

Low proportion of mupirocin resistance in *S. aureus* isolates collected from four Belgian national wide surveillances between 2005 and 2014.

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OBJECTIVES

Mupirocin is a topical antimicrobial that has been widely used for decolonisation strategies of *Staphylococcus aureus*. Resistance to mupirocin were rapidly reported reducing the effectiveness of eradication regimens. Two categories of resistance to mupirocin are described; low level resistance (MLLR) (MIC 2-256 mg/L), resulting of independent spontaneous mutation events in the gene encoding isoleucyl-tRNA synthetase (*ileS*), and high level resistance (MHLR) (MIC \geq 512 mg/L) acquired by a plasmid-encoded *mupA* gene or by a novelty described *mupB* gene. The aim of the study was to determine the prevalence of mupirocin resistance and to explore resistance mechanisms among a large collection of *S. aureus* isolates (n = 1971).

METHODS

Bacterial strains

A total of 1244 MRSA isolates and 727 MSSA isolates, collected from four nationwide surveillances conducted between 2005 and 2014 (n=100-116 Belgian hospitals), were analysed for their resistance to mupirocin. Identification and oxacillin resistance were confirmed by PCR.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was realised by agar dilution or broth microdilution method. MHLR was confirmed by E-Test.

Detection of mupirocin resistance

A new multiplex PCR was performed for the detection of the *mupA*/*mupB* genes involved in MHLR. MLLR was further analysed by sequencing a fragment of the *ileS* gene.

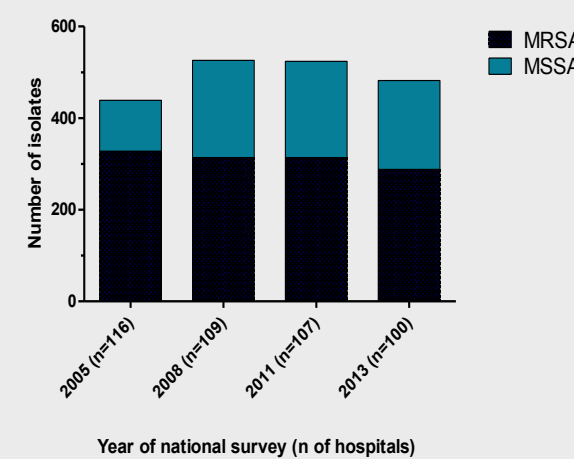
Target identification	Primer	Séquence (5' – 3')	Amplicon size (bp)	Reference
Mupirocin high level of resistance (<i>mupB</i>)	mupB-F	CTA GAA GTC GAT TTT GGA GTA G	674	Seah et al., AAC 2012, 56:1916-20
	mupB -R	AGT GTC TAA AAT GAT AAG ACG ATC		
Mupirocin high level of resistance (<i>mupA</i>)	mupA-F	CCC ATG GCT TAC CAG TTG A	1650	Ramsey et al., AAC 1996, 40:2820-3
	mupA-R	CCA TGG AGC ACT ATC CGAA		
<i>S. aureus</i> -specific region of the thermonuclease gene	nuc-F	GCG ATT GAT GGT GAT ACG GTT	279	Brakstad et al., APMIS 1993, 101:681-8
	nuc-R	AGC CAA GCC TTG ACG AAC TAA AGC		
Mupirocin low level of resistance	mupLLR-F	CGG AAT TAA GTT TCC CAG CA	472	This study
	mupLLR-R	TCA AAG TTT TCA TAG TTG TTAATC GT		

Molecular typing

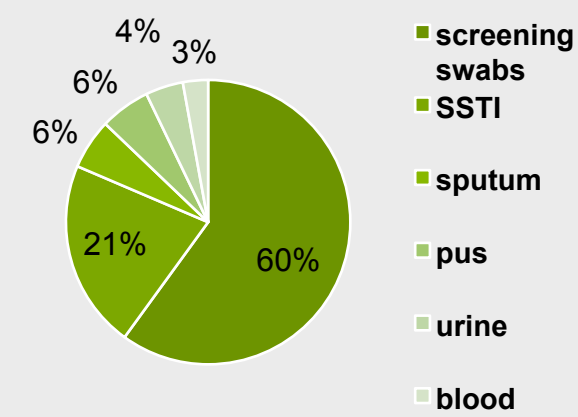
All strains were *spa* typed and grouped into *spa* CCs using the Ridom StaphType software.

RESULTS

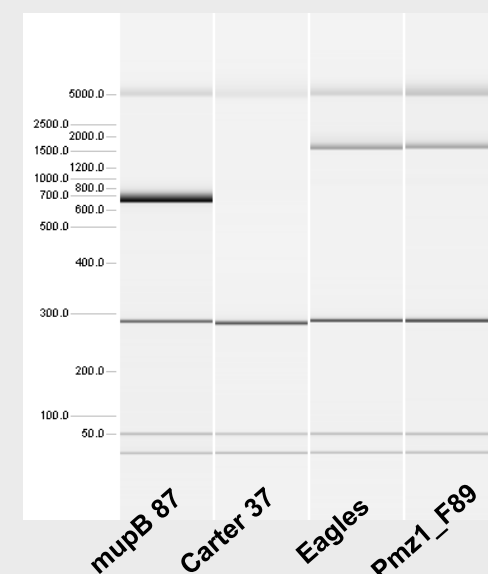
Strain collection from four Belgian national wide surveillances



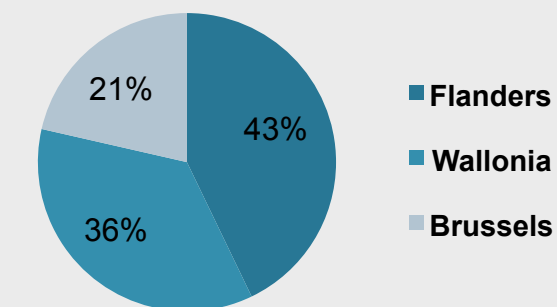
Origin of the strains



Electrophoresis profiles of MHLR for the control strains

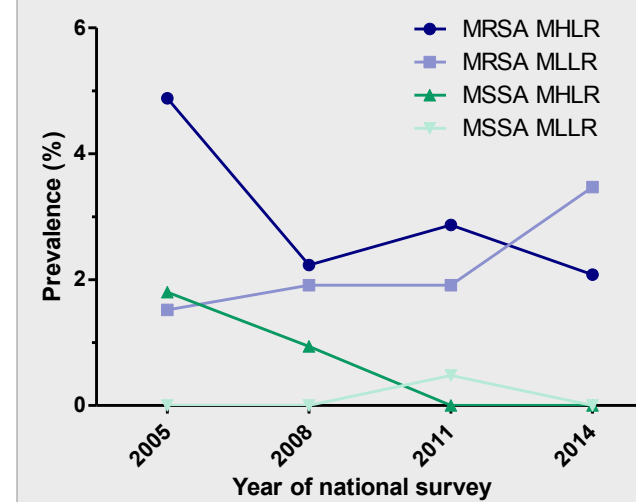


Control strains were included in each test run : Carter (LLMR and *mupA*-negative), Eagles and F89 (both HLMR and *mupA*-positive) and MUP87 (HLMR and *mupB*-positive). The expected sizes of the amplified DNA fragments were 279, 1650 and 674 bp for the *nuc*, *mupA* and *mupB* genes, respectively.



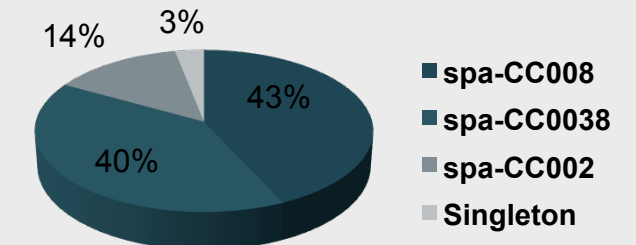
Year of national survey	Total	MIC > 1 mg/L	MHLR (MIC \geq 512 mg/L)	MLLR (MIC > 1 and \leq 256 mg/L)	Positive for <i>mupA</i>	Positive for <i>mupB</i>	V588F mutation in the <i>ileS</i> gene	V631F mutation in the <i>ileS</i> gene
2005	MRSA	328	21	16	5	16	0	5
	MSSA	111	2	2	0	2	0	0
2008	MRSA	314	13	7	6	7	0	6
	MSSA	212	2	2	0	2	0	0
2010-2011	MRSA	314	15	9	6	9	0	5
	MSSA	210	1	0	1	0	1	0
2013-2014	MRSA	288	16	6	10	6	0	10
	MSSA	194	0	0	0	0	0	0
From 2005 to 2014	MRSA	1244	65 (5.2%)	38 (3.0%)	27 (2.2%)	38 (3.0%)	0	26 (2.1%)
	MSSA	727	5 (0.7%)	4 (0.6%)	1 (0.1%)	4 (0.6%)	0	1 (0.1%)

Evolution of mupirocin resistance among the 4 national surveillance



More than 90 % of resistant *S. aureus* strains belonged to three major *spa*-clonal complex corresponding to major epidemic MRSA genotypes found in Belgian hospitals.

The proportion of MHLR versus MLLR significantly varied from 2005 to 2014, whereas the prevalence of resistance was similar. During the survey performed in 2005, 76% of collected resistant isolates harboured MHLR while 24% had MLLR. The ratio changed in 2014 with 37% MHLR and 63% MLLR isolates ($p = 0.02$).



CONCLUSIONS

➤ This first Belgian nationwide 10-year surveillance shows a low prevalence of mupirocin resistance among MRSA (5.2%) and MSSA (0.7%) circulating in hospitals.

➤ Resistance in *S. aureus* was mainly mediated by the presence of *mupA* gene (59.9%) and the V588F point mutation in *ileS* gene (38.6%).

➤ Mupirocin resistant strains belonged to major epidemic clones found in Belgium, highlighting the importance of monitoring resistance to topical agents used in decolonisation programs.

REFERENCES

Seah C, Alexander DC, Louie L *et al.* MupB, a new high-level mupirocin resistance mechanism in *Staphylococcus aureus*. AAC 2012; 56(4): 1916-20.

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