# Biofilms:
# Role on Pathogenesis and Treatment of UTIs.

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# Medical devices-related infections

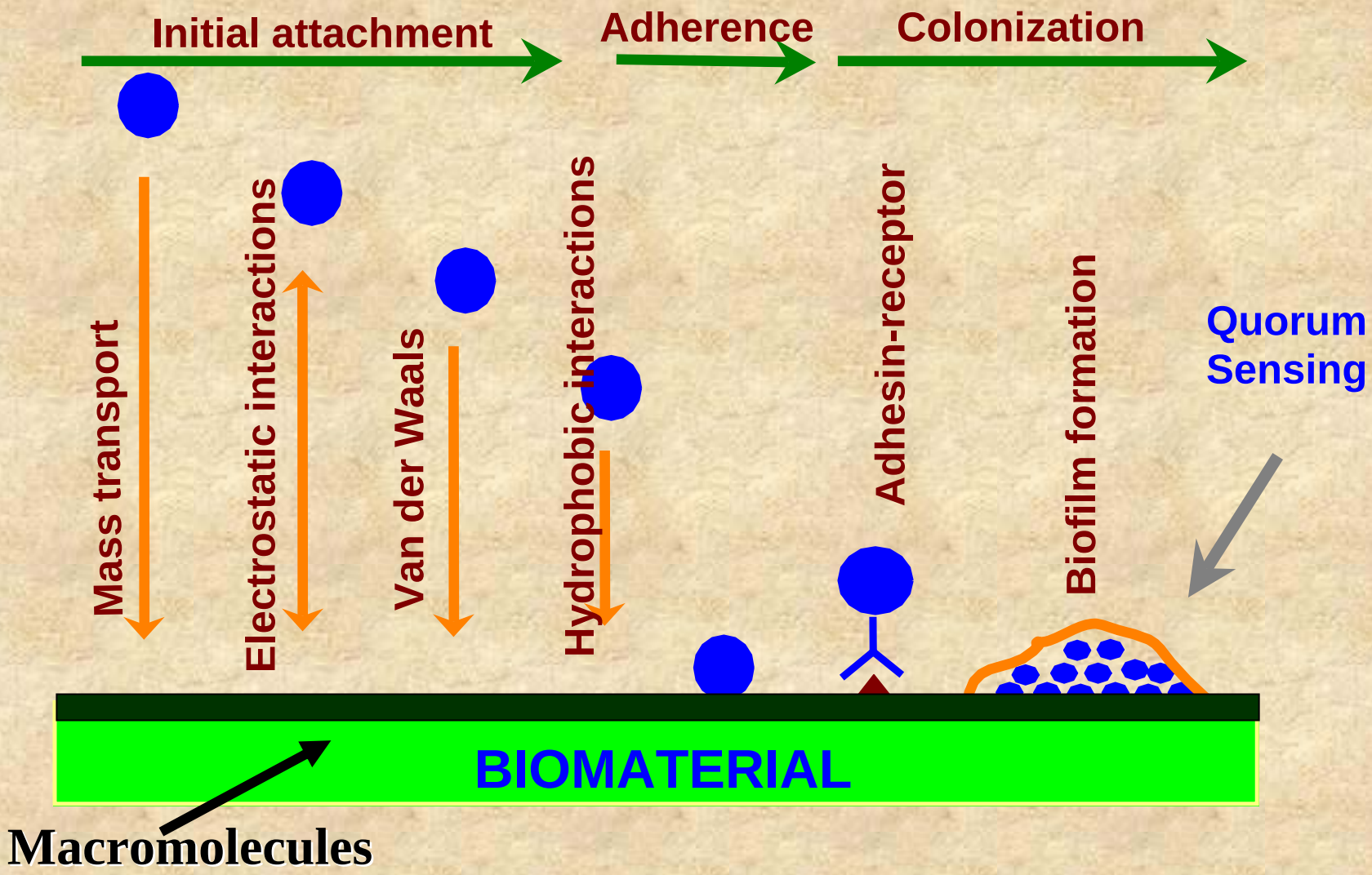
1. 35 millions/year in USA
2. Most frequent complication.
3. Increase mortality
4. Increase Hospital stay
5. Increase costs x 5-7

# Medical devices-related infections: USA.

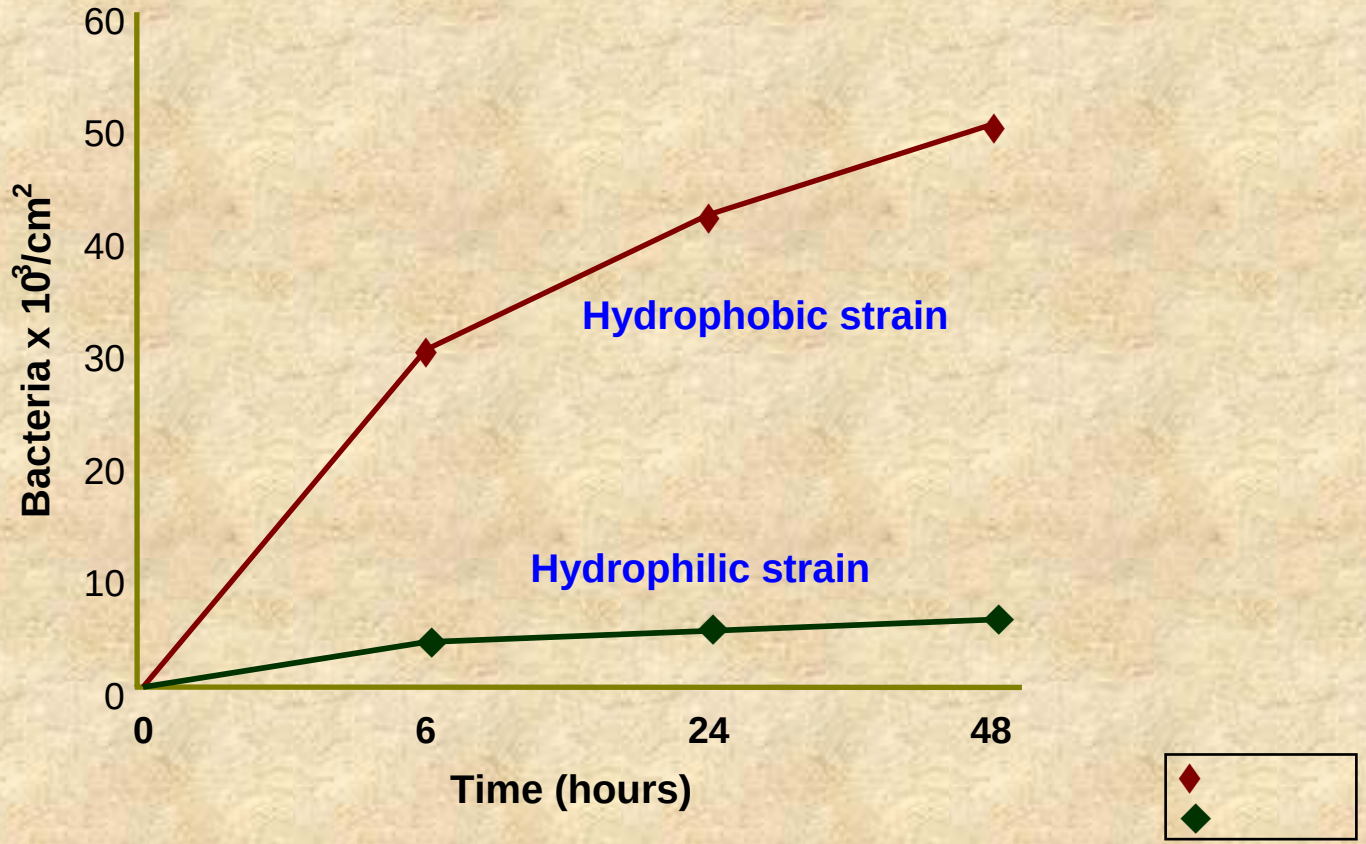
| Device | # /year | Infection | Mortality |
|---|---|---|---|
| Intravascular catheter | 200.000.000 | <0,1-7% | 15-20% |
| Urinary catheter | 5.000.000 | 5-10% | |
| Shunts CSF | 80.000 | 10-15% | |
| Pacemakers | 60.000 | 0-3% | 2% |
| Prosthetic valves | 100.000 | 1-5% | 34% |
| Joint prothesis | 350.000 | <1-2% | 2,5% |
| Dental implants | 436.000 | 15% | |

Wadvogel et al. 2000

# Biofilm formation

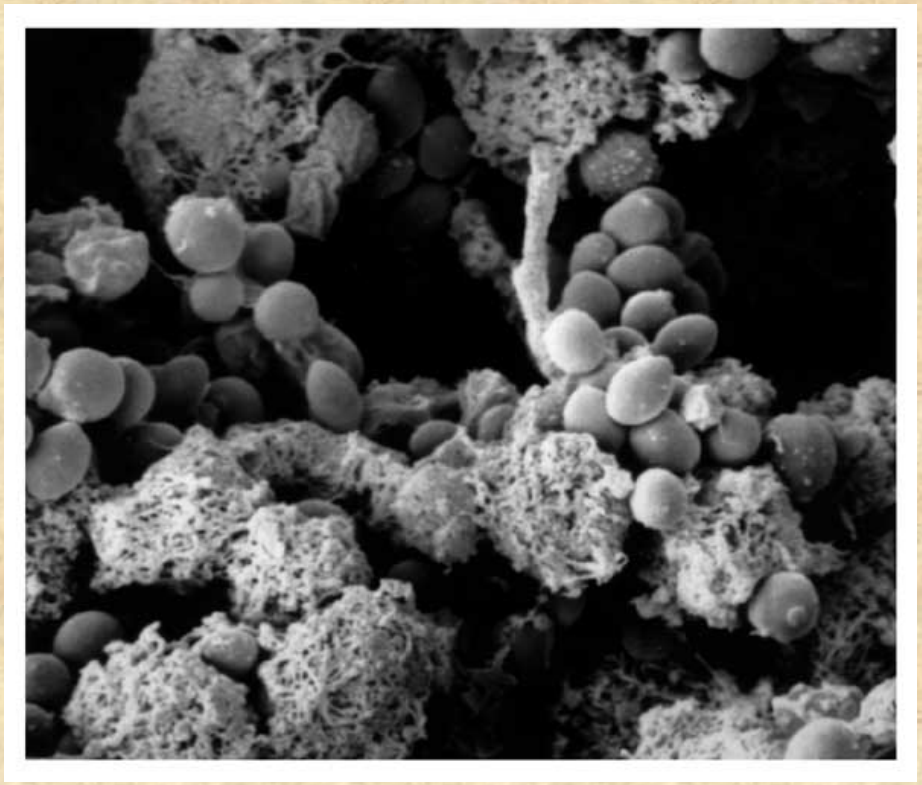


# Adherence de *S. epidermidis* to Teflon catheters

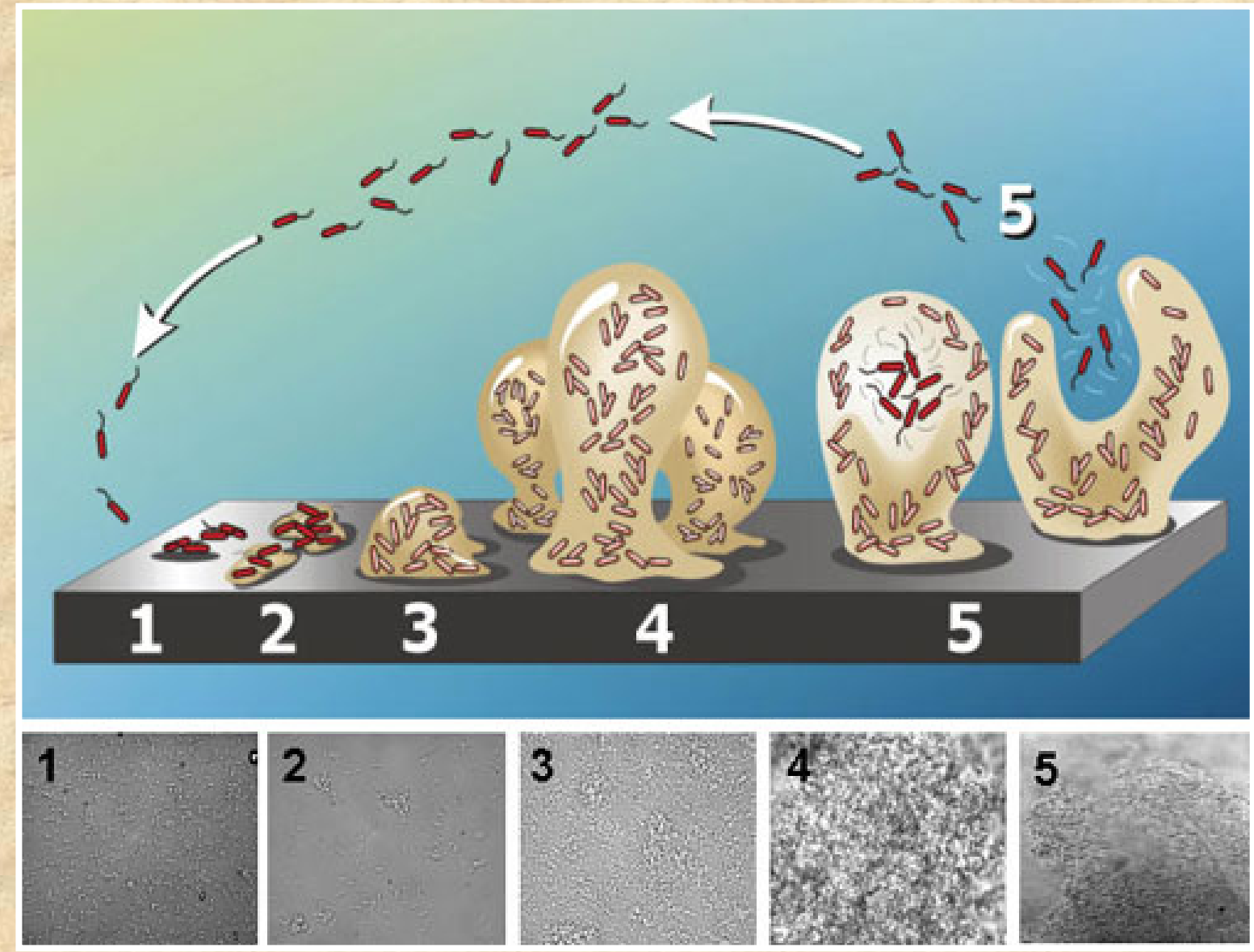



Pascual et al. Eur. J. Clin. Microbiol. (1986).

# Bacterial biofims

# Bacterial biofilms dynamics



# Bacterial adherence to catheters

Attached bacteria x $10^5/cm^2$

| Catheter | *S. aureus* | *S. epidermidis* | *E. coli* | *P. aeruginosa* |
|---|---|---|---|---|
| Siliconized latex | 27 ±3 | 18 ±2 | 19 ±2 | 24 ±3 |
| PVC | 42 ±5 | 23 ±3 | 18 ±2 | 41 ±4 |
| Teflon | 11 ±1 | 7 ±0,4 | 3 ±0,1 | 10 ±1 |
| Poliurethane | 15 ±1 | 4 ±0,6 | 9 ±0,1 | 13 ±1 |
| Vialon | 7 ±1 | 5 ±0,3 | 4 ±0,2 | 11 ±1 |

Pascual A et al. J. Med Microbiol. 1991.

# Siliconized latex eluted substances used as nutrients by *P. aeruginosa* and toxic for other bacteria

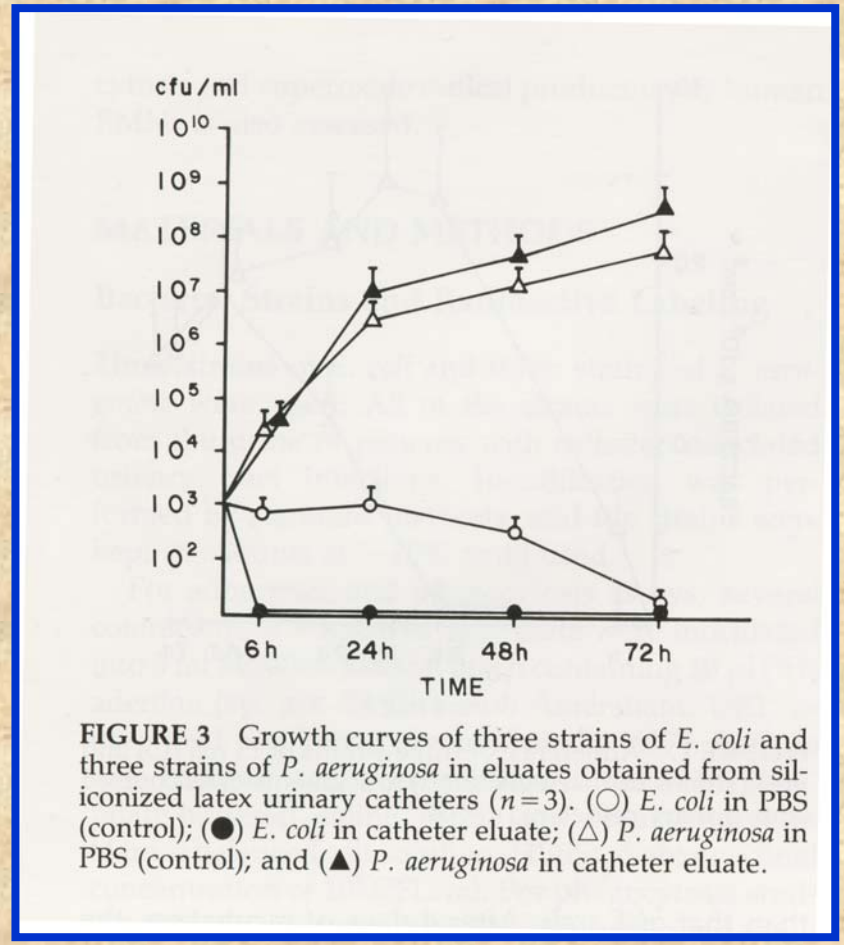



**FIGURE 3** Growth curves of three strains of *E. coli* and three strains of *P. aeruginosa* in eluates obtained from siliconized latex urinary catheters ($n=3$). (○) *E. coli* in PBS (control); (●) *E. coli* in catheter eluate; (△) *P. aeruginosa* in PBS (control); and (▲) *P. aeruginosa* in catheter eluate.

**Pascual A et al. Diag Microbiol Infect Dis. 1991.**

# Effect of biomaterials on host defense mechanisms

1. PMNs disfunctions
2. Complement activation
3. Inflammatory response
4. Systemic effects

# Effect of biomaterials on bactericidal mechanisms of human PMNs

nmol superóxide/mg protein

| Biomaterial | PMA | Zimosan |
|---|---|---|
| Control | 196 | 86 |
| Siliconized latex | 62* | 32* |
| PVC | 104* | 50* |
| Teflon | 127* | 51* |
| Poliurethane | 180* | 87* |
| Vialon | 167* | 80 |

*p<0.05

Pascual A. et al. Eur J Clin Microbiol. 1990

# Mechanisms of antimicrobial resistance in bacterial biofilms

1. Biofilm impermeability: vancomycin, aminoglycosides.
2. Altered growth rate of sessile bacteria: betalactams.
3. Biofilm microenvironment antagonizes activity: aminoglycosides, macrolides, tetracyclines.
4. Resistance mechanisms expressed in sessile bacteria: ?
5. Horizontal dissemination in biofilm: plasmids.
6. Communication systems: *quorum sensing*. Aminoglycosides.
7. Biomaterial-induced resistance: carbapenems.

Rodriguez JM, Pascual A. Rev Med Microbiol. In press

# Activity of 8 antimicrobials against *S. epidermidis*: planktonic Vs sessile.

MBCbiofilm/MBCplanktonic

| Antimicrobial | Slime - | Slime + |
|---|---|---|
| Amicacin | >256 | >128 |
| Clindamycin | >1024 | >128 |
| Cloxacilin | >512 | >256 |
| Ciprofloxacin | >1024 | >256 |
| Vancomycin | >256 | 32 |
| Teicoplanin | >64 | >16 |
| Rifampin | >32768 | >8192 |

*p<0.05

Pascual A et al. J Hosp Infect. 1993

# Activity of glycopeptides (4 x MBC) against *S. epidermidis* biofilms.

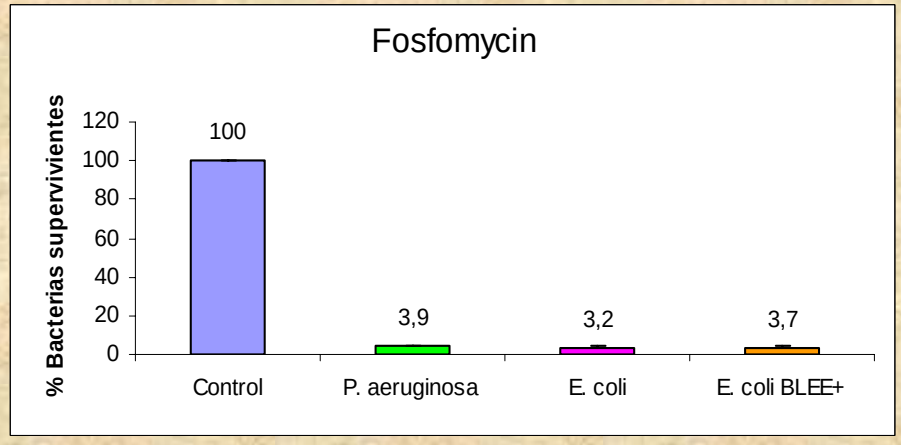
Bacteria x $10^3$/catheter segment

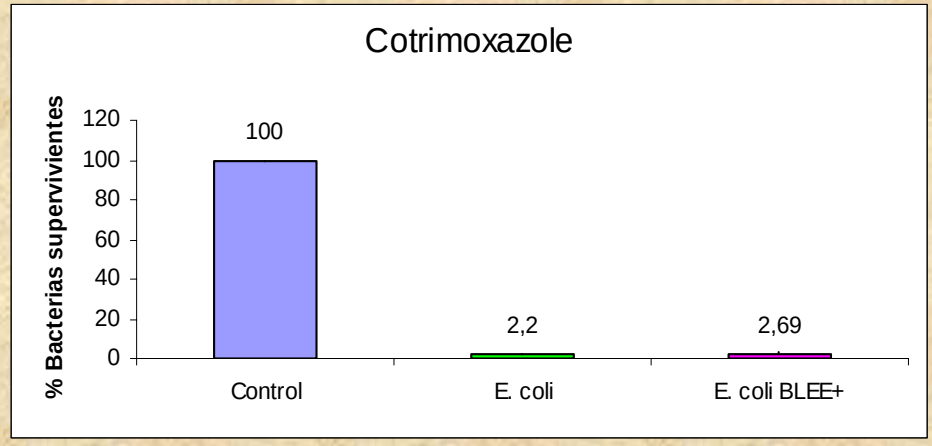
| Antimicrobial | Teflon | Poliurethane |
|---|---|---|
| Vancomycin | 1860 | 3270 |
| Teicoplanin | 363 | 316 |
| V + Amicacin | 0,2 | 242 |
| V + Rifampin | 0,6 | 0 |
| T + Amicacin | 3,6 | 0 |
| T + Rifampin | 3,1 | 0 |

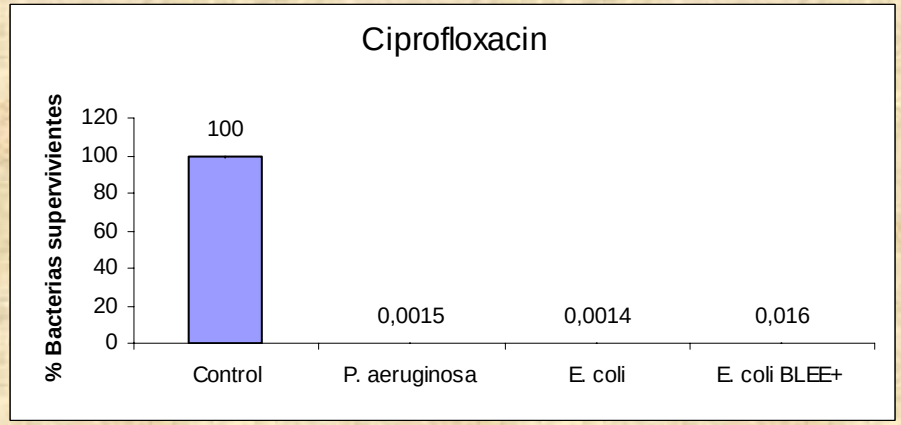
*$p<0.05$

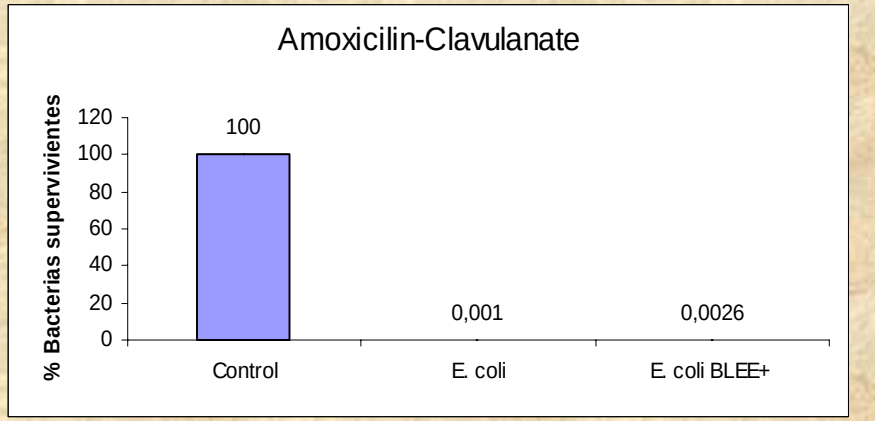
Pascual et al. Eur J Clin Microbiol. 1994

# Activity of 4 antimicrobials against bacterial biofilms on SLC.




**24 hour-biofilms. Antimicrobial concentration: 10 x MIC (24 hour incubation)**

# Penetration of antimicrobials through bacterial biofilms

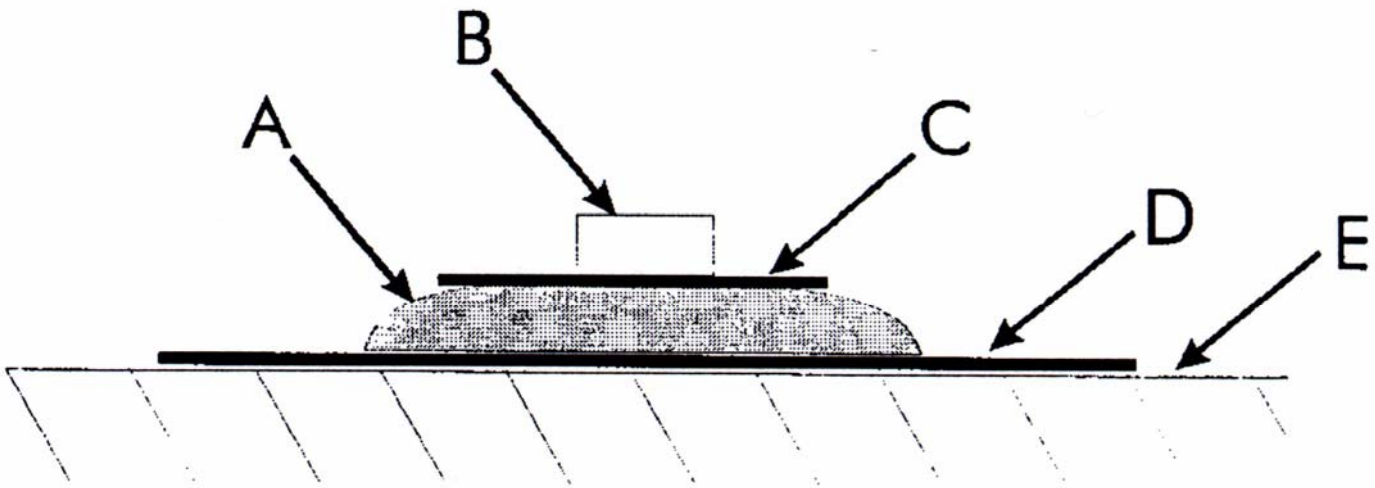
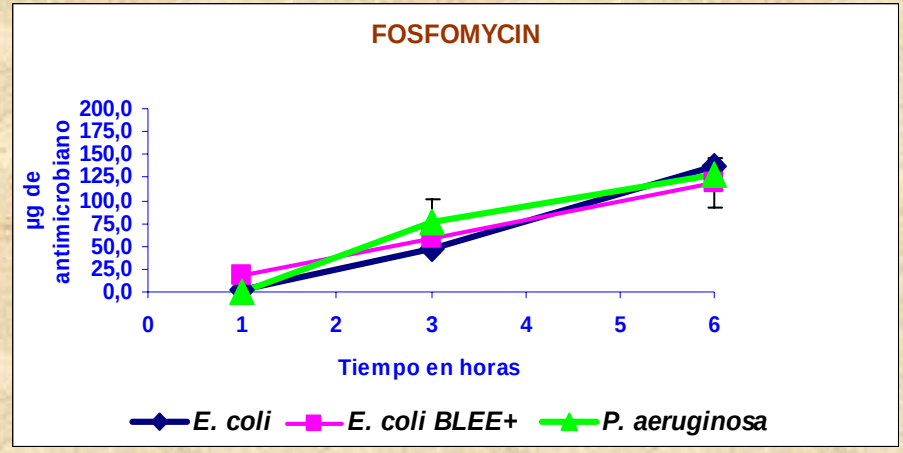
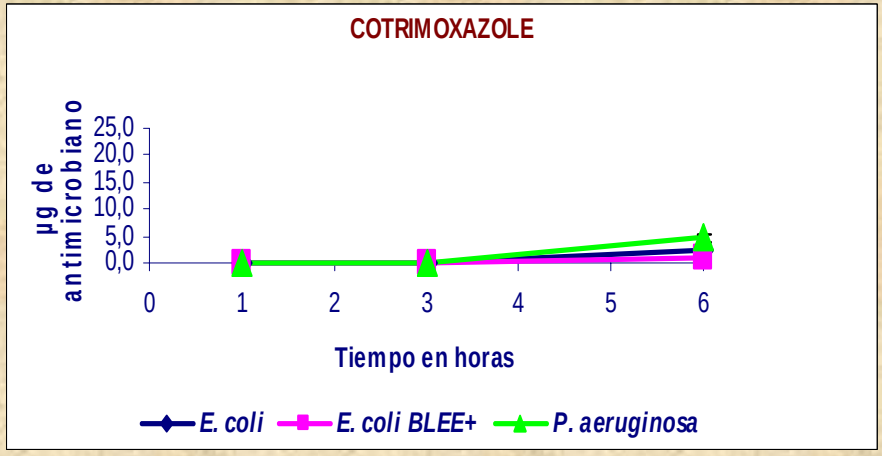


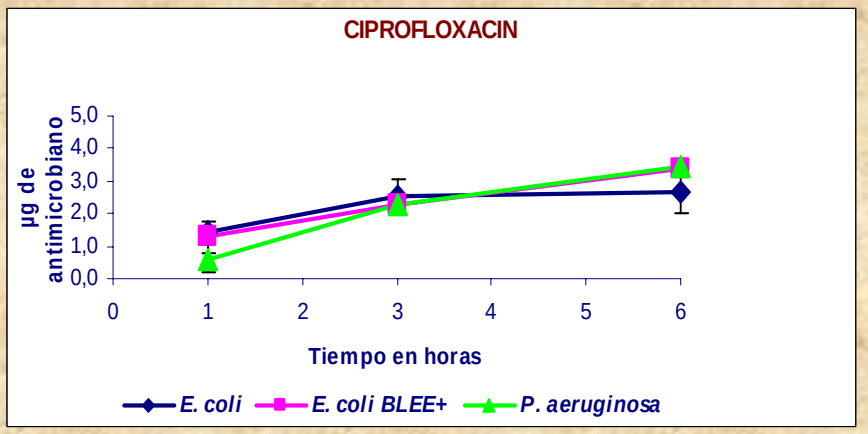

FIG. 1. Schematic depiction of the experimental system used to track the penetration of antibiotics through the biofilms. The biofilm (A) was developed on a 25-mm-diameter microporous polycarbonate membrane (D) resting on agar culture medium. A 13-mm-diameter microporous polycarbonate membrane (C) was placed on top of the biofilm. A moistened concentration disk (B) was placed on top of the 13-mm-diameter membrane. The entire unit, components A through D, was transferred to antibiotic-containing agar (E) with sterile forceps.

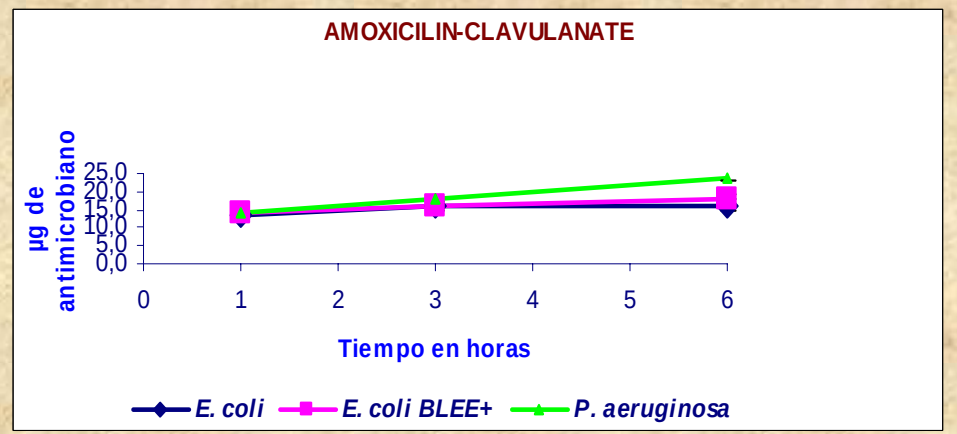
**Anderl et al. AAC 2000**

# Penetration of 4 antimicrobials through bacterial biofilms on polycarbonate membranes.

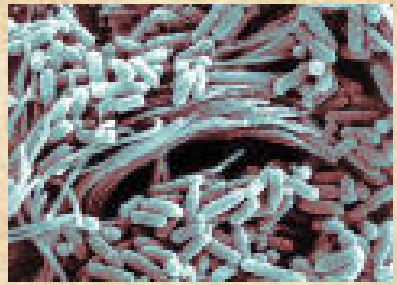


# *P. aeruginosa* y Siliconized latex urinary catheters (SLC)



- ***P. aeruginosa*** **is a main pathogen in SLC-related UTIs.**
- ***P. aeruginosa*** **adherence to SLC is greater than that to other bomaterials.**
- **SLC elutes substances used by *P. aeruginosa* as nutrient.**

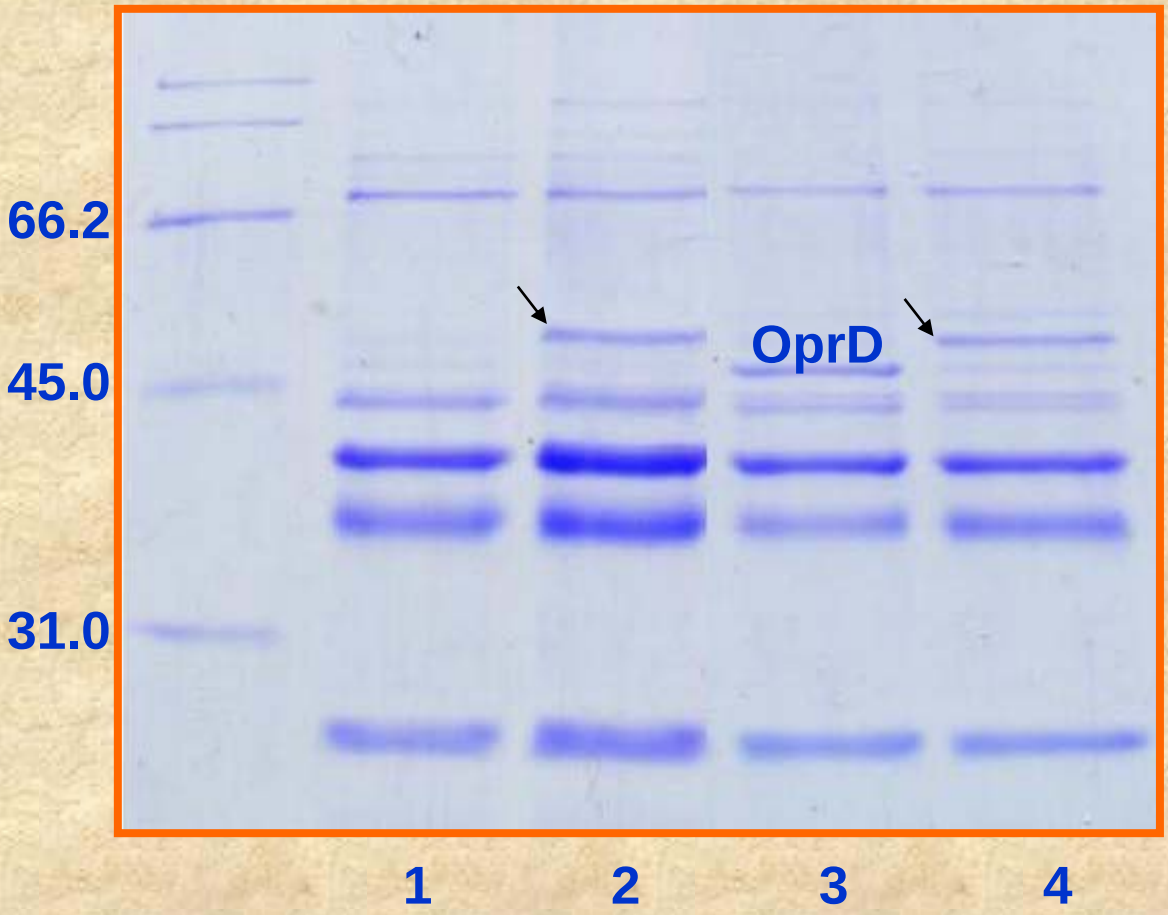
# In the presence of SLC *P. aeruginosa* turns resistant to some antimicrobials

**Table 2:** Effect of siliconized latex urinary catheters on the inhibitory activity of antimicrobial agents against *Pseudomonas aeruginosa*. Data are expressed as the ratio of the MIC in the presence of catheter material to the MIC in the absence of catheter material.

| Strains | MIC in catheter presence/MIC in catheter absence | | | | |
|---|---|---|---|---|---|
| | Amikacin | Ceftazidime | Ciprofloxacin | Norfloxacin | Meropenem |
| HUS-2 | 4 | 2 | 2 | 2 | 32 |
| HUS-3 | 4 | 1 | 1 | 1 | 16 |
| HUS-6 | 8 | 1 | 2 | 1 | 32 |

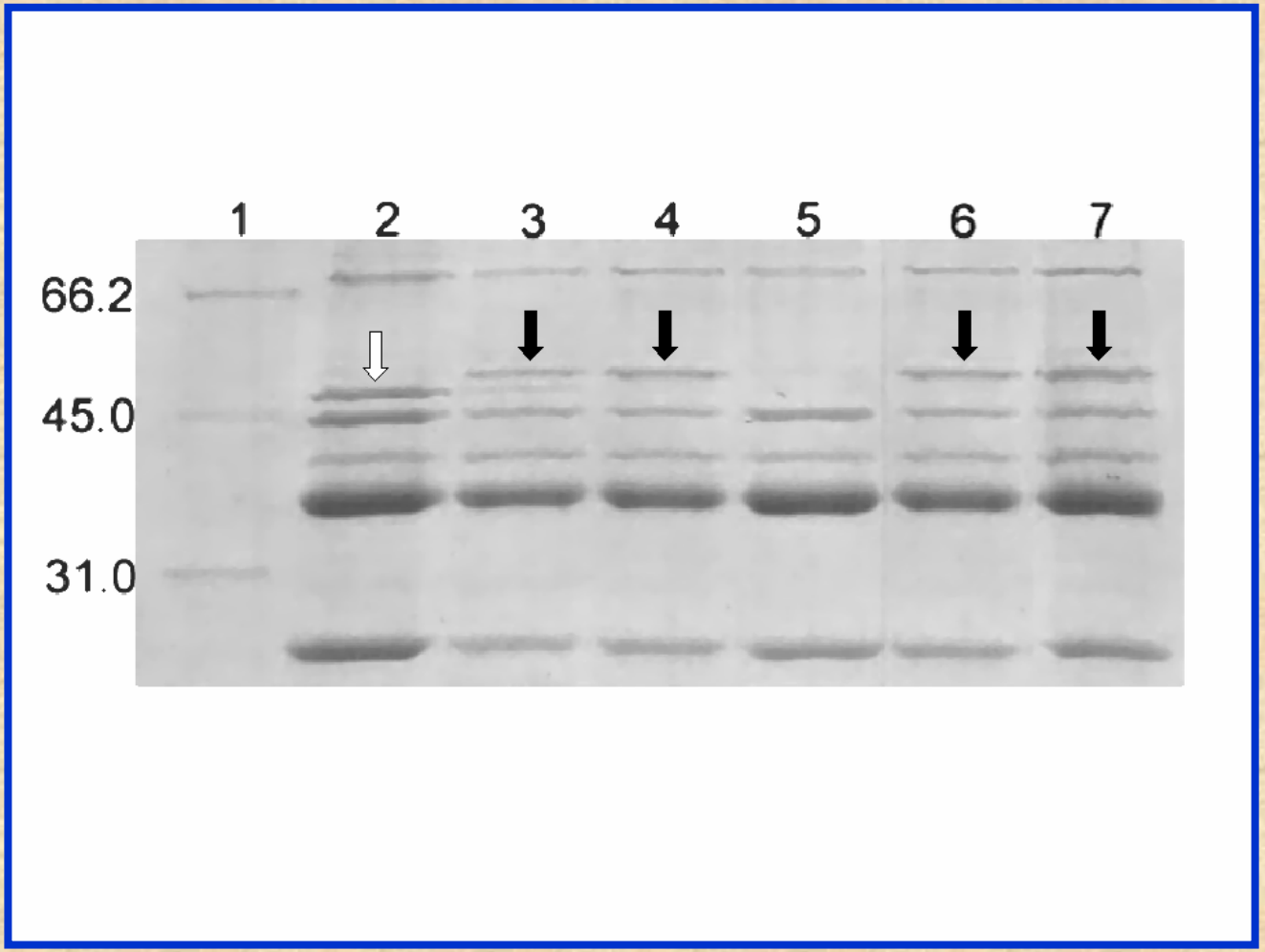
**Pascual A. et al. J. Med Microbiol 1997**

# OMPs de *P. aeruginosa*: Effect of SLC "eluates"



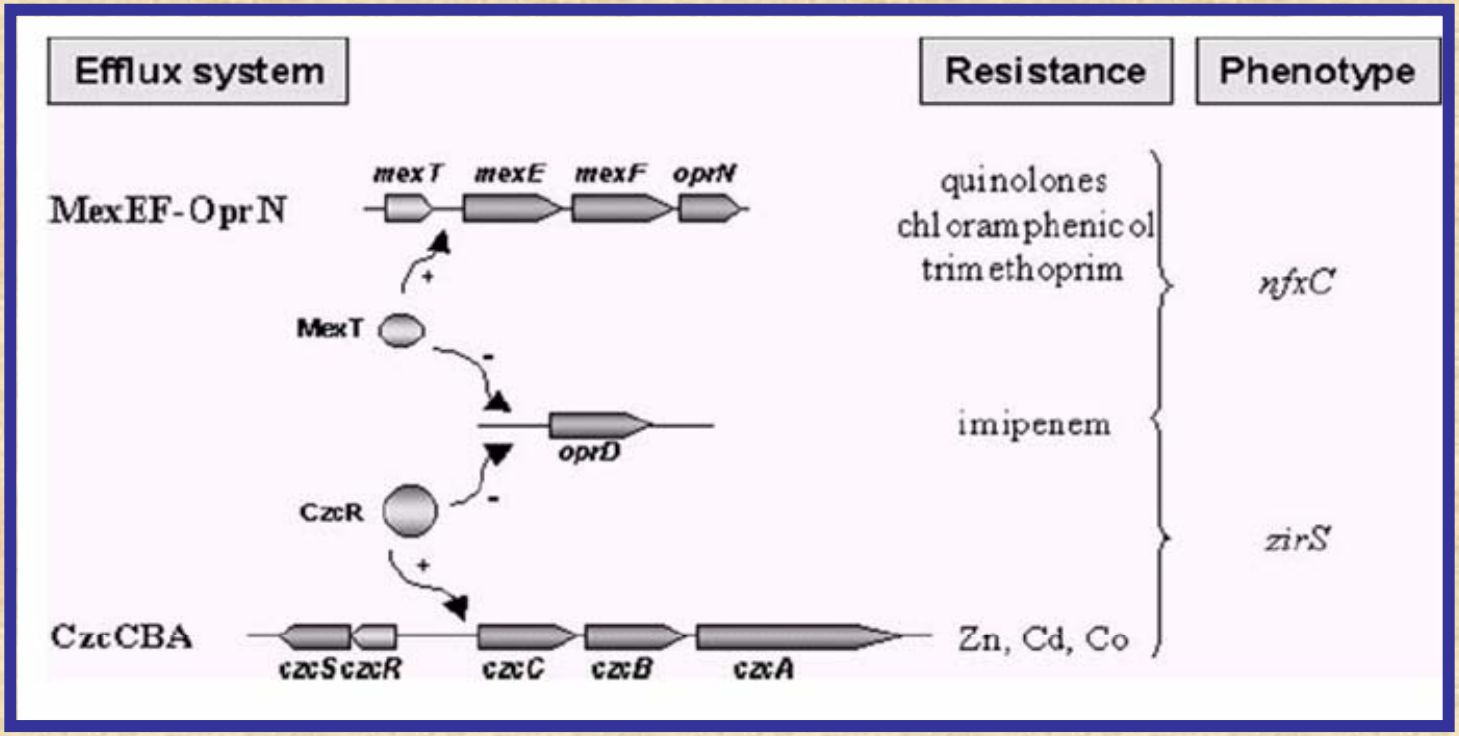

A. Pascual et al. Antimicrob Agents Chemother. 1999

# Zinc eluted from siliconized latex-urinary catheters decreased OprD expression, causing carbapenem resistance in *P. aeruginosa*.



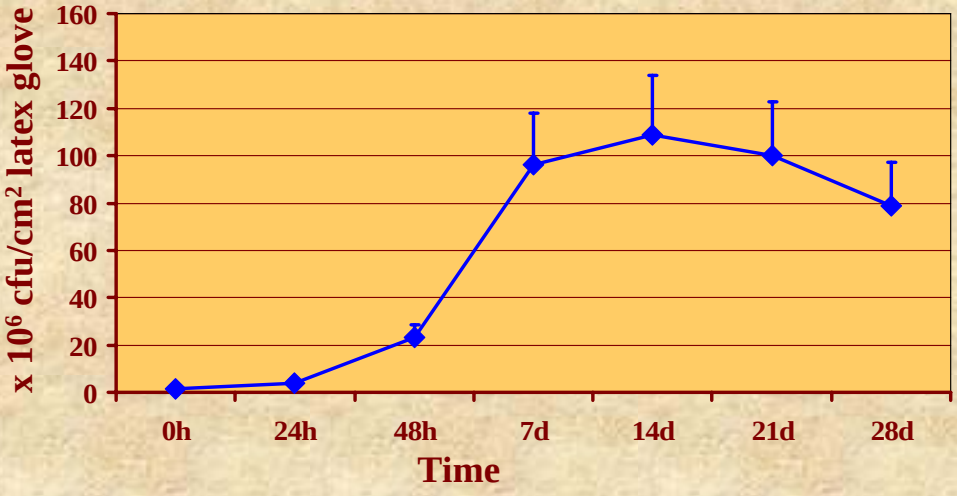

Pascual A. et al. Antimicrob Agents Chemother. 2003

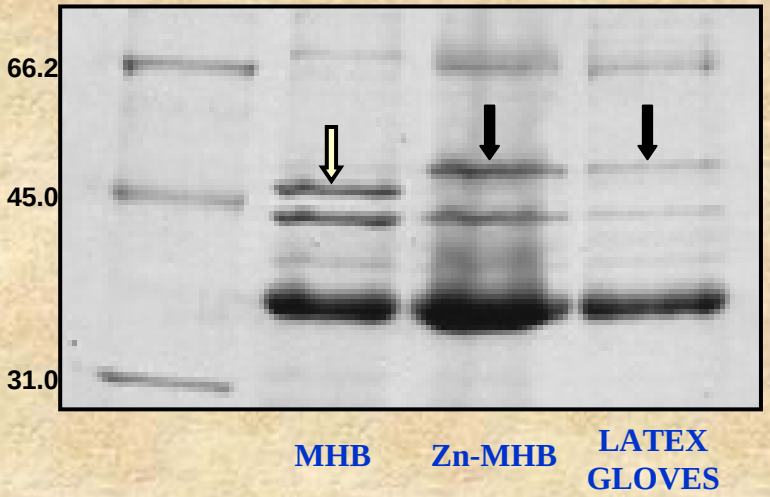
# CzcCBA: Efflux pump responsible of co-resistance to carbapenems and heavy metals in *P. aeruginosa*.




Perron K. et al. J Biol Chem 2004; 279: 8761-8768.

# Survival of *Pseudomonas aeruginosa* on latex gloves




Pascual A. et al. J. Antimicrob. Chemother. 2006.

# Experimental foreign body-associated peritonitis in mice. Siliconized latex Vs Silicone. Strain: *P. aeruginosa* PAO1.

| Catheter | Inoculum log cfu/ml | Liver Log cfu/g | Splain Log cfu/g | Catheter Log cfu | EXITUS |
|---|---|---|---|---|---|
| Siliconized latex 24 h | 6.98±0.23 | 8,24±1,21 | 8,23±1,61 | 6,90±0,87 | 80% |
| Silicone 24 h | 6.98±0.23 | 8,52±0,88 | 8,24±1,63 | 7,33±0,46 | 80% |
| Siliconized latex 24h+IMP | 6.89±0.35 | 3,04±0,80 | 4,01±0,91 | 3,82*±2,02 | 0% |
| Silicone 24 h+IMP | 6.98±0.23 | 3,11±1,03 | 4,06±1,04 | 2,27±1,71 | 6% |

* p>0.05 compared to Silicone

**Zinc-induced imipenem resistance in *P. aeruginosa* affects the bacterial survival at local level but not at systemic level.**

Pascual A. et al. Submitted

# Strategies for biofilm prevention in urinary catheters

1. Avoid the use of Siliconized latex urinary catheters (silicone).
2. Anti quorum sensing agents (furanones).
3. Iron-scavenging catheters.
4. Bacterial interference.

# *Thanks for your attention*