The National Reference Centre (NRC) for *S. aureus* of Université Libre de Bruxelles (ULB) provides the following tasks:

- Identification and antimicrobial susceptibility testing of *Staphylococcus sp*. strains using:
  - ❖ Phenotypic methods: protein profiles (Maldi-TOF), biochemical tests, minimal inhibitory concentration (MIC)
  - ❖ Genotypic methods: detection by PCR of *nuc* gene (*S. aureus* identification), *mec*A and *mecC* genes (coding for resistance to oxacillin), *mup*A gene (coding for mupirocin resistance), *cfr* gene (coding for resistance to linezolid) and genes coding for resistance to macrolides-lincosamides-streptogramines (MLS), tetracyclines and aminoglycosides.
- Detection of genes coding for exfoliatins A, B and D, Panton-Valentine leucocidin (PVL), Toxic Shock Syndrome Toxin (TSST-1), enterotoxins (*se*a to *se*e, *se*g to *se*i and *se*r to *se*t) and enterotoxin-like (*sel*i, *sel*k to *sel*q and *sel*u).
- Molecular typing: pulsed field gel electrophoresis (PFGE) after genomic macrorestriction, multi-locus sequence typing (MLST), *spa* sequence typing, characterisation of the staphylococcal cassette chromosome *mec* (SCC*mec*), determination of *agr* group and detection of the arginine catabolic mobile element (ACME) - *arc*A gene.

These analyses are performed on clinical staphylococcal isolates causing diagnostic problems or collected during epidemiological investigations. Request forms are available on websites of the NRC (http://www.mrsa.be) or ISP-WIV (https://nrchm.wiv-isp.be).

The Microbiology laboratory including the NRC - *S. aureus* is accredited according to standard ISO15189. The list of accredited analyses is available on the BELAC website (http://economie.fgov.be/belac.jsp).

Characterisation of atypical clinical strains

In 2014, the NRC identified and/or determined the antimicrobials susceptibility of 113 clinical staphylococcal isolates. None MRSA strain showed a decreased susceptibility to glycopeptides (GISA). Among the 80 isolates received for confirmation of oxacillin resistance, three (4%) cryptic MRSA isolates, containing *mec*A gene but presenting phenotypic susceptibility to oxacillin (MIC < 2 µg/mL), were found. On the other side, eight (10%) isolates resistant to oxacillin (MIC > 2 µg/mL) but lacking *mec*A gene were detected. The *mec*C gene was found in three (4%) isolates. *Staphylococcus* containing *mec*C gene are difficult to detect by routine laboratory methods, particularly by conventional PCRs. Resistance to mupirocin was determined by MIC and *mup*A detection for 32 isolates, among these 12 (37%) showed a high level resistance to mupirocin (MIC>524 µg/mL) and the presence of *mup*A gene.

Toxin detection and characterisation of community-acquired (CA) *S. aureus* strains

In 2014, 408 isolates of *S.aureus* including 181 MRSA and 227 MSSA were sent to the NRC for toxins detection (Figure 1).

Seventy-eight (43%) MRSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). These MRSA isolates were principally recovered from skin lesions, in particular from skin abscess, soft tissues or furunculous (n=53) but also from deep fluids (n=7), screenings (n=7),blood cultures (n=2 or unknown (n=9)).

By molecular typing, most of PVL positive MRSA isolates (n=56, 72%) belonged to one of the three following clones: ST8-SCC*mec* IV (n=43), European clone ST80-SCC*mec* IV (n=8) and ST30-SCC*mec* IV (Southwest Pacific clone) (n=5) (Figure 2). Thirty-five of the 43 (81%) isolates belonging to the clone ST8-SCC*mec* IV contained the pathogenicity island ACME characteristic of MRSA USA300. Among these 78 CA-MRSA isolates, 8 isolates were identified as belonging to the Taiwan ST59 clone.

Figure 1: Number of MRSA and MSSA isolates received for PVL detection, 2005-2014

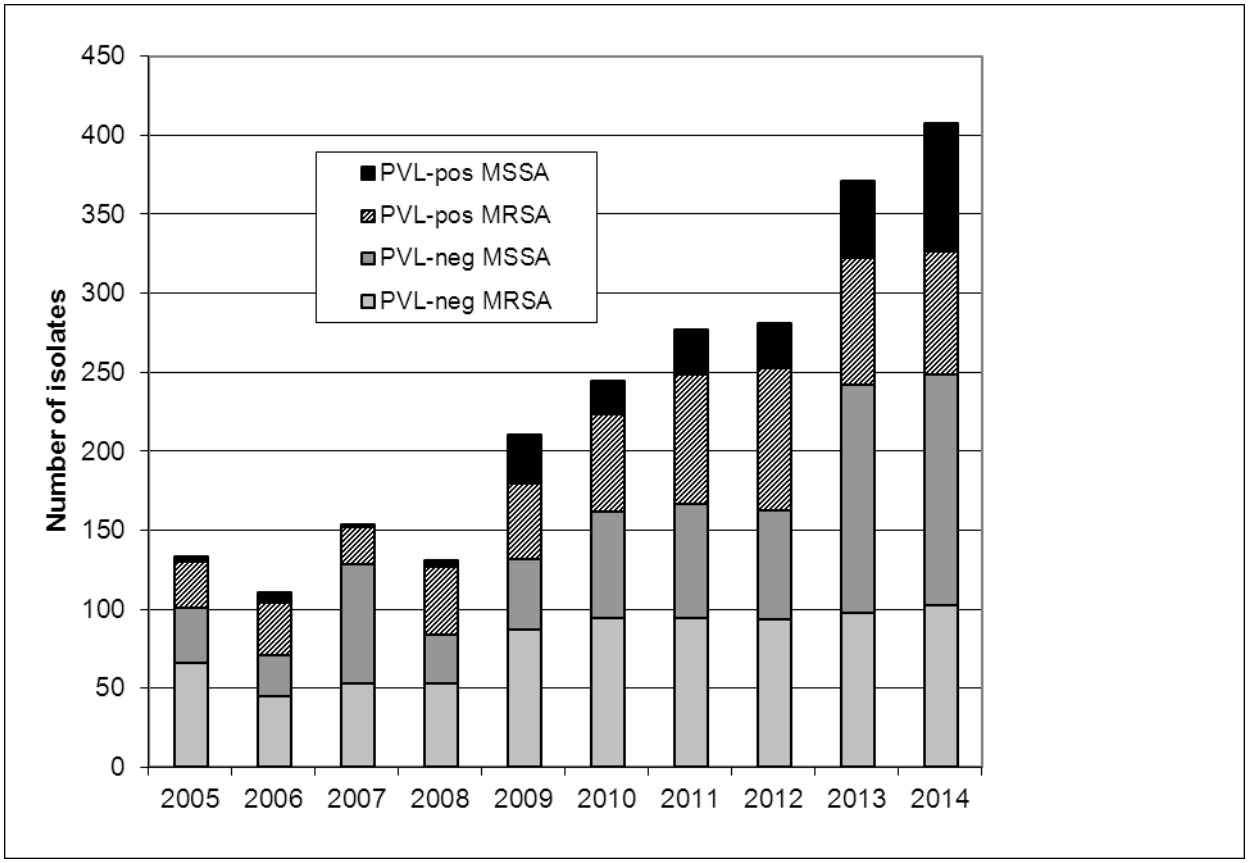
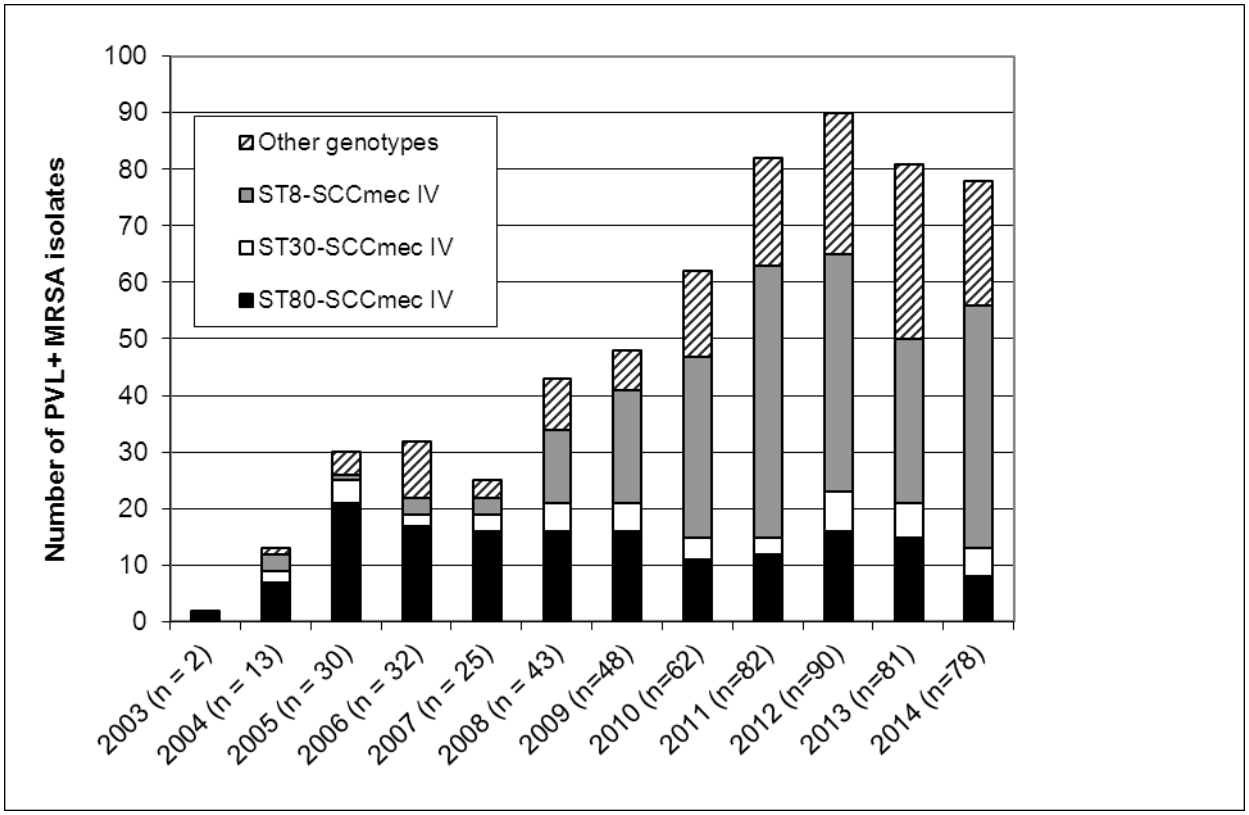


Figure 2: Evolution of major genotypes recovered from PVL positive CA-MRSA, 2003-2014



Eighty-one (36%) MSSA isolates contained *luk*S-*luk*F genes coding for Panton-Valentine leucocidin (PVL). Molecular typing of these 81 PVL positive MSSA isolates revealed more genomic diversity than for MRSA isolates. The most frequent genotypes were those belonging to clones ST30 (n=12), ST121 (n=6) ST80 (n=2) and ST1 (n=2).

TSST-1 toxin was detected in 13 MRSA (7%) and 33 MSSA (15%) isolates. TSST-1 positive isolates were recovered from skin lesions (n=23), deep fluids (n=8), screenings (n=6), blood cultures (n=3), urines (n=3) or other sites (n=3). Molecular typing showed that majority of TSST-1 positive isolates belonged to ST30 (41%) or ST22 (22%).

Genes coding for exfoliatins A and B were found in 5 (MSSA isolates recovered from skin lesions and belonging to clone ST121 corresponding to the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC). Gene coding for exfoliatin A alone was recovered in 4 MSSA isolates belonging to clones ST121, ST88 and ST109. Gene coding for exfoliatin B alone was found in one MSSA isolate belonging to clone ST121.

Table 1: Percentage of antimicrobial resistance of MRSA isolates received for toxin detection

Antimicrobials	Antimicrobial resistance of MRSA isolates (%)		
	PVL positive (n=78)	PVL negative (n=103)	Total (n=181)
Erythromycin	67	48	57
Clindamycin	21	47	36
Ciprofloxacin	34	51	44
Gentamycin	3	14	9
Tobramycin	5	36	23
Kanamycin	67	51	58
Minocycline	0	16	9
Tetracycline	30	50	41
Rifampin	0	0	0
Cotrimoxazole	3	1	2
Linezolid	0	0	0
Fusidic acid	11	7	8
Mupirocin	0	1	<1

Table 2: Percentage of antimicrobial resistance (%) of MSSA isolates received for toxin detection

Antimicrobials	Antimicrobial resistance of MSSA isolates (%)		
	PVL positive (n=79)	PVL negative (n=142)	Total (n=221)
Erythromycin	23	25	24
Clindamycin	13	20	17
Ciprofloxacin	6	3	4
Gentamycin	2	0	<1
Tobramycin	8	1	3
Kanamycin	13	3	6
Minocycline	3	0	<1
Tetracycline	3	4	3
Rifampin	0	0	0
Cotrimoxazole	4	<1	2
Linezolid	0	0	0
Fusidic acid	9	6	7
Mupirocin	0	0	0

Typing for epidemiological investigations

In 2014, molecular typing using *spa* typing and/or PFGE analysis was performed on 477 *S. aureus* isolates including 218 MRSA and 259 MSSA. Among these, 54 MRSA isolates and 55 MSSA isolates were sent for epidemiological investigation of local outbreaks (n=25, 15 MRSA outbreaks and 10 MSSA outbreaks).

The 54 MRSA isolates recovered from 11 hospitals were classified into 9 distinct genotypes. The most frequently recovered genotypes were those previously found in our Belgian hospitals: ST8-SCC*mec* IV found in 26% of MRSA isolates recovered from 5 hospitals, ST5-SCC*mec* II or IV found in 24% of MRSA isolates recovered from 5 hospitals and ST45-SCCmec IV found in 13% of MRSA isolates recovered from 3 hospitals. Genotype ST1-SCC*mec* IV corresponding to clone USA400 was recovered from an outbreak in a single hospital. Molecular typing allowed confirmation of horizontal transmission of MRSA isolates in 10 of the 15 clusters investigated.

The 55 MSSA isolates recovered from 10 hospitals showed high diversity with 18 distinct genotypes. The most frequent genotypes were those recovered from MRSA isolates, ST5 (15%), ST30 (11%) and ST8 (9%).

European Antimicrobial Resistance Surveillance Network (EARS-Net) program on *S. aureus* isolated from blood cultures

Since 2005, all MRSA and MSSA bacteraemia are subject to mandatory reporting on a request form including clinical and microbiological data in collaboration with the Public Health Scientific Institut (Scientifique de Santé Publique) (WIV-ISP).

In 2013, 1612 patients with a first *S. aureus* bacteraemia episode (per trimester) were recorded. Among these patients, 272 cases of MRSA bacteraemia were confirmed corresponding to a proportion of 16.9% of MRSA (Table 3). Data for 2014 are not yet available.

Data from all countries participating to the EARS-Net program were available on the website:
http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx

Table 3: EARS-Net Data on oxacillin resistance of *S. aureus* isolated from blood cultures in Belgium, 1999-2013

year	Number			Total	Percentage		
	S	I	R	N	S	I	R
1999	340	0	105	445	76.4	0.0	23.6
2000	520	0	137	657	79.1	0.0	20.9
2001	729	0	213	942	77.4	0.0	22.6
2002	783	0	309	1092	71.7	0.0	28.3
2003	798	0	336	1134	70.4	0.0	29.6
2004	819	0	408	1227	66.7	0.0	33.3
2005	719	0	329	1048	68.6	0.0	31.4
2006	670	0	188	858	78.1	0.0	21.9
2007	656	0	199	855	76.7	0.0	23.3
2008	719	0	187	906	79.4	0.0	20.6
2009	748	0	200	948	78.9	0.0	21.1
2010	840	0	217	1057	79.5	0.0	20.5
2011	1440	0	304	1744	82.6	0.0	17.4
2012	1308	0	260	1568	83.4	0.0	16.6
2013	1340	0	272	1612	83.1	0.0	16.9

Analyse of *S. aureus* from animal origin

Eighteen (8%) (18/ 218) MRSA isolates belonging to clone ST398, called livestock-associated MRSA and corresponding to strains from animal origin, were detected in hospitalised or ambulant patients in Flanders (n=16), Wallonia (n=1) and Brussels (n=1). These MRSA were isolated from screenings (n=7), skin lesions (n=3), blood cultures (n=3) or other sites (n=5). Available data allowed to bring out that seven patients had direct contact with animals (farmers, vets).

Ten (4%) (10/ 259) MSSA isolates belonging to clone ST398 were identified. These MSSA ST398 were isolated from skin lesions (n=6), screenings (n=3) and deep fluid (n=1). However, ST398 MSSA strains are not associated with livestock contacts.

None toxins was detected in these MRSA and MSSA ST398 strains.

National surveillance of MRSA, MSSA and coagulase negative Staphylococcus (CoNS) in acute-care hospitals

In 2013, a national surveillance survey was conducted in acute-care Belgian hospitals (October 2013 - March 2014). In this study, in addition of MRSA (n=3) and MSSA (n=2) isolates, a clinically relevant CoNS isolated from blood cultures was also collected by laboratories. A clinical relevant CoNS is defined as a CoNS isolated from a single patient from at least two pairs of blood cultures obtained during a single bacteraemic episode, belonging to the same species and with identical antimicrobial profiles.

Except for antimicrobial susceptibility of MRSA and MSSA isolates that will be presented in this report, all data were previously described in the NRC *S. aureus* report 2013, published in 2014.

Antimicrobial susceptibility

For MRSA and MSSA, MICs to 19 antimicrobials were determined by microdilution method using sensititre (TREK Diagnostic Systems) according to CLSI guidelines (2014).

Table 4 shows antimicrobial susceptibility results of the 288 MRSA isolates collected in 2013 compared to data from previous surveillance surveys. All MRSA were susceptible to glycopeptides and linezolid. All but one MRSA isolates were susceptible to

tigecycline. More than 90% of MRSA were susceptible to gentamycin (96%), minocycline (92%), rifampin (99%), cotrimoxazole (98%) and mupirocin (93%). Majority of MRSA were susceptible to fusidic acid (88%) and tetracycline (79%). Resistance to MLS was relatively frequent, with 44% of resistance to erythromycin and 29% to clindamycin. For aminoglycosides, resistance to kanamycin (40%) and tobramycin (39%) was more frequent than resistance to gentamycin (4%). Eighty-five percent of MRSA isolates were resistant to ciprofloxacin.

In 2013, compared to previous surveillance surveys, MRSA isolates were slightly more susceptible to MLS and ciprofloxacin. In contrast, MRSA became more resistant to tetracycline, fusidic acid and mupirocin.

Evolution of antimicrobial resistance other than β-lactames is shown for MSSA in Table 5. Majority of MSSA isolates remained susceptible to antimicrobials tested (>90%), except for MLS.

Table 4: Evolution of antimicrobial resistance of MRSA during national surveillance surveys, 2003 – 2013

Antibiotics	**2003 (n=511)**		**2005 (n =335)**		**2008 (n=314)**		**2011 (n=313)**		**2013 (n=288)**	
(Critique Concentrations in µg/ml)	**% R**	**% I**	**% R**	**% I**	**% R**	**% I**	**% R**	**% I**	**% R**	**% I**
Oxacillin (2-4)	100	0.0	99.9	0.0	100	0.0	99	0.0	99	0.0
Vancomycin (4-32)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Teicoplanin (8-32)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythromycin (0.5-8)	59.3	0.0	64.0	0.0	59.3	0.0	53	1	44	<1
Clindamycin (0.5-4)	38.6	0.0	37.6	0.2	38.6	0.0	40	0.0	29	<1
Ciprofloxacin (1-4)	97.8	0.6	94.5	0.4	97.8	0.6	93	<1	85	1
Gentamycin (4-16)	4.9	0.0	11.2	0.2	4.9	0.0	1	1	4	<1
Tobramycin (4-16)	44.4	0.8	45.3	1.3	44.4	0.8	38	2	38	1
Kanamycin (16-64)	37.8	2.9	NT	NT	37.8	2.9	35	6	39	1
Minocycline (4-16)	4.1	1.6	4.6	2.0	4.1	1.6	1	4	8	<1
Tetracycline (4-16)	9.6	0.8	NT	NT	9.6	0.8	8	4	21	<1
Tigecycline (≤0.5)	0.0	0.0	NT	NT	0.0	0.0	0.0	0.0	<1	0.0
Rifampicine (1-4)	1.4	1.6	3.5	2.0	1.4	1.6	<1	<1	0.0	<1
Cotrimoxazole (2-4)	0.0	0.0	0.7	0.0	0.0	0.0	1	0.0	2	0.0
Linezolid (≤ 4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fusidic acid (2-32)	0.8	0.6	0.7	5.8	0.8	0.6	6	0.0	12	0.0
Mupirocin (2-1024)	3.5	2.9	3.5	6.8	3.5	2.9	4	2	7	0.0

NT, not tested

Table 5: Evolution of antimicrobial resistance of MSSA during national surveillance surveys, 2003 – 2013

Antibiotics (Critique Concentrations in µg/ml)	2003 (n = 102)		2005 (n = 216)		2008 (n = 211)		2011 (n=210)		2013 (n =196)	
	% R	% I	% R	% I	% R	% I	%R	%I	% R	% I
Oxacilline (2-4)	NT	NT	0.0	0.0	0.0	0.0	0.0	0.0	<1	0.0
Vancomycine (4-32)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Teicoplanine (8-32)	NT	NT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythromycin (0.5-8)	21.6	0.0	20.4	1.4	17.5	4.2	25	2	22	4
Clindamycin (0.5-4)	3.9	0.0	4.6	0.0	3.3	0.0	6	0.0	5	<1
Ciprofloxacin (1-4)	NT	NT	7.8	5.0	9.0	0.4	6	0.0	9	<1
Gentamycin (4-16)	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tobramycin (4-16)	2.0	2.0	0.4	0.4	0.4	0.4	1	0.0	1	0.0
Kanamycin (16-64)	2.0	1.0	0.9	0.0	0.9	0.0	1	<1	1	0.0
Minocycline (4-16)	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	<1	0.0
Tetracycline (4-16)	4.0	0.0	3.6	0.0	3.3	0.9	5	0.0	2	0.0
Rifampin (1-4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<1	0.0
Cotrimoxazole (2-4)	0.0	0.0	1.3	0.0	0.0	0.0	1	0.0	<1	0.0
Linezolid (≤ 4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fusidic acid (2-32)	1.0	0.0	0.4	3.1	1.9	1.9	10	0.0	9	0.0
Mupirocin (2-1024)	0.0	0.0	1.4	0.5	1.4	0.0	0	<1	2	0.0

NT, not tested

Conclusions

In 2014, the number of PVL- positive MRSA cases per year remained stable compared to 2013, 78 cases in 2014 versus 81 cases in 2013. The antimicrobial resistance profile of these PVL- positive MRSA remained unchanged. The proportion of CA-MRSA belonging to clone ST8-SCC*mec* IV increased compared to 2013 (36% to 55%) but percentage of clone USA-300 remained stable (80%). A diminution of the proportion of CA-MRSA isolates belonging to clone ST80-SCC*mec* IV was observed (from 19% to 10%).

The number of MSSA isolates received for toxin detection increased compared to the 3 previous years (227 in 2014 versus 97 to 192 in 2011- 2013). The proportion of PVL-positive MSSA cases is also in augmentation with 36% (n=81) detected in 2014 compared to 25% – 29% identified from 2011 to 2013. High diversity of genotypes was observed including the most frequent clones ST30 (15%) and ST121 (7%).

Genotyping of MRSA from local outbreak in hospitals showed that MRSA isolates belonged mainly to nosocomial epidemic clones ST8-SSC*mec* IV, ST45-SCC*mec* IV and ST5 SCC*mec* IV ou II.

Antimicrobial resistance profiles of MRSA and MSSA isolates collected during the national surveillance study conducted in Belgian hospitals in 2013-2014 showed that glycopeptides, tigecycline and linezolid showed excellent activity against MRSA and MSSA isolates. A high proportion of MRSA were resistant to fluoroquinolones (95%) and MLS (44%). Except resistance to erythromycin (22%) and fusidic acid (9%), MSSA isolates remained susceptible to majority of antimicrobials tested.

As in 2013, the livestock-associated MRSA clone ST398 was recovered in persons living principally in Flanders and with direct contact with animals like farmers or vets. These MRSA from animal origin was sporadically (8%) isolated in Belgian hospitals. MSSA strains, presenting the same genotypic characteristics than MRSA ST398, were also rarely (4%) recovered from persons without livestock contact.

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