Silver as a putative health concern

Lotte Jakobsen\textsuperscript{1,2}, Karen A. Krogfelt\textsuperscript{1} & Niels Frimodt-Møller\textsuperscript{1,2}
Statens Serum Institut\textsuperscript{1} & Hvidovre Hospital\textsuperscript{2}
## Use of silver: in and out of medicine

### Medical:
- Topical antimicrobial agent in burns
- Topical use for tonsillitis
- Bandages for trauma and diabetic wounds
- Silver coated catheters and medical devices
- Dental silver amalgams
- Arsphenamine – iv treatment for syphilis

### Non-medical:
- Desinfect water, e.g. Legionella, anthrax
- Sterilize drinking water, e.g. space shuttles
- Growth promoter in agriculture
- Additive in foods, traditional medicine
- Coating of clothing etc, e.g. sports fabrics, sleeping bags, socks
- Coating supermarket surfaces for meat storage
Silver as antibacterial in household products

**Silver Wash**

**What makes Silver Wash superior?**

When water is supplied, 99.9% pure silver is electrolyzed to create Silver Nano ions or Ag+. These ions mix with your clothes, bond with fabric fibers at molecular level, and eliminate up to 99.9% of germs, further guaranteeing an anti-bacterial effect for up to 30 days after washing. Samsung’s Silver Nano Washers come with enough silver to protect you and your family for 10 long years!

**Sterilization result of fabric after 72 hours**

<table>
<thead>
<tr>
<th>Silver Nano Health System™</th>
<th>Conventional</th>
</tr>
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<td>Antibacterial Coating</td>
<td></td>
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Traditionally used in clinical settings -> today also outside the clinics!!
Reports of silver resistance

Salmonella typhimurium resistant to silver nitrate, chloramphenicol and ampicillin

Silver-resistant Enterobacteriaceae from hospital patients

Instability and linkage of silver-resistance in E. cloacae

Gentamicin- and silver-resistant Pseudomonas

Plasmid-determined silver resistance in Ps. stutzeri

Plasmid mediated silver resistance in A. baumannii

Plasmid mediated resistance to silver ions in E. coli

Lancet 1975

Can J Microbiol 1979

J Clin Pathol 1976

BMJ 1979

J Bacteriol 1984

Biometals 1994

Indian J Med Res 1985
Modes of resistance

- Efflux mechanisms (encoded by sil genes)

- Silver binding peptides (silE)

  Gupta et al, Microbiol 2001

- Peptide-mediated tolerance

  Sedlak et al, Appl Environ Microbiol, Epub ahead of print
Diversity of silver resistance genes

Comparison of the:
- pMG101 sil determinant
- *E. coli* K-12 and O157:H7 *agr* homologues.

-> up to 4% variation

-> wide distribution of homologues

Gupta et al. *Microbiology* (2001)
Diversity of silver resistance genes

Located on plasmid->
horizontal gene transfer of silver resistance!

Gupta et al. *Microbiology* (2001)
Development of resistance \textit{in vitro}

Table I. Antimicrobial susceptibility of \textit{S. epidermidis}: MICs and MBCs before and after 20 passages through subinhibitory concentration of the drugs

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (mg/L)</th>
<th>MBC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-passage</td>
<td>post-passage</td>
</tr>
<tr>
<td>MIN\textsuperscript{a}</td>
<td>0.078</td>
<td>0.156</td>
</tr>
<tr>
<td>RIF\textsuperscript{a,b}</td>
<td>0.02</td>
<td>&gt;500</td>
</tr>
<tr>
<td>RIF\textsuperscript{b,c}</td>
<td>0.012</td>
<td>&gt;500</td>
</tr>
<tr>
<td>MIN + RIF\textsuperscript{a} (1:1\textsuperscript{d})</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>MIN + RIF\textsuperscript{c} (1:1\textsuperscript{d})</td>
<td>0.015</td>
<td>0.25</td>
</tr>
<tr>
<td>TC\textsuperscript{a}</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>CHA\textsuperscript{a}</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>PCMX\textsuperscript{a}</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>PHMB\textsuperscript{a}</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>CHA + TC\textsuperscript{a} (3:1\textsuperscript{d})</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>CHA + AgSD\textsuperscript{a,c} (3:1\textsuperscript{d})</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

MIN, minocycline; RIF, rifampicin; TC, triclosan; CHA, chlorhexidine acetate; PCMX, \textit{p}-chloro-\textit{m}-xylenol; PHMB, polyhexamethylene bis-biguanide; AgSD, silver sulphadiazine.

\textsuperscript{a}Tested against ATCC strain.
\textsuperscript{b}After 10 passages.
\textsuperscript{c}Tested against clinical isolate H.
\textsuperscript{d}Ratios based on drug levels in catheters and concentrations are the total of the two drugs combined.

10 passages

Tambe et al. JAC 2001
Glansen er ved at gå af nanosølvet

Med nanoteknologien fremmarch anvendes sølv i stigende grad i hverdags som bakteriedræbende middel. Men nu slår svenske og americanske myndigheder fast, at sølv må blive tilbage, da det ikke har været tilstrækkeligt testet.

Af Thomas Lemke, fredag, 16. oktober 2009

I februar 2006 udsendte Samsung en pressemelding om, at der indgik sølv i deres vaskemaskine, Silver Wash. I pressemeddelelsen gav man andre produkter, der også indeholdt sølv.

I pressemeddelelsen afholdt hverdags i 2005 blev der vendt tilbage til en helt nyt og revolutionerende teknologi.

POLITIKEN.DK

GUIDE: Find de skæve og føde film på Cph:Dox
MERE END RENT: Vaskeputer er fyldt med hemmelig kemikali

Ingen kender konsekvenserne af nanoteknologi

Der er postet 239 millioner forskningskroner i nanoteknologi i år. Men ingen ved, om den nye tids teknologi giver kræft, hjerte-kar-sygdomme eller ødelægger miljøet.
Susceptibility to silver nitrate in common human pathogens in Denmark

S. aureus (bacteremia):
- MSSA, 1972-2007 \( N = 130 \)
- MRSA, 2001-06 \( N = 70 \)
  (Various mec-types)
- Total \( N = 200 \)

E. coli
- Human bacteremia \( N = 34 \)
- Human UTI \( N = 34 \)
- Human volunteers \( N = 34 \)
- Chicken \( N = 34 \)
- Chicken meat \( N = 34 \)
- Pigs \( N = 34 \)
- Pork \( N = 34 \)
- Total \( N = 238 \)

For each group, strains were chosen to vary in antibiotic susceptibility, from no - to multiple-resistant.
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MSSA, 1972-2007  N = 130
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   (Various mec-types)
Total  N = 200
E. coli
  Human bacteremia  N = 34
  Human UTI  N = 34
  Human volunteers  N = 34
  Chicken  N = 34
  Chicken meat  N = 34
  Pigs  N = 34
  Pork  N = 34
  Total  N = 238

Control silver-nitrate resistant isolates from Åsa Melhus, MIC ≥ 128 mg/L
Prevalence of resistance to silver in a Burns unit

117 bacterial non-duplicate clinical isolates from 71 patients:

Only one isolate, an *Enterobacter cloacae*, was resistant with an MIC of silver of >5440 μg/mL.

Ip et al, J Hosp Infect 2006
Silver-palladium surfaces inhibit biofilm formation

Or do they?
In the case of a high load of a silver resistant E. coli J53 pMG101 biofilm occurred upon a layer of surface-associated dead bacteria

Chiang et al, Appl Environ Microbiol 2009
Cross-resistance

Silver resistant mutant of E. coli selected after stepwise exposure to silver nitrate or silver sulfadiazine -> low-level cross-resistance to cephalosporins and HgCl2

<table>
<thead>
<tr>
<th>Strain</th>
<th>AgNO₃ (µg/ml)</th>
<th>AgSD (µg/ml)</th>
<th>HgCl₂ (µg/ml)</th>
<th>Cephaloridine (µg/ml)</th>
<th>Cephalothin (µg/ml)</th>
<th>Cefepime (µg/ml)</th>
<th>Cefpirom (µg/ml)</th>
<th>Tetracycline (µg/ml)</th>
<th>Chloramphenicol (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>8 (16)</td>
<td>16</td>
<td>1.4</td>
<td>4</td>
<td>8</td>
<td>0.03</td>
<td>0.06</td>
<td>0.8</td>
<td>5</td>
</tr>
<tr>
<td>116AgNO₃R</td>
<td>&gt;1,024(64)</td>
<td>&gt;1,024</td>
<td>6.4</td>
<td>16</td>
<td>32</td>
<td>0.13</td>
<td>0.25</td>
<td>0.8</td>
<td>6</td>
</tr>
<tr>
<td>496</td>
<td>8 (16)</td>
<td>16</td>
<td>1.4</td>
<td>4</td>
<td>8</td>
<td>0.06</td>
<td>0.06</td>
<td>1.0</td>
<td>4</td>
</tr>
<tr>
<td>496AgNO₃R</td>
<td>&gt;1,024(64)</td>
<td>1,024</td>
<td>6.4</td>
<td>8</td>
<td>32</td>
<td>0.13</td>
<td>0.13</td>
<td>1.3</td>
<td>5</td>
</tr>
<tr>
<td>496AgSD</td>
<td>&gt;1,024(64)</td>
<td>&gt;1,024</td>
<td>12.8</td>
<td>16</td>
<td>32</td>
<td>0.13</td>
<td>0.13</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>B1</td>
<td>8 (16)</td>
<td>16</td>
<td>1.4</td>
<td>16</td>
<td>32</td>
<td>0.13</td>
<td>0.13</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>B1AgNO₃R</td>
<td>&gt;1,024(64)</td>
<td>&gt;1,024</td>
<td>2.8</td>
<td>128</td>
<td>64</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>B1AgSD</td>
<td>&gt;1,024(64)</td>
<td>&gt;1,024</td>
<td>1.4</td>
<td>256</td>
<td>64</td>
<td>0.50</td>
<td>1.0</td>
<td>1.0</td>
<td>2</td>
</tr>
</tbody>
</table>

* MICs of CoSO₄ (440 µg/ml), CrCl₃ (1,335 µg/ml), and CuSO₄ (1,250 µg/ml) were identical for all strains, except for B1AgNO₃R, which showed a twofold higher value. MICs of MnSO₄ (1,690 µg/ml) and ZnCl₂ (170 µg/ml) were also identical for all strains, except that of MnSO₄ for B1AgSDR (211 µg/ml) and that of ZnCl₂ for 496 (340 µg/ml). The MIC of Na₂HAsO₄ was identical (500 µg/ml) for the two strains tested, 116 and 116AgNO₃R.

* The values in parentheses are the MICs determined in LB broth without NaCl.

* MICs of this compound were unchanged, in every case, in LB broth without NaCl.

Li et al, J Bacteriol 1997
IncHI2 plasmid from extraintestinal pathogenic *E. coli*

<table>
<thead>
<tr>
<th>Genes</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>terY3Y2XY1W, terZABCDEF</td>
<td>Potassium tellurite</td>
</tr>
<tr>
<td>silESRCBAP</td>
<td>Silver nitrate</td>
</tr>
<tr>
<td>pcoEABCDRSE</td>
<td>Copper sulfate</td>
</tr>
<tr>
<td>aadA</td>
<td>Streptomycin</td>
</tr>
<tr>
<td>aac3-VI</td>
<td>Gentamicin</td>
</tr>
<tr>
<td>tetAR</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>qacE 1</td>
<td>Benzylkonium chloride</td>
</tr>
<tr>
<td><em>SulI</em></td>
<td>Sulfisoxazole</td>
</tr>
</tbody>
</table>

Co-resistance

Johnson et al, Antimicrobial Chemother 2006
**Characteristics of silver resistance isolates**

Table III. Characteristic of strains with phenotypic and/or genetic resistance to silver in the study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Silver-resistance genes</th>
<th>No. of passages&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Other properties</th>
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</thead>
<tbody>
<tr>
<td><em>E. cloacae</em> SM0700965 II</td>
<td>+ + +</td>
<td>NA</td>
<td>Cefotaxime I</td>
</tr>
<tr>
<td><em>E. cloacae</em> S4279/06</td>
<td>+ + +</td>
<td>2</td>
<td>D mutant</td>
</tr>
<tr>
<td><em>E. cloacae</em> S0707396</td>
<td>+ − +</td>
<td>3</td>
<td>D mutant</td>
</tr>
<tr>
<td><em>E. coli</em> B0709322</td>
<td>− − −</td>
<td>Unstable</td>
<td>Colistin R</td>
</tr>
<tr>
<td><em>E. coli</em> S0506373</td>
<td>− − −</td>
<td>8</td>
<td>ESBL positive</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> B0716185</td>
<td>+ + +</td>
<td>NI</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em> CCUG 54718</td>
<td>+ + +</td>
<td>2</td>
<td>ESBL positive, outbreak strain</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> AI2884</td>
<td>+ − +</td>
<td>NI</td>
<td></td>
</tr>
</tbody>
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<sup>a</sup>For induction of silver-resistance and its stability.

<sup>b</sup>After silver exposure.

NA: not applicable; NI: no induction; D: derepressed, ESBL: extended spectrum beta-lactamase; I: indeterminate; R: resistant.

Sütterlin et al, Acta Derm Venereol 2012
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<tr>
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117 bacterial non-duplicate clinical isolates from 71 patients

Only one isolate, an *Enterobacter cloacae*, was resistant (MIC > 5440 μg/mL)

-> extended-spectrum beta-lactamase (ESBL) producer, and was multi-drug resistant (only susceptible to imipenem)

Ip et al, J Hosp Infect 2006
Silver resistance linked to E. coli ST131

**ST131**: associated with the CTX-M-15 extended-spectrum beta-lactamase, has emerged internationally as a multidrug-resistant pathogen causing serious infections

**Plasmid**: a hybrid between a ST131 plasmid and a Klebsiella pneumonia plasmid

Plasmid was associated with a major nosocomial outbreak

Resistance to b-lactams, aminoglycosides, tetracyclines, trimethoprim, sulphonamides, macrolides, silver, copper and arsenic.

Sandegren et al, J Antimicrobial Chemother 2012
Argyria induced by silver

Argyria – deposit of silver in tissues e.g. skin

53-year old man in good general health

8-month progressive gray hyperpigmentation

Denied using any prescription medications

Induced by silver-containing dietary supplement

Bowden et al, J Cutan Pathol 2011

Tonsillitis - silver nitrate is used topically: Cumulative dosage needed to produce argyria ~ 6 g

Syphilis – silver arsphenamine is used IV: Argyria becomes clinically apparent after the exposure to 8 doses ~ a total dose of silver of 1.84 g
Silver: conclusions

• Resistance occur in human pathogenic bacteria

• Cross- and co-resistance have been shown:
  – > selection by other antimicrobials likely

• At the moment, prevalence of silver resistance is low

• Silver – a health concern??
  – Increase awareness -> i.e. monitoring of resistance (and consumption) needed to avoid future spread
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