ANNUAL REPORT ON STAPHYLOCOCCUS AUREUS BACTERAEMIA CASES IN DENMARK 2012 (part I)

Staphylococcus aureus Bacteraemia Annual Report, Part I

The annual report is published in two parts. Part I includes bacteriological characteristics of *Staphylococcus aureus* isolates (typing and antimicrobial susceptibility testing), and Part II additionally describes patient characteristics (age, gender, site of acquisition, co-morbidities and secondary manifestations). Since 1957 clinical and epidemiological information for the majority of patients with *S. aureus* bacteraemia (SAB) in Denmark have been registered at the Staphylococcus Laboratory at Statens Serum Institut (SSI).

Members of the Danish *Staphylococcus aureus* bacteraemia group

Paal Skytt Andersen, MSc PhD
Thomas Benfield, MD DMSc
Niels Frimodt-Møller, MD DMSc
Allan Garlik Jensen, MD DMSc
Anders Rhod Larsen, MSc PhD
Andreas Petersen, MSc PhD
Henrik C. Schønheyder, Prof. MD DMSc
Peter Skinhøj, Prof. MD DMSc
Robert Skov, MD
Henrik Westh, MD DMSc

Acknowledgement

The following Departments of Clinical Microbiology have collected and sent the SAB isolates reported in this annual report:

Esbjerg
Herlev
Herning
Hillerød
Hvidovre
Nykøbing Falster
Odense
Rigshospitalet
Slagelse
Statens Serum Institut
Vejle
Viborg
Aarhus
Aalborg

Lone Ryste Hansen Kildevang, Julie Hindsberg Nielsen, and Stine Frese-Madsen, Staphylococcus Laboratory, are thanked for excellent technical assistance.

Summary

This annual report is based on data from the 1,528 bacteraemia cases submitted to the Staphylococcus Laboratory by 14 Danish Departments of Clinical Microbiology in 2012.

Typing of SAB isolates

A total of 483 different *spa* types were demonstrated in SAB isolates from 2012. The five predominant *spa* types were t230 (5.9%), t127 (4.5%), t084 (4.3%), t002 (3.9%) and t015 (3.0%). CC45 was the largest clonal complex including 17.7% of all isolates, followed by CC30 (14.1%), CC15 (10.3%), CC5 (7.3%) and CC1 (6.7%).

Antimicrobial susceptibility

The present annual report includes susceptibility testing using disk diffusion performed according to the methodology and breakpoints of EUCAST. The following antimicrobial agents were tested:

Penicillin, cefoxitin, erythromycin, clindamycin, tetracycline, kanamycin, rifampin, fusidic acid, norfloxacin, linezolid, mupirocin and trimethoprim-sulfametoxazole.

Resistance frequencies for 2012 are shown with 2007-2011 for comparison in Table 3. The proportion of methicillin resistant *S. aureus* (MRSA) was 1.2%, which is comparable to previous years. Fusidic acid resistance remained at a high level (14.4%). For the other antimicrobials, frequencies were at similar levels as previous years (Table 3 and Figure 2). Resistance varied between different *spa* types (Table 4). Most notably were 79.4% of *spa* type t127 resistant to fusidic acid (average for all isolates were 14.4%).

Panton-Valentine Leukocidin (PVL)

The genes encoding Panton Valentine Leukocidin (*lukS/F-PV*) were detected in 12/1,528 (0.8%) of SAB isolates.

Materials and methods

Staphylococcus aureus bacteraemia (SAB) isolates

SAB isolates were referred from the regional Departments of Clinical Microbiology (DCM) to the Staphylococcus Reference Laboratory, SSI. Isolates from the same patients were regarded as belonging to separate episodes if the intervening period was more than one month.

spa typing and PCR detection of resistance and virulence genes

The Spa gene was amplified and sequenced and all isolates were investigated for the presence of the *mecA* and *mecC* genes and the Panton-Valentine leukocidin genes by PCR (Stegger et al 2012). The isolates were analysed and *spa* types were annotated using Bionumerics 6.1 (Applied Maths, Sint-Martens-Latem, Belgium) and Ridom StaphType 1.4 (Ridom GmbH, Würzburg, Germany). *spa* types were assigned to multi locus sequence typing (MLST) clonal complexes (CC).

Antimicrobial susceptibility testing

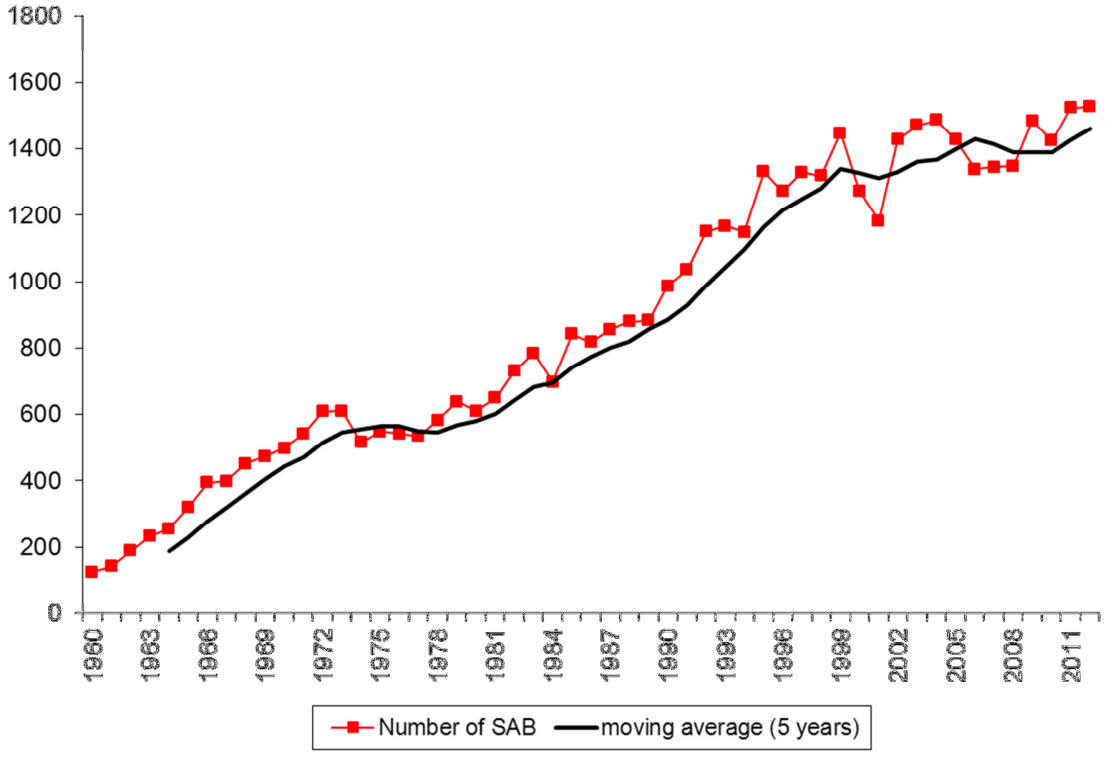
Susceptibility testing was performed by disc diffusion according to EUCAST methodology using discs from Oxoid (Ballerup, DK) on Muller Hinton Agar (SSI, Copenhagen, DK). The following antibiotics were tested: Erythromycin, clindamycin, kanamycin, rifampicin, penicillin, cefoxitin, fusidic acid, norfloxacin, linezolid, tetracycline, trimethoprim-sulfametoxazole and mupirocin. Intermediate zone diameters were reported as susceptible. Differences in resistance prevalence were examined with 2x2 contingency table and Fisher's exact test.

MRSA were screened for glycopeptide resistance using spot test on agar plates containing teicoplanin (5 mg/L) (Fitzgibbon et al. 2007). Isolates demonstrating 10 or more colony forming units in the spot were subjected to the Etest macro method. Brain Heart Infusion agar plates (BD) were inoculated with a McFarland 2 suspension, incubated at 35-36°C in atmospheric air. Etest values were read after 24 and 48 hours. Isolates with MIC- values ≥ 8 mg/L for both vancomycin and teicoplanin or an MIC ≥ 12 mg/L for teicoplanin were tested with the PAP-AUC method (Walsh et al. 2001)

Results

A total number of 1,528 SAB cases was received in 2012. This is the highest number during the whole period (Figure 1). The 5-years moving average demonstrates a steady increase from 1960 to present but in the last decade the increase has been less pronounced. In total 1,437 patients were diagnosed with SAB of whom 71 had two episodes of SAB and nine had three or more episodes. This corresponds to an incidence rate of SAB of 26.9 (patients)/100,000 inhabitants and 28.6 (cases)/100,000 inhabitants.

Figure 1. Number of *S. aureus* bacteraemia (SAB) cases 1960-2012 and 5 years moving average



Typing

spa types were obtained from 1,499/1,528 isolates (98%). The remaining isolates were indeterminate either because the isolates were *spa* negative or had deviating repeats. In total, 483 different *spa* types were identified, with ten predominant *spa* types comprising 34% of the isolates (Table 1). Nine of the ten most predominant types in 2012 were also among the most frequent in 2011 (Table 1).

Table 1. Predominant *spa* types among Danish *S. aureus* bacteraemia isolates in 2011 and 2012

Rank 2012	*spa* type	MLST CC	Number of isolates in 2012 (percent of total isolates)	Rank in 2011 (percent of total isolates)
1	t230	CC45	90 (5.9)	1 (5.3)
2	t127	CC1	68 (4.5)	2 (4.9)
3	t084	CC15	66 (4.3)	3 (4.7)
4	t002	CC5	60 (3.9)	5 (2.6)
5	t015	CC45	46 (3.0)	4 (3.0)
6	t012	CC30	45 (2.9)	6 (2.3)
7	t008	CC8	44 (2.9)	9 (2.1)
8	t021	CC30	41 (2.7)	8 (2.2)
9	t091	CC7	40 (2.6)	7 (2.2)
10	t701	CC8	25 (1.6)	15 (1.4)

Annotation to multi locus sequence type clonal complex (MLST CC) inferred from *spa*-repeats was possible for 1,276 isolates (84 %). The isolates were grouped into 26 different MLST CC groups (Table 2). CC45 was as in the previous years the most prevalent group constituting 17.7% of the isolates and contained a total of 58 different *spa* types. In Figure 2 the prevalence of the ten most common clonal complexes in 2012 is shown during 2007-2012. The proportion of CC45 was 21-23% in 2007-2011 but decreased in 2012. The other major CC groups showed no larger changes in prevalence during 2007-2012.

Figure 2. Prevalence of the ten most common clonal complexes in 2012 during 2007-2012.

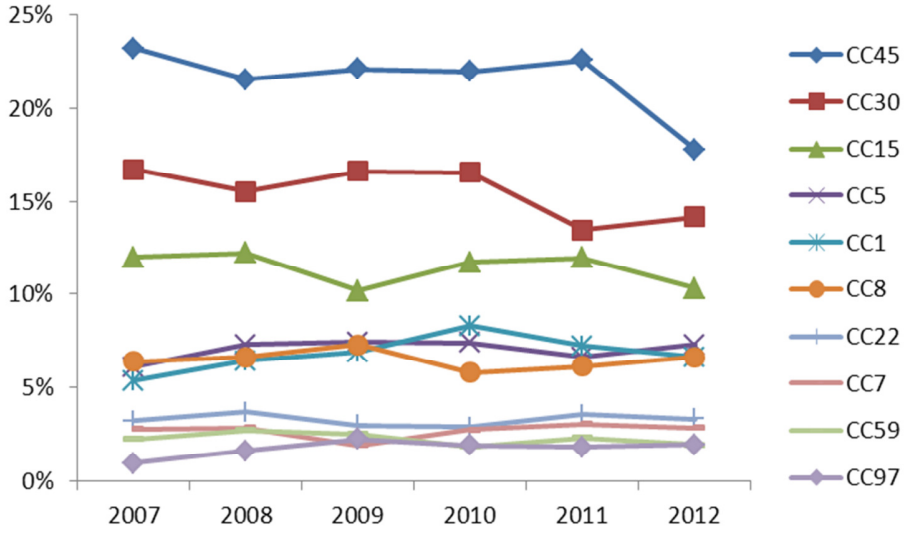


Table 2. Frequencies of multi locus sequence type (MLST) clonal complex (CC) based on *spa* types.

MLST CC	Number of isolates 2012 (% of total)	% of total in 2011	Number of different *spa* types	Dominating *spa* type(s)
CC45	271 (17.7)	22.2	58	t230, t015, t065, t026, t050
CC30	215 (14.1)	13.3	58	t012, t021, t363
CC15	157 (10.3)	11.6	28	t084
CC5	112 (7.3)	6.7	25	t002
CC1	102 (6.7)	7.2	13	t127
CC8	101 (6.6)	6.0	18	t008, t701
CC22	51 (3.3)	3.6	16	t005
CC7	44 (2.9)	3.1	5	t091
CC59	30 (2.0)	2.4	6	t216
CC97	29 (1.9)	1.8	6	t267
CC101	22 (1.4)	0.9	5	t056
CC509	21 (1.4)	1.2	4	t375
others*	121			
unknown or unassigned	252			

* CC20, CC25, CC12, CC398, CC121, CC182, CC72, CC88, CC130, CC9, CC80, CC151, ST152/377 and CC50

Antimicrobial Susceptibility Testing

Resistance to the tested antimicrobials in 2012 is shown in Table 3. Results from 2007-2011 are provided for comparison. The proportion of MRSA was 1.2% which is comparable to the levels in recent years. The new *mecC* gene was demonstrated in one of the 19 MRSA SAB (*spa* type t1773). Three of the MRSA isolates were positive in the teicoplanin spot test but the MIC for teicoplanin was 3 mg/L for all isolates, determined by Etest macro method. Resistance to fusidic acid continued to be high (14.4%, highest rate recorded). Erythromycin resistance was 6.4% and clindamycin was 5.9%. Resistance to the remaining antimicrobials were below 5%, with the exception of penicillin (74.2%) (Table 3). The proportion of isolates that was susceptible to all antimicrobials was 20.9%. Resistance to at least one other antimicrobial in addition to penicillin increased from 14.6% to 25.2% (Table 3). In Figure 3 resistance percentages of Danish *S. aureus* bacteraemia isolates 1980-2012 are shown together with percentage of isolates sensitive to all tested antimicrobials.

Table 3. Antimicrobial resistance (%) among Danish *S. aureus* bacteraemia isolates 2007-2012

Resistance to	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Penicillin	78.2	77.4	75.4	74.6	76.8	74.2
Cefoxitin	0.6	1.3	1.6	1.4	1.4	1.2
Erythromycin	4.3	4.5	6.7	5.4	6.5	6.4
Clindamycin	3.2	3.7	5.9	4.3	5.8	5.9
Tetracycline	2.0	2.5	2.2	2.7	1.6	2.5
Kanamycin	0.6	1.0	1.1	1.1	0.5	1.0
Rifampicin	0.6	0.4	0.3	0.7	0.1	0.5
Fusidic acid	9.0	8.9	8.6	12.9	13.2	14.4
Norfloxacin	1.1	2.2	2.0	3.4	3.6	4.2
Linezolid	0	0	0	0	0	0
Mupirocin	0.5	0.6	0.4	0.4	0.1	0.1
Trimethoprim-sulfametoxazole	NT	NT	NT	NT	0.3	1.1
Multi-resistance						
Penicillin + 1	11.9	14.7	15.3	22.5	14.6	25.2
Penicillin + 2	3.7	4.6	6.1	6.6	5.8	8.6
Penicillin + 3	1.6	1.0	1.8	2.2	1.3	2.1
Sensitive to all antimicrobials	18.0	16.5	21.6	19.1	18.6	20.9

NT= Not Tested

The resistance frequencies for the most frequent *spa* types are listed in Table 4. The frequency of resistance seems in part to depend on the genetic background of the isolates. Thus, resistance to penicillin was significantly higher in isolates belonging to t012, t084, and t021 compared with all isolates and lower in t230 and t002. Fusidic acid resistance was unevenly distributed between the most prevalent *spa* types: range 4.5% to 79.4% for t127 (all isolates 14.4%).

Table 4. Antimicrobial resistance (%) in major *spa* types

spa type	t230	t127	t084	t002	t015	t012	t008	t021	t091	t701
Number of isolates	90	68	66	60	46	45	44	41	40	25
Antimicrobial										
Penicillin	62.2	66.2	92.4	61.7	78.3	100	70.5	92.7	85.0	72.0
Erythromycin	2.2	7.4	3.0	10	8.7	0	15.9	4.9	7.5	0
Clindamycin	2.2	5.9	3.0	10	6.5	0	15.9	0	7.5	0
Tetracycline	1.1	0	6.1	0	2.2	0	0.0	7.3	2.5	0
Fusidic acid	7.8	79.4	4.5	5.0	15.2	6.7	38.6	4.9	5.0	32.0
Norfloxacin	2.2	2.9	3.0	3.3	4.3	2.2	2.3	4.9	2.5	4.0

Figure 3. Resistance percentages of Danish *S. aureus* bacteraemia isolates (1980-2012) and proportion of isolates sensitive to all tested antimicrobials.

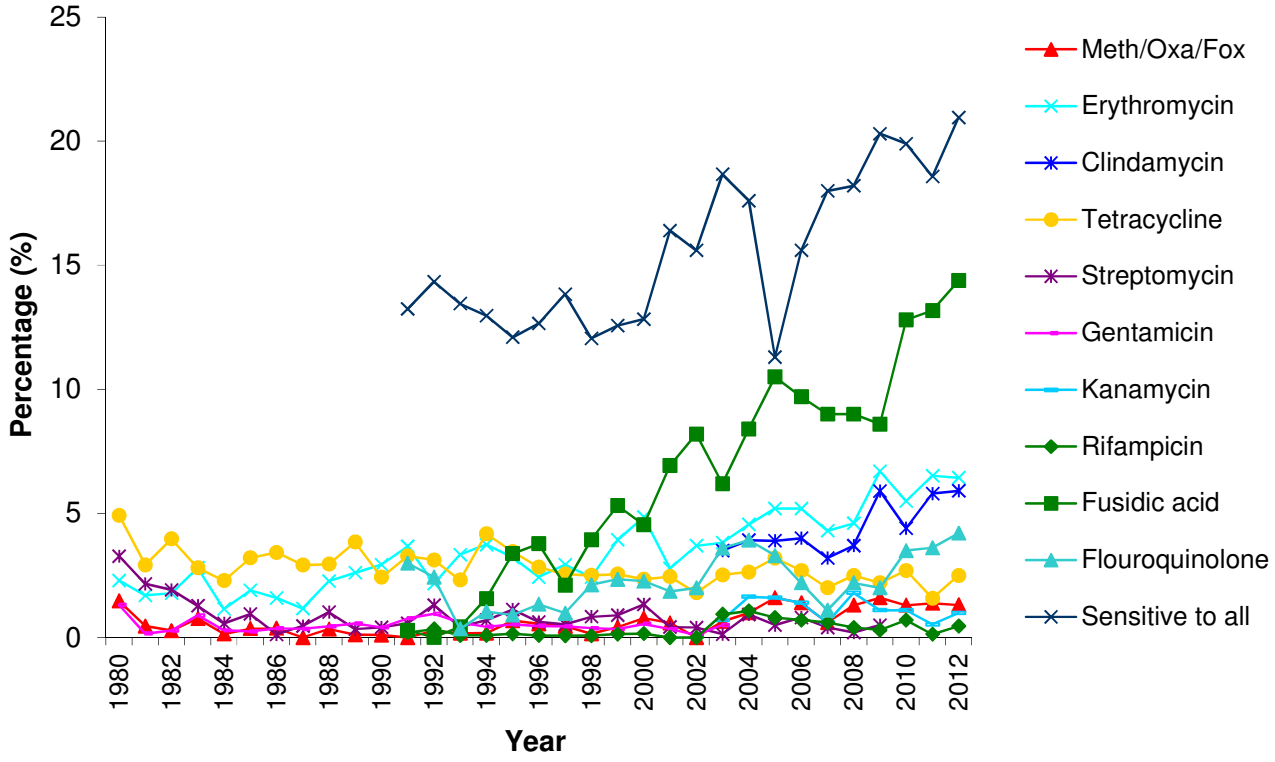


Figure note: Due to the long time span over which susceptibility has been monitored the antimicrobials tested have changed. Thus, in 2003 kanamycin replaced gentamicin, norfloxacin replaced ciprofloxacin and cefoxitin replaced oxacillin, which had replaced methicillin in 2001. Penicillin, linezolid, mupirocin and trimethoprim-sulfamethoxazole are not shown.

Panton-Valentine Leukocidin (PVL)

The genes encoding Panton Valentine Leukocidin (*lukS/F-PV*) were detected in 12/1,528 (0.8%) of SAB isolates. Three of the isolates were MRSA (two of *spa* type t044 and one of t019). Among the 9 PVL positive MSSA two isolates were t005, two were t021, two were t645 and one each of t105, t355 and t433.

References

Fitzgibbon MM, Rossney AS and O'Connell B. 2007. Investigation of reduced susceptibility to glycopeptides among methicillin-resistant *Staphylococcus aureus* isolates from patients in Ireland and evaluation of agar screening methods for detection of heterogeneously glycopeptide-intermediate *S. aureus*. J Clin Microbiol. 45:3263-3269.

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

Walsh TR, Bolmström A, Qwärnström A, Ho P, Wootton M, Howe RA, MacGowan AP and Diekema D. 2001. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J Clin Microbiol. 39:2439-2444.

Staphylococcus Laboratory
Artillerivej 5
2300 Copenhagen S
Denmark www.ssi.dk