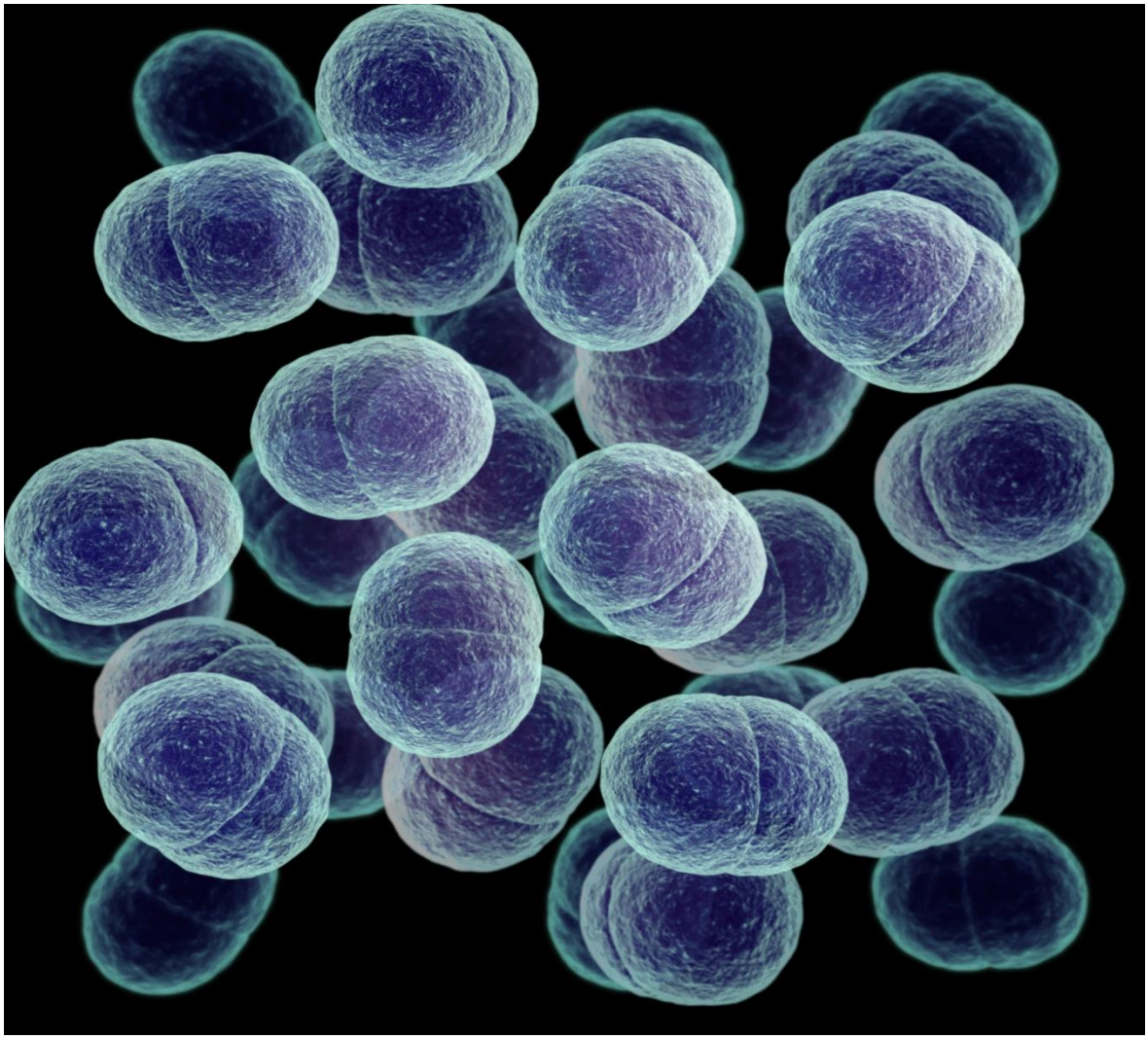
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2016



This report describes the laboratory and clinical characteristics of the 1,981 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2016. 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
Viborg
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, Ditte Marie Brix and Stine Frese-Madsen are thanked for technical assistance.

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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 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)). SAB per participating DCM is presented in order to demonstrate geographic differences. 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://sau-reus.mlst.net/).

1.3 Antimicrobial susceptibility testing

The Staphylococcal Laboratory performed susceptibility testing of 560 isolates (~28%) 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. For ceftobiprole no breakpoints were available. *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 1,981 SAB cases were recorded in 2016 (Figure 1); hereof 1,807 primary (91%) and 174 subsequent episodes. MRSA was identified from 41 cases (2.1%). This corresponds to an incidence rate of SAB of 34.7/100,000 inhabitants/year (Figure 2) and an incidence of MRSA-SAB of 0.72/100,000 inhabitants/year. The number of bacteraemia was almost identical to the numbers in 2014 and 2015 (Figure 1). There was an excess of men (62% vs. 38% women) among the cases of SAB in 2016. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

Figure 1. Number of SAB cases 1960-2016.

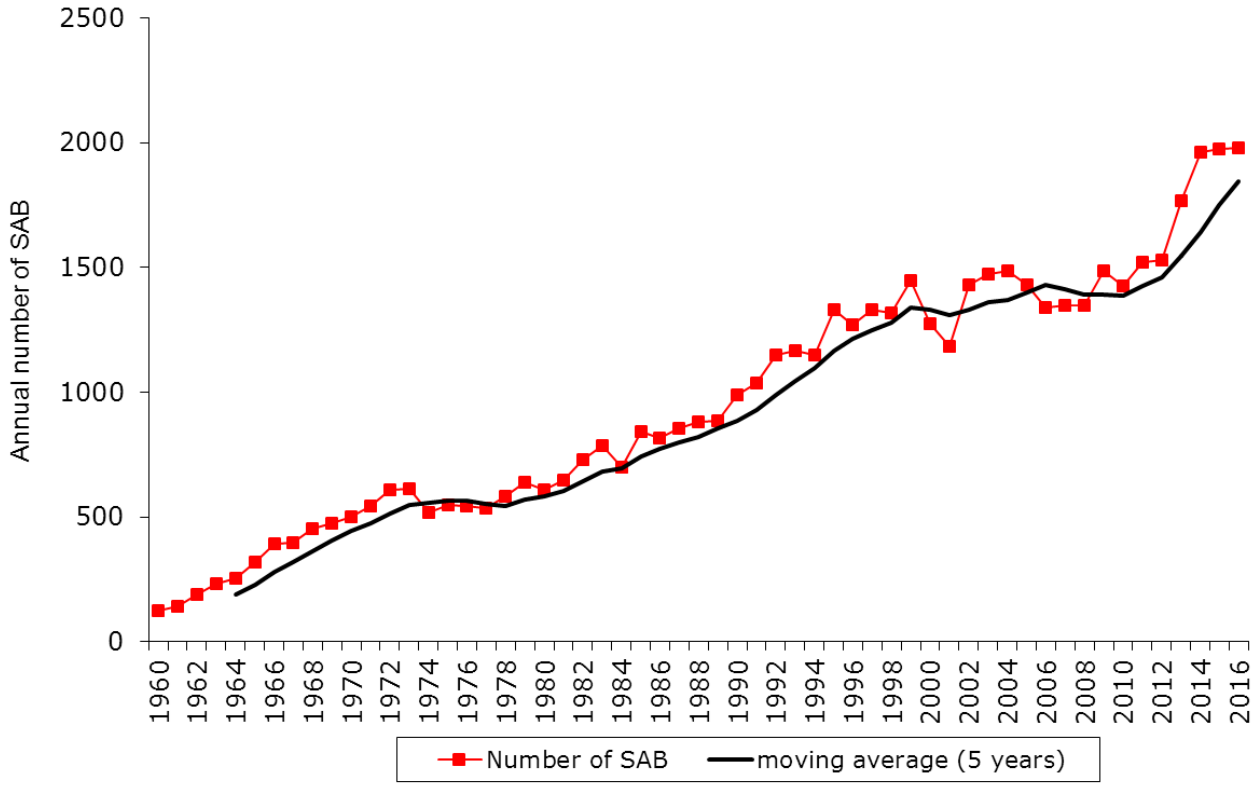
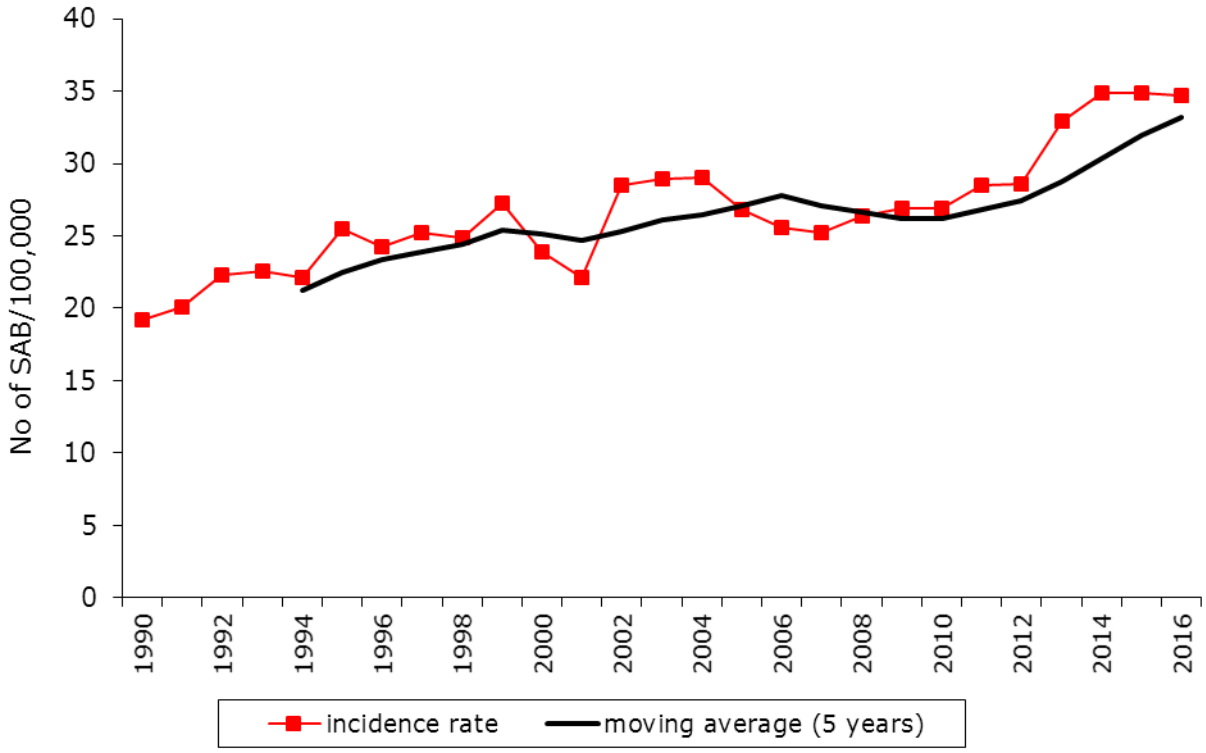


Figure 2. Incidence rate of SAB per 100,000 inhabitants during 1990-2016.



2.2 Geographic variation

The incidence of SAB varied from 23 to 43 cases/100,000 in the catchment population for the individual DCMs (Table 2). Esbjerg DCM demonstrated the highest incidence. The merger of several DCMs during the period hampers comparisons to previous years. See the notes for Table 2 for more details.

Table 2. Number of SAB cases in 2016 and SAB/100,000 inhabitants in each geographic area covered by the Departments of Clinical Microbiology (DCM) 2012-2016.

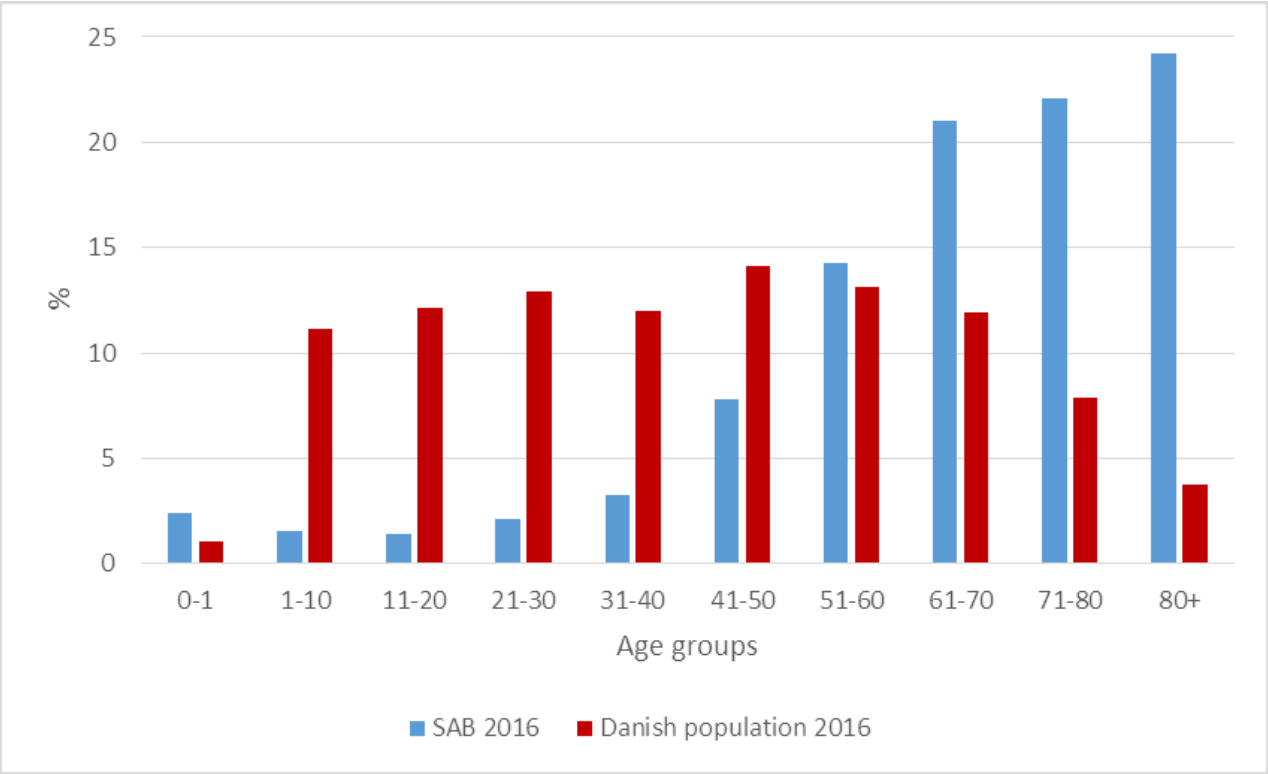
	Number of SAB	SAB/100,000 inhabitants				
DCM	2016	2012	2013	2014	2015	2016
Aalborg	199	34	31	38	38	38
Aarhus	370	28	30	33	31	30
Vejle	101	16	19	21	22	23
Esbjerg	85	29	34	28	33	43
Sønderborg	60	<1	<1	11	16	26
Odense	192	27	39	46	41	39
Slagelse	302	33	37	38	36	36
Greater Copenhagen	672	37	36	40	39	38

Notes for Table 2:
The catchment population for Slagelse changed in 2010 and from 2013 most isolates from Nykøbing F were handled by Slagelse. Likewise, Hillerød terminated their service in March 2013 and thereafter all samples were handled by Herlev. Most samples from Region Midt was handled by Skejby (Aarhus) from 2016 and onwards. The incidence rates for Aarhus, Slagelse and Greater Copenhagen have thus been calculated according to the population within the new catchment areas. Sønderborg did not participate in the years 2012 – 2013 and started to submit isolates from the second half of 2014.

2.3 Age

SAB primarily affected older people and more than 80% of the SAB patients in 2016 were older than 50 years and 24% were older than 80 years (Figure 3). In 2016 the general Danish population only included 3.7% persons older than 80 years and the incidence of SAB among people above 80 years of age (224.1/100,000 inhabitants/year) was six times higher than for the rest of the population (34.7/100,000 inhabitants/year).

Figure 3. Age distribution of S. aureus *bacteraemia patients and the general Danish population in 2016 (%).*



2.4 Case fatality

The 30-day all-cause case fatality was 21.5% in 2016 (Table 3) and did not differ from the previous 20 years (Figure 4). Case fatality was low between 1-40 years, increased from the age group of 41-50 years, and patients above 80 years had a case fatality rate of 38.4% (Table 3), almost twice as high as the average.

Table 3. Case fatality among Danish SAB patients in 2016 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	30	28	42	64	154	282	417	438	479	1981
No. case fatality	0	1	0	2	0	13	39	81	105	184	425
% case fatality	0	3.3	0	4.8	0	8.4	13.9	19.4	24.0	38.4	21.5

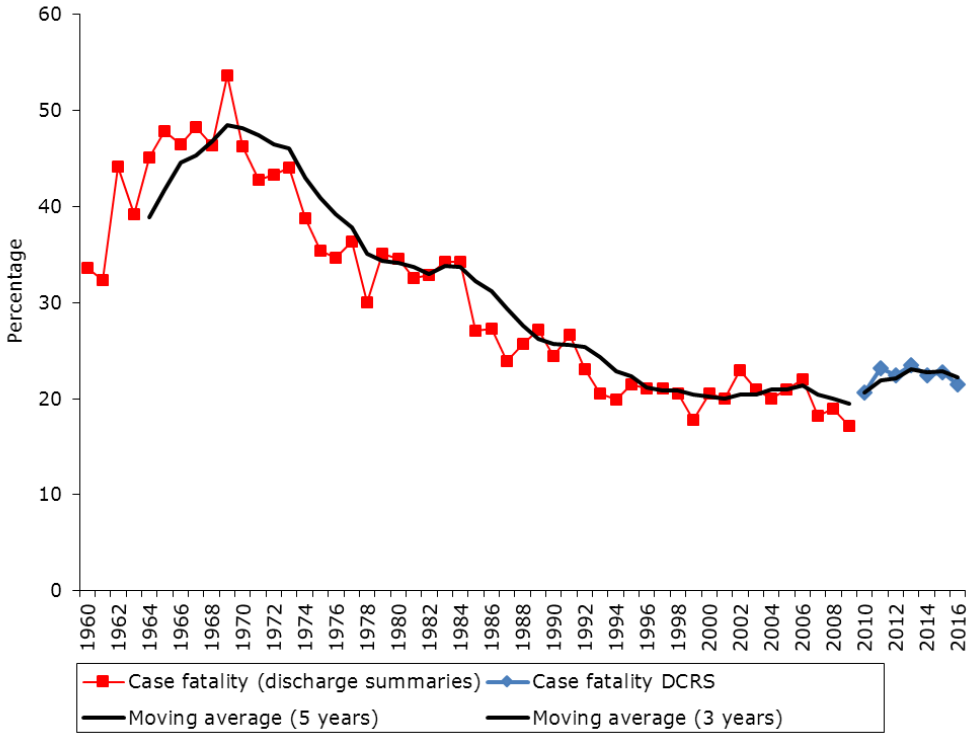


Figure 4. 30-day all-cause case fatality (number and %) of Danish SAB patients 1960-2016. Until 2009, data was extracted from discharge notes. From 2010 and onwards 30-day all-cause case fatality was extracted from the Danish Civil Registration System (DCRS).

Thirty-day case fatality among MRSA-cases was lower but not significantly different from the MSSA-case fatality (12.2% vs. 21.6%, p=0.18, Fischer's exact test). The distribution of the most prevalent *spa* types and CC groups among the 425 isolates from cases dying within 30 days did not differ from the overall distribution of *spa* types. No significant variation from the overall case fatality could be established for individual *spa* types and CC groups. Thus, the outcome of SAB did not seem to depend on the specific type of *S. aureus* causing infection.

2.5 Acquisition

Based on data from NPR, 486 cases (24.5%) had an onset of infection two or more days after admission to a hospital (HA). The corresponding percentage was 25.3% in 2015 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.

2.6 Secondary infections

During admission, 239 cases (12.1%) had a secondary infection registered and after three months, the number was 489 cases, corresponding to 24.7%. Table 4 demonstrates the secondary infections observed during admission and three months after SAB onset. Endocarditis was the most prevalent secondary infection, followed by spondylitis, prosthetic infection and arthritis (Table 4). Tenosynovitis, myositis, and abdominal abscesses were all registered in less than 1%. Table 5 shows a comparison of secondary infections for the years 2011 to 2016. No major changes has been observed in the period.

Table 4. Secondary infections (%) among Danish SAB patients in 2016, recorded during admission and 3 months after.

	CNS	Endocarditis	Arthritis	Spondylitis	Osteomyelitis	Prosthetic infection
During admission	0.7	5.5	2.0	1.4	1.2	1.9
3 months follow-up	1.8	11.4	3.4	5.5	2.3	3.7

Table 5. The most common secondary infections (%) among Danish SAB patients in 2011-2016, recorded 3 months after admission.

Secondary infection	2011	2012	2013	2014	2015	2016
Endocarditis	11.4	10.1	10.9	10.0	11.5	11.4
Spondylitis	5.3	5.6	5.8	5.3	5.6	5.5
Prosthetic infection	4.1	5.1	4.1	4.4	3.8	3.7
Arthritis	3.5	3.3	3.1	2.7	3.5	3.4
Osteomyelitis	2.4	2.9	2.6	3.3	2.4	2.3
CNS	2.9	2.4	2.3	2.3	1.8	1.8

2.7 Comorbidities

598 cases (30%) had no comorbidities registered, while 709 cases (36%) had a comorbidity index score of 1-2, and 674 cases (34%) had a score of more than 2. Table 6 presents comorbidity based on the Charlson index. Malignancy (24.5%), diabetes without chronic complication (24.5%), and cerebrovascular disease (18.0%) were the most frequently registered comorbidities among SAB patients in 2016.

Table 6. Number and percentage of comorbidities among SAB patients 2016, with percentages for 2013 - 2016 for comparison.

Comorbidity	2016 Number	2013 %	2014 %	2015 %	2016 %
AIDS/HIV	12	0.4	0.2	0.3	0.6
Any malignancy	486	26.8	25.6	24.8	24.5
Metastatic solid tumor	106	5.7	5.3	5.6	5.4
Diabetes without chronic complication	485	25.9	24.5	24.8	24.5
Diabetes with chronic complication	272	14.8	13.9	13.7	13.7
Dementia	86	4.9	4.4	4.6	4.3
Hemiplegia or paraplegia	20	1.4	1.8	1.6	1.0
Cerebrovascular disease	357	20.1	19.1	17.0	18.0
Myocardial infarction	171	10.5	12.3	10.2	8.6
Congestive heart failure	348	18.0	19.7	18.0	17.6
Chronic pulmonary disease	340	16.9	18.4	18.5	17.2
Peptic ulcer disease	150	8.3	7.5	7.3	7.6
Mild liver disease	191	9.2	9.3	8.4	9.6
Moderate or severe liver disease	92	4.7	5.2	3.1	4.6
Renal disease	344	20.1	19.6	20.0	17.4
Rheumatic disease	116	5.6	5.8	4.7	5.9
Peripheral vascular disease	332	15.9	16.9	16.5	16.8
Drug abuse	57	2.4	3.1	2.7	2.9

2.8 Typing

spa types were obtained for 1,957 isolates (98.8%). In total, 610 different *spa* types were identified, with ten *spa* types accounting for 32% of the isolates (Table 7). The same ten *spa* types were the most prevalent in 2015 but with some differences in ranking. A total of 423 *spa* types were only encountered once. Putative assignment to MLST CC was possible for 1,614 isolates (81%). 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 38% of the

SAB isolates in 2016 while the 10 most prevalent constituted 73% (Table 8). Twenty-three SAB isolates were *pvl* positive (1.2%), of which six were methicillin-resistant *S. aureus* (MRSA; *spa* types three t019/CC30 and one of each t044/CC80, t437/CC59, and t1028/CC80). The *pvl* positive isolates were distributed among 17 different *spa* types (one isolate could not be typed) and seven MLST CC groups; seven isolates had an unresolved relationship with MLST typing.

2.8.1 CC398

CC398 MRSA isolates have been associated with a reservoir in livestock. CC398 constituted 41 SAB cases (2.1%) in 2016 of which 7 were MRSA. Twelve belonged to *spa* type t034 (hereof four MRSA), nine belonged to *spa* type t571, nine to t1451 and the remaining belonged to seven other *spa* types. None of the seven SAB CC398 MRSA cases had direct or indirect contact to livestock. A single SAB CC398 MRSA patient died within 30 days of diagnosis. Since 2007, 7 SAB patients with CC398 MRSA died within 30 days.

Table 7. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2016. Corresponding numbers and prevalences for the four previous years are shown for comparison.

spa type	2012	2013	2014	2015	2016
t002	59 (3.9)	73 (4.1)	88 (4.5)	84 (4.3)	87 (4.4)
t084	62 (4.1)	73 (4.1)	66 (3.4)	89 (4.5)	83 (4.2)
t127	68 (4.5)	81 (4.6)	103 (5.2)	96 (4.9)	81 (4.1)
t230	90 (5.9)	93 (5.3)	100 (5.1)	82 (4.2)	81 (4.1)
t091	40 (2.6)	41 (2.3)	63 (3.2)	60 (3.0)	62 (3.1)
t012	45 (2.9)	52 (2.9)	66 (3.4)	57 (2.9)	62 (3.1)
t008	42 (2.7)	41 (2.3)	46 (2.3)	36 (1.8)	55 (2.8)
t021	41 (2.7)	43 (2.4)	31 (1.6)	46 (2.3)	39 (2.0)
t015	46 (3.0)	53 (3.0)	51 (2.6)	53 (2.7)	38 (1.9)
t701	25 (1.6)	28 (1.6)	35 (1.8)	45 (2.3)	37 (1.9)

Table 8. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2015. Corresponding numbers and prevalences for the four previous years are shown for comparison.

Clonal complex	2012	2013	2014	2015	2016
CC45	269 (17.6)	294 (16.6)	343 (17.5)	330 (16.7)	305 (15.4)
CC30	214 (14.0)	245 (13.8)	239 (12.2)	243 (12.3)	241 (12.2)
CC15	150 (9.8)	175 (9.9)	204 (10.4)	197 (10.0)	209 (10.6)
CC5	111 (7.3)	134 (7.6)	175 (8.9)	169 (8.6)	171 (8.6)
CC1	102 (6.7)	132 (7.5)	165 (8.4)	169 (8.6)	149 (7.5)
CC8	99 (6.5)	119 (6.7)	142 (7.2)	143 (7.2)	138 (7.0)
CC7	44 (2.9)	51 (2.9)	74 (3.8)	69 (3.5)	72 (3.6)
CC22	50 (3.3)	43 (2.4)	64 (3.3)	58 (2.9)	64 (3.2)
CC97	28 (1.8)	28 (1.8)	40 (2.0)	48 (2.4)	47 (2.4)
CC398	12 (0.8)	21 (1.2)	31 (1.6)	25 (1.3)	41 (2.1)

2.9 Antimicrobial susceptibility testing

Table 9 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 2016.

Resistance to penicillin increased to 75.4% which is at the same level as during the last decade with exception from 2015 (71.1%). Resistance to fusidic acid decreased to 12.1%, the lowest in seven years and after a decade of steadily increase. All tested SAB isolates were susceptible to linezolid and mupirocin in 2016. The proportion of isolates susceptible to all antibiotics was 18.8%. The proportion of resistance to at least one antimicrobial in addition to penicillin was 21.4% and the proportion of resistance to at least two and three additional antimicrobials were 5.4% and 1.8%, respectively.

Table 9. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2016 (n=560)

		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	75.4		23.8	0.9	75.4							
Erythromycin	7.2						92.3	0.6	0.4	6.8		
Clindamycin	5.9§				98.8	0.4		0.9				
Fusidic acid	12.1					85.4	2.5	2.1	10			
Tetracycline	2.3						97.0	0.7	0	2.3		
Norfloxacin	3.7								96.3	1.6	2.1	
Rifampicin	0.4				99.5	0.2		0.4				
Linezolid	0							99.8	0.2			
Kanamycin	1.4										98.6	1.4
TMP/SXT*	0.4							99.3	0.4	0.2	0.2	
Ceftaroline	0					98.6	1.4					
Ceftobiprole	NA					98.0	2.0					
Daptomycin	4.6					78.6	16.8	4.6				
Gentamicin	0.6						99.5	0.4	0.2			
Mupirocin	0					99.5	0.5					
Vancomycin	0						97.9	2.1				

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 2016 (n=560) (%).

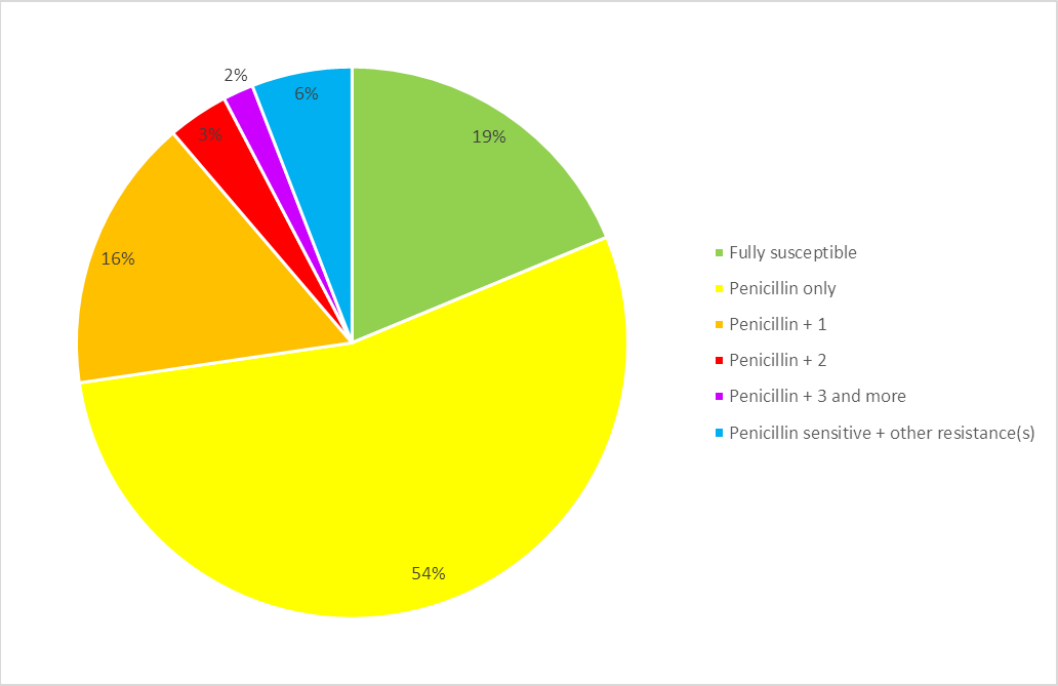
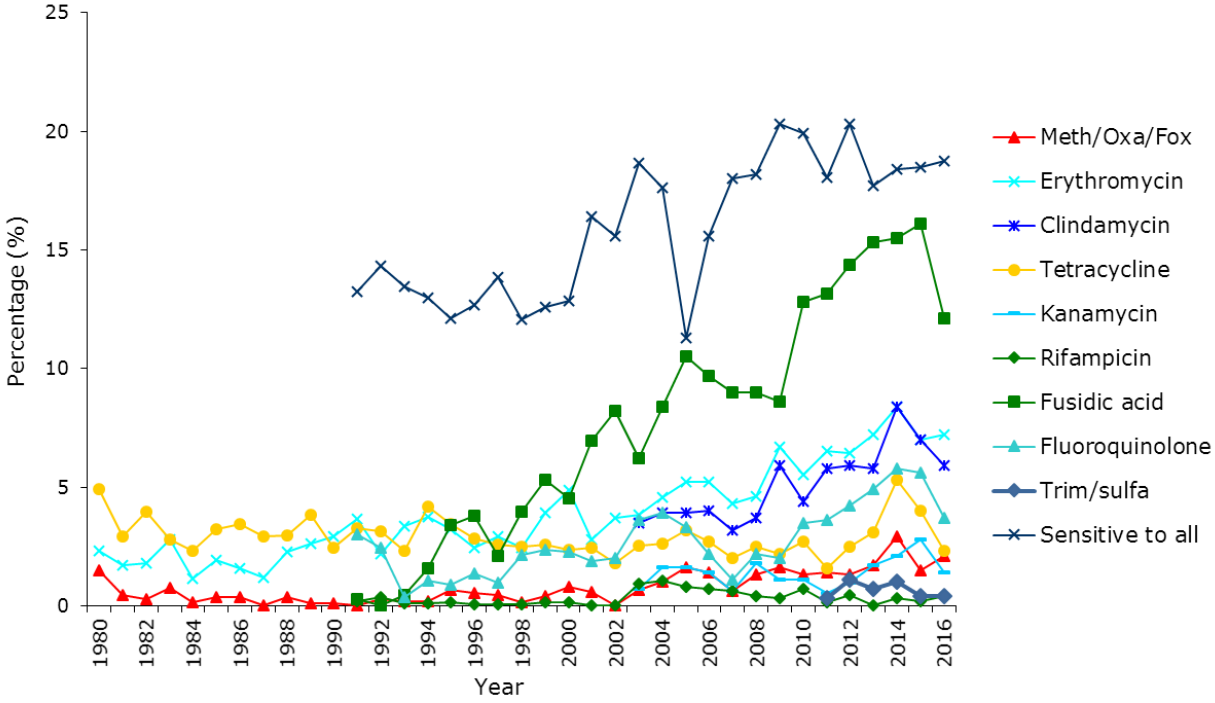


Figure 6. Prevalence of antimicrobial resistance in Danish SAB isolates (1980-2016). Resistance to penicillin is not shown.



3. Conclusions

The number of recorded SAB cases remained at the same level as in 2015 after a steep increase from 2012 to 2014. The number and ratio of MRSA cases increased compared to 2015 but were lower than the high number registered in 2014 (56 cases, 2.9%). Case fatality among MRSA SAB cases (12.2%) was lower but not statistically significant from MSSA SAB cases (21.6%). Resistance to fusidic acid decreased in 2016 to 12.1% compared to 16.1% in 2015 which partly may be explained by fewer isolates of *spa* type t127 of which most are resistant to this drug. Of importance 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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