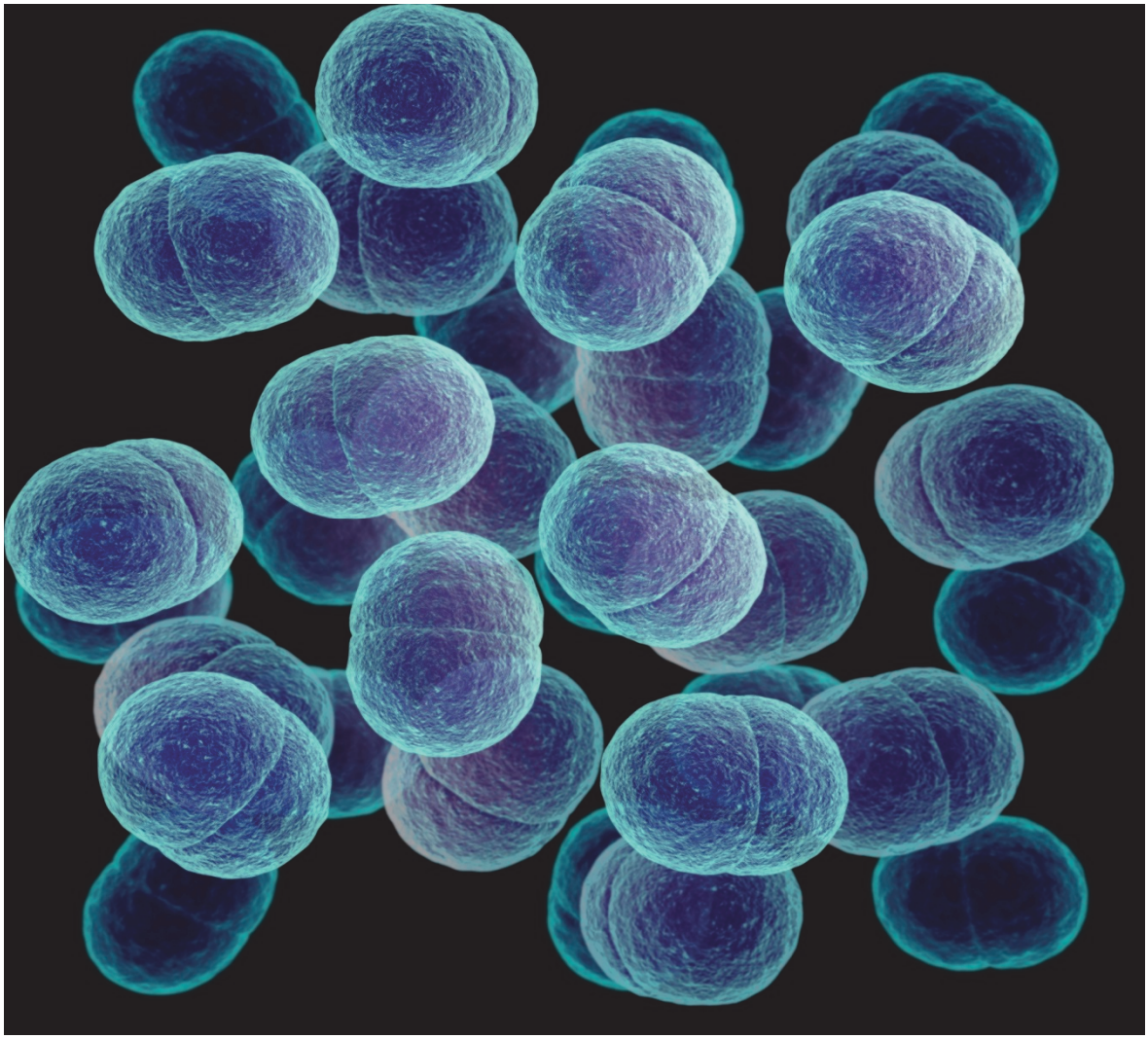
STATENS SERUM INSTITUT

Staphylococcus aureus bacteraemia

Cases in Denmark 2013



This report describes the laboratory and clinical characteristics of the 1,769 submitted cases of *Staphylococcus aureus* bacteraemia (SAB) in Denmark 2013. SAB has been surveyed in Denmark since 1957 by strain characterization and collection of clinical and epidemiological information by the Staphylococcus Laboratory at Statens Serum Institut 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
Herning
Hillerød
Hvidovre
Nykøbing F
Odense
Rigshospitalet
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 Kildevang Hansen, Alexandra Medina, Mille Weismann Poulsen and Stine Frese-Madsen are thanked for technical assistance.

Authors: Andreas Petersen, Robert L. Skov & Anders R. Larsen
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

EUCAST: The European Committee on Antimicrobial Susceptibility Testing

HA: Hospital acquired

ICD-10: International Classification of Diseases

*luk*F/S-pv: Genes encoding the Panton-Valentine leukocidin

*mec*A: The gene encoding for methicillin resistance

*mec*C: The newly described variant of the *mec*A gene

MLST: Multi locus sequence typing

MRSA: Methicillin-resistant *Staphylococcus aureus*

MSSA: Methicillin-susceptible *Staphylococcus aureus*

NPR: The Danish National Patient Register ('Landspatientregistret')

SAB: *Staphylococcus aureus* bacteraemia

spa: The gene encoding the staphylococcal protein A

1. Materials and Methods

1.1 *Staphylococcus aureus* bacteraemia (SAB) episodes

One *S. aureus* isolate per bacteraemia episode was referred to the Staphylococcus Laboratory from the Departments of Clinical Microbiology in Denmark as part of an ongoing collaboration starting in 1957. Subsequent isolates from the same patient were only included if the period between two received isolates was more than one month (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 occacions 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 after 3 months of 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 were listed in the 2010 SAB report (www.ssi.dk/bakteriaemirapport2010). 30 day mortality was calculated based on data extracted from the Danish Civil Registration System ('CPR-registret', Pedersen *et al*. (2006)).
In order to demonstrate geographic differences, SAB per participating DCM is presented. Demographic data from Denmark were obtained through the homepage of Statistics Denmark (http://www.statistikbanken.dk/bef5).

1.2 Typing

All isolates were typed by sequencing of the *spa* gene, which encodes the staphylococcal protein A. The *spa* gene is present in almost all *S. aureus* isolates and offers a high level of discrimination (Harmsen *et al*., 2003). The used method simultaneously detects the *spa*, *mec*A, *mec*C and *luk*F/S-pv genes (PVL) (Stegger *et al*. 2012). *spa* types were annotated using Bionumerics 6.1 (Applied Maths, Sint-Martens-Latem, Belgium) and RidomStaphType 1.4 (Ridom GmbH, Würzburg, Germany). *spa* types were approximated to multi locus sequence typing (MLST) clonal complexes (CC), using the MLST homepage and eBURST (http://saureus.mlst.net/).

1.3 Antimicrobial susceptibility testing

Susceptibility testing was performed on 962 isolates (54%) by MIC determination using a custom-made panel from TREK Diagnostics. The antimicrobials tested and the ranges included are presented in Table 1. EUCAST breakpoints were used for interpretation of antimicrobial resistance. For kanamycin 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	0.12-1 and D-test
Tetracycline	0.5-4
Tigecycline	0.06-1
Rifampicin	0.06-1
Gentamycin	0.25-2
Kanamycin	Screen: 16
Fusidic acid	0.12-16
Sulfametoxazole/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

1,769 SAB cases were submitted in 2013 (Figure 1). This corresponds to an incidence rate of SAB of 32.9/100,000 inhabitants/year (Figure 2). The number of bacteraemia cases increased by 16% compared to 2012 (Figure 1). Whether this increase reflects an actual increase in cases or a better compliance of voluntary participation is unclear. The number of participating DCMs was the same in both 2012 and 2013. There was an excess of men (63% vs. 37% women) among the cases of SAB in 2013. This proportion has been relatively constant comprising 60%-64% during the last 20 years.

Figure 1. Number of SAB cases 1960-2013. The moving average demonstrates increasing numbers throughout the period.

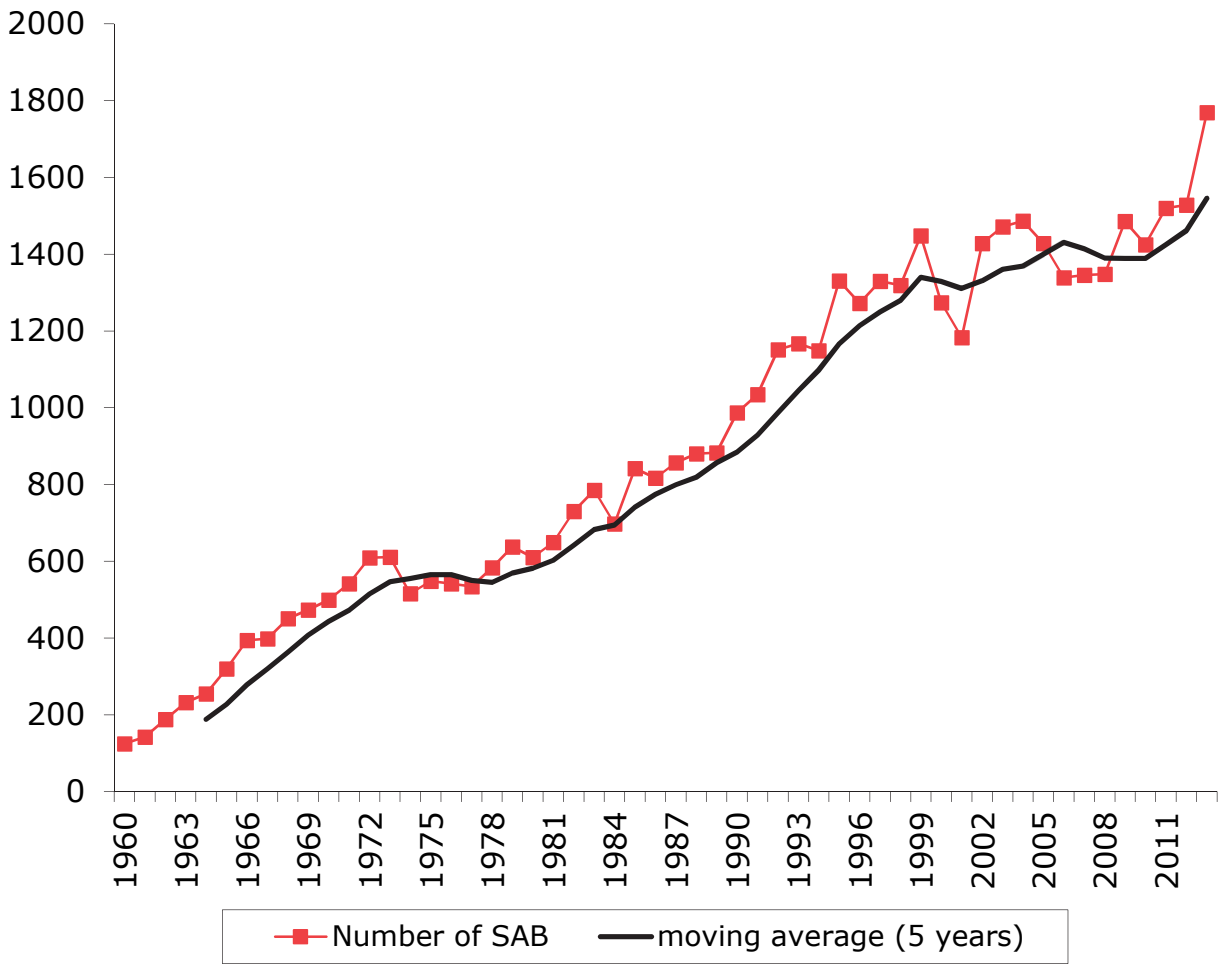
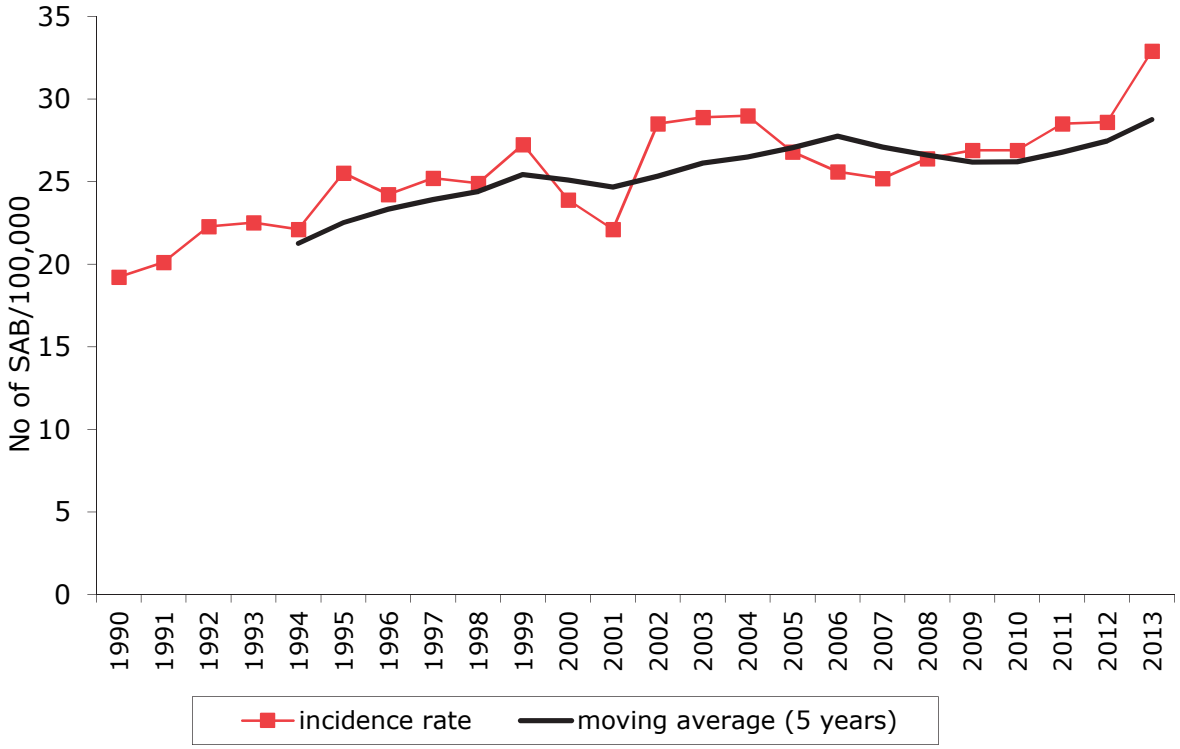


Figure 2. Incidence rate of SAB per 100,000 inhabitants during 1990-2013.



2.2 Geographic variation

The incidence of SAB varied from 19 to 39 cases/100,000 in the catchment population for the DCMs (Table 2). The highest incidence was observed for Odense DCM. The incidence for Viborg and Odense DCMs increased the most from 2012 to 2013. The merger of several DCMs during 2013 makes comparisons to previous years not straight-forward. For more details, see the note for Table 2.

Table 2. Number of SAB cases in 2013 and SAB/100,000 inhabitants in each region covered by the Departments of Clinical Microbiology 2009-2013.

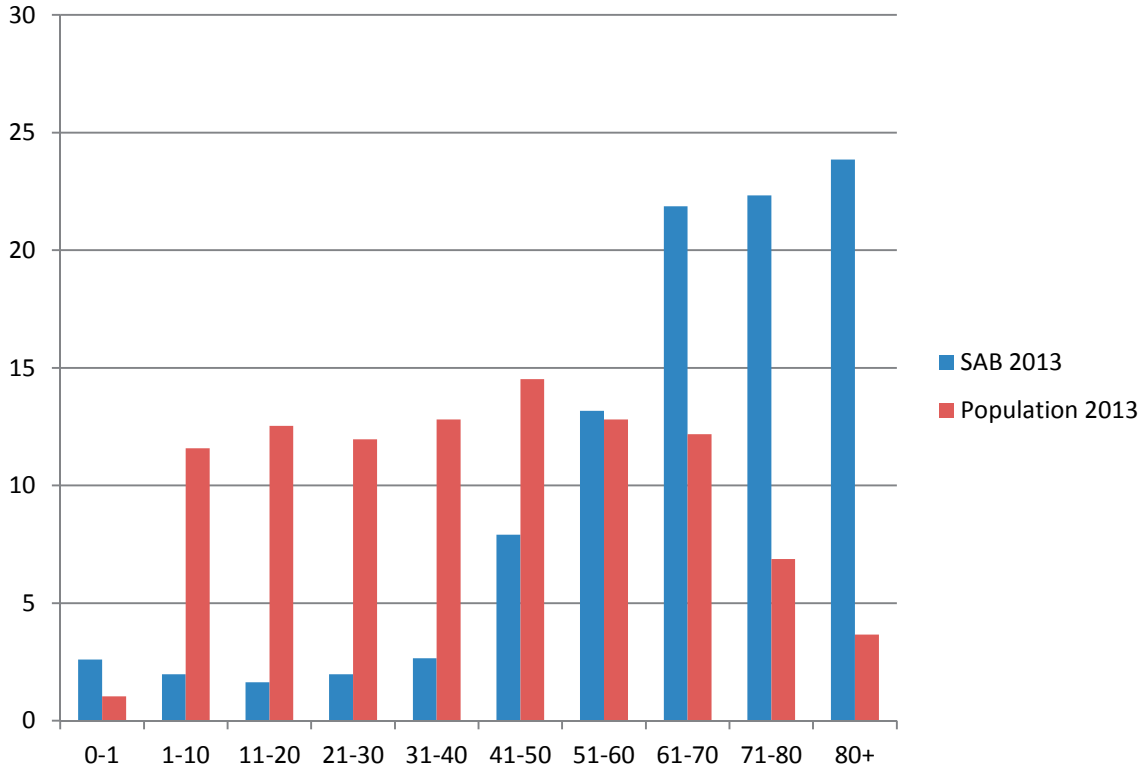
	Number of SAB	SAB/100,000 inhabitants				
DCM	2013	2009	2010	2011	2012	2013
Aalborg	158	23	28	34	34	31
Aarhus	216	27	26	28	28	30
Viborg	66	31	29	32	20	32
Herning	67	20	20	25	18	24
Vejle	81	10	16	17	16	19
Esbjerg	66	24	29	28	29	34
Sønderborg	(2)	18	(3)	(<1)	(<1)	(<1)
Odense	191	33	33	35	27	39
Hillerød	33	26	25	23	27	see note
Greater Copenhagen	585	34	30	32	37	36
Slagelse	302	(30)	22	27	33	37
Nykøbing F	2	28	29	21	9	see note

Notes for Table 2:
The catchment population for Slagelse DCM changed in 2010 and in 2013 most isolates from Nykøbing F were handled by Slagelse. Likewise, Hillerød DCM terminated their service in March 2013 and thereafter all samples were handled by Herlev. The incidence rates for Slagelse and Greater Copenhagen have thus been calculated according to the new practises. Sønderborg DCM has only submitted isolates sporadically during the last four years.

2.3 Age

SAB is primarily affecting older people and more than 80% of the SAB patients in 2013 were older than 50 years and almost 24% were older than 80 years (Fig 3). In 2013 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 (205.6/100,000 inhabitants/year) was more than six times higher than for the rest of the population (32.9/100,000 inhabitants/year).

Figure 3. Age distribution of SAB patients in 2013 and the general Danish population (%).



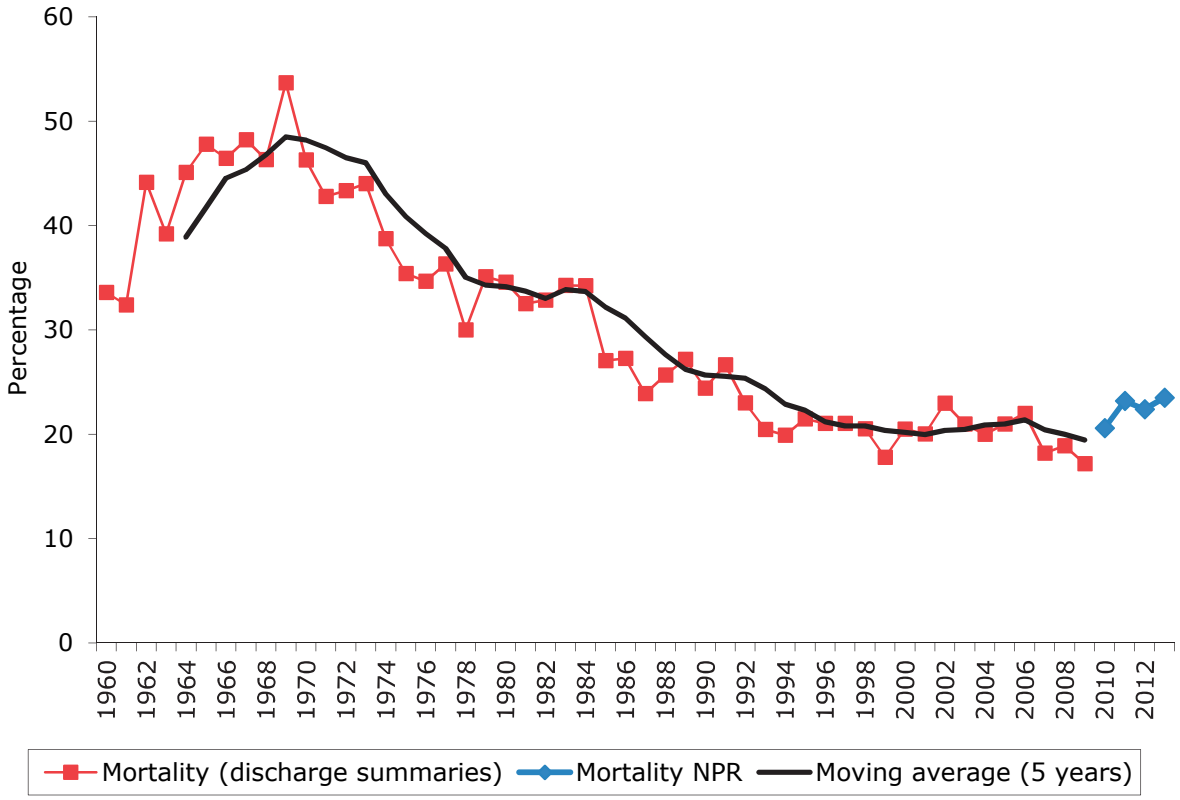
2.4 Mortality

The 30 day mortality was 23.5% in 2013 (Table 3) and did not differ from mortality rates from the previous 20 years (Figure 4). No 30 day mortality was demonstrated for ages 0-30. The 30-day mortality increased with age from the age group of 31-40 years and patients above 80 years had a mortality rate of 41.9% (Table 3).

Table 3. Mortality among Danish SAB patients in 2013 by age group and in total.

Age group	0-1	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	80+	Total
No of SAB	46	35	29	35	47	140	233	387	395	422	1769
30 day mortality	0	0	0	0	1	16	35	66	121	177	416
%	0.0	0.0	0.0	0.0	2.1	11.4	15.0	17.1	30.6	41.9	23.5

Figure 4. Mortality (%) of Danish SAB patients 1960-2013. Until 2009 mortality was extracted from discharge notes. From 2010 and onwards mortality was extracted from The Danish National Patient Register (NPR).



The distribution of the most prevalent *spa* types and CC groups among the 416 isolates from cases with 30-day mortality did not differ from the overall distribution of *spa* types. No significant variation from the overall mortality rate could be established for individual *spa* types and CC groups. Only six of the 416 isolates contained the PVL gene. Thus, the outcome of SAB did not seem to be dependent on the specific type of *S. aureus* causing infection.

2.5 Acquisition

Based on data from NPR 506 cases (28.6%) had an onset of infection two or more days after admission (HA). The corresponding number was 29.5% in 2012 and has been steadily decreasing since 2002 (from 41%).

2.6 Secondary infections

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, prosthesis and arthritis (Table 4). Tenosynovitis, myositis, and abdominal abscesses were all registered in less than 1%. Table 5 shows a comparison of secondary infection for the years 2010 to 2013. 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 2013, recorded during admission and 3 months after.

	CNS	Endocarditis	Arthritis	Spondylitis	Osteomyelitis	Prosthesis
During admission	1.4	6.2	1.6	2.2	1.2	2.5
3 months after SAB	2.3	10.9	3.1	5.8	2.6	4.1

Table 5. The most common secondary infections (%) among Danish SAB patients in 2010-2013, recorded 3 months after admission.

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

2.7 Comorbidities

Comorbidity based on the Charlson index is presented in Table 6. Malignancy (26.8%), diabetes without chronic complication (25.9%), and renal disease and cerebrovascular disease (both 20.1%) were the most frequently registered comorbidities. 475 cases (31%) had no comorbidities registered, while 707 cases (46%) had a comorbidity index score of 1-2 and 587 cases (38%) had a score of more than 2.

Table 6. Number and percentage of comorbidities among SAB patients 2013, with percentages for 2011 and 2012 for comparison.

Comorbidity	Number 2013	% 2013	% 2011	% 2012
AIDS/HIV	7	0.4	0.4	0.5
Any malignancy	474	26.8	23.5	23.4
Metastatic solid tumor	100	5.7	5.1	5.4
Diabetes without chronic complication	458	25.9	22.1	23.6
Diabetes with chronic complication	261	14.8	12.8	12.2
Dementia	86	4.9	3.5	4.1
Hemiplegia or paraplegia	24	1.4	2.0	1.5
Cerebrovascular disease	356	20.1	15.6	15.8
Myocardial infarction	185	10.5	10.7	10.7
Congestive heart failure	319	18.0	18.0	17.5
Chronic pulmonary disease	299	16.9	16.0	16.0
Peptic ulcer disease	147	8.3	8.5	8.3
Mild liver disease	162	9.2	9.5	9.1
Moderate or severe liver disease	83	4.7	4.3	4.1
Renal disease	355	20.1	19.7	20.0
Rheumatic disease	99	5.6	4.9	4.9
Peripheral vascular disease	282	15.9	13.7	15.1
Drug abuse	42	2.4	3.2	2.4

2.8 Typing

spa types were obtained from 1,723 isolates (97.4%). In total, 508 different *spa* types were identified, with ten *spa* types accounting for 33% of the isolates (Table 7). A total of 328 *spa* types were only encountered once. Putative assignment to MLST CC was possible for 1,442 isolates (82%). 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 40% of the SAB isolates in 2013 while the 10 most prevalent constituted 71% (Table 8). CC398 MRSA isolates have been associated with a reservoir in livestock. CC398 constituted 21 SAB cases (1.2%) in 2013 of which 4 were MRSA. Ten belonged to *spa* type t034 (three MRSA), five belonged to *spa* type t571, three to t1451, two to t011 (one MRSA) and one to t6575.

Table 7. Number and prevalence of the ten most prevalent *spa* types among Danish SAB episodes in 2013. Corresponding numbers and prevalences for the four previous years are shown for comparison.

spa type	2009	2010	2011	2012	2013
t230	91 (6.1)	86 (6.1)	81 (5.3)	90 (5.9)	93 (5.3)
t127	44 (3.0)	62 (4.4)	75 (4.9)	68 (4.5)	81 (4.6)
t002	57 (3.9)	55 (3.9)	40 (2.6)	59 (3.9)	73 (4.1)
t084	56 (3.8)	66 (4.7)	72 (4.7)	62 (4.1)	73 (4.1)
t015	40 (2.7)	55 (3.9)	46 (3.0)	46 (3.0)	53 (3.0)
t012	59 (4.0)	51 (3.6)	33 (2.2)	45 (2.9)	52 (2.9)
t021	45 (3.0)	23 (1.6)	33 (2.2)	41 (2.7)	43 (2.4)
t091	21 (1.4)	26 (1.8)	34 (2.2)	40 (2.6)	41 (2.3)
t008	34 (2.3)	32 (2.3)	32 (2.1)	42 (2.7)	41 (2.3)
t701	16 (1.1)	16 (1.1)	21 (1.4)	25 (1.6)	28 (1.6)

Table 8. Number and prevalence of the ten most prevalent CC groups among Danish SAB episodes in 2013. Corresponding numbers and prevalences for the four previous years are shown for comparison.

Clonal complex	2009	2010	2011	2012	2013
CC45	325 (22.0)	306 (21.6)	338 (22.2)	269 (17.6)	294 (16.6)
CC30	244 (16.5)	229 (16.1)	200 (13.2)	214 (14.0)	245 (13.8)
CC15	151 (10.2)	162 (11.4)	177 (11.6)	150 (9.8)	175 (9.9)
CC5	108 (7.3)	107 (7.5)	102 (6.7)	111 (7.3)	134 (7.6)
CC1	95 (6.4)	113 (8.0)	110 (7.2)	102 (6.7)	132 (7.5)
CC8	107 (7.2)	85 (6.0)	92 (6.1)	99 (6.5)	119 (6.7)
CC7	29 (2.0)	39 (2.7)	47 (3.1)	44 (2.9)	51 (2.9)
CC22	45 (3.0)	41 (2.9)	54 (3.6)	50 (3.3)	43 (2.4)
CC97	33 (2.2)	28 (2.0)	28 (1.8)	28 (1.8)	39 (2.2)
CC25	20 (1.4)	16 (1.1)	26 (1.7)	16 (1.0)	29 (1.6)

PVL was demonstrated among 27 SAB isolates (1.5%), of these were two methicillin-resistant *S. aureus* (MRSA; spa type t019). The PVL positive isolates were distributed among 17 different *spa* types and seven MLST CC groups; six isolates had an unresolved relationship with MLST typing. Of the 21 PVL positive isolates with an assigned CC group, 12 (57%) belonged to CC30.

2.9 Antimicrobial susceptibility testing

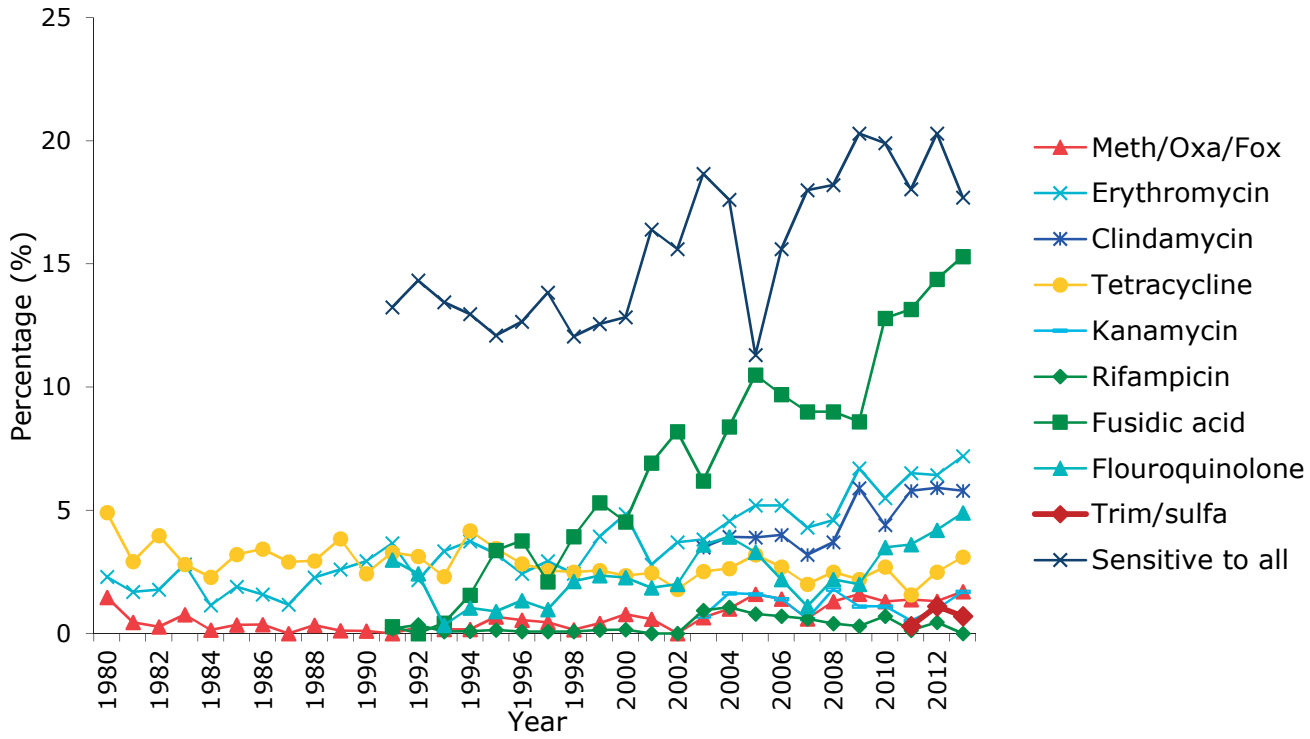
The prevalences of resistance to the tested antimicrobials are shown in Table 9. The susceptibility testing was performed by micro broth dilution in contrast to disk diffusion in previous years. A validation between the two methods demonstrated very good comparability (Larsen *et al.* 2014) and in Figure 5 resistance prevalences are shown from 1980 to 2013. The number of MRSA cases was 30 (1.7%). This proportion is comparable to proportions in recent years. All SAB isolates were susceptible to linezolid in 2013. Resistance to fusidic acid continued to increase (15.3%) compared to 2012. The proportion of isolates that was susceptible to all antibiotics was 17.7%. The proportion of resistance to one antimicrobial in addition to penicillin was 21.5% and the proportion of resistance to two and three additional antimicrobials were 7.9% and 3.8%, respectively.

Table 9. Distribution (%) of MICs (mg/L) and resistance (%) in SAB 2013 (n=962)

Antimicrobial	Resistance (%)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
Penicillin	76.0	10.4	11.4	2.2	76.0							
Cefoxitin	1.7								98.3	1.7		
Erythromycin	7.2				22.8	68.9	1.0	0.1	0.8	6.3		
Clindamycin	5.8			94.6	3.4	0.6	0.2	1.1				
Fusidic acid	15.3			11.5	55.3	15.8	2.1	1.7	2.7	2.5	6.3	2.0
Tetracycline	3.1					81.8	14.4	0.6	0.4	2.7		
Norfloxacin	4.9						72.2	20.7	2.2	1.1	3.7	
Rifampicin	0		99.8	0.2								
Linezolid	0						1.5	66.8	31.7			
Kanamycin	1.7										98.3	1.7
TMP/SXT*	0.7					95.8	1.8	1.0	0.6	0.7		
Ceftaroline	0.2			7.9	83.1	7.3	1.6	0.2				
Ceftobiprole	NA			0.3	36.7	59.3	3.2	0.5				
Daptomycin	0.5					85.6	13.9	0.4	0.1			
Gentamycin	1.7				51.5	39.7	7.2	1.1	0.5			
Moxifloxacin	1.6				97.2	0.8	0.4	0.8	0.7			
Mupirocin	0.1			6.3	77.3	15.9	0.3	0.1				
Teicoplanin	0						97.9	2.1				
Tigecycline	0		40.9	50	8.5	0.6						
Vancomycin	0					3.1	93.6	3.3				

** Trimethoprim/sulfamethoxazole. 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.*

Figure 5: Resistance patterns (1980-2013). Resistance to penicillin is not shown.



3. 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.

Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol. 41(12):5442-8.

Larsen AR, Petersen A, Skov R. 2014. Comparison of a custom made TREK panel with EUCAST disc diffusion for antimicrobial susceptibility testing of *Staphylococcus aureus*. Abstract at the 24th European Conference on Clinical Microbiology and Infectious Diseases, May 10 – 13, Barcelona, Spain.

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