.6	5.8	4.0	3.4	3.0	1.3

CNS=disease in the central nervous system

Table 4. The most common secondary infections (%) among Danish SAB patients in 2013-2018, recorded 3 months after admission.

Secondary infection	2013	2014	2015	2016	2017	2018
Endocarditis	10.9	10.0	11.5	11.4	10.6	11.6
Spondylitis	5.8	5.3	5.6	5.5	4.9	5.8
Prosthetic infection	4.1	4.4	3.8	3.7	5.1	4.0
Arthritis	3.1	2.7	3.5	3.4	4.3	3.4
Osteomyelitis	2.6	3.3	2.4	2.3	2.1	3.0
CNS	2.3	2.3	1.8	1.8	1.6	1.3

CNS=disease in the central nervous system

2.6 Comorbidities

SAB primarily affects people who are diagnosed with other diseases. In 2018, 705 cases (31%) had no comorbidities registered, while 874 cases (38%) had a comorbidity index score of 1-2, and 697 cases (31%) had a score of more than 2. Table 5 presents comorbidity based on the Charlson index. Diabetes without chronic complication (25.4%), malignancy (23.2%), and renal disease (18.2%) were the most frequently registered comorbidities among SAB patients in 2018. 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.

Table 5. Number and percentage of comorbidities among Danish SAB patients 2018, with percentages for 2015 - 2017 for comparison.

Comorbidity	2018 Number	2015 %	2016 %	2017 %	2018 %
AIDS/HIV	5	0.3	0.6	0.7	0.2
Any malignancy	529	24.8	24.5	24.9	23.2
Metastatic solid tumor	102	5.6	5.4	5.0	4.5
Diabetes without chronic complication	578	24.8	24.5	26.5	25.4
Diabetes with chronic complication	331	13.7	13.7	14.9	14.5
Dementia	96	4.6	4.3	4.5	4.2
Hemiplegia or paraplegia	40	1.6	1.0	2.1	1.8
Cerebrovascular disease	410	17.0	18.0	19.8	18.0
Myocardial infarction	201	10.2	8.6	9.7	8.8
Congestive heart failure	397	18.0	17.6	21.1	17.4
Chronic pulmonary disease	402	18.5	17.2	20.1	17.7
Peptic ulcer disease	141	7.3	7.6	7.9	6.2
Mild liver disease	185	8.4	9.6	11.4	8.1
Moderate or severe liver disease	87	3.1	4.6	5.6	3.8
Renal disease	415	20.0	17.4	21.1	18.2
Rheumatic disease	123	4.7	5.9	6.2	5.4
Peripheral vascular disease	315	16.5	16.8	17.4	13.8
Drug abuse	57	2.7	2.9	3.3	2.5

2.7 Typing

spa typing was successful for 2,260 isolates (99.3%). A total of 630 different *spa* types were identified, and ten *spa* types accounted for 34% of the isolates (Table 6). The same ten *spa* types were the most prevalent in 2016 and 2017 but with some differences in ranking. A total of 442 *spa* types (70% of all *spa* types) were only found once. Putative assignment to MLST CC was possible for 1,940 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 2018 while the 10 most prevalent constituted 76% (Table 7). Twenty-nine SAB isolates were *pvl* positive (1.3%), of which five were MRSA (*spa* types three t005/CC22, one t008/CC8 and one t044/CC80). The *pvl* positive isolates were distributed among 25 different *spa* types and eleven MLST CC groups; six isolates had an unresolved relationship with MLST typing.

2.7.1 CC398

CC398 MRSA isolates have been associated with a reservoir in livestock. CC398 constituted 51 SAB cases (2.2%) in 2018 of which 8 were MRSA. Seventeen belonged to *spa* type t571, thirteen to *spa* type t034, twelve to t1451 and the remaining belonged to seven other *spa* types. Three of the SAB CC398 MRSA had direct contact to livestock. Two of the SAB CC398 MRSA patients died within 30 days of diagnosis. Since 2007, nine SAB patients with CC398 MRSA have died within 30 days.

Table 6. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2018. Corresponding numbers and prevalences for the four previous years are shown for comparison.

spa type	2014	2015	2016	2017	2018
t127	103 (5.2)	96 (4.9)	81 (4.1)	128 (6.1)	137 (6.0)
t084	66 (3.4)	89 (4.5)	83 (4.2)	75 (3.6)	105 (4.6)
t002	88 (4.5)	84 (4.3)	87 (4.4)	81 (3.8)	101 (4.4)
t230	100 (5.1)	82 (4.2)	81 (4.1)	70 (3.3)	81 (3.6)
t091	63 (3.2)	60 (3.0)	62 (3.1)	85 (4.0)	85 (4.0)
t012	66 (3.4)	57 (2.9)	62 (3.1)	65 (3.1)	70 (3.1)
t021	31 (1.6)	46 (2.3)	39 (2.0)	52 (2.5)	61 (2.7)
t008	46 (2.3)	36 (1.8)	55 (2.8)	44 (2.1)	53 (2.3)
t015	51 (2.6)	53 (2.7)	38 (1.9)	43 (2.0)	49 (2.2)
t701	35 (1.8)	45 (2.3)	37 (1.9)	51 (2.4)	40 (1.8)

Table 7. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2018. Corresponding numbers and prevalences for the four previous years are shown for comparison.

Clonal complex	2014	2015	2016	2017	2018
CC45	343 (17.5)	330 (16.7)	305 (15.4)	292 (13.9)	330 (14.5)
CC30	239 (12.2)	243 (12.3)	241 (12.2)	269 (12.8)	289 (12.7)
CC15	204 (10.4)	197 (10.0)	209 (10.6)	200 (9.5)	230 (10.1)
CC1	165 (8.4)	169 (8.6)	149 (7.5)	208 (9.9)	224 (9.8)
CC5	175 (8.9)	169 (8.6)	171 (8.6)	176 (8.4)	193 (8.5)
CC8	142 (7.2)	143 (7.2)	138 (7.0)	142 (6.7)	163 (7.2)
CC22	64 (3.3)	58 (2.9)	64 (3.2)	80 (3.8)	89 (3.9)
CC7	74 (3.8)	69 (3.5)	72 (3.6)	94 (4.5)	86 (3.8)
CC97	40 (2.0)	48 (2.4)	47 (2.4)	48 (2.3)	67 (2.9)
CC398	31 (1.6)	25 (1.3)	41 (2.1)	47 (2.2)	51 (2.4)

2.8 Antimicrobial susceptibility testing

Table 8 shows the prevalence of resistance to the antimicrobials tested. The resistance profiles are shown in Figure 5. Figure 6 shows selected resistance prevalences from 1980 to 2018.

Resistance to penicillin was 72.2% (71.9% in 2017). Resistance to fusidic acid increased to 16.7%, the highest recorded after two years of lower prevalence. All tested SAB isolates were susceptible to ceftaroline, ceftobiprole, linezolid, mupirocin, trimethoprim/sulfametoxazole and vancomycin in 2018. The proportion of isolates susceptible to all antibiotics was 21.4%. The proportion of resistance to at least one antimicrobial in addition to penicillin was 20.4% and the proportion of resistance to at least two and three additional antimicrobials were 6.9% and 2.2%, respectively.

Table 8. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2018 (n=504)

		Minimal inhibitory concentration (MIC, mg/L)										
Antimicrobial	**Resistance (%)**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
Penicillin	72.2		26.4	1.4	72.2							
Erythromycin	5.2						94.6	0.2	0	5.2		
Clindamycin	4.2§				98.2	0.6	0.4	0.8				
Fusidic acid	16.7					81.2	2.2	0.6	16.1			
Tetracycline	3.2						96.4	0.4	0.2	3.0		
Norfloxacin	4.0								96.0	2.0	2.0	
Rifampicin	0.2				99.6	0.2	0	0.2				
Linezolid	0							97.8	2.2	0		
Kanamycin	2.0										98.0	2.0
TMP/SXT*	0							99.4	0.6	0		
Ceftaroline	0					99.8	0.2	0				
Ceftobiprole	0					98.0	2.0	0	0			
Daptomycin	0.6					80.2	19.2	0.6				
Gentamicin	1.0						99.0	0.2	0.8			
Mupirocin	0					99.4	0.6	0				
Vancomycin	0						98.0	2.0	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.*

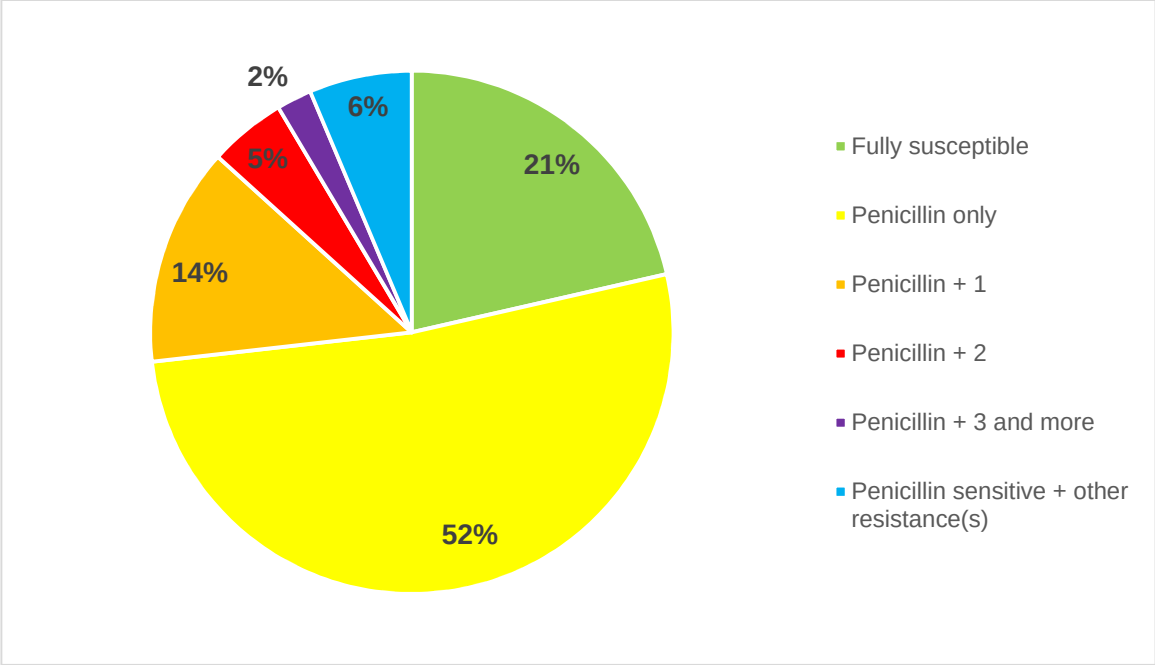

Figure 5. Resistance profiles of a subset of Danish SAB isolates 2018 (n=504) (%).

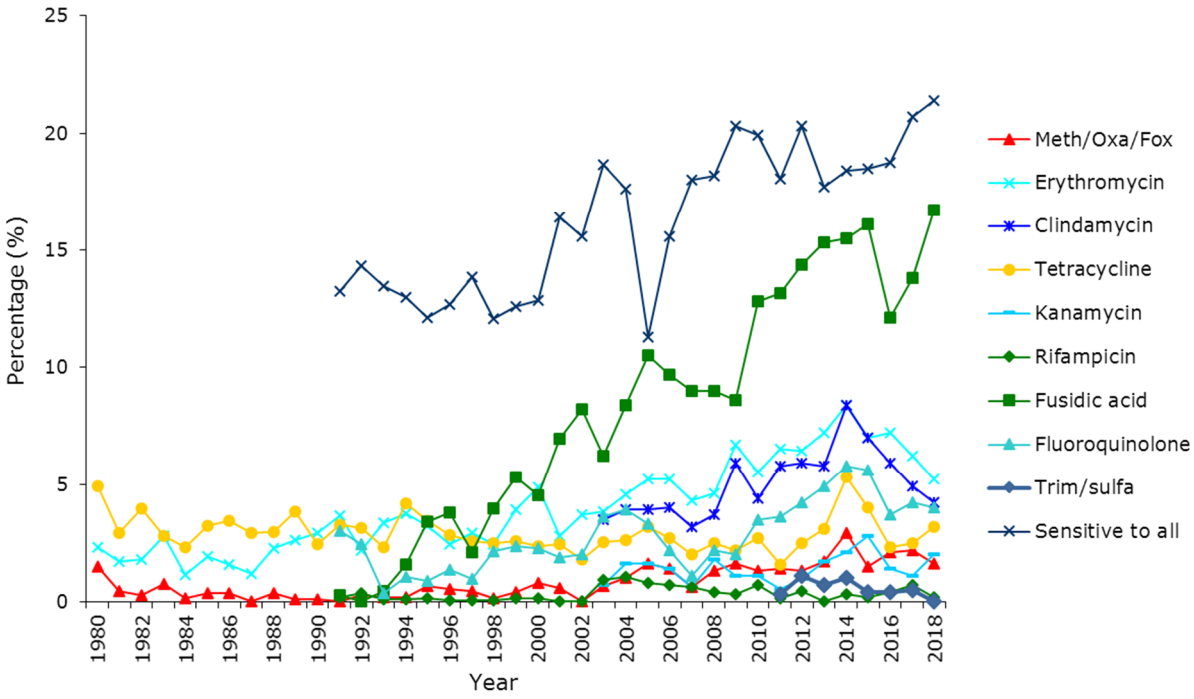

Figure 6. Prevalence of selected antimicrobial resistance in Danish SAB isolates (1980-2018). Resistance to penicillin is not shown.

3. Conclusions

The number of recorded SAB cases increased in 2018 and the long-term trends demonstrate increasing numbers and incidence. The prevalence of MRSA cases was 1.6%, lower than in the previous two years (both above 2%). Case fatality among MRSA SAB cases (18.9%) was lower but not statistically significant from MSSA SAB cases (22.5%). Almost three quarters 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.

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Appendix 1. ICD-10 codes used to extract and categorise secondary infections

CNS	Endocarditis	Osteomyelitis
DG00	DI33	DM860
DG003	DI330	DM861
DG009	DI339	DM862
DG009A	DI38	DM863
DG01	DI389	DM864
DG019	DI398	DM865
DG060	Arthritis	DM865A
DG060A	DM000	DM866
DG060B	DM000A	DM868
DG060C	DM000B	DM868A
DG060D	DM009	DM869
DG060E	DM013	DM869A
DG060F	Spondylitis	DM869B
DG061A	DM46	DM869C
DG061B	DM462	Prosthesis
DG061C	DM465	DT826
DG062	DM465A	DT826A
DG062A	DM468	DT827
DG062B	DM469	DT827A
DG062C	DM493	DT827B
DG089	DM493A	DT827I
DG089A	Tendosynovitis	DT827P
DG089B	DM650	DT828
DG089C	DM651	DT845
DG089D	DM680	DT845A
DG089E		DT846
DG089F		DT846A
DG089G		DT847
DG089H	Myositis	
DG089I	DT857	
DG089J	DM600	
DG089K	DM600A	
DG089L	DM608A1	
DG089M		
DG089N		

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