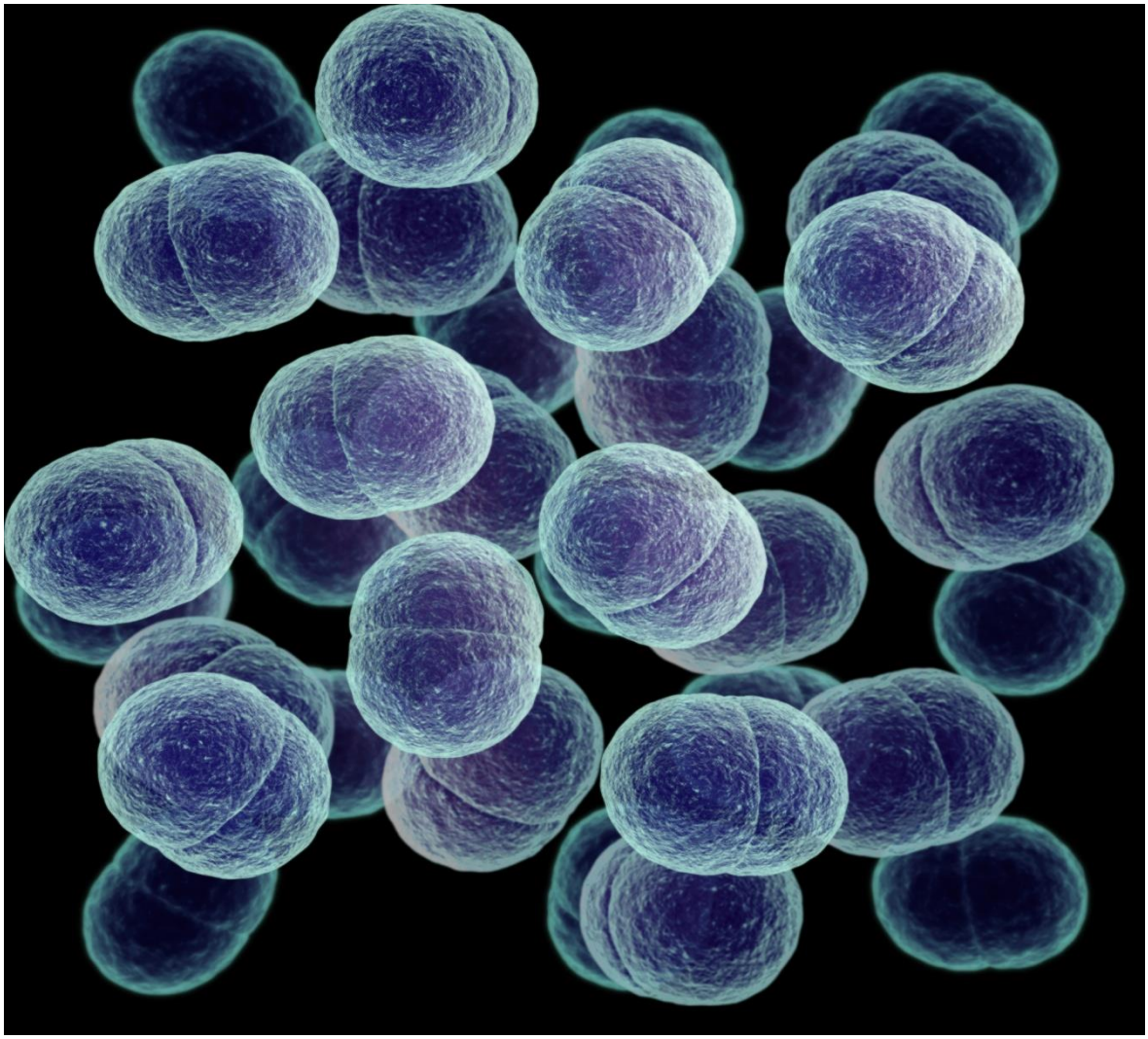
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2017



This report describes the laboratory and clinical characteristics of the 2,104 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2017. 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.

Esbjerg
Herlev
Hvidovre
Odense
Rigshospitalet (København)
Slagelse
Sønderborg
Vejle
Aalborg
Aarhus

The localization 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 and Stine Frese-Madsen are thanked for technical assistance.

Authors: Andreas Petersen and Anders R. Larsen
Publisher: National Reference Laboratory for Antimicrobial Resistance (NRL-AMR)
Responsible institution: Statens Serum Institut
Design: Statens Serum Institut

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 leukocidin
mecA: The gene encoding for methicillin resistance
mecC: Variant of the *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). The 2010 SAB report (www.ssi.dk/bakteriaemirapport2010) listed the ICD-10 codes used to identify secondary infections. 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 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 (http://saureus.mlst.net/).

1.3 Antimicrobial susceptibility testing

The Staphylococcal Laboratory performed susceptibility testing of 551 isolates (26%) 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) break-points. 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

A total of 2,104 SAB cases were recorded in 2017 (Figure 1); hereof 1,889 primary (90%) and 215 subsequent episodes. Methicillin-resistant *S. aureus* (MRSA) was identified from 47 cases (2.2%). This corresponds to an incidence rate of SAB of 36.6/100,000 inhabitants (Figure 2) and an incidence of MRSA-SAB of 0.80/100,000 inhabitants. In the first decade of the new millennium the number of cases were relatively stable while an increase has been recorded during the last 5 years (Figure 1). There was an excess of men (64% vs. 36% women) among the cases of SAB in 2017. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

Figure 1. Number of SAB cases 1960-2017.

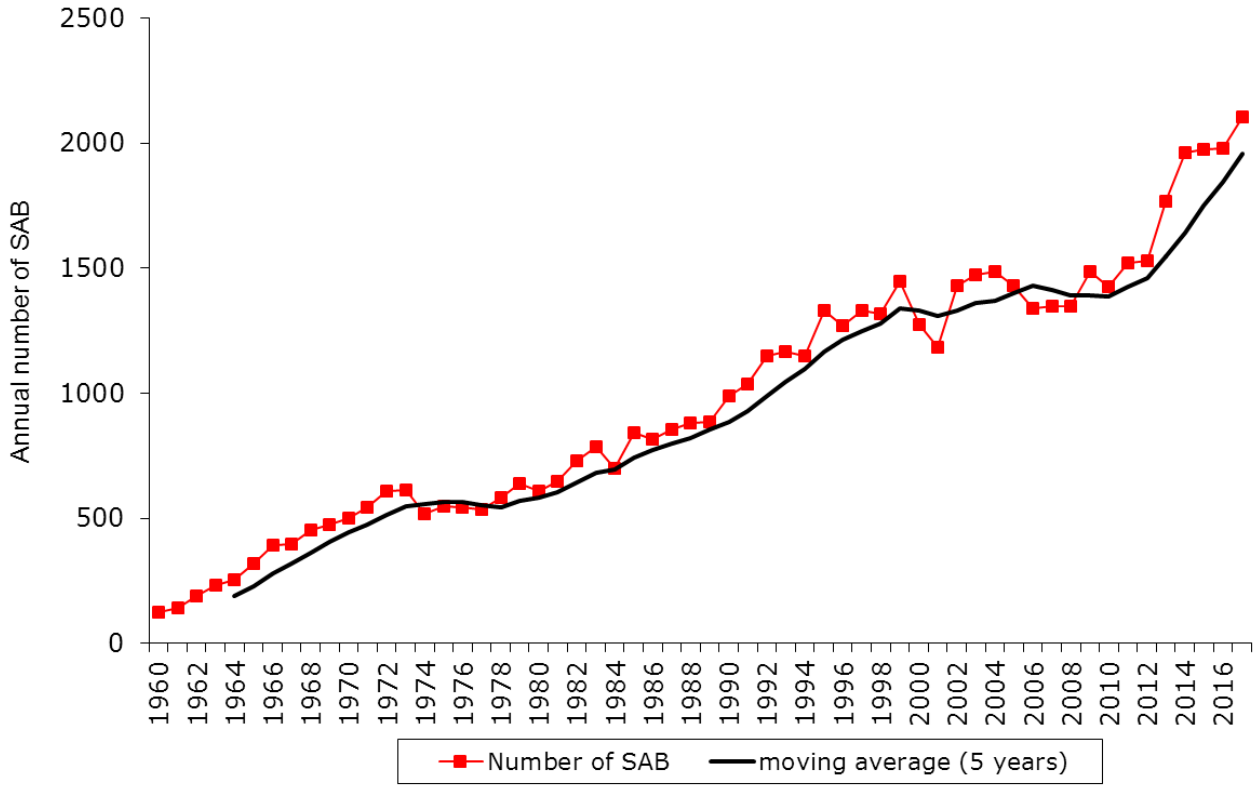
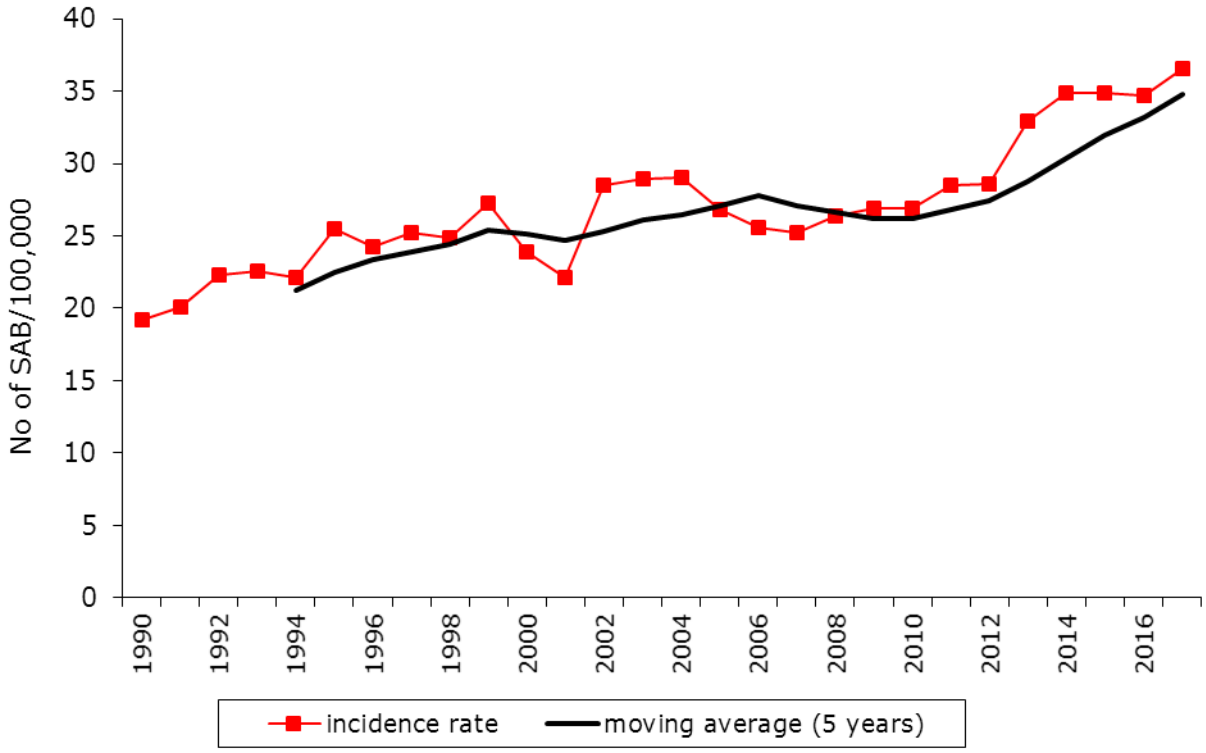


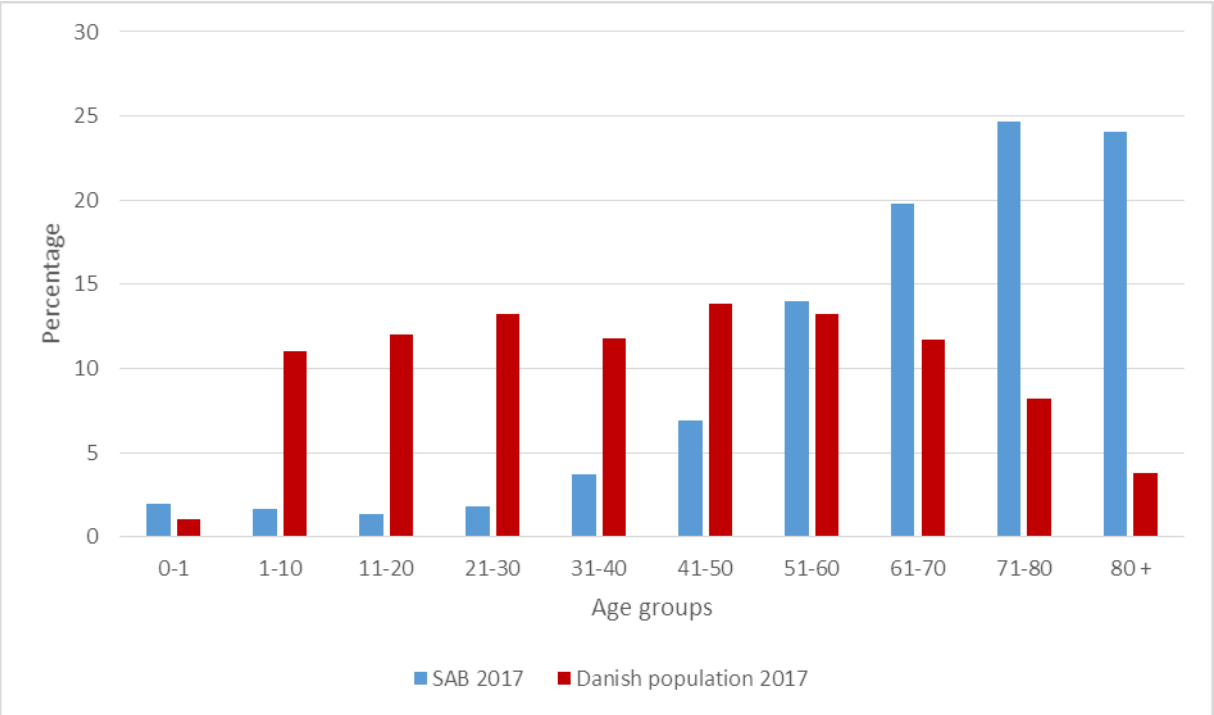
Figure 2. Incidence rate of SAB per 100,000 inhabitants during 1990-2017.



2.2 Age

SAB primarily affected older people and more than 80% of the SAB patients in 2017 were older than 50 years and 24% were older than 80 years (Figure 3). In 2017 the general Danish population only included 3.8% persons older than 80 years and the incidence of SAB among people above 80 years of age (224.1/100,000 inhabitants) was six times higher than for the rest of the population (34.7/100,000 inhabitants).

Figure 3. Age distribution of S. aureus *bacteraemia patients and the general Danish population in 2017 (%).*



2.3 Case fatality

The 30-day all-cause case fatality was 23.1% in 2017 (Table 2) and did not differ from the previous 20 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 43.7% (Table 2), almost twice as high as the average. There was no difference in 30-day all-cause case fatality between men and women.

Table 2. Case fatality among Danish SAB patients in 2017 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	42	35	29	38	78	146	294	417	519	506	2104
No. case fatality	3	1	0	0	7	15	40	67	132	221	486
% case fatality	7.1	2.9	0	0	9.0	10.3	13.6	16.1	25.4	43.7	23.1

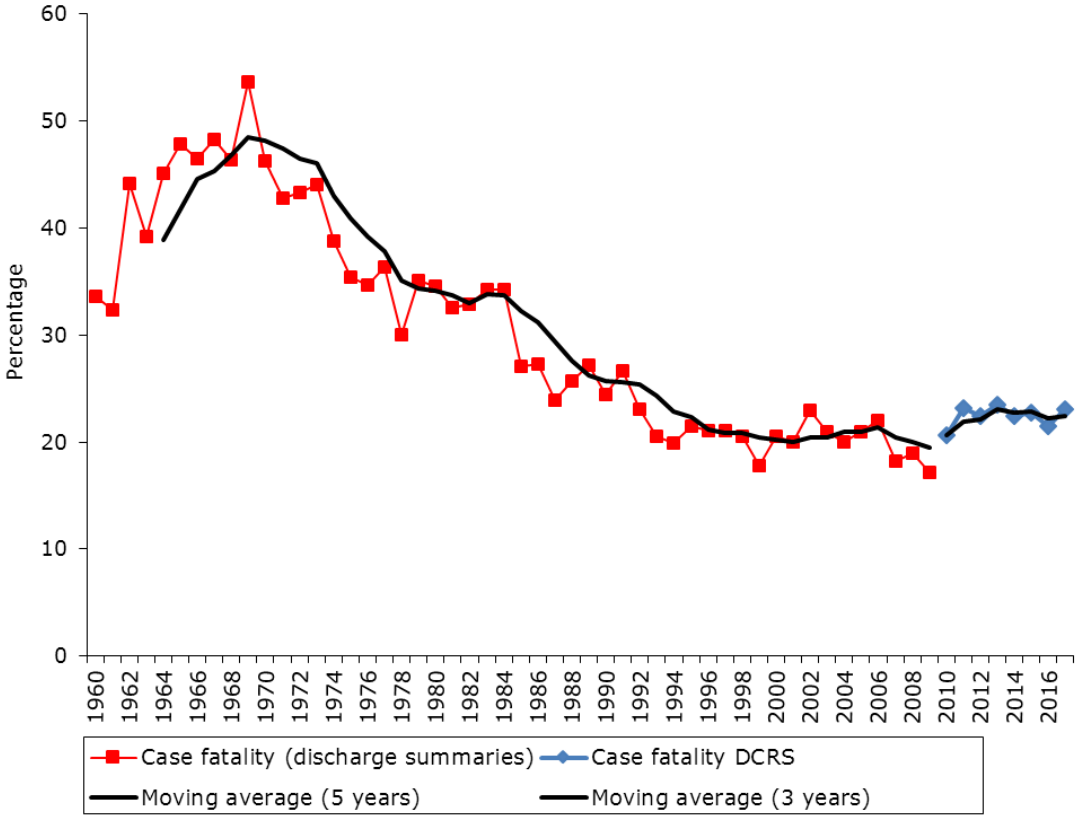


Figure 4. 30-day all-cause case fatality (%) of Danish SAB patients 1960-2017. Until 2009, data was extracted from discharge notes. From 2010 and onwards 30-day all-cause case fatality was extracted from the Danish Civil Registration System (DCRS).

Thirty-day case fatality among MRSA-cases was almost the same as the MSSA-case fatality (19.6% vs. 23.2%, p=0.72, Fischer's exact test). The distribution of the most prevalent *spa* types and CC groups among the 486 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.

2.4 Acquisition

Based on data from NPR, 543 cases (25.8%) had an onset of infection two or more days after admission to a hospital (HA). The corresponding percentage was 24.5% in 2016 and has been steadily decreasing since 2002 (from 41%). Assignment of acquisition to health-care related cases with a community onset was not possible with data from NPR. This category constituted an increasing part of the cases up until 2009.

2.5 Secondary infections

During admission, 226 cases (10.7%) had a secondary infection registered and after three months, the number was 542 cases, corresponding to 25.8%. Table 3 demonstrates the secondary infections observed during admission and three months after SAB onset. Endocarditis was the most prevalent secondary infection, followed by prosthetic infection, spondylitis, and arthritis (Table 3). Myositis and abdominal abscesses were all registered in less than 1%. Table 4 shows a comparison of secondary infections for the years 2012 to 2017. No major changes has been observed in the period.

Table 3. Secondary infections (%) among Danish SAB patients in 2017, recorded during admission and 3 months after.

	CNS	Endocarditis	Arthritis	Spondylitis	Osteomyelitis	Prosthetic infection
During admission	0.6	3.3	2.1	1.4	0.9	2.3
3 months follow-up	1.6	10.6	4.3	4.9	2.1	5.1

Table 4. The most common secondary infections (%) among Danish SAB patients in 2012-2017, recorded 3 months after admission.

Secondary infection	2012	2013	2014	2015	2016	2017
Endocarditis	10.1	10.9	10.0	11.5	11.4	10.6
Spondylitis	5.6	5.8	5.3	5.6	5.5	4.9
Prosthetic infection	5.1	4.1	4.4	3.8	3.7	5.1
Arthritis	3.3	3.1	2.7	3.5	3.4	4.3
Osteomyelitis	2.9	2.6	3.3	2.4	2.3	2.1
CNS	2.4	2.3	2.3	1.8	1.8	1.6

2.6 Comorbidities

560 cases (27%) had no comorbidities registered, while 741 cases (35%) had a comorbidity index score of 1-2, and 803 cases (38%) had a score of more than 2. Table 5 presents comorbidity based on the Charlson index. Diabetes without chronic complication (26.5%), malignancy (24.9%), and congestive heart failure and renal disease (both 21.1%) were the most frequently registered comorbidities among SAB patients in 2017.

Table 5. Number and percentage of comorbidities among Danish SAB patients 2017, with percentages for 2014 - 2016 for comparison.

Comorbidity	2017 Number	2014 %	2015 %	2016 %	2017 %
AIDS/HIV	14	0.2	0.3	0.6	0.7
Any malignancy	523	25.6	24.8	24.5	24.9
Metastatic solid tumor	106	5.3	5.6	5.4	5.0
Diabetes without chronic complication	541	24.5	24.8	24.5	26.5
Diabetes with chronic complication	314	13.9	13.7	13.7	14.9
Dementia	95	4.4	4.6	4.3	4.5
Hemiplegia or paraplegia	45	1.8	1.6	1.0	2.1
Cerebrovascular disease	416	19.1	17.0	18.0	19.8
Myocardial infarction	204	12.3	10.2	8.6	9.7
Congestive heart failure	444	19.7	18.0	17.6	21.1
Chronic pulmonary disease	423	18.4	18.5	17.2	20.1
Peptic ulcer disease	167	7.5	7.3	7.6	7.9
Mild liver disease	239	9.3	8.4	9.6	11.4
Moderate or severe liver disease	118	5.2	3.1	4.6	5.6
Renal disease	443	19.6	20.0	17.4	21.1
Rheumatic disease	130	5.8	4.7	5.9	6.2
Peripheral vascular disease	367	16.9	16.5	16.8	17.4
Drug abuse	70	3.1	2.7	2.9	3.3

2.7 Typing

spa types were obtained for 2,093 isolates (99.5%). In total, 623 different *spa* types were identified, with ten *spa* types accounting for 33% of the isolates (Table 6). The same ten *spa* types were the most prevalent in 2016 but with some differences in ranking. A total of 429 *spa* types were only encountered once. Putative assignment to MLST CC was possible for 1,757 isolates (84%). 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 28 MLST CC groups were assigned. The three most prevalent CC groups constituted 37% of the

SAB isolates in 2017 while the 10 most prevalent constituted 74% (Table 7). Twenty-six SAB isolates were *pvl* positive (1.2%), of which seven were MRSA (*spa* types two t019/CC30, two t044/CC80, one t005/CC22, one t657/CC97 and one not typeable). The *pvl* positive isolates were distributed among 19 different *spa* types (one isolate could not be typed, see above) and eleven MLST CC groups; five 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 47 SAB cases (2.2%) in 2017 of which 4 were MRSA. Fourteen belonged to *spa* type t571, eleven to *spa* type t1451, ten to t034 1451 and the remaining belonged to five other *spa* types. Three of the SAB CC398 MRSA had contact to livestock; two patients had family members working with pigs and one patient had contact to horses. None of the SAB CC398 MRSA patient died within 30 days of diagnosis. Since 2007, seven 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 2017. Corresponding numbers and prevalences for the four previous years are shown for comparison.

spa type	2013	2014	2015	2016	2017
t127	81 (4.6)	103 (5.2)	96 (4.9)	81 (4.1)	128 (6.1)
t091	41 (2.3)	63 (3.2)	60 (3.0)	62 (3.1)	85 (4.0)
t002	73 (4.1)	88 (4.5)	84 (4.3)	87 (4.4)	81 (3.8)
t084	73 (4.1)	66 (3.4)	89 (4.5)	83 (4.2)	75 (3.6)
t230	93 (5.3)	100 (5.1)	82 (4.2)	81 (4.1)	70 (3.3)
t012	52 (2.9)	66 (3.4)	57 (2.9)	62 (3.1)	65 (3.1)
t021	43 (2.4)	31 (1.6)	46 (2.3)	39 (2.0)	52 (2.5)
t701	28 (1.6)	35 (1.8)	45 (2.3)	37 (1.9)	51 (2.4)
t008	41 (2.3)	46 (2.3)	36 (1.8)	55 (2.8)	44 (2.1)
t015	53 (3.0)	51 (2.6)	53 (2.7)	38 (1.9)	43 (2.0)

Table 7. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2017. Corresponding numbers and prevalences for the four previous years are shown for comparison.

Clonal complex	2013	2014	2015	2016	2017
CC45	294 (16.6)	343 (17.5)	330 (16.7)	305 (15.4)	292 (13.9)
CC30	245 (13.8)	239 (12.2)	243 (12.3)	241 (12.2)	269 (12.8)
CC1	132 (7.5)	165 (8.4)	169 (8.6)	149 (7.5)	208 (9.9)
CC15	175 (9.9)	204 (10.4)	197 (10.0)	209 (10.6)	200 (9.5)
CC5	134 (7.6)	175 (8.9)	169 (8.6)	171 (8.6)	176 (8.4)
CC8	119 (6.7)	142 (7.2)	143 (7.2)	138 (7.0)	142 (6.7)
CC7	51 (2.9)	74 (3.8)	69 (3.5)	72 (3.6)	94 (4.5)
CC22	43 (2.4)	64 (3.3)	58 (2.9)	64 (3.2)	80 (3.8)
CC97	28 (1.8)	40 (2.0)	48 (2.4)	47 (2.4)	48 (2.3)
CC398	21 (1.2)	31 (1.6)	25 (1.3)	41 (2.1)	47 (2.2)

2.8 Antimicrobial susceptibility testing

Table 8 demonstrates 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 2017.

Resistance to penicillin decreased to 71.9% (75.4% in 2016) which is at the same level as in 2015 (71.1%) and lower compared to the last decade. Resistance to fusidic acid increased to 13.8%, but was lower compared to the years 2012-2015. All tested SAB isolates were susceptible to ceftaroline, ceftobiprole, linezolid and vancomycin in 2017. The proportion of isolates susceptible to all antibiotics was 20.7%. The proportion of resistance to at least one antimicrobial in addition to penicillin was 17.6% and the proportion of resistance to at least two and three additional antimicrobials were 5.8% and 2.2%, respectively.

Table 8. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2017 (n=551)

		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	71.9		26.7	1.5	71.9							
Erythromycin	6.2						93.8	0	0	6.2		
Clindamycin	4.9§				99.3	0	0	0.7				
Fusidic acid	13.8					84.6	1.6	0.7	13.0			
Tetracycline	2.5						97.5	0.7	0	1.8		
Norfloxacin	4.2								95.8	0.7	3.4	
Rifampicin	0.7				99.3	0.2	0	0.5				
Linezolid	0							97.5	2.5	0		
Kanamycin	1.1										98.9	1.1
TMP/SXT*	0.5							99.5	0.4	0	0.2	
Ceftaroline	0					99.1	0.9	0				
Ceftobiprole	0					98.5	1.5	0	0			
Daptomycin	0.9					86.2	12.9	0.9				
Gentamicin	1.1						98.9	0.7	0.4			
Mupirocin	0.2					99.8	0	0	0.2			
Vancomycin	0						98.7	1.3	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 2017 (n=551) (%).

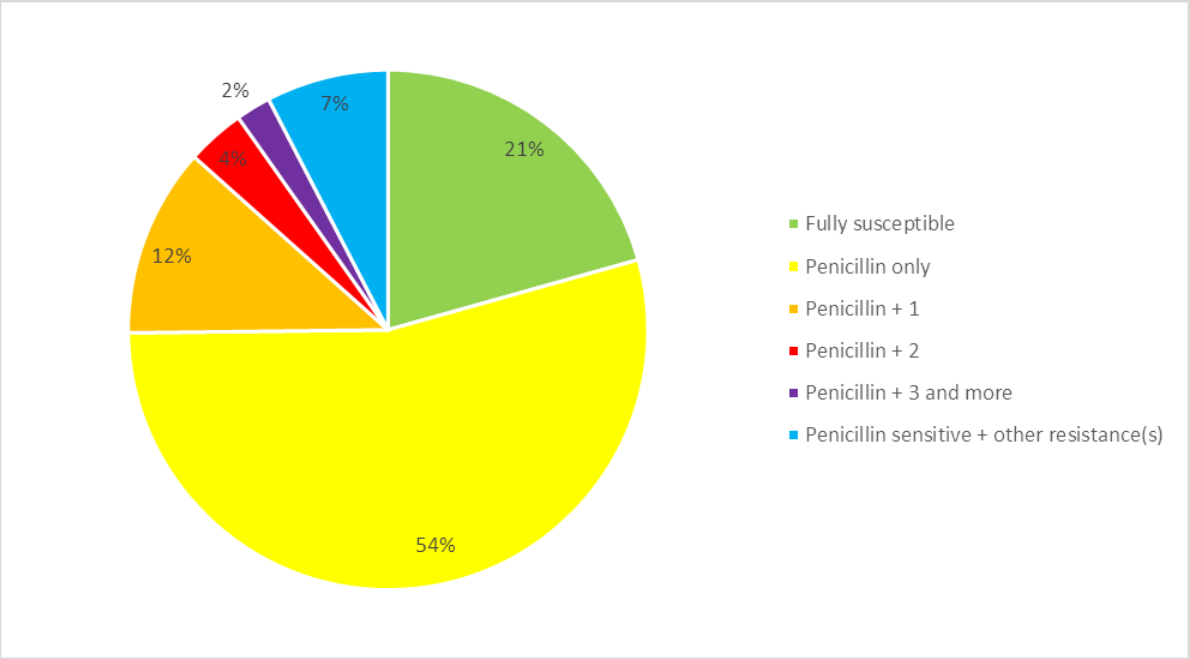
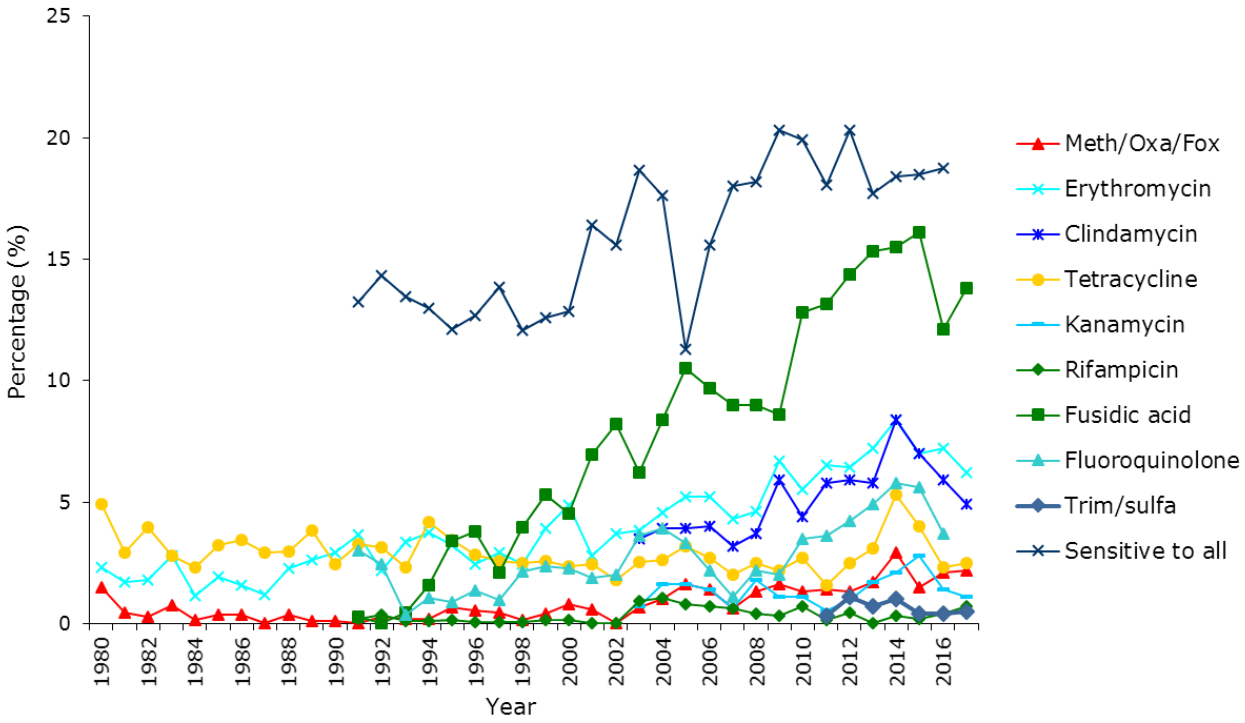


Figure 6. Prevalence of selected antimicrobial resistance in Danish SAB isolates (1980-2017). Resistance to penicillin is not shown.



3. Conclusions

The number of recorded SAB cases increased in 2017 after three years of stagnation. The long-term trends demonstrates increasing numbers and incidence. The prevalence of MRSA cases were similar to 2016 but still above 2%. Case fatality among MRSA SAB cases (19.6%) was lower but not statistically significant from MSSA SAB cases (23.2%). 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.

4. References

Charlson ME, Pompei P, Ales KL, MacKenzie CR 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 40(5):373-83.

Elixhauser A, Steiner C, Harris DR, Coffey RM 1998. Comorbidity measures for use with administrative data. Med Care. 36(1):8-27.

Lynge E, Sandegaard JL, Rebolj M. 2011. The Danish National Patient Register. Scand J Public Health. 39(7 Suppl):30-3.

Pedersen CB, Gøtzsche H, Møller JØ Mortensen PB 2006. The Danish Civil Registration System. Dan Med Bull 53:441-9

Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, Januel JM, Sundararajan V 2011. Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol. 173(6):676-82

Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi JC, Saunders LD, Beck CA, Feasby TE, Ghali WA. 2005. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. Med Care. 43(11):1130-9.

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. 2012. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mec*A or the new *mec*A homologue *mec*A(LGA251). Clin Microbiol Infect. 18 (4):395-400

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