Polyhexanide MIC profiles after topical decolonization of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage

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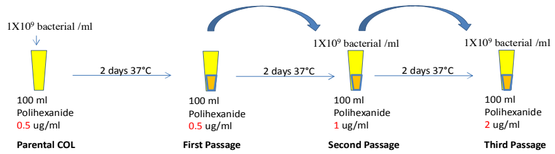
Background

- MRSA colonization is associated with increased risk of acquisition of MRSA infection and act as a reservoir for cross-transmission.
- Decolonization prevents infection and transmission (1,2)
- Antiseptics such as chlorexidine and mupirocine can be used for patient decolonization. However, increasing resistance of MRSA to these agents is observed.
- Polihexanide is an alternative molecule, however development of resistance to polihexanide has not been tested.

The aims of this study was to determine the polyhexanide MIC profiles of MRSA clinical isolates following *in vivo* polyhexanide (Prontoderm®) decolonization and to determine the emergence of *in vitro* resistance to polyhexanide.

Methods

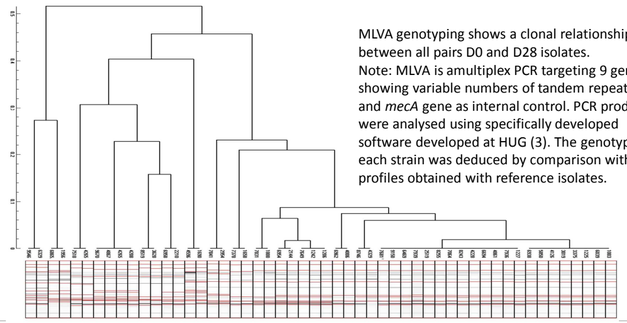
- A collection of 54 MRSA strains from patients colonized with MRSA were obtained from a randomized, placebo-controlled study (See oral session O115).
- Patients were treated with Polyhexanide for 10 days and screened for MRSA carriage at day 2 and day 28 following decolonization.
- Selected strains at day 0 (D0) and 28 days (D28) after polyhexanide decolonization were subjected to molecular genotyping (MLVA) and chlorhexidine and polyhexanide macrodilution MIC determinations as follows: 1.5×10^6 bacteria were added to 1ml of MHB containing or not different polihexanide concentrations (0.25, 0.5, 1 and 2 ug/ml). MIC was determined after 48h of incubations at 37°C. Polihexanide was supplied by the manufacturer (BBraun).
- In addition, we assessed *in vitro* emergence of resistance to polyhexanide by stepwise exposure in broth culture.
  S. aureus strains were serially passage onto increasing concentrations of polihexanide as follows:



After each passage bacteria were collected and macrodilution MIC was determined.

Results

1. MLVA genotyping analysis



MLVA genotyping shows a clonal relationship between all pairs D0 and D28 isolates.
Note: MLVA is amultiplex PCR targeting 9 genes showing variable numbers of tandem repeats and *mecA* gene as internal control. PCR products were analysed using specifically developed software developed at HUG (3). The genotype of each strain was deduced by comparison with profiles obtained with reference isolates.

2. Polihexanide and chlorexidine MIC values

Strain	MIC Chlorexidine	MIC Polihexanide	Strain	MIC Chlorexidine	MIC Polihexanide
Strain D0 nose			*Strain D28 nose*		
1227	4	0.5	*5156*	4	0.5 / 2
1690	< 0.5	0.5	*4596*	1 / 0.5	1
4126	4	0.5	*7939*	4	1 / 0.5
4265	< 0.5	0.5	*7518*	< 0.5	0.5
4329	4	0.25	*8746*	2 / 4	0.5
4861	4	0.5	*8243*	2	0.5
6039	4	0.5	*9150*	4	0.5 / 1
6329	1	0.5	*9546*	1	0.5
6958	< 0.5	0.5	*2210*	< 0.5	0.5 / 1
7064	4	0.5 / 1	*1206*	4	0.5
7649	4	0.5 / 1	*2144*	1	0.5 / 1
7841	4	0.25	*2954*	4	0.5
3019	4	1 / 0.5	*5850*	< 0.5	0.5
4228	4	0.5	*7681*	2	1 / 0.5
4867	1	1	*7936*	4	0.5
5642	2	0.5 / 1	*9987*	4	0.5
6865	< 0.5	1	*1956*	< 0.5	0.5 / 1
6954	4	0.5	*1242*	4	0.25
6962	4	0.5	*4006*	4	0.5
7378	2	0.5 / 1	*1658*	2	0.5
8255	2	0.25	*2519*	2	0.25
8519	< 0.5	0.5 / 0.25	*3628*	1	1
9670	2	1	*4208*	1	1
1225	4	0.5 / 0.25	*6938b*	4	0.5
3375	4	0.5	*6694*	1	0.5
6469	4	0.5	*1003*	4	0.5
7831	2	1 / 0.5	*1800*	4	0.25

- None of the strains showed resistance level >1 µg/ml to polihexanide
- Resistance level against chlorexidine varied between 0.5-4 µg/ml
- Around 80% of our collection is low-level resistance to mupirocin
- The majority of our D28 isolates show neither chlorexidine nor polihexanide MIC changes compared to isolates at D0
- No correlation between chlorexidine and polihexanide resistance was observed
- No cross-resistance between polihexanide and chlorhexidine was observed

3. Preliminary analysis of in *vitro* emergence of resistance to polihexanide and chlorexidine

A. Do sub-inhibitory concentrations of Polihexanide *in vitro* can select for MRSA strains with increased polihexanide MICs?

Strain	Macrodilution MIC Polihexanide (ug/ml)
Parental COL	0.25-0.5
First passage	0.25
Second passage	2
Third passage	2

Prolonged passages on subinhibitory concentrations of polihexanide permitted selection of bacteria growing only until 2 ug/ml of polihexanide. Further passages do not increase MIC levels.

B. Is there cross-resistance between chlorexidine and polihexanide?

Table 1.

	MIC Chlorexidine Macro	Qac genes (A and B)
134947 J0	4	-
134947 J20	8	-
128822 J0	8	+
128822 J20	8	+
COL J0	2	-
COL J20	8	-

Table 2.

	MIC Chlorhexidine 48h	MIC Polihexanide 48h
134947 J0	4.0	1.0
134947 J20	8.0	1.0
128822 J0	8.0	0.5
128822 J20	8.0	1.0
COL J0	2.0	0.5
COL J20	8.0	0.5
Parental COL J0	2.0	0.5
COL P3	2.0	2.0

Different MRSA strains were exposed to ten *in vitro* passages (2 days each) in sub-inhibitory of chlorhexidine concentrations. This allowed selection of bacteria growing on 8 ug/ml of chlorhexidine (Table 1). Analysis of polihexanide MIC of these strains showed no correlation of *in vitro* induced chlorhexidine and polihexanide MIC. Accordingly, bacteria showing *in vitro* induced polihexanide MIC do not show increased chlorexidine MIC (Table 2).

Conclusion

- Polihexanide is a potential alternative molecule for MRSA decolonization
- Level of resistance against Polihexanide is very low (≤ 1 µg/ml) in our population
- *In vitro* exposition of MRSA to polihexanide shows minor effect on MIC
- *In vitro* exposition of MRSA to chlorexidine for prolonged periods shows MIC increases to 8 µg/ml
- Increase in MIC level against chlorexidine does not impact MIC level against polihexanide
- Polihexanide could be use for decolonisation of chlorexidine resistant MRSA strains

REFERENCES
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