Exposure of *Pseudomonas aeruginosa* to sub-MIC concentrations of chlorhexidine (CHX) leads to increased resistance, marked phenotypic changes, overexpression of MexG, and cross resistance to antipseudomonal antibiotics

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Background and Aim

- Biocides are widely used in health-related activities as a convenient means of disinfection and protection against bacterial contamination
- Yet, they carry a risk of resistance to them-selves as well as cross-resistance to antibiotics *
- Chorhexidine is widely usedOur aim was to examine whether exposure of *P. aeruginosa* to sub-MIC concentrations of chlohexidine could
  - lead to resistance to chlorhexidine
  - modify the bacterial phenotype,
  - cause increased expression of efflux transporters,
  - and trigger cross-resistance to anti-pseudomonal antibiotics.

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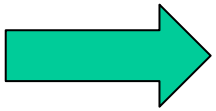
* Meyer & Cookson, J Hosp Infect. 2010, 76:200-5).

Methods (1)

- 6 fully susceptible and 18 multi-resistant isolates of Pseudomonas aeruginosa (from pateints suffering from ventilator-associated pneumonia and from burned patients
- with initial MIC of chlorhexidine ≤ 32 mg/l (wild type)
- Exposure to chlorhexidine at 0.5 x its MIC with daily measurement of MIC (microdilution) and readjustment of the chlorhexidine concentration to 0.5 x the new MIC value, for up to 14 days

  ➔ "trained bacteria" …
- followed by 10 subcultures on chlorhexidine-free agar

  ➔ revertants



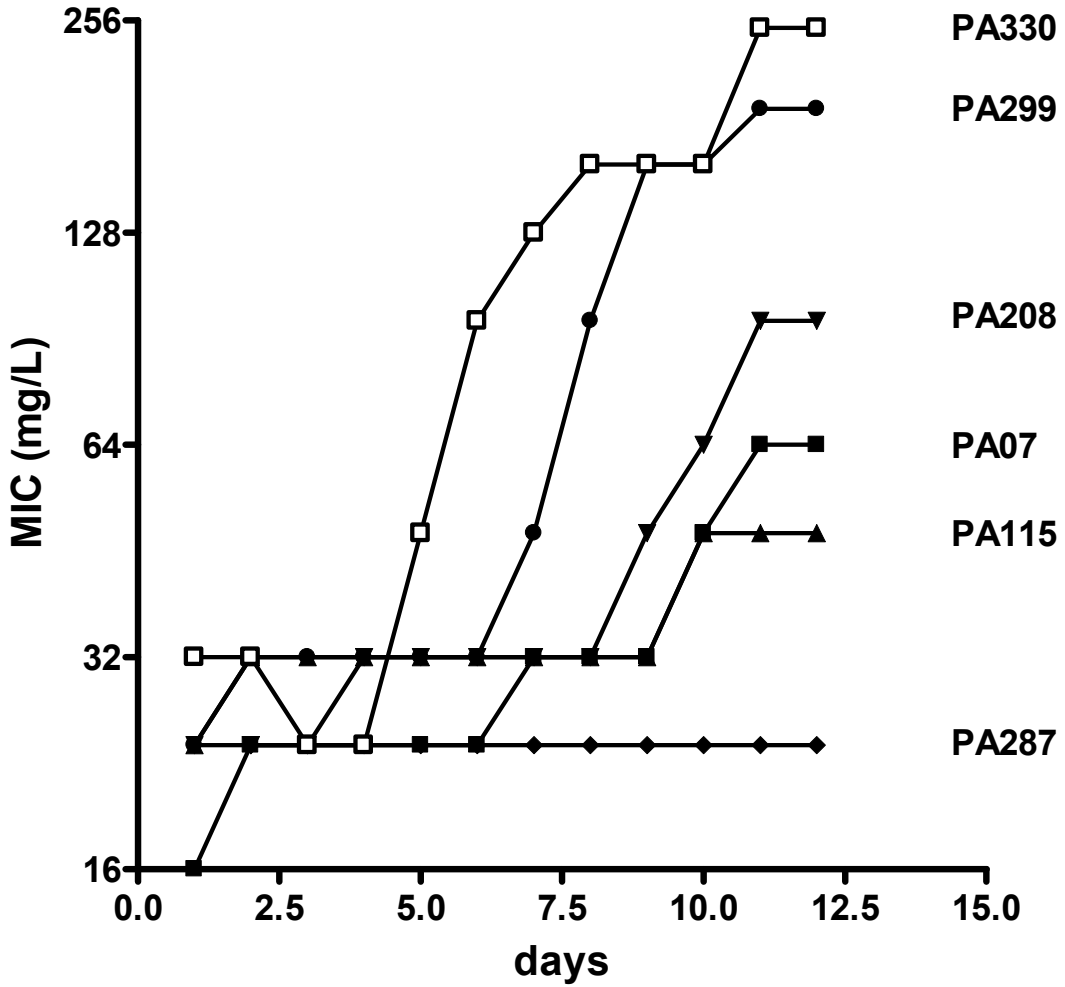
This protocol is similar to what has been used previously to study the emergence of resistance of *S. pneumoniae* to fluoroquinolones(Avrain et al., JAC, 2007 60:965-972)

Methods (2)

- Bacteria were examined for
  - change in chlorhexidine MIC,
  - swarming (agar plates containing CHX at 0.5 MIC),
  - pyoverdine production (405/660 nm absorbance ratio), and
  - susceptibility to antipseudomonal antibiotics (MIC; CLSI methods)
  - expression of 3 genes (*mexA*, *mexX*, *mexG*) part of clusters encoding 3 major efflux transporets (RT-PCR).
- Clonality during the whole duration of the selection/reversion process was checked by Repetitive Extragenic Palindromic-PCR [DiversiLab®] with treshold set at > 95% similarity [Riou et al. IJAA 2010, 36:513-22]).

Results: 1. Change in CHX MIC

- All isolates tested showed an increased in chlorhexidine MIC (2 to > 8-fold and up to > 256 mg/L) after 13 days exposure
- The graph shows data for 6 selected strains).



Results: 2. Effect of PaβN (efflux inhibitor *)

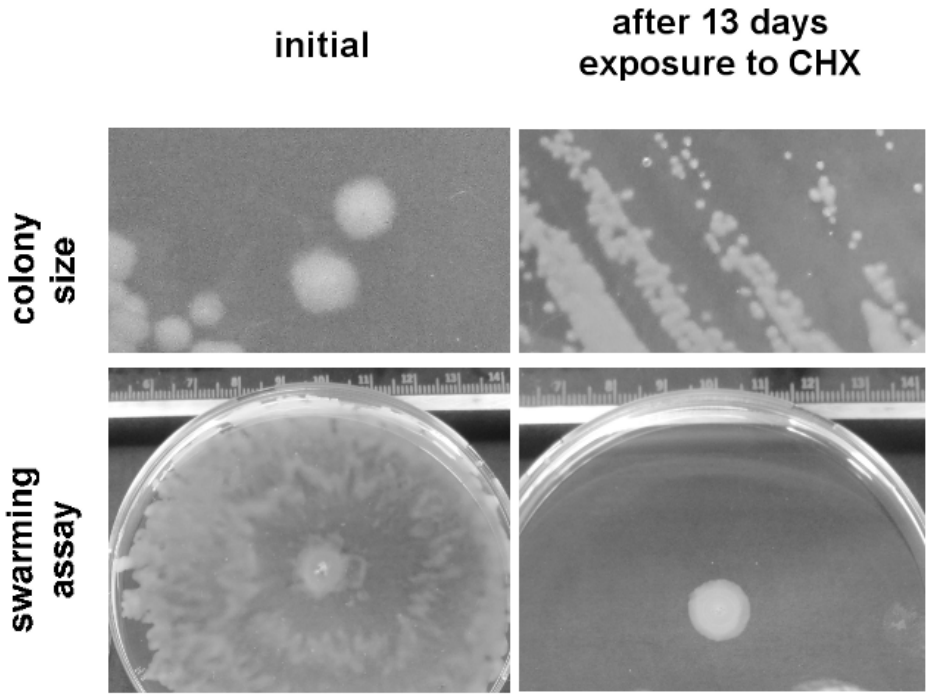
Strains	MIC (mg.L)			
	initial	chlorhexidine-exposed		revertant
		-PAβN	+PAβN	
PA299	24	192	64	24
PA115	24	48	32	24
PA287	12	24	16	24
PA330	32	256	32	24
PA07	16	64	16	48
PA208	24	96	24	48

* MC-207,110 - Lomovskaya et al. Antimicrob Agents Chemother. 2001 Jan;45(1):105-16.

Results: 3: bacterial morphology and properties

Most chlorhexidine-exposed isolates showed

- reduction in colony size,
- marked reduction of swarming, and
- almost complete suppression of pyoverdin production



Typical change in colony size and swarming abilities after 13 days of exposure to 0.5 MIC

Results: 4. Cross resistance

Increase in MIC for antibiotics (from n=24):

- n= 3 for amikacin (2 to 4 x)
- n=1 for piperacillin (2 to > 8-x),
- n=3 for cefepime (2 to 4-x), and
- n= 5 for ciprofloxacin (2 to 16-x)

drug	initial	CHLD-exp		revertants
		-PAβN	+PAβN	
amikacin	4	16	16	16
	4	16	16	8
pip/tazo	8	16	4	8
	4	**>128**	**>128**	**>128**
cefepime	4	**16**	**16**	**16**
	8	**16**	8	16

Figures in **bold red** are MICs > EUCAST "R" brkpt

Results: 5. Efflux overexoverexpression

- Variable overexpression of *mexA*, *mexX*
- constant overexpression of *mexG* *
- Reversion was partial only.

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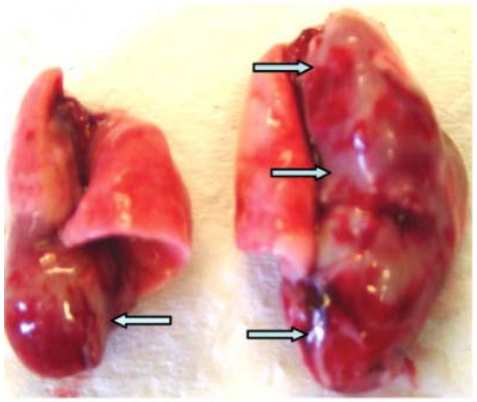
* MexGHI-OpmD facilitates cell-to-cell communication, confers resistance to vanadium, promotes virulence and growth in *P. aeruginosa* but increases susceptibility to many antibiotics [Aendekerk et al. Microbiology 2002, 148:2371-81]



Δ *mexI* **WT**

Conclusions

- Exposure of *Pseudomonas aeruginosa* to non-lethal concentrations of chlorhexidine fosters the development of strains
  - with reduced susceptibility to chlorhexidine it-self, and
  - with cross-resistance to antibiotics.
- A possible mechanism is the overexpression of transporter(s) with a special role of MexGHI
- However, the multiple genotypic and phenotypic alterations observed in thee strains need to be critically assessed
- The data call for caution against using chlorhexidine at non-lethal concentrations.

Disclosures

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