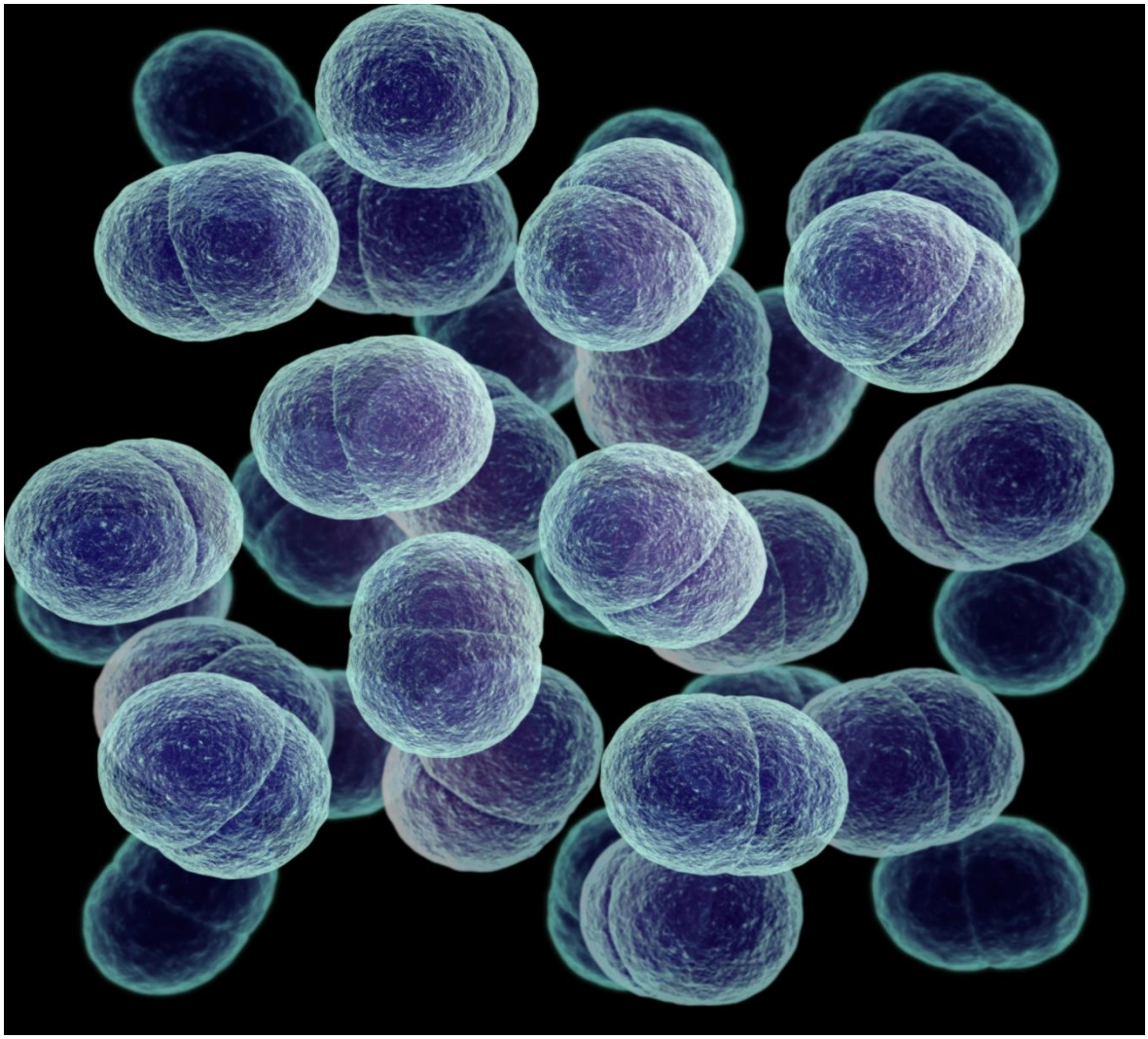
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2015



This report describes the laboratory and clinical characteristics of the 1,973 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2015. 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, 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 detects 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 on every second isolate during May – July and September – December, 2015, in total 502 isolates (~25%) 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 EUCAST breakpoints. For kanamycin and norfloxacin the breakpoints of 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 1,973 SAB cases were recorded in 2015 (Figure 1); hereof 1,782 primary and 191 subsequent episodes. MRSA was identified from 29 cases (1.5%). This corresponds to an incidence rate of SAB of 34.9/100,000 inhabitants/year and an incidence of MRSA-SAB of 0.51/100,000 inhabitants/year (Figure 2). The number of bacteraemia was almost identical to the number in 2014 (Figure 1). The increase in the number of SAB cases in the past years appears to be caused by an increase in cases among individuals older than 80 years. There was an excess of men (63% vs. 37% women) among the cases of SAB in 2015. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

Figure 1. Number of SAB cases 1960-2015.

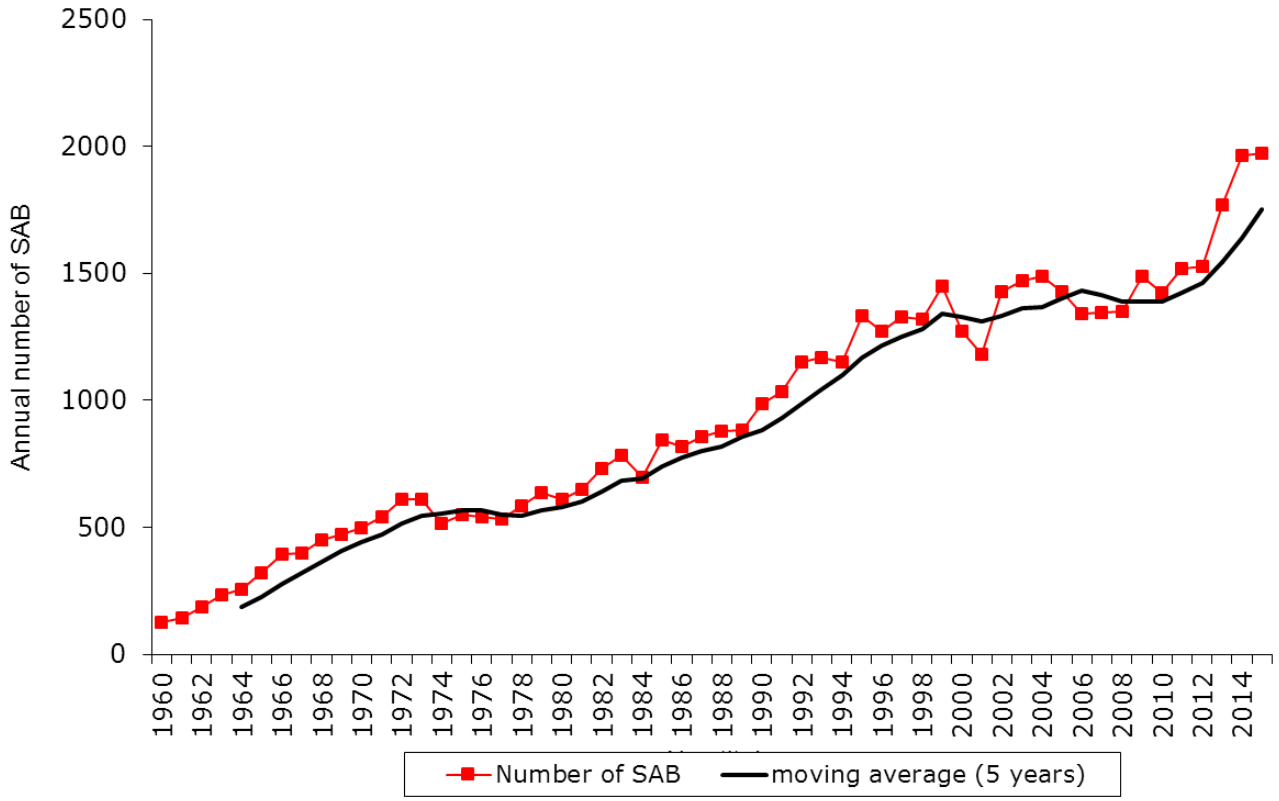
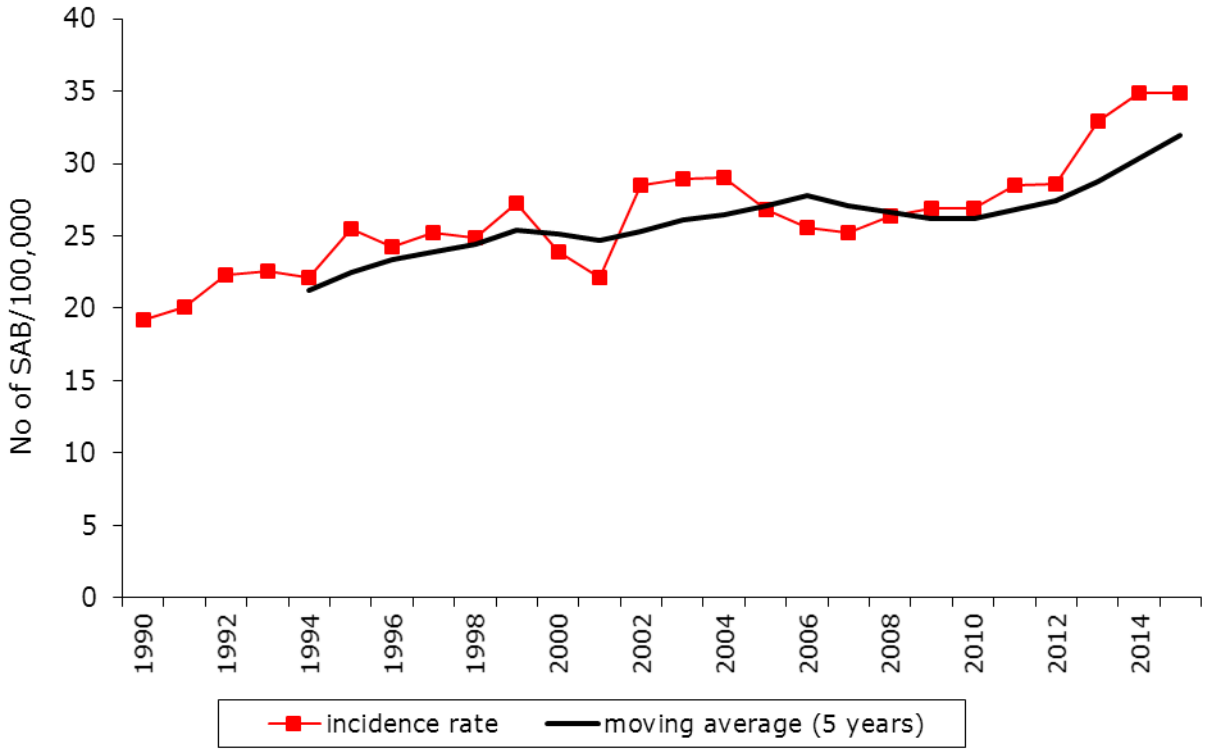


Figure 2. Incidence rate of SAB per 100,000 inhabitants during 1990-2015.



2.2 Geographic variation

The incidence of SAB varied from 16 to 41 cases/100,000 in the catchment population for the individual DCMs (Table 2). Odense DCM demonstrated the highest incidence. The merger of several DCMs during 2013 and 2014 hampers comparisons to previous years. See the note for Table 2 for more details.

Table 2. Number of SAB cases in 2015 and SAB/100,000 inhabitants in each geographic area covered by the Departments of Clinical Microbiology 2011-2015.

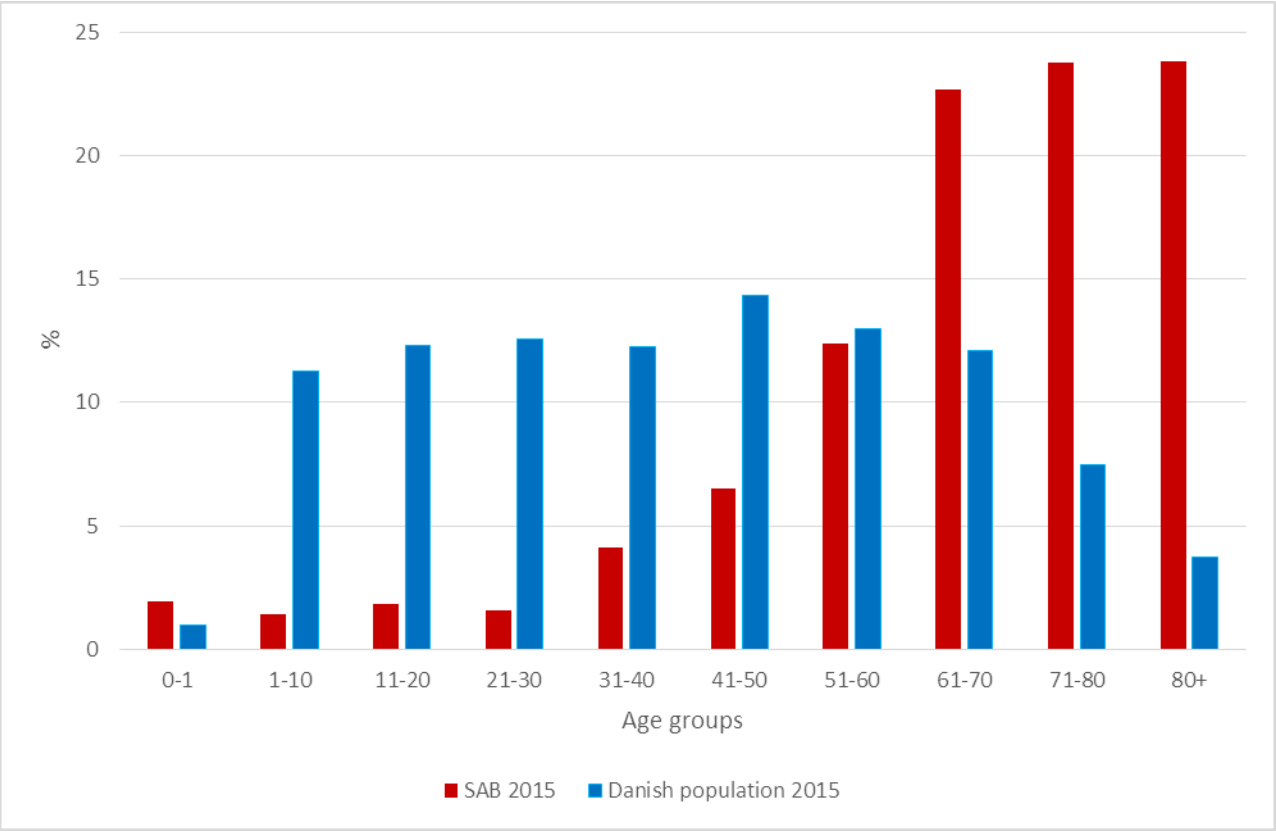
	Number of SAB	SAB/100,000 inhabitants				
DCM	2015	2011	2012	2013	2014	2015
Aalborg	196	34	34	31	38	38
Aarhus	228	28	28	30	33	31
Viborg	160	32	20	32	28	33
Vejle	95	17	16	19	21	22
Esbjerg	65	28	29	34	28	33
Sønderborg	37	<1	<1	<1	11	16
Odense	198	35	27	39	46	41
Slagelse	296	27	33	37	38	36
Greater Copenhagen	698	32	37	36	40	39

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 cases from Herning were handled by Viborg in 2014. The incidence rates for Viborg, Slagelse and Greater Copenhagen have thus been calculated according to the population within the new catcment areas. Sønderborg did not participate in the years 2011 – 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 2015 were older than 50 years and almost 24% were older than 80 years (Figure 3). In 2015 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 (223.5/100,000 inhabitants/year) was more than six times higher than for the rest of the population (34.9/100,000 inhabitants/year).

Figure 3. Age distribution of S. aureus *bacteraemia patients and the general Danish population in 2015 (%).*



2.4 Case fatality

The 30-day case fatality was 22.7% in 2015 (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 42.1% (Table 3) almost twice as high as the average.

Table 3. Case fatality among Danish SAB patients in 2015 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	38	28	36	31	81	129	244	447	469	470	1973
No. 30-day case fatality	4	2	0	0	1	7	32	80	127	198	448
% case fatality	10.5	7.1	0	0	1.2	5.4	13.1	17.9	27.1	42.1	22.7

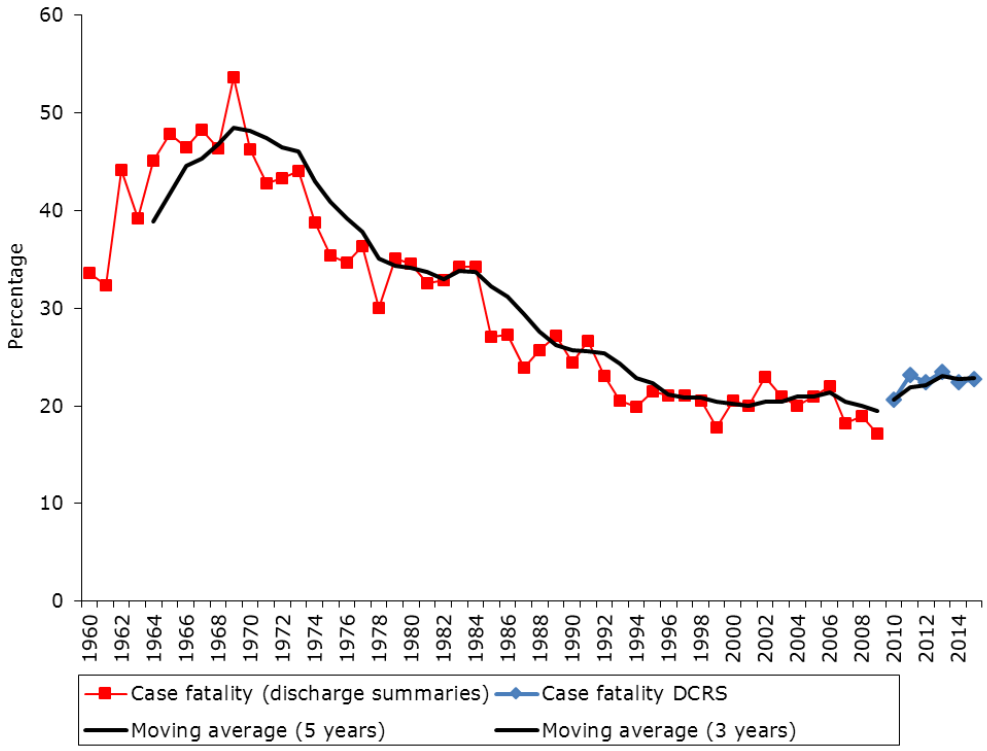


Figure 4. 30-day case fatality (number and %) of Danish SAB patients 1960-2015. Until 2009, data was extracted from discharge notes. From 2010 and onwards 30-day case fatality was extracted from the Danish Civil Registration System (DCRS).

Thirty-day case fatality among MRSA-cases was not different from the average case fatality (24.1% vs. 22.7%). The distribution of the most prevalent *spa* types and CC groups among the 448 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, 500 cases (25.3%) had an onset of infection two or more days after admission to a hospital (HA). The corresponding percentage was 27.6% in 2014 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, 245 cases (12.4%) had a secondary infection registered and after three months, the number was 495 cases, corresponding to 25.1%. 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 2010 to 2015. The source of vital statistics changed in 2010, which was the first year where patient information was obtained from NPR.

Table 4. Secondary infections (%) among Danish SAB patients in 2015, recorded during admission and 3 months after.

	CNS	Endocarditis	Arthritis	Spondylitis	Osteomyelitis	Prosthetic infection
During admission	0.8	5.5	1.8	1.6	1.2	1.9
3 months follow-up	1.8	11.5	3.5	5.6	2.4	3.8

Table 5. The most common secondary infections (%) among Danish SAB patients in 2011-2015, recorded 3 months after admission.

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

2.7 Comorbidities

613 cases (31%) had no comorbidities registered, while 687 cases (35%) had a comorbidity index score of 1-2, and 673 cases (34%) had a score of more than 2. Table 6 presents comorbidity based on the Charlson index. Malignancy (24.8%), diabetes without chronic complication (24.8%), and renal disease (20.0%) were the most frequently registered comorbidities.

Table 6. Number and percentage of comorbidities among SAB patients 2015, with percentages for 2012 - 2014 for comparison.

Comorbidity	2015 Number	2012 %	2013 %	2014 %	2015 %
AIDS/HIV	5	0.5	0.4	0.2	0.3
Any malignancy	489	23.4	26.8	25.6	24.8
Metastatic solid tumor	110	5.4	5.7	5.3	5.6
Diabetes without chronic complication	489	23.6	25.9	24.5	24.8
Diabetes with chronic complication	270	12.2	14.8	13.9	13.7
Dementia	91	4.1	4.9	4.4	4.6
Hemiplegia or paraplegia	31	1.5	1.4	1.8	1.6
Cerebrovascular disease	336	15.8	20.1	19.1	17.0
Myocardial infarction	201	10.7	10.5	12.3	10.2
Congestive heart failure	356	17.5	18.0	19.7	18.0
Chronic pulmonary disease	365	16.0	16.9	18.4	18.5
Peptic ulcer disease	144	8.3	8.3	7.5	7.3
Mild liver disease	166	9.1	9.2	9.3	8.4
Moderate or severe liver disease	61	4.1	4.7	5.2	3.1
Renal disease	395	20.0	20.1	19.6	20.0
Rheumatic disease	93	4.9	5.6	5.8	4.7
Peripheral vascular disease	326	15.1	15.9	16.9	16.5
Drug abuse	53	2.4	2.4	3.1	2.7

2.8 Typing

spa types were obtained for 1,948 isolates (98.7%). In total, 567 different *spa* types were identified, with ten *spa* types accounting for 33% of the isolates (Table 7). A total of 364 *spa* types were only encountered once. Putative assignment to MLST CC was possible for 1,645 isolates (83%). 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 25 MLST CC groups were assigned. The three most prevalent CC groups constituted 39% of the SAB isolates in 2015 while the 10 most prevalent constituted 75% (Table 8). Twenty-five SAB

isolates were *pvl* positive (1.3%), of which five were methicillin-resistant *S. aureus* (MRSA; *spa* types two t891/CC22 and one of each t008/CC8, t1028/CC80, and t5041/CC88. The *pvl* positive isolates were distributed among 22 different *spa* types and eleven MLST CC groups; four 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 25 SAB cases (1.3%) in 2015 of which 3 were MRSA. Eleven belonged to *spa* type t034 (three MRSA), ten belonged to *spa* type t571, and four to t1451. None of three 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 6 SAB patients with CC398 MRSA patients died within 30 days.

Table 7. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2015. Corresponding numbers and prevalences for the four previous years are shown for comparison.

spa type	2011	2012	2013	2014	2015
t127	75 (4.9)	68 (4.5)	81 (4.6)	103 (5.2)	96 (4.9)
t084	72 (4.7)	62 (4.1)	73 (4.1)	66 (3.4)	89 (4.5)
t002	40 (2.6)	59 (3.9)	73 (4.1)	88 (4.5)	84 (4.3)
t230	81 (5.3)	90 (5.9)	93 (5.3)	100 (5.1)	82 (4.2)
t091	34 (2.2)	40 (2.6)	41 (2.3)	63 (3.2)	60 (3.0)
t012	33 (2.2)	45 (2.9)	52 (2.9)	66 (3.4)	57 (2.9)
t015	46 (3.0)	46 (3.0)	53 (3.0)	51 (2.6)	53 (2.7)
t021	33 (2.2)	41 (2.7)	43 (2.4)	31 (1.6)	46 (2.3)
t701	21 (1.4)	25 (1.6)	28 (1.6)	35 (1.8)	45 (2.3)
t008	32 (2.1)	42 (2.7)	41 (2.3)	46 (2.3)	36 (1.8)

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	2011	2012	2013	2014	2015
CC45	338 (22.2)	269 (17.6)	294 (16.6)	343 (17.5)	330 (16.7)
CC30	200 (13.2)	214 (14.0)	245 (13.8)	239 (12.2)	243 (12.3)
CC15	177 (11.6)	150 (9.8)	175 (9.9)	204 (10.4)	197 (10.0)
CC5	102 (6.7)	111 (7.3)	134 (7.6)	175 (8.9)	169 (8.6)
CC1	110 (7.2)	102 (6.7)	132 (7.5)	165 (8.4)	169 (8.6)
CC8	92 (6.1)	99 (6.5)	119 (6.7)	142 (7.2)	143 (7.2)
CC7	47 (3.1)	44 (2.9)	51 (2.9)	74 (3.8)	69 (3.5)
CC22	54 (3.6)	50 (3.3)	43 (2.4)	64 (3.3)	58 (2.9)
CC97	28 (2.0)	28 (1.8)	28 (1.8)	40 (2.0)	48 (2.4)
CC59	36 (2.4)	30 (2.0)	25 (1.4)	48 (2.4)	43 (2.2)

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 2015.

Resistance to penicillin has decreased during the last decade and was at its lowest in 2015 (71.1%). Resistance to fusidic acid was 16.1% and has increased gradually from 8-10% in the period 2005-2009. All SAB isolates were susceptible to linezolid in 2015. The proportion of isolates susceptible to all antibiotics was 18.3%. The proportion of resistance to at least one antimicrobial in addition to penicillin was 23.5% and the proportion of resistance to at least two and three additional antimicrobials were 7.4% and 3.2%, respectively.

Table 9. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2015 (n=502)

Antimicrobial	Resistance (%)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Penicillin	71.1		26.5	2.4	71.1							
Cefoxitin	1.5								98.5	1.5		
Erythromycin	7.0						91.2	1.8	1.0	6.0		
Clindamycin	7.0§				97.2	1.4		1.4				
Fusidic acid	16.1					80.7	3.2	1.6	14.5			
Tetracycline	4.0						95.4	0.6	1.4	2.6		
Norfloxacin	5.6								94.4	2.6	3.0	
Rifampicin	0.2				99.4	0.4		0.2				
Linezolid	0							94.4	5.6			
Kanamycin	2.8										97.2	2.8
TMP/SXT*	0.4							98.8	0.8	0.4		
Ceftaroline	0					97.8	2.2					
Ceftobiprole	NA					97.6	2.0	0.2		0.2		
Daptomycin	2.8					85.1	12.2	2.4	0.4			
Gentamicin	3.0						97.0	2.4	0.6			
Mupirocin	0.4					99.4	0.2		0.4			
Vancomycin	0.0						98.2	1.8				

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 2015 (n=502) (%).

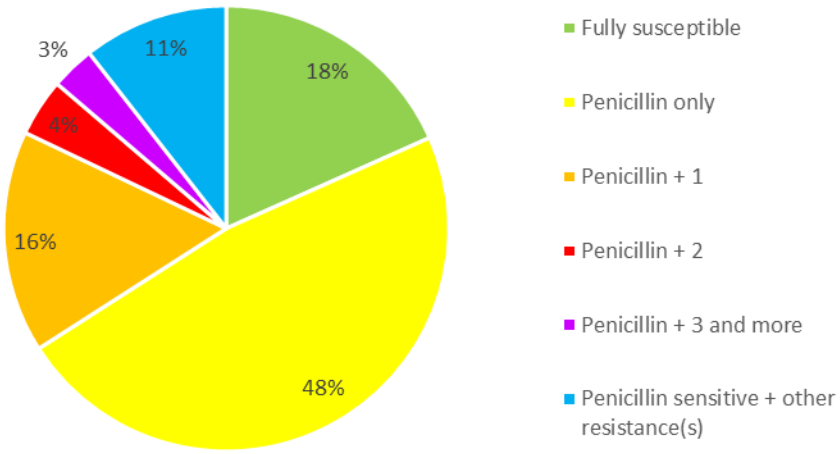
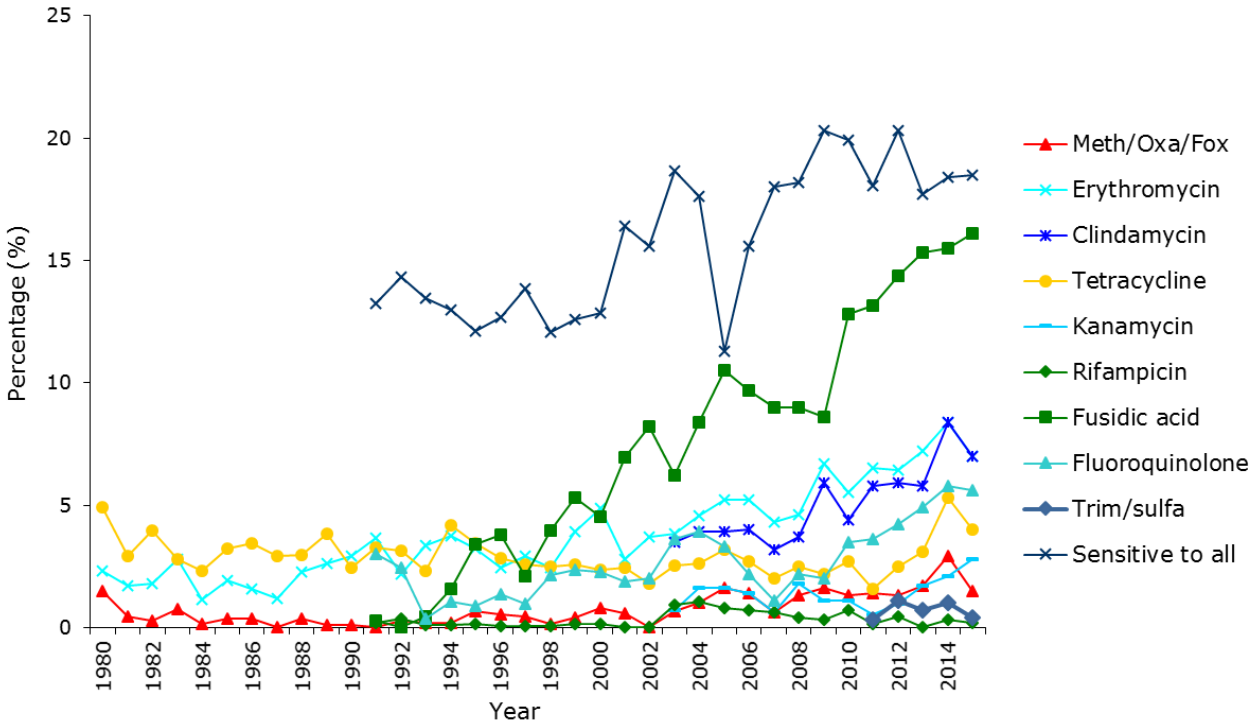


Figure 6. Prevalence of antimicrobial resistance in Danish SAB isolates (1980-2015). Resistance to penicillin is not shown.



3. Conclusions

The number of recorded SAB cases stabilized in 2015 after a steep increase from 2012 to 2014. By comparison with SAB cases recorded in the Danish Microbiological database (MIBA) it was found that the compliance of submissions to the national SAB surveillance in the period 2010-2015 was very high (94-97%).

The number and ratio of MRSA cases was at the same level as in the previous decade, indicating that the high number registered in 2014 (56 cases, 2.9%) does not depict a continuous increase. Case fatality among MRSA SAB cases does not seem to differ from MSSA SAB cases. Resistance to fusidic acid has increased in recent years. *spa* type t127 has become the most numerous *spa* type among SAB isolates and contributes to this trend as most t127 isolates carry the *fusC* gene. With respect to SAB, fusidic acid resistance is no major problem as this antimicrobial is not used as monotherapy and alternatives are available. Of importance almost two-thirds 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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