FINAL STUDY REPORT

Borg study

Susceptibility and resistance induction to Staphefekt SA.100

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1. Introduction and Summary

Micreos has developed the endolysin Staphefekt SA.100, capable of killing *Staphylococcus aureus* by disrupting the structural integrity of its cell wall. Staphefekt SA.100 targets cell wall structures, pentaglycin bridges, that are specific to *S. aureus*, independent of its antibiotic resistance profile, making it possible to target MRSA. Furthermore. endolysins target highly preserved structures that cannot be changed easily by the bacteria.
This makes endolysins like Staphefekt SA.100 ideal candidates for antimicrobial therapy, as antibiotic resistant strains can be targeted, while resistance induction against the endolysin is highly unlikely.

To test the sensitivity of clinically relevant S. aureus strains for Staphefekt SA.100, the minimal inhibitory concentrations (MIC's) were measured in clinical isolates of MSSA and MRSA derived from blood cultures, surgical wounds, burn wounds and nasal carriage. MIC's ranged from 8-64 µg/ml, and no difference was observed between MSSA and MRSA.

Induction of resistance was tested with one strain of MSSA and MRSA in duplo by passing 18 incubation cycles with a sub-optimal concentration of Staphefekt SA.100, mupirocin and lysostaphin and monitoring the MIC's at every cycle. No induction of resistance was observed with both strains for Staphefekt SA.100 over time, while a significant increase of MIC's was observed with mupirocin (4-8 fold increase) and lysostaphin (>4000 fold increase).

In conclusion, the endolysin Staphefekt SA.100 was equally effective in killing both MSSA and MRSA and resistance to the endolysin could not be induced by *in vitro* exposition.

2. Materials and Methods

Minimal inhibitory concentrations (MICs) of Staphefekt SA.100 for MSSA and MRSA

To determine the sensitivity to Staphefekt SA.100, minimal inhibitory concentrations (MICs) of SA.100 were determined in 52 clinical isolates of *S. aureus* from different body sites and infections. The isolates included 28 strains of MSSA and 24 strains of MRSA.

MICs were determined by incubating fresh bacterial strains at 35ºC overnight in a 96-wells plate at $1x10^6$ CFU/ml, exposing them to a 2-fold dilution series of Staphefekt SA.100 in Tryptic Soy Broth (TSB), ranging from 1 µg/ml to 256 µg/ml SA.100.

After incubation for 16-18 hours at 35 ºC, bacterial growth was assessed visually per well. The MIC was the lowest concentration at which no growth of the bacterium was observed.

Induction of resistance against SA.100

Induction of resistance against Staphefekt SA.100 was evaluated in one MSSA and one MRSA strains in duplicate by passing 18 incubation cycles with a sub-inhibitory concentration (½ MIC) of Staphefekt SA.100, the antibiotic mupirocin and the lysozyme lysostaphine, and monitoring the MICs at every cycle as described above.

Every cycle consisted of a separate MIC determination, with Staphefekt SA.100, mupirocin and Lysostaphin. Between every cycle, the cells in the wells at ½ MIC (the well with the highest Staphefekt concentration still showing growth) were transferred to 5 ml TSB, incubated for 4 hours at 35ºC and spun down as described above. The spun down cells served as target cells in the new MIC determination in the next cycle.

3. Results and Discussion

Equal susceptibility of MSSA and MRSA to Staphefekt SA.100

The MICs of Staphefekt SA.100 for MSSA and MRSA ranged from 8 to 64 µg/ml. (figure 1). No significant difference was observed between the MIC distributions of MSSA and MRSA, indicating that MSSA and MRSA are equally susceptible to Staphefekt SA.100.

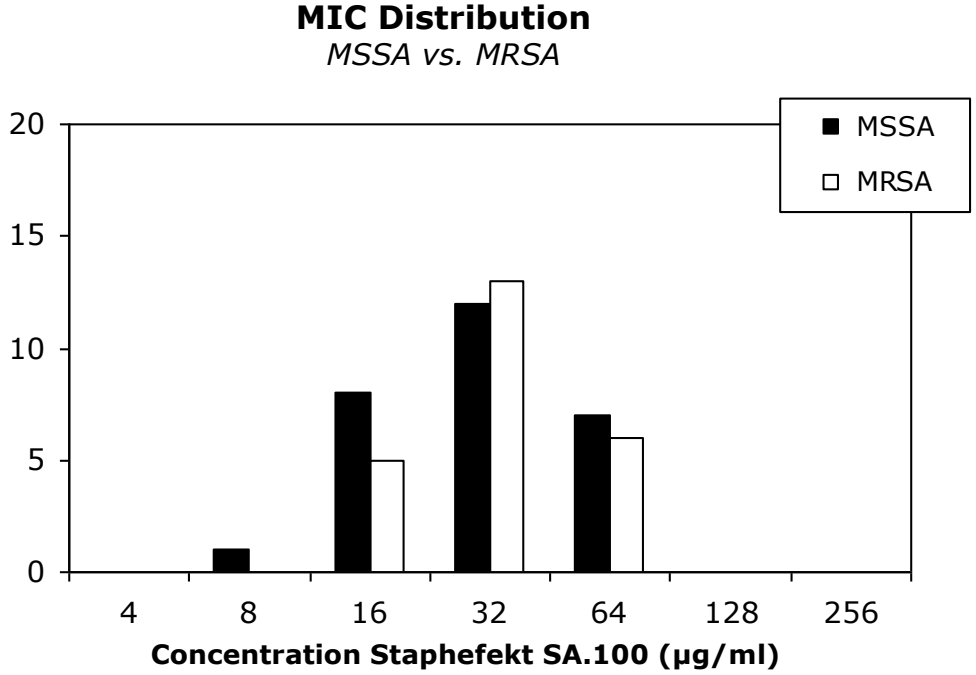

Figure 1. Distribution of minimal inhibitory concentrations (MIC's) of Staphefekt SA.100 for MSSA and MRSA. *Twenty eight strains of MSSA and twenty four strains of MRSA were incubated overnight at 1x106 CFU/ml in a 2-fold dilution series of Staphefekt SA.100 in TSB, ranging from 1 to 256 µg/ml. The MIC was the lowest concentration at which no growth of S. aureus was observed. No significant difference was observed between the MIC distributions of MSSA and MRSA, indicating that MSSA and MRSA are equally susceptible to Staphefekt SA.100.*

No induction of resistance against Staphefekt SA.100 in MSSA and MRSA

After 18 cycles of repeated exposure, no induction of resistance was observed in either MSSA and MRSA strain against Staphefekt SA.100, while MICs of mupirocin and lysostaphine gradually increased in all four experiments.
Figure 2 shows a representative experiment in the MRSA strain, all data are given in appendix 1.

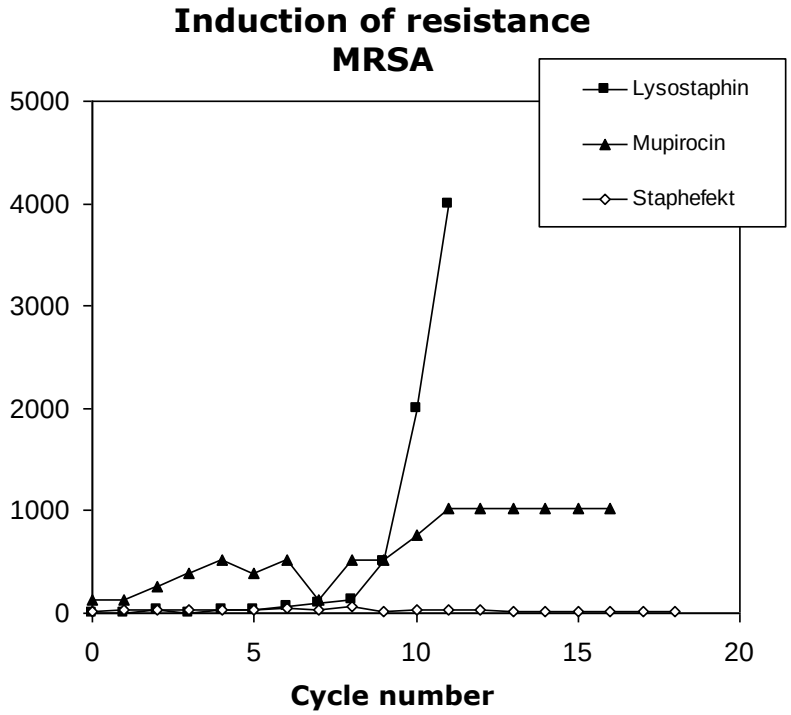

Figure 2. Induction of resistance against Staphefekt SA.100. *Induction of resistance was evaluated for a MSSA and MRSA strain in duplo by passing 18 incubation cycles with a sub-optimal concentration (½ MIC) of Staphefekt SA.100, lysostaphin and mupirocin and monitoring the MIC's at every cycle. Both strains showed similar results. As an example, the results of the MRSA strain is depicted (µg/ml for Staphefekt SA.100 and mupirocin; ng/ml for lysostaphin). No induction of resistance was observed in both the MSSA and MRSA strain with Staphefekt SA.100, while a significant increase in MIC was observed with mupirocin (4-8 fold increase) and lysostaphin (>4000 fold increase).*

4. Conclusion

Staphefekt SA.100 showed to be equally effective in killing the MSSA and MRSA strains. In contrast to the antibiotic mupirocin and the lysostaphine enzyme, no resistance could be induced to Staphefekt SA.100. These characteristics make Staphefekt SA.100 a promising candidate for sustainable antimicrobial therapy against *S. aureus.*

Appendix I.

Induction of resistance against Staphefekt SA.100: MICs during repeated exposure.

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	16µg/ml	8µg/ml	16µg/ml	32µg/ml
	1/2 MIC	8µg/ml	4µg/ml	8µg/ml	16µg/ml
Lysostaphin	MIC	15,6nM	31,25nM	3,9nM	7,8nM
	1/2 MIC	7,8nM	18,6nM	2nM	3,9nM
Mupirocin	MIC	x	x	x	x
	1/2 MIC	x	x	x	x

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	16µg/ml	16µg/ml	32µg/ml	32µg/ml
	1/2 MIC	8µg/ml	8µg/ml	16µg/ml	16µg/ml
Lysostaphin	MIC	15,6nM	31,3nM	2nM	3,9nM
	1/2 MIC	7,8nM	15,6nM	1nM	2nM
Mupirocin	MIC	40ng/ml	40ng/ml	40ng/ml	40ng/ml
	1/2 MIC	8ng/ml	8ng/ml	8ng/ml	8ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	8µg/ml	8µg/ml	32µg/ml	32µg/ml
	1/2 MIC	4µg/ml	4µg/ml	16µg/ml	16µg/ml
Lysostaphin	MIC	31,3nM	62,5nM	31,3nM	31,3nM
	1/2 MIC	15,6nM	31,3nM	15,6nM	15,6nM
Mupirocin	MIC	128ng/ml	128ng/ml	128ng/ml	128ng/ml
	1/2 MIC	64ng/ml	64ng/ml	64ng/ml	64ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	32µg/ml	32µg/ml	32µg/ml	32µg/ml
	1/2 MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
Lysostaphin	MIC	62,5nM	62,5nM	7,8nM	7,8nM
	1/2 MIC	31,3nM	31,3nM	3,9nM	3,9nM
Mupirocin	MIC	128ng/ml	128ng/ml	128ng/ml	128ng/ml
	1/2 MIC	64ng/ml	64ng/ml	64ng/ml	64ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	32µg/ml	32µg/ml	32µg/ml	32µg/ml
	1/2 MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
Lysostaphin	MIC	125nM	62,5nM	31,3nM	31,3nM
	1/2 MIC	62,5nM	31,3nM	15,6nM	15,6nM
Mupirocin	MIC	256ng/ml	256ng/ml	256ng/ml	256ng/ml
	1/2 MIC	128ng/ml	128ng/ml	128ng/ml	128ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	32µg/ml	32µg/ml	64µg/ml	32µg/ml
	1/2 MIC	16µg/ml	16µg/ml	32µg/ml	16µg/ml
Lysostaphin	MIC	62,5nM	62,5nM	62,5nM	62,5nM
	1/2 MIC	31,3nM	31,3nM	31,3nM	31,3nM
Mupirocin	MIC	128ng/ml	128ng/ml	512ng/ml	512ng/ml
	1/2 MIC	64ng/ml	64ng/ml	256ng/ml	256ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	16µg/ml	16µg/ml	32µg/ml	32µg/ml
	1/2 MIC	8µg/ml	8µg/ml	16µg/ml	16µg/ml
Lysostaphin	MIC	31,3nM	31,3nM	62,5nM	125nM
	1/2 MIC	15,6nM	15,6nM	31,3nM	62,5nM
Mupirocin	MIC	256ng/ml	256ng/ml	512ng/ml	256ng/ml
	1/2 MIC	128ng/ml	128ng/ml	256ng/ml	128ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	16µg/ml	16µg/ml	64µg/ml	64µg/ml
	1/2 MIC	8µg/ml	8µg/ml	32µg/ml	32µg/ml
Lysostaphin	MIC	31,3nM	31,3nM	125nM	125nM
	1/2 MIC	15,6nM	15,6nM	62,5nM	62,5nM
Mupirocin	MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml
	1/2 MIC	128ng/ml	128ng/ml	256ng/ml	256ng/ml

		MSSA	MSSA	MRSA	MRSA
Staphefekt	MIC	32µg/ml	32µg/ml	16µg/ml	16µg/ml
	1/2 MIC	16µg/ml	16µg/ml	8µg/ml	8µg/ml
Lysostaphin	MIC	31,3nM	31,3nM	>500nM	>500nM
	1/2 MIC	15,6nM	15,6nM	>500nM	>500nM
Mupirocin	MIC	64ng/ml	64ng/ml	128ng/ml	128ng/ml
	1/2 MIC	32ng/ml	32ng/ml	64ng/ml	64ng/ml

			MSSA	MSSA	MRSA	MRSA
3-2-2014	Staphefekt	MIC	32µg/ml	32µg/ml	32µg/ml	32µg/ml
		1/2 MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
	Lysostaph	MIC	62,5nM	62,5nM	>2000nM	>2000nM
		1/2 MIC	31,3nM	31,3nM	>2000nM	>2000nM
	Mupirocin	MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml
		1/2 MIC	128ng/ml	128ng/ml	256ng/ml	256ng/ml

			MSSA	MSSA	MRSA	MRSA
4-2-2014	Staphefekt	MIC	32µg/ml	32µg/ml	32µg/ml	32µg/ml
		1/2 MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
	Lysostaph	MIC	62,5nM	62,5nM	>4000nM	>4000nM
		1/2 MIC	31,3nM	31,3nM		
	Mupirocin	MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml
		1/2 MIC	128ng/ml	128ng/ml	256ng/ml	256ng/ml

			MSSA	MSSA	MRSA	MRSA
5-2-2014	Staphefekt	MIC	16µg/ml	16µg/ml	32µg/ml	32µg/ml
		1/2 MIC	8µg/ml	8µg/ml	16µg/ml	16µg/ml
	Lysostaph	MIC	31,3nM	31,3nM	x	x
		1/2 MIC	15,6nM	15,6nM		
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	512ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	256ng/ml

			MSSA	MSSA	MRSA	MRSA
6-2-2014	Staphefekt	MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
		1/2 MIC	8µg/ml	8µg/ml	8µg/ml	8µg/ml
	Lysostaph	MIC	31,3nM	15,6nM	x	x
		1/2 MIC	15,6nM	7,8nM		
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	1024ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml

			MSSA	MSSA	MRSA	MRSA
7-2-2014	Staphefekt	MIC	16µg/ml	16µg/ml	32µg/ml	16µg/ml
		1/2 MIC	8µg/ml	8µg/ml	16µg/ml	8µg/ml
	Lysostaph	MIC	62,5nM	62,5nM	x	x
		1/2 MIC	31,3nM	31,3nM		
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	1024ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml

			MSSA	MSSA	MRSA	MRSA
12-2-2014	Staphefekt	MIC	32µg/ml	32µg/ml	16µg/ml	32µg/ml
		1/2 MIC	16µg/ml	16µg/ml	8µg/ml	16µg/ml
	Lysostaph	MIC	62,5nM	62,5nM		
		1/2 MIC	31,3nM	31,3nM		
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	1024ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml

			MSSA	MSSA	MRSA	MRSA
17-2-2014	Staphefekt	MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
		1/2 MIC	8µg/ml	8µg/ml	8µg/ml	8µg/ml
	Lysostaph	MIC	125nM	125nM	x	x
		1/2 MIC	62,5nM	62,5nM		
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	1024ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml

			MSSA	MSSA	MRSA	MRSA
18-2-2014	Staphefekt	MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
		1/2 MIC	8µg/ml	8µg/ml	8µg/ml	8µg/ml
	Lysostaph	MIC	>500nm	>500nm	x	x
		1/2 MIC				
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	1024ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml

			MSSA	MSSA	MRSA	MRSA
19-2-2014	Staphefekt	MIC	16µg/ml	16µg/ml	16µg/ml	16µg/ml
		1/2 MIC	8µg/ml	8µg/ml	8µg/ml	8µg/ml
	Lysostaph	MIC	>4000nm	>4000nm	x	x
		1/2 MIC				
	Mupirocin	MIC	512ng/ml	512ng/ml	1024ng/ml	1024ng/ml
		1/2 MIC	256ng/ml	256ng/ml	512ng/ml	512ng/ml