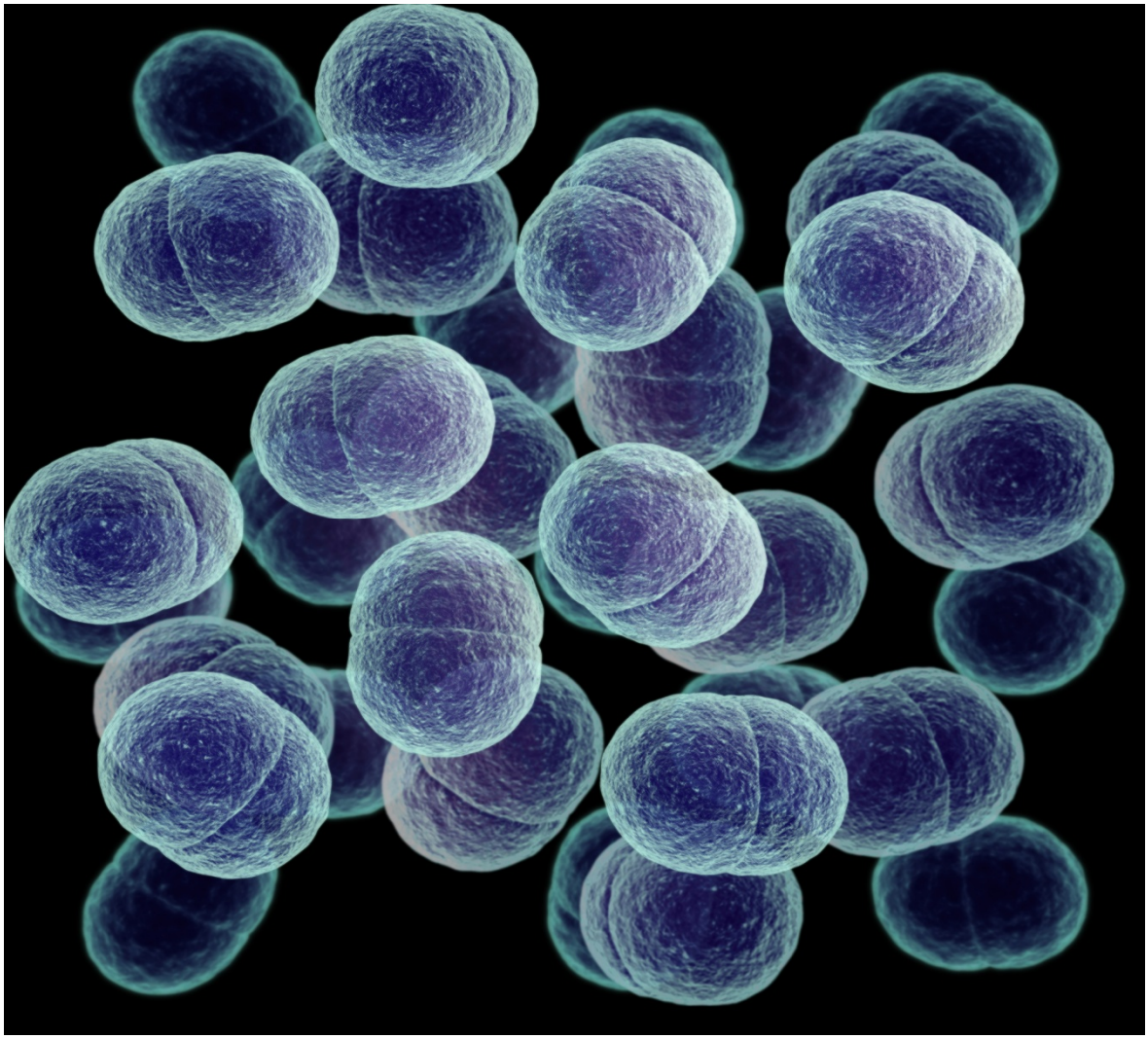
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2014



This report describes the laboratory and clinical characteristics of the 1,964 cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark in 2014. 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 and Stine Frese-Madsen are thanked for technical assistance.

Authors: Andreas Petersen, Robert L. Skov & Anders R. Larsen and members of the Danish *Staphylococcus aureus* bacteraemia group: Thomas Benfield, Henrik C. Schønheyder, Peter Skinhøj and Henrik Westh.
Publisher: Reference Laboratory for Antimicrobial Resistance and Staphylococci
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: The newly described 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 records from The Danish National Patient Register (NPR, Lynge *et al*. 2011) were extracted for each patient with SAB. The registry 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. 30-day mortality 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. The homepage of Statistics Denmark (http://www.statistikbanken.dk/bef5) was the source of demographic data.

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, 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 January – March and May – June, 2014, in total 381 isolates (~20%) by MIC determination using a custom-made panel (DKSSP, 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	0.03-0.12
Cefoxitin	screen: 4 and 6
Oxacillin	0.25-4
Ceftaroline	0.12-4
Ceftobiprole	0.12-8
Erythromycin	0.25-4
Clindamycin, including induction	0.12-1 and D-test
Tetracycline	0.5-4
Tigecycline	0.06-1
Rifampicin	0.06-1
Gentamicin	0.25-2
Kanamycin	Screen: 16
Fusidic acid	0.12-16
Sulfamethoxazole/Trimethoprim	0.5/9.5-4/76
Linezolid	1-8
Mupirocin	0.12-2 and screen: 256
Vancomycin	0.5-8
Teicoplanin	1-8
Daptomycin	0.5-2
Norfloxacin	1-8
Moxifloxacin	0.25-2

2. Results

2.1 Patient information

1,964 SAB cases were recorded in 2014 (Figure 1); hereof 57 cases (2.9%) were MRSA. This corresponds to an incidence rate of SAB of 34.9/100,000 inhabitants/year (Figure 2). The number of bacteraemia cases increased by 11% compared to 2013 (Figure 1). The proportion of MRSA represents a steep increase compared to recent years. This is the second year in a row with a markedly increased number of recorded cases. The number of participating DCMs increased by one in 2014 but the increase in cases cannot solely be explained by one more participating DCM. 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. An increased focus on sepsis may also partly explain the increase. There was an excess of men (63% vs. 37% women) among the cases of SAB in 2014. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

Figure 1. Number of SAB cases 1960-2014.

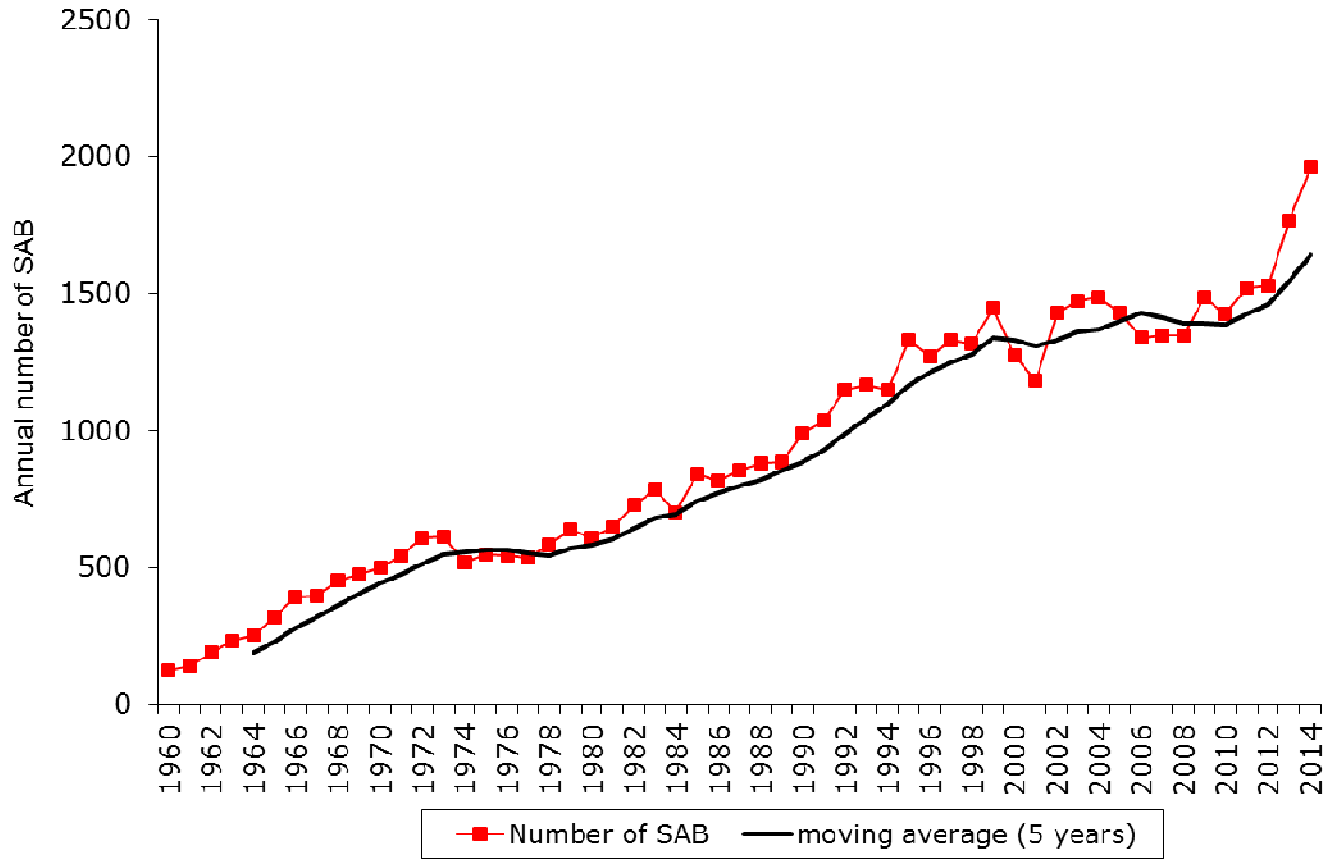
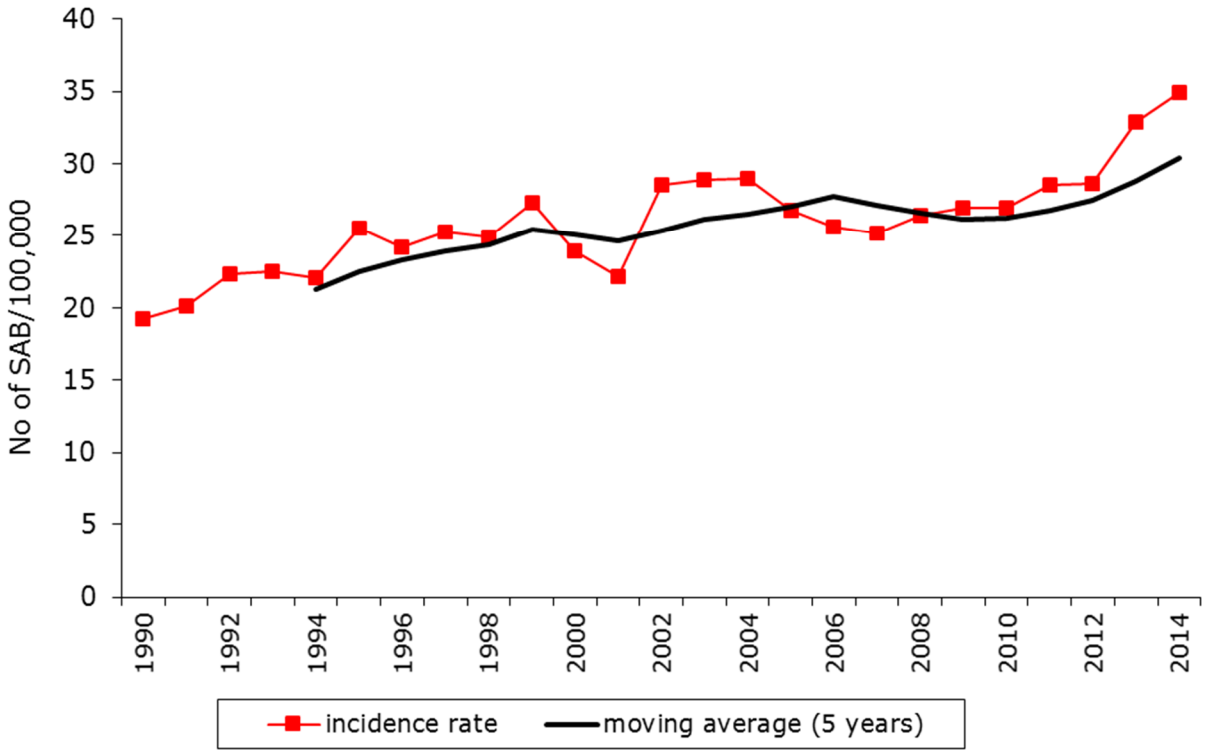


Figure 2. Incidence rate of SAB per 100,000 inhabitants during 1990-2014.



2.2 Geographic variation

The incidence of SAB varied from 21 to 46 cases/100,000 in the catchment population for the DCMs (Table 2). Odense DCM demonstrated the highest incidence. The incidence for Odense and Aalborg DCMs increased the most from 2013 to 2014. 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 2014 and SAB/100,000 inhabitants in each geographic area covered by the Departments of Clinical Microbiology 2010-2014.

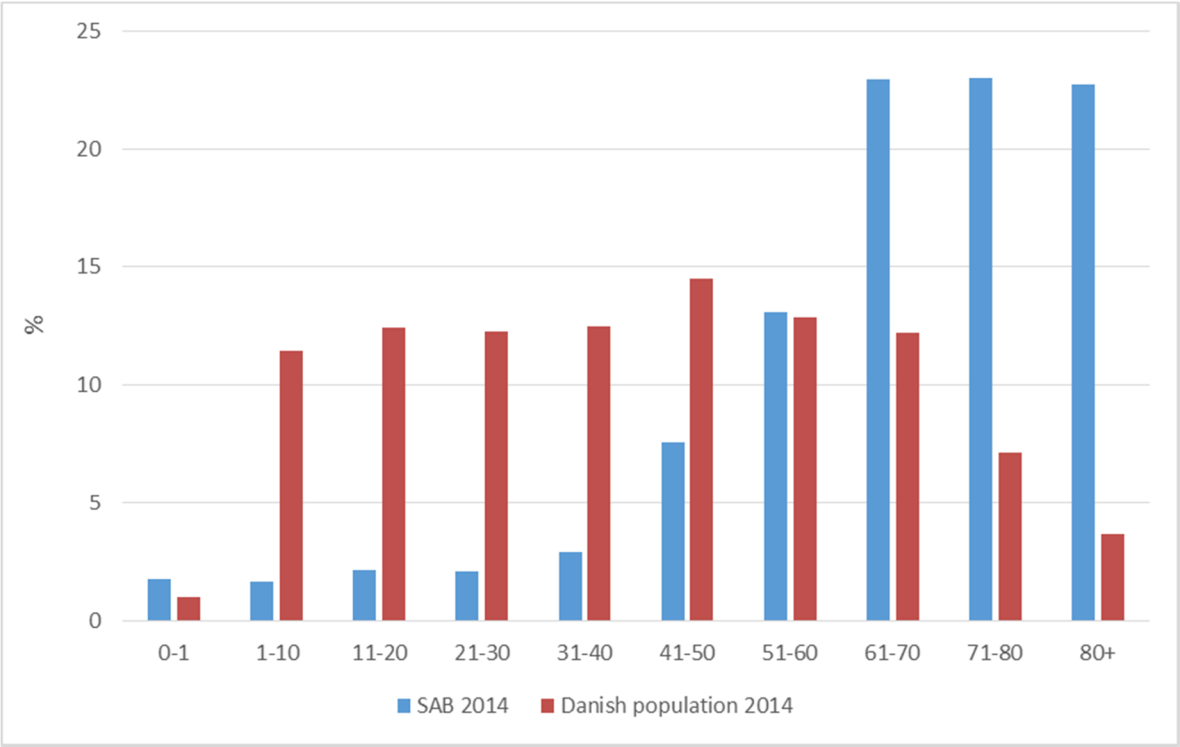
	Number of SAB	SAB/100,000 inhabitants				
DCM	2014	2010	2011	2012	2013	2014
Aalborg	194	28	34	34	31	38
Aarhus	237	26	28	28	30	33
Viborg	136	29	32	20	32	28
Vejle	88	16	17	16	19	21
Esbjerg	55	29	28	29	34	28
Sønderborg	25	3	<1	<1	<1	11
Odense	224	33	35	27	39	46
Slagelse	308	22	27	33	37	38
Greater Copenhagen	697	30	32	37	36	40

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 new practises. Sønderborg did not participate in the years 2010 – 2013 and started to submit isolates from the second half of 2014.

2.3 Age

SAB is primarily affecting older people and more than 80% of the SAB patients in 2014 were older than 50 years and almost 23% were older than 80 years (Figure 3). In 2014 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 (215.8/100,000 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 2014 (%).*



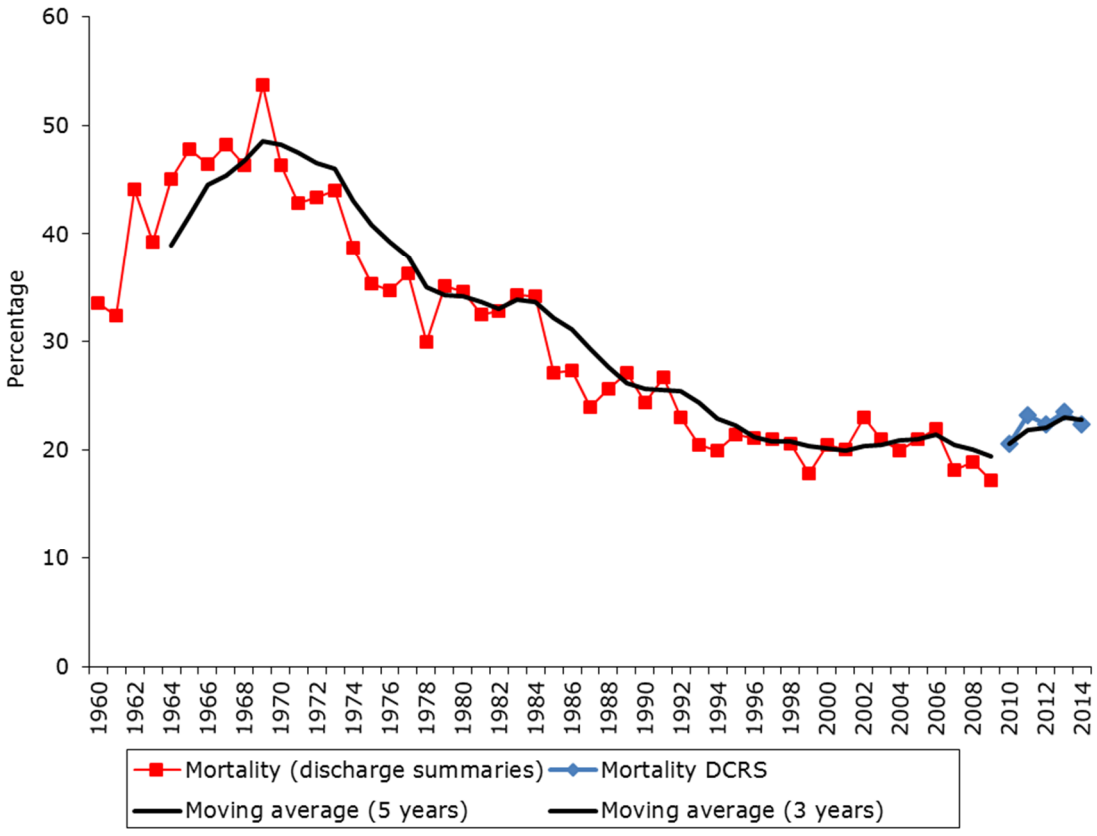
2.4 Mortality

The 30-day mortality was 22.4% in 2014 (Table 3) and did not differ from the previous 20 years (Figure 4). Mortality was low between 1-40 years; no 30-day mortality was demonstrated for the age group 11-20 years. The 30-day mortality increased with age from the age group of 41-50 years, and patients above 80 years had a mortality rate of 40.8% (Table 3). Mortality in this age group was almost twice as high as the average mortality.

Table 3. Mortality among Danish SAB patients in 2014 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	30	42	41	57	148	257	451	452	448	1964
No. 30-day mortality	3	1	0	1	1	13	43	84	108	183	437
% mortality	7.9	3.3	0.0	2.4	1.8	8.8	16.7	18.6	23.9	40.8	22.4

Figure 4. Mortality (number and %) of Danish SAB patients 1960-2014. Until 2009, mortality was extracted from discharge notes. From 2010 and onwards 30-day mortality was extracted from the Danish Civil Registration System (DCRS).



Mortality among MRSA-cases was not different from the average mortality (23.2% vs. 22.4%). The distribution of the most prevalent *spa* types and CC groups among the 437 isolates from cases dying within 30 days did not differ from the overall distribution of *spa* types. No significant variation from the overall mortality 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 542 cases (27.6%) had an onset of infection two or more days after admission to a hospital (HA). The corresponding percentage was 28.6% in 2013 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. Unpublished data suggests a continuing increase of these cases.

2.6 Secondary infections

During admission, 233 cases (11.9%) had a secondary infection registered and after three months, the number was 486 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 2010 to 2014. 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 2014, recorded during admission and 3 months after.

	CNS	Endocarditis	Arthritis	Spondylitis	Osteomyelitis	Prosthetic infection
During admission	0.7	4.5	1.3	1.2	1.8	2.4
3 months follow-up	2.3	10.0	2.7	5.3	3.3	4.4

Table 5. The most common secondary infections (%) among Danish SAB patients in 2010-2014, recorded 3 months after admission.

Secondary infection	2010	2011	2012	2013	2014
Endocarditis	7.9	11.4	10.1	10.9	10.0
Spondylitis	4.5	5.3	5.6	5.8	5.3
Prosthetic infection	3.7	4.1	5.1	4.1	4.4
Arthritis	3.5	3.5	3.3	3.1	2.7
Osteomyelitis	3.3	2.4	2.9	2.6	3.3
CNS	2.3	2.9	2.4	2.3	2.3

2.7 Comorbidities

561 cases (29%) had no comorbidities registered, while 705 cases (36%) had a comorbidity index score of 1-2 and 698 cases (35%) had a score of more than 2. Table 6 presents comorbidity based on the Charlson index. Malignancy (25.6%), diabetes without chronic complication (24.5%), and congestive heart failure (19.7%) were the most frequently registered comorbidities.

Table 6. Number and percentage of comorbidities among SAB patients 2014, with percentages for 2012 and 2013 for comparison.

Comorbidity	Number 2014	% 2012	% 2013	% 2014
AIDS/HIV	4	0.5	0.4	0.2
Any malignancy	502	23.4	26.8	25.6
Metastatic solid tumor	104	5.4	5.7	5.3
Diabetes without chronic complication	482	23.6	25.9	24.5
Diabetes with chronic complication	273	12.2	14.8	13.9
Dementia	87	4.1	4.9	4.4
Hemiplegia or paraplegia	36	1.5	1.4	1.8
Cerebrovascular disease	376	15.8	20.1	19.1
Myocardial infarction	242	10.7	10.5	12.3
Congestive heart failure	386	17.5	18.0	19.7
Chronic pulmonary disease	361	16.0	16.9	18.4
Peptic ulcer disease	148	8.3	8.3	7.5
Mild liver disease	182	9.1	9.2	9.3
Moderate or severe liver disease	103	4.1	4.7	5.2
Renal disease	385	20.0	20.1	19.6
Rheumatic disease	114	4.9	5.6	5.8
Peripheral vascular disease	331	15.1	15.9	16.9
Drug abuse	61	2.4	2.4	3.1

2.8 Typing

spa types were obtained for 1,950 isolates (99.3%). In total, 545 different *spa* types were identified, with ten *spa* types accounting for 33% of the isolates (Table 7). A total of 349 *spa* types were only encountered once. Putative assignment to MLST CC was possible for 1,691 isolates (86%). 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 40% of the SAB isolates in 2014 while the 10 most prevalent constituted 76% (Table 8).

Twenty-one SAB isolates were *pvl* positive (1.1%), of which six were methicillin-resistant *S. aureus* (MRSA; *spa* types two t008/CC8 and one of each t044/CC80, t068/CC8, t437/CC59 and t1028/CC80). The *pvl* positive isolates were distributed among 18 different *spa* types and ten 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 31 SAB cases (1.6%) in 2014 of which 8 were MRSA. Thirteen belonged to *spa* type t034 (seven MRSA), ten belonged to *spa* type t571 (one MRSA), four to t1451, two to t011 and one each of t2582 and t3625. Four of the eight (50%) SAB CC398 MRSA cases had direct or indirect contact to livestock. Phylogenetic studies of whole-genome sequences of the 31 CC398 SAB cases demonstrated that 14 belonged to a basal, human clade, while 17 belonged to a derived, livestock-associated (LA) clade (unpublished data). All eight CC398 MRSA SAB cases belonged to the derived LA clade, while 14 of the 23 CC398 MSSA SAB cases belonged to the basal human clade and 9 to the derived LA clade.

Table 7. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2014. Corresponding numbers and prevalences for the four previous years are shown for comparison.

spa type	2010	2011	2012	2013	2014
t127	62 (4.4)	75 (4.9)	68 (4.5)	81 (4.6)	103 (5.2)
t230	86 (6.1)	81 (5.3)	90 (5.9)	93 (5.3)	100 (5.1)
t002	55 (3.9)	40 (2.6)	59 (3.9)	73 (4.1)	88 (4.5)
t084	66 (4.7)	72 (4.7)	62 (4.1)	73 (4.1)	66 (3.4)
t012	51 (3.6)	33 (2.2)	45 (2.9)	52 (2.9)	66 (3.4)
t091	26 (1.8)	34 (2.2)	40 (2.6)	41 (2.3)	63 (3.2)
t015	55 (3.9)	46 (3.0)	46 (3.0)	53 (3.0)	51 (2.6)
t008	32 (2.3)	32 (2.1)	42 (2.7)	41 (2.3)	46 (2.3)
t701	16 (1.1)	21 (1.4)	25 (1.6)	28 (1.6)	35 (1.8)
t065	21 (1.5)	25 (1.6)	18 (1.2)	23 (1.3)	33 (1.7)

Table 8. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2014. Corresponding numbers and prevalences for the four previous years are shown for comparison.

Clonal complex	2010	2011	2012	2013	2014
CC45	306 (21.6)	338 (22.2)	269 (17.6)	294 (16.6)	343 (17.5)
CC30	229 (16.1)	200 (13.2)	214 (14.0)	245 (13.8)	239 (12.2)
CC15	162 (11.4)	177 (11.6)	150 (9.8)	175 (9.9)	204 (10.4)
CC5	107 (7.5)	102 (6.7)	111 (7.3)	134 (7.6)	175 (8.9)
CC1	113 (8.0)	110 (7.2)	102 (6.7)	132 (7.5)	165 (8.4)
CC8	85 (6.0)	92 (6.1)	99 (6.5)	119 (6.7)	142 (7.2)
CC7	39 (2.7)	47 (3.1)	44 (2.9)	51 (2.9)	74 (3.8)
CC22	41 (2.9)	54 (3.6)	50 (3.3)	43 (2.4)	64 (3.3)
CC59	25 (1.8)	36 (2.4)	30 (2.0)	25 (1.4)	48 (2.4)
CC97	33 (2.2)	28 (2.0)	28 (1.8)	28 (1.8)	40 (2.0)

2.9 Antimicrobial susceptibility testing

Table 9 demonstrates the prevalences of resistance to the antimicrobials tested. The resistance profiles is shown in Figure 5. Figure 6 shows selected resistance prevalences from 1980 to 2014. All SAB isolates were susceptible to linezolid in 2014. Resistance to fusidic acid was at the same level as in 2013 (15.3%) but has increased from 8-10% in the period 2005-2009. Resistance to erythromycin, clindamycin, tetracycline, and fluoroquinolones have increased since 2010 (Figure 6). The proportion of isolates susceptible to all antibiotics was 18.4%. The proportion of resistance to at least one antimicrobial in addition to penicillin was 29.1% and the proportion of resistance to at least two and three additional antimicrobials were 16.0% and 8.9%, respectively.

Figure 5. Resistance profiles of a subset of Danish SAB isolates 2014 (n=381) (%).

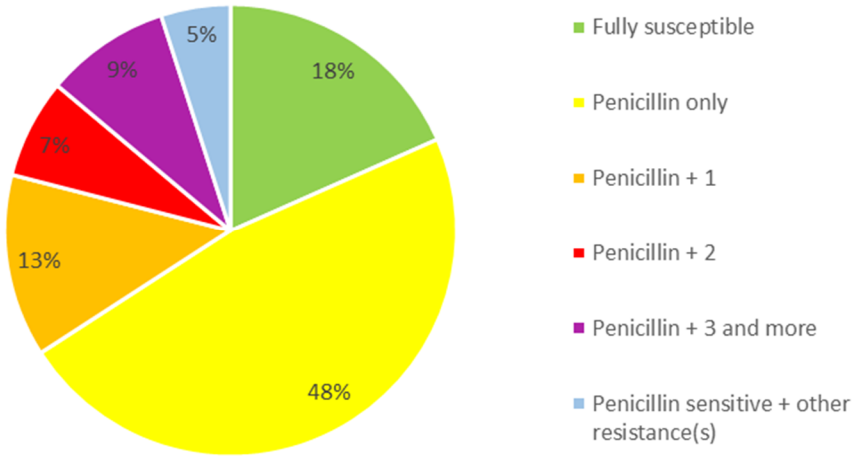


Figure 6: Prevalence of antimicrobial resistance in Danish SAB isolates (1980-2014). Resistance to penicillin is not shown.

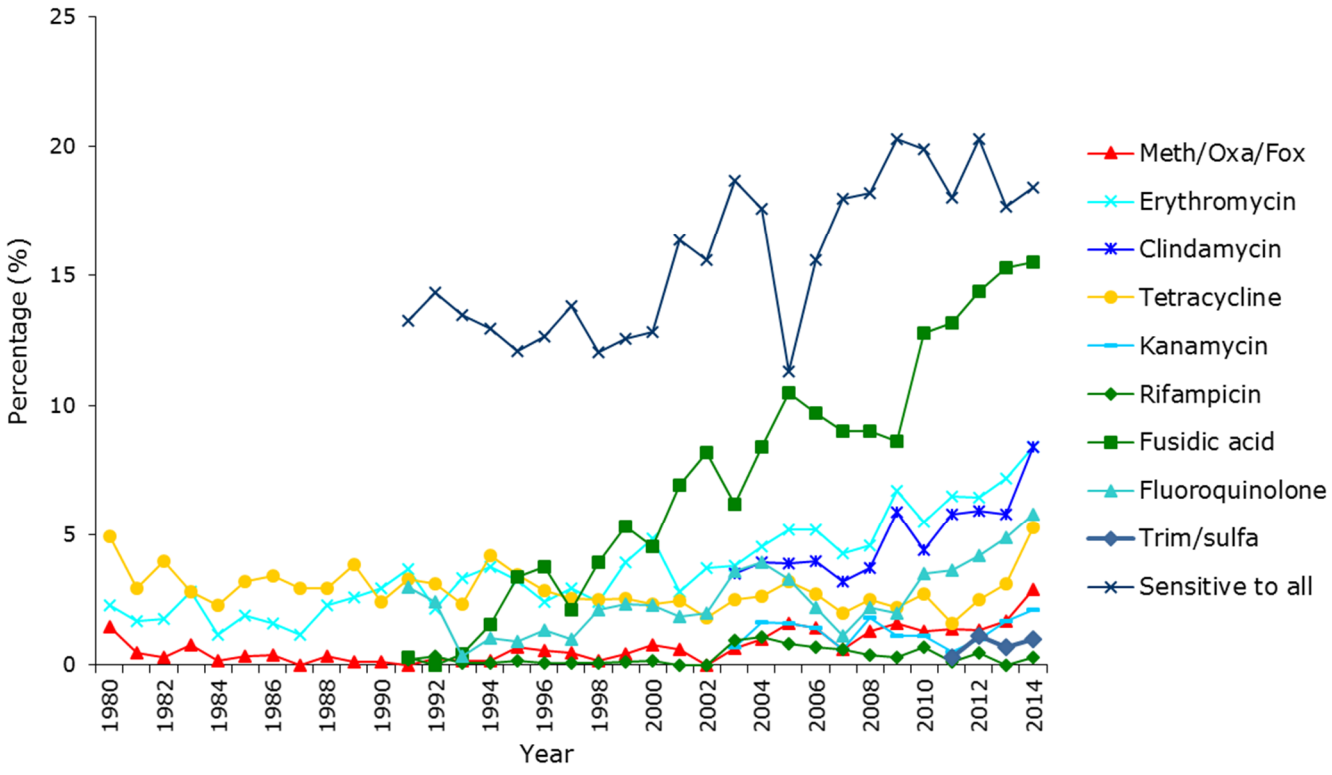


Table 9. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2014 (n=381)

Antimicrobial	Resistance (%)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Penicillin	76.6	15.2	7.1	1.0	76.6							
Cefoxitin	2.9								97.1	2.9		
Erythromycin	8.4				3.9	85.8	1.8		0.3	8.1		
Clindamycin	8.4			56.4	40.4	0.5		2.6				
Fusidic acid	15.5			16.5	52.5	13.6	1.8	1.8	2.6	3.7	6.0	1.3
Tetracycline	5.3					61.7	32.8	0.3	0.3	5.0		
Norfloxacin	5.8						66.4	26.5	1.3	0.5	5.2	
Rifampicin	0.3		98.4	1.3			0.3					
Linezolid	0.0						1.0	52.8	46.2			
Kanamycin	2.1										97.9	2.1
TMP/SXT*	1.0					96.1	1.6	1.0	0.3	1.0		
Ceftaroline	0.3			3.4	70.6	21.8	3.9	0.3				
Ceftobiprole	NA			0.8	24.1	67.5	6.6	1.0				
Daptomycin	1.9					73.0	26.0	1.0				
Gentamicin	2.9				13.4	64.3	19.4	2.1	0.8			
Moxifloxacin	3.7				95.3	0.5	0.5	0.8	2.9			
Mupirocin	0.5			3.9	54.1	40.7	0.8		0.5			
Teicoplanin	0.0						96.6	3.4				
Tigecycline	0.3		2.9	58.8	37.3	0.8	0.3					
Vancomycin	0.0					2.4	90.3	7.3				

** Trimethoprim/sulfamethoxazole; MIC expressed as the trimethoprim concentration. For clindamycin inducible resistance is included. 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.*

3. Conclusions

The number of recorded SAB cases increased markedly for the second year in a row. No single event may fully explain the increase but better compliance with the voluntary collaboration, increased focus on sepsis in the clinical setting, improved culture methods, and an increasing number of cases among the elderly are factors likely to contribute to the recorded increase.

The number and ratio of MRSA cases increased markedly (56 cases, 2.9%). Even though the increase is worrisome and needs to be monitored in the coming years, mortality among MRSA SAB cases does not seem to differ from MSSA SAB cases. Resistance to in particular fusidic acid but also erythromycin, clindamycin, tetracycline, and fluoroquinolones have increased in recent years. Almost two-thirds of all 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