# New insights into the antimicrobial properties of copper touch surfaces to reduce healthcare acquired infection

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# July 2004 Report from the Infectious Disease Society of America and update:

- 1.7- 2.0 million people infected in U.S. hospitals each year (approx 5% of those admitted)
- 99,000 of those infected die (4th leading cause of death)
- 70% of infections are resistant to at least one drug
- In 2012 estimate cost at $47 billion per annum
- CDC estimates HCAI add 208% to hospital bill; ~ $48,000 per patient
- Trends toward the increasing number of infections and increasing drug resistance show no signs of abating

# Copper Symbols since Antiquity

**Egyptian Ankh**

***Eternal Life***

銅

**Chinese Medicine**

*increase the flow of 'chi' (life energy)*

**Alchemy**

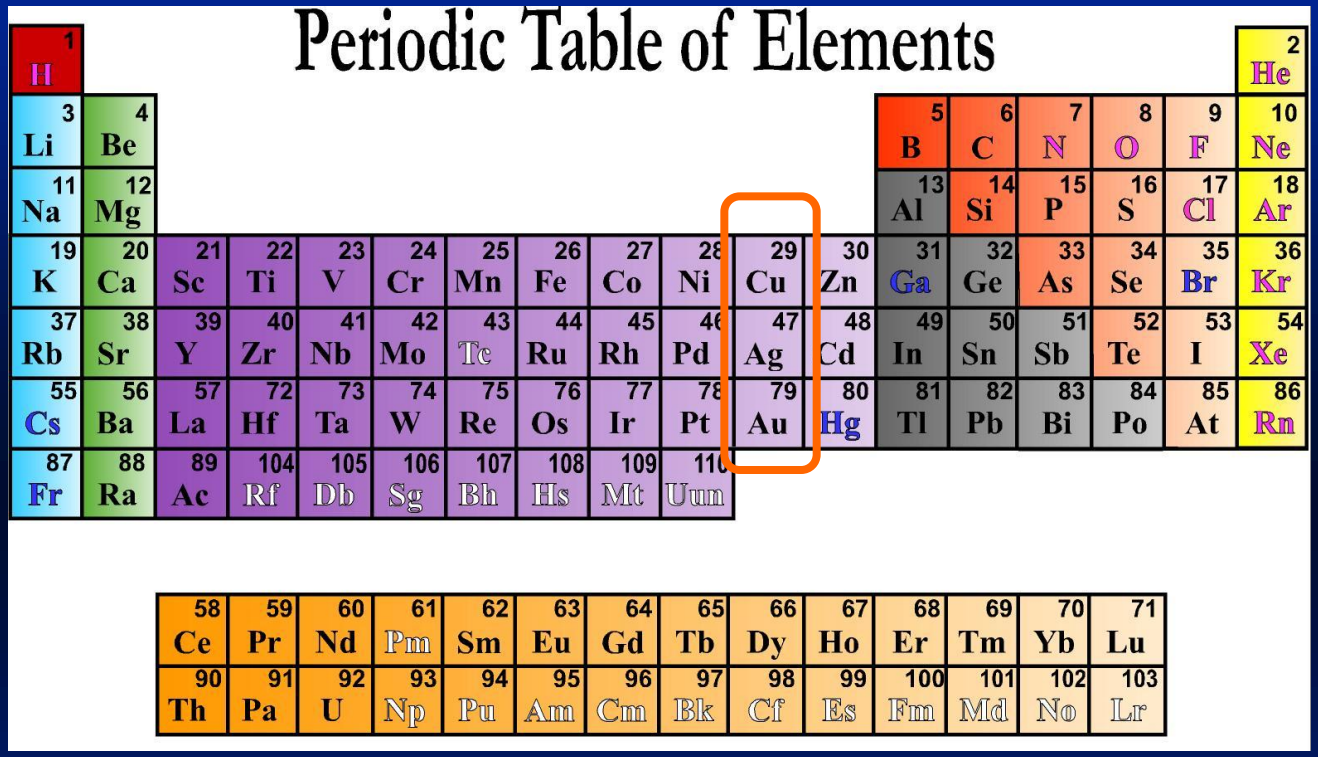
planetary symbol for Venus, protect against evil

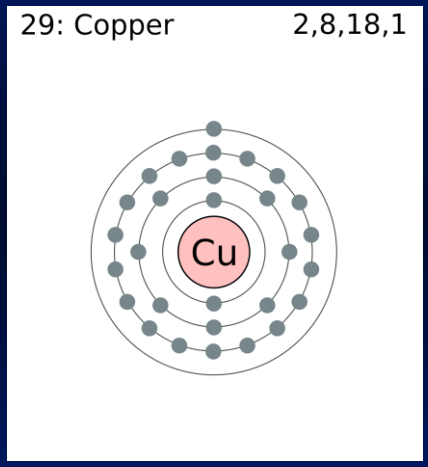
# Copper Used to Protect Human Health in the Past

銅

- Egypt (2000 BC) - Sterilize drinking water and wounds
- Greece, Hippocrates (400 BC) – Treat leg ulcers related to varicose veins
- Aztecs – Copper oxide and malachite  for skin conditions
- France (1850 AD) – Copper workers found to be immune during Cholera epidemic
- USA, Phildadelphia Hospital Study (1983 AD) – Low *E. coli* count on brass doorknobs
- Punjab, India (2005 AD) – *E. coli* eliminated in 24 hours in water-filled brass containers (TamraJal used for 5000 years)

# Copper Chemistry



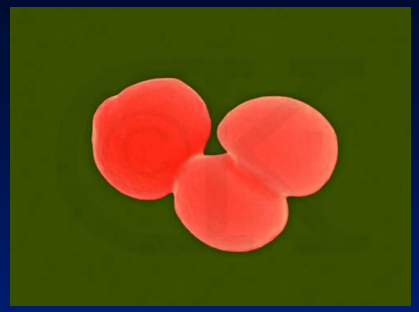




Cu(0) $4s^1\ 3d^{10}$
Cu(I) $4s^0\ 3d^{10}$
Cu(II) $4s^1\ 3d^9$

Good for redox reactions

# ANTIMICROBIAL COPPER

- **Water-borne pathogens in biofilms**
  - *Legionella pneumophila, Helicobacter pylori*
  - *E. coli* O157
- **Food-borne pathogens on surfaces**
  - *E. coli* O157, *Salmonella*
  - *Listeria monocytogenes*
- **Hospital-acquired pathogens**
  - MRSA, VRE, *C. difficile*,
  - *A. baumannii, K. pneumoniae* NDM-1 *etc*
  - Viruses e.g. Influenza H1N1, Adenovirus
  - Fungi- *Candida, Aspergillus* (HVAC systems)



# HOSPITALS PRESENT COMPLEX ENVIRONMENTS


Water circuits, shower and cooling tower aerosols; medical and dental water aerosols;

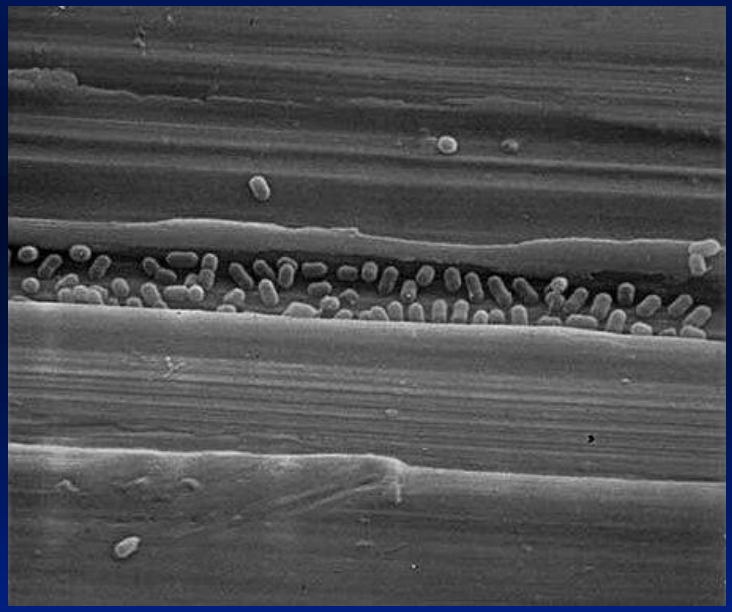
**patient, staff and visitor aerosols, touch surfaces**

# Hospital-Acquired (Nosocomial) Infections

Hospitals are dangerous places for
hand transmission and cross contamination!

# SURFACE CLEANING PROBLEMS

most surfaces are not smooth
- have draw marks, scratches etc where pathogens hide



Stainless steel is not easy to clean, as claimed; risk of cross-contamination

Hand hygiene compliance (e.g. hand rubs or soap) is poor, even professionals

# TERMINAL CLEANING PROBLEMS

- **French, G. L., Otter, J. A., Shannon, K. P., Adams, N. M., Watling, D. & Parks, M. J. (2004).** Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. *J Hosp Infect* **57**, 31-37

- Terminal cleaning (defined as environmental cleaning after discharge of an infectious patient) is ineffective in eradicating MRSA

- 74% of environmental swabs yielded MRSA before cleaning and 66% afterwards.

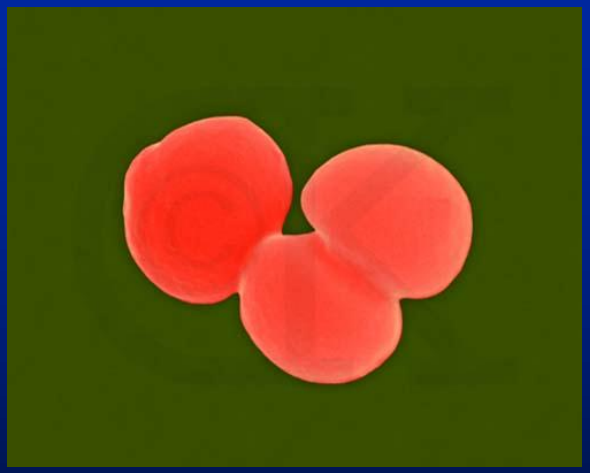
- Evidence indicates the need for more passive preventative measures with regards to reducing MRSA populations on commonly touched surfaces.

# Antimicrobial Copper Experiments

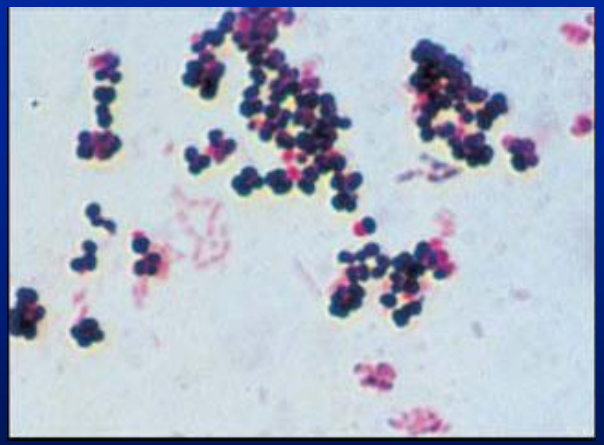
- Stainless steel used throughout hospitals
- Compare with:
- Copper alloys:
- Nickel
- Zinc
- Silver

| | | |
|---|---|---|
| C19700 | copper | 100% Cu |
| C24000 | brass | 80% Cu; 20% Zn |
| C77000 | nickel silver | 55% Cu; 27% Zn; 18% Ni |
| C70600 | Copper nickel | 87% Cu; 1% Zn; 10% Ni; 1% Fe; 1% Mn |
| C51000 | Tin bronze | 95% Cu; 5% Sn; |
| Y90 | | 78% Cu;12% Zn; 3% Ni; 7% Mn |

# Wet contact model



# MRSA



- Methicillin Resistant *Staphylococcus aureus* *"super bug"*
- Major human pathogen responsible for a range of infections.

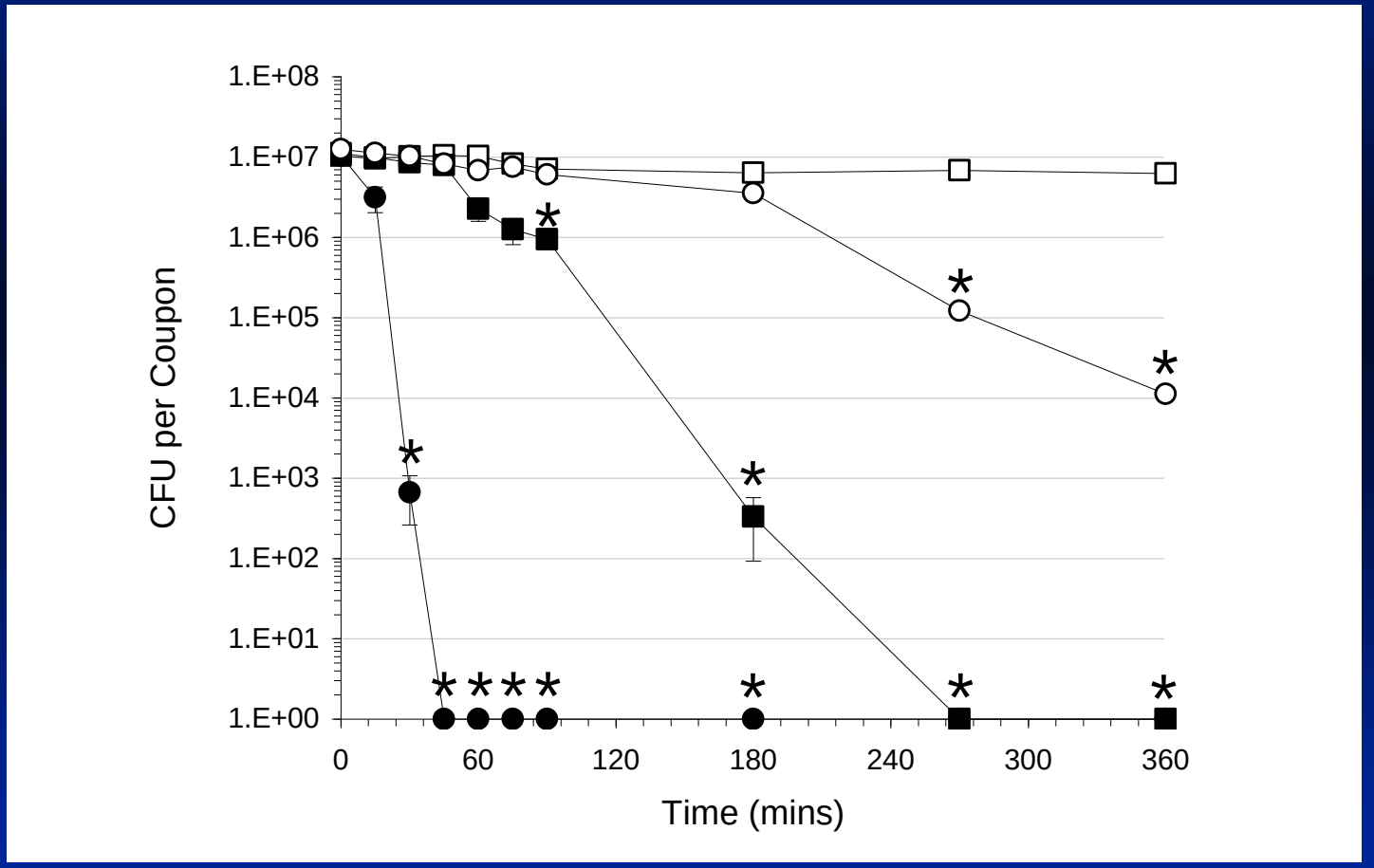
- Essentially resistant to all β–lactam antibiotics, due to the possession of the mecA gene encoding the low-affinity penicillin binding protein.

- Major problem in critical care facilities (studies show prevalence on door handles)
- Epidemic strains of MRSA (EMRSA) spread from hospital to hospital.
- EMRSA 15 and 16: 95% of all UK hospital infections (5000 deaths p.a)

- Emerging VISA and VRSA strains! – Japan, USA, Europe

# MRSA on Stainless Steel (□), C19700 (●), C24000 (■) and C77000 (○) at 20°C

## Wet test simulating coughs, sneezes etc (20 µL inoculum)

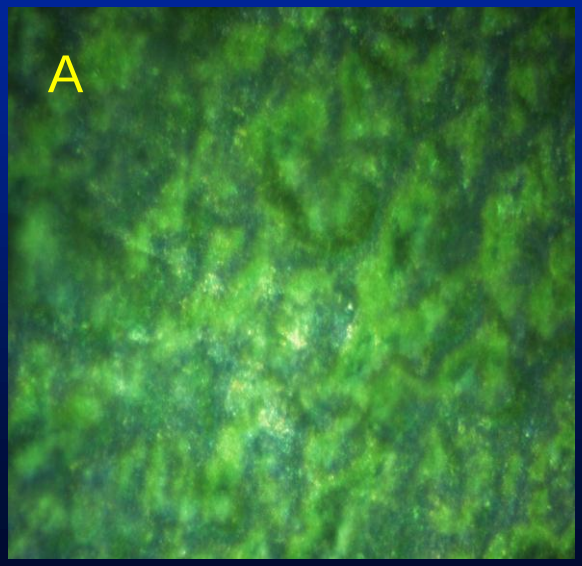


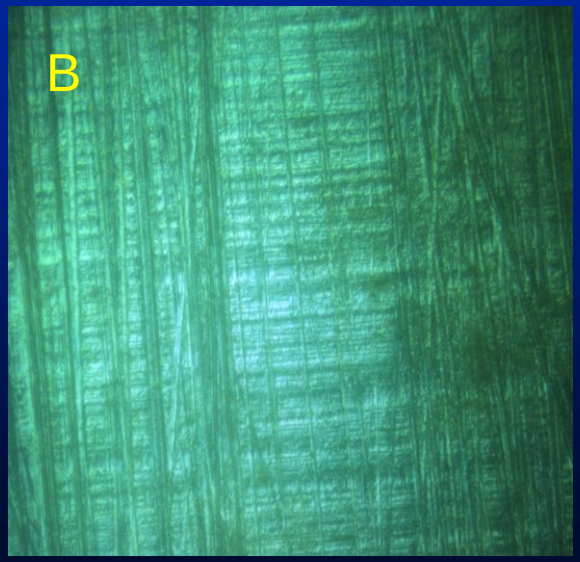

* Indicates p=<0.05 compared to zero time controls

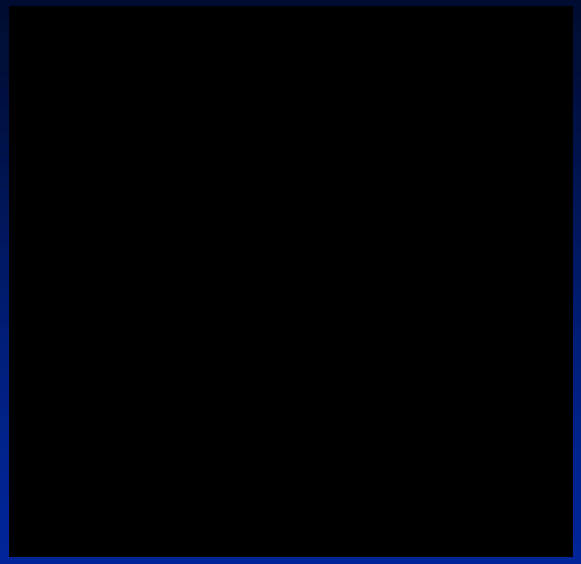
# Further evidence to confirm cell death

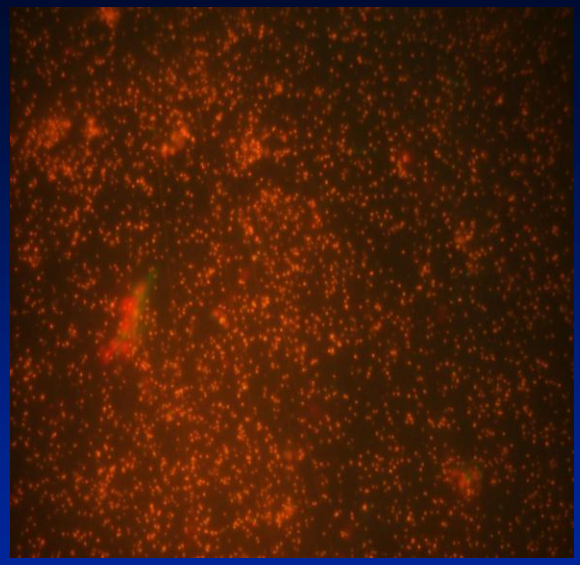
- Are cells sub-lethally damaged but still viable?
- can also show this effect by using viability stains such as CTC (5-cyano-2,3-ditoyl tetrazolium chloride)
- is a monotetrazolium dye
- when biologically reduced, produces a fluorescent, insoluble formazan
- viable cells will fluoresce bright red.  Use patented biofilm microscope to view on surfaces

# EDIC and Epifluorescence analysis for live/dead cells





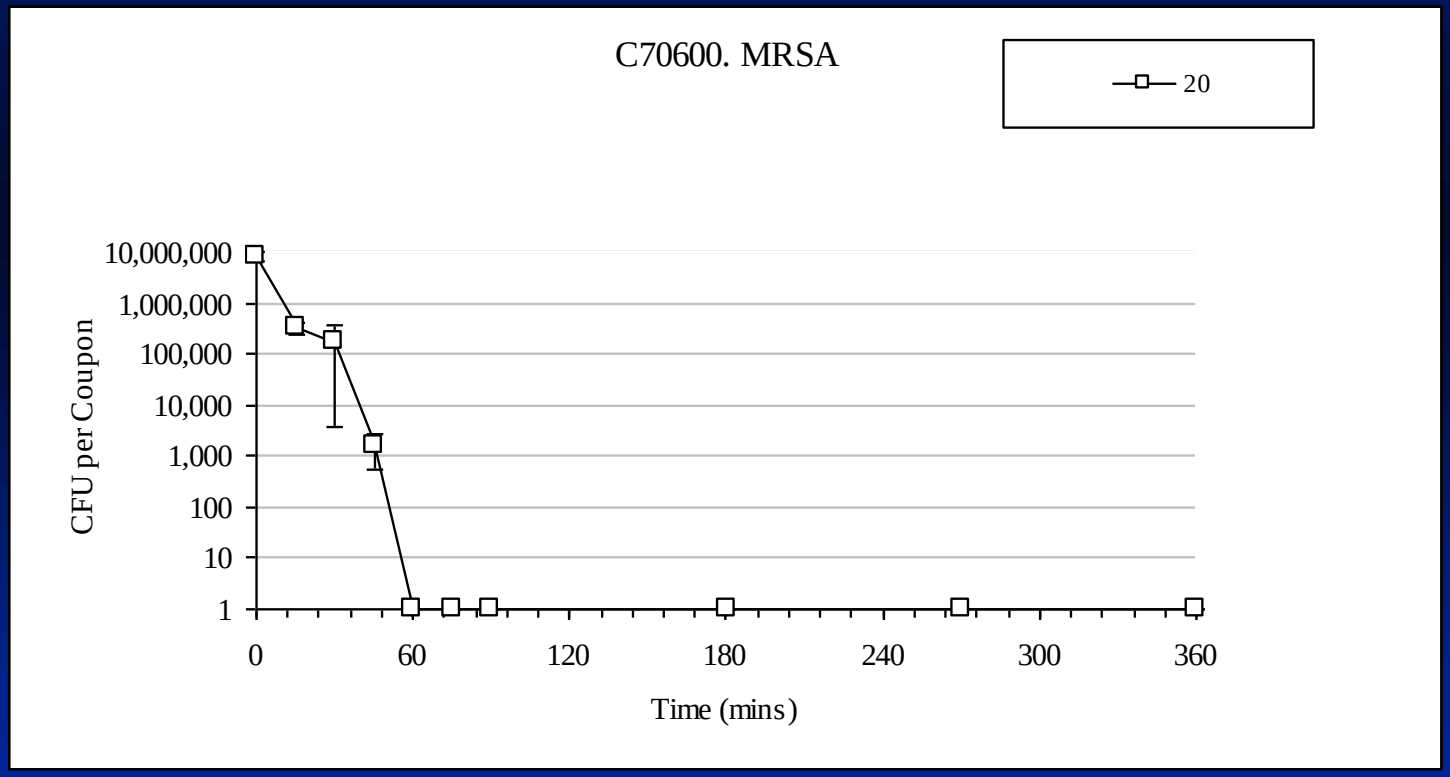
CTC
respiration

Copper (45 min)

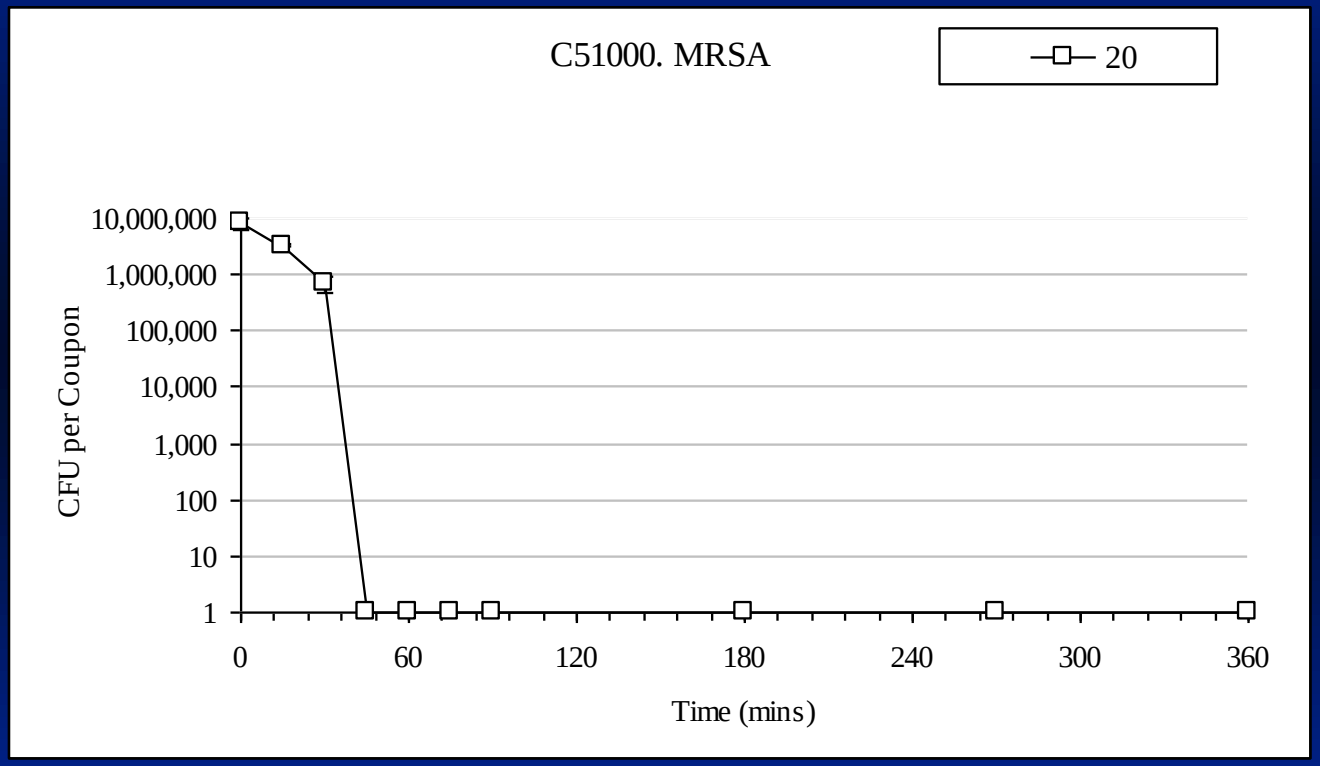
Stainless steel (3 days)

# NEW GENERATION ALLOYS

## C70600 (87% Cu; 1% Zn; 10% Ni; 1% Fe; 1% Mn)



# C51000 (95% Cu; 5% Sn)



# Reduced Bioburden Testing with MRSA

*Journal of Hospital Infection* 2002, **51**, pp140-143.

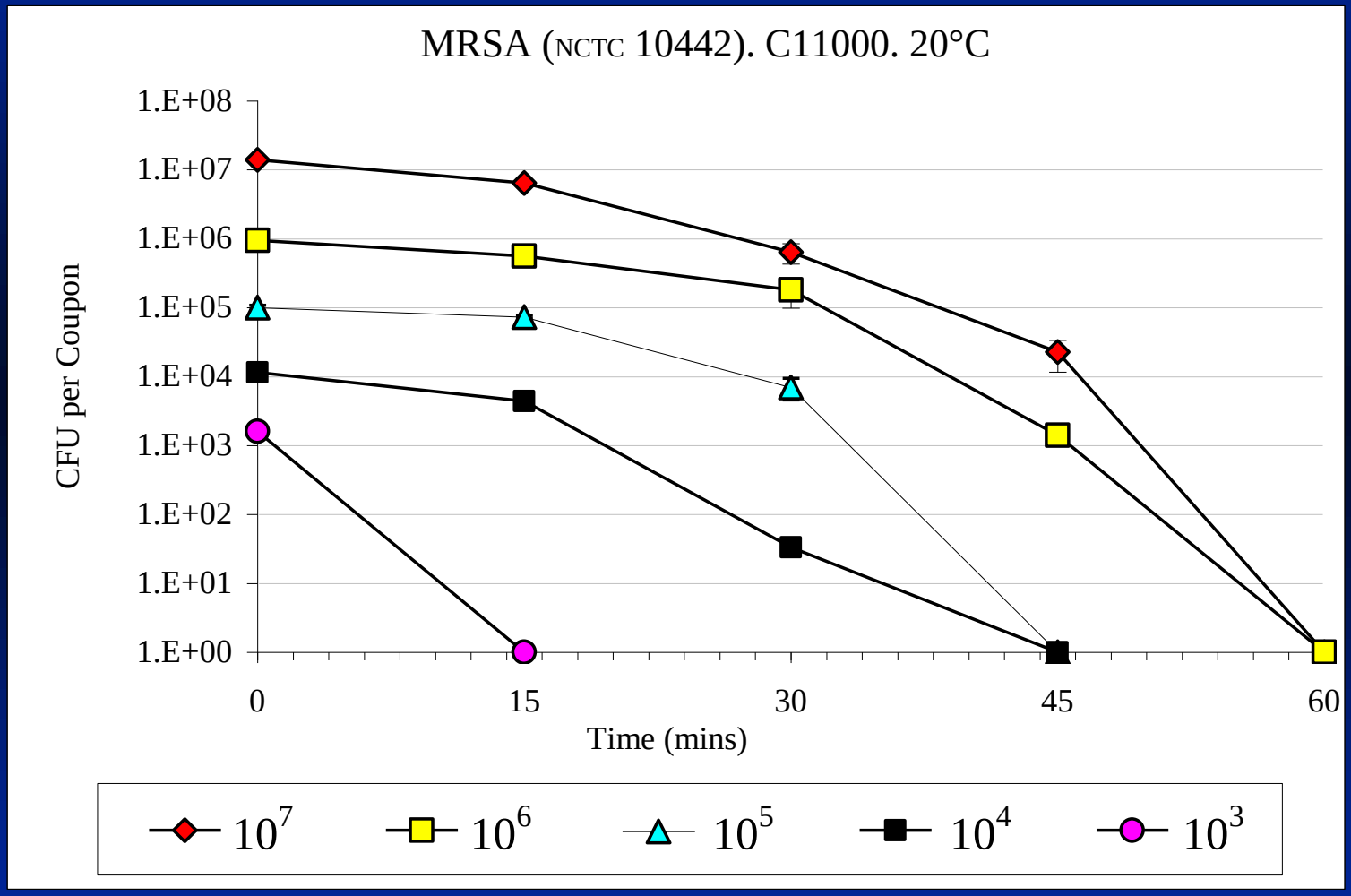
- University hospital: 1 – 6 x $10^3$ per door handle
- i.e. significantly <$10^2$ cfu $cm^{-2}$

Southampton Research:

- MRSA viability on stainless steel is in days
- $10^7$ MRSA viability on 1 $cm^2$ copper C11000 section is 60 min.
- What time can be achieved with a reduced inoculum of $10^5$, $10^4$ and $10^3$ MRSA cells?

# Inoculum Testing with MRSA on 100% Cu

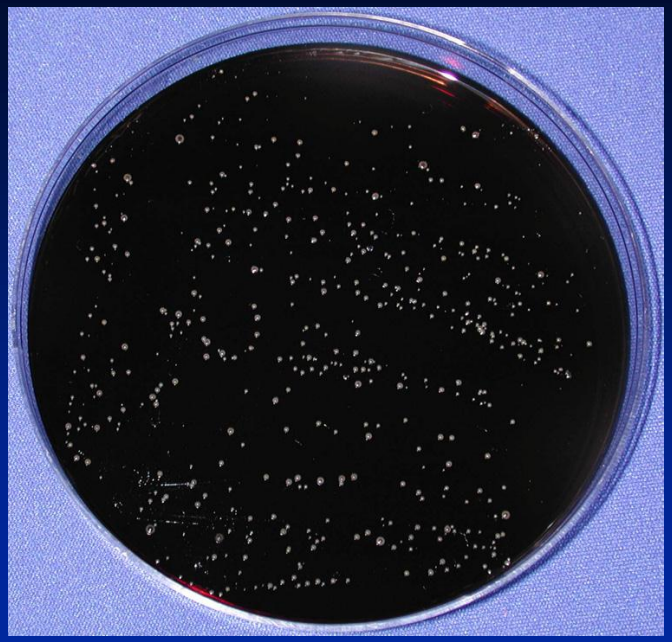
Wet test simulating coughs, sneezes etc




n = 3

# Concerns about nosocomial enterococcal infections

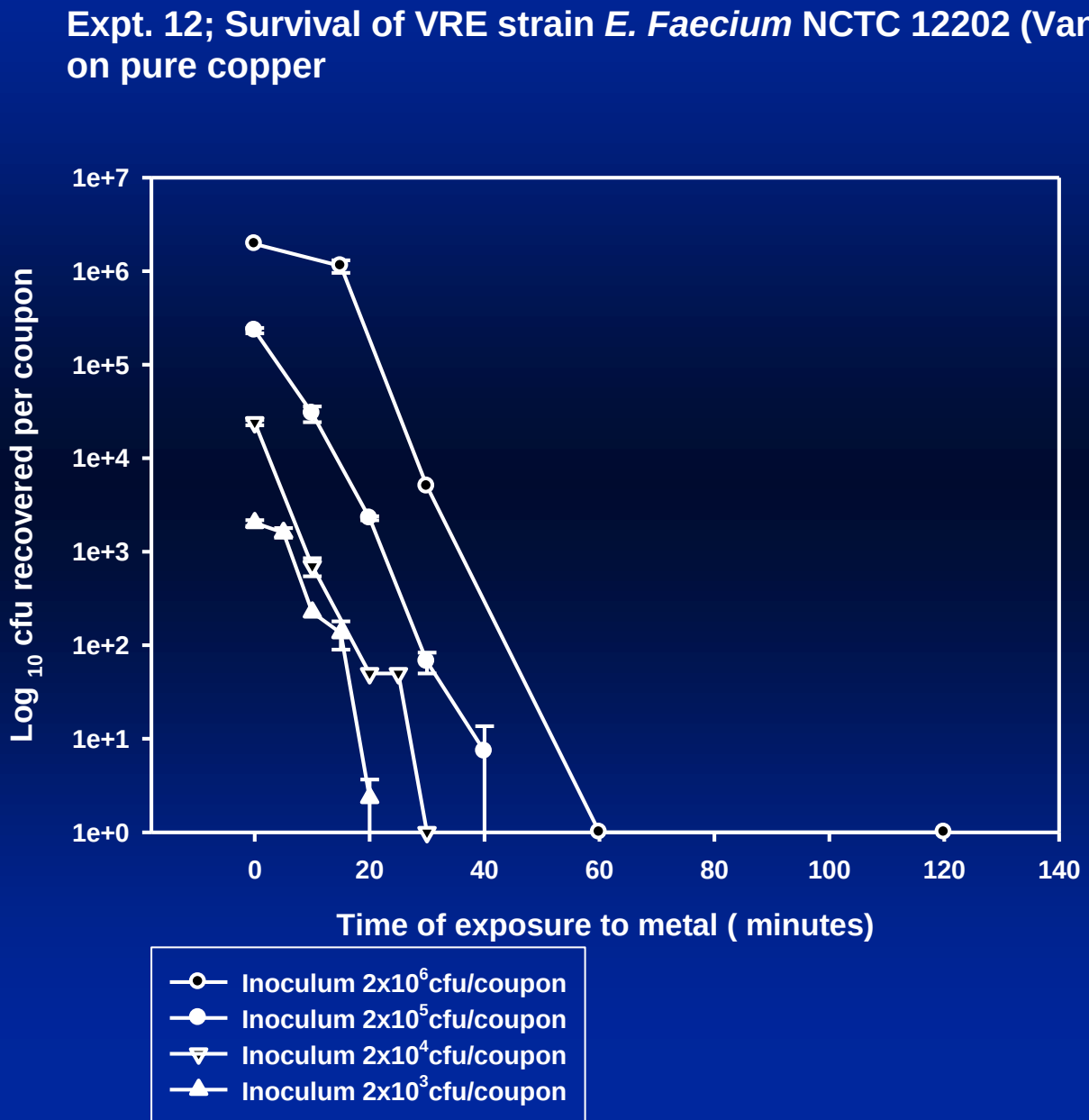
- Vancomycin resistant enterococci (VRE) isolated not only from hospital personnel and patients but also from many hospital surfaces including bedrails, telephones, call buttons, door knobs, toilet seats and bedside tables



Culture plate showing gross contamination of a call button with vancomycin-resistant *Enterococcus* (VRE)

Eckstein et al 2007

# Survival of VRE *E. faecium* on copper

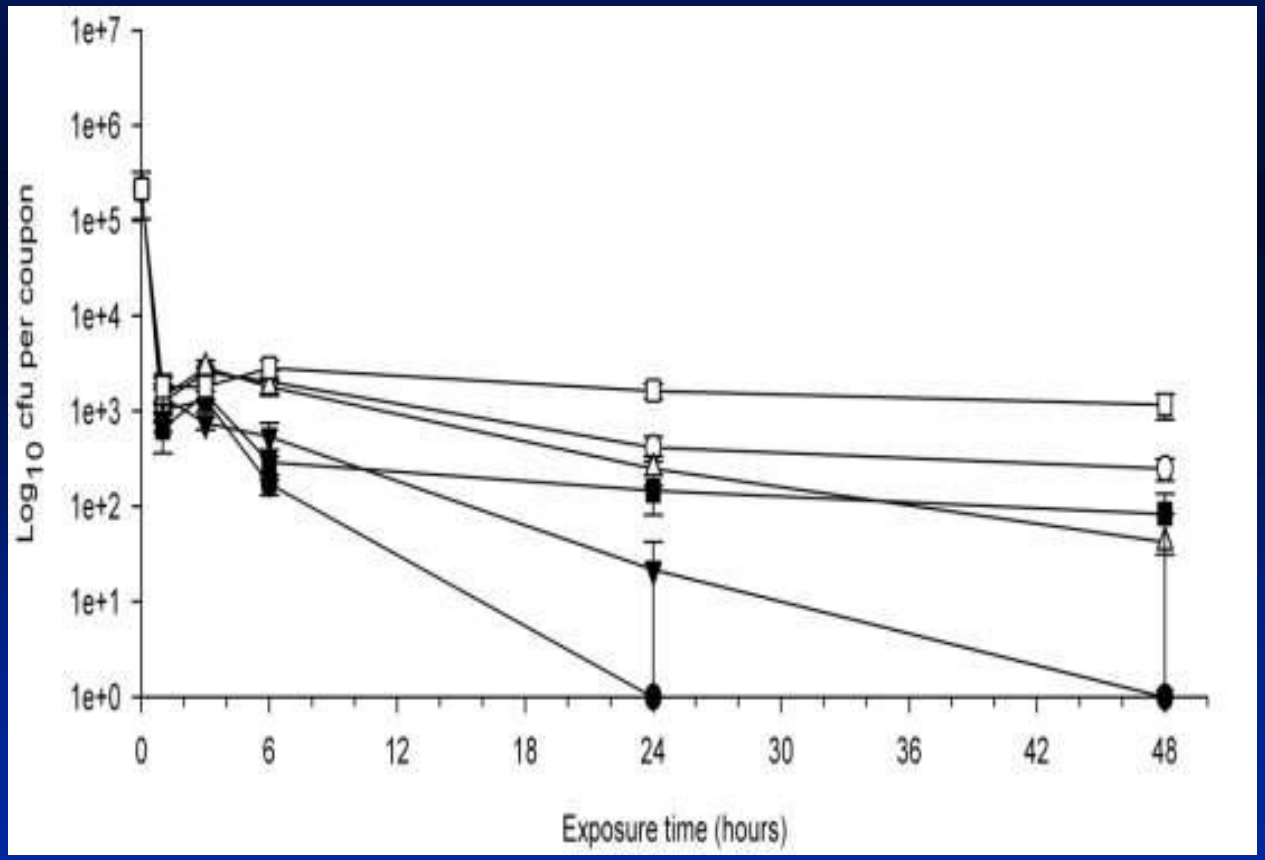



Reduced inoculum lowers survival

# *Clostridium difficile* forms resilient spores


- Most important cause of hospital-acquired diarrhoea.
- Anaerobic bacterium that is present in the gut of healthy adults and infants.
- Antibiotics disturb balance of bacteria in the gut, *C. difficile* multiplies rapidly and produces toxins which cause illness.
- Infection is usually spread on the hands or contact with environmental surfaces (e.g. floors, bedpans, toilets).
- Spores resistant to handrubs
- *Mortality rate* as high as 25% in elderly patients who are frail (Aberra, 2013).

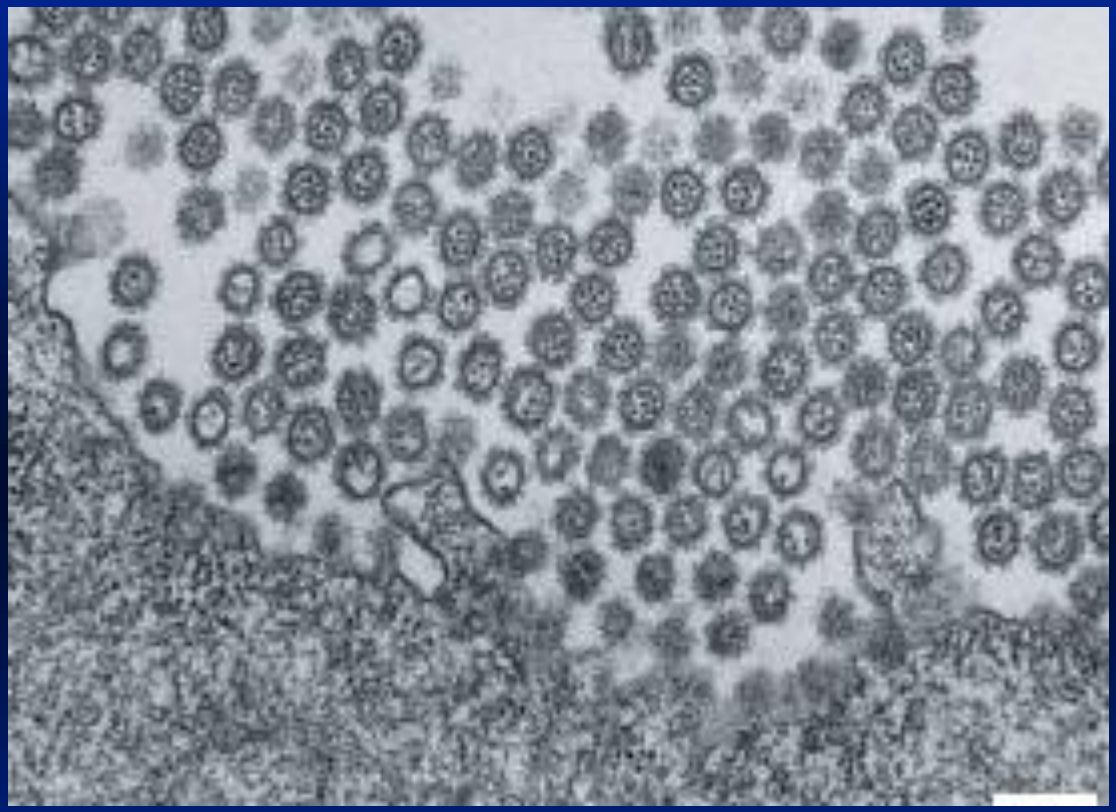
# Survival of *C. difficile* cells and spores




**Spores**

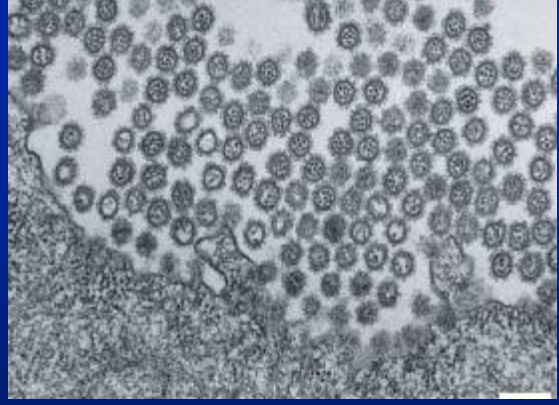
C11000 (●),
C26000 (○),
C51000 (▼),
C70600 (Δ),
C75200 (■),
S30400 (□) at 22ºC.

# Influenza A

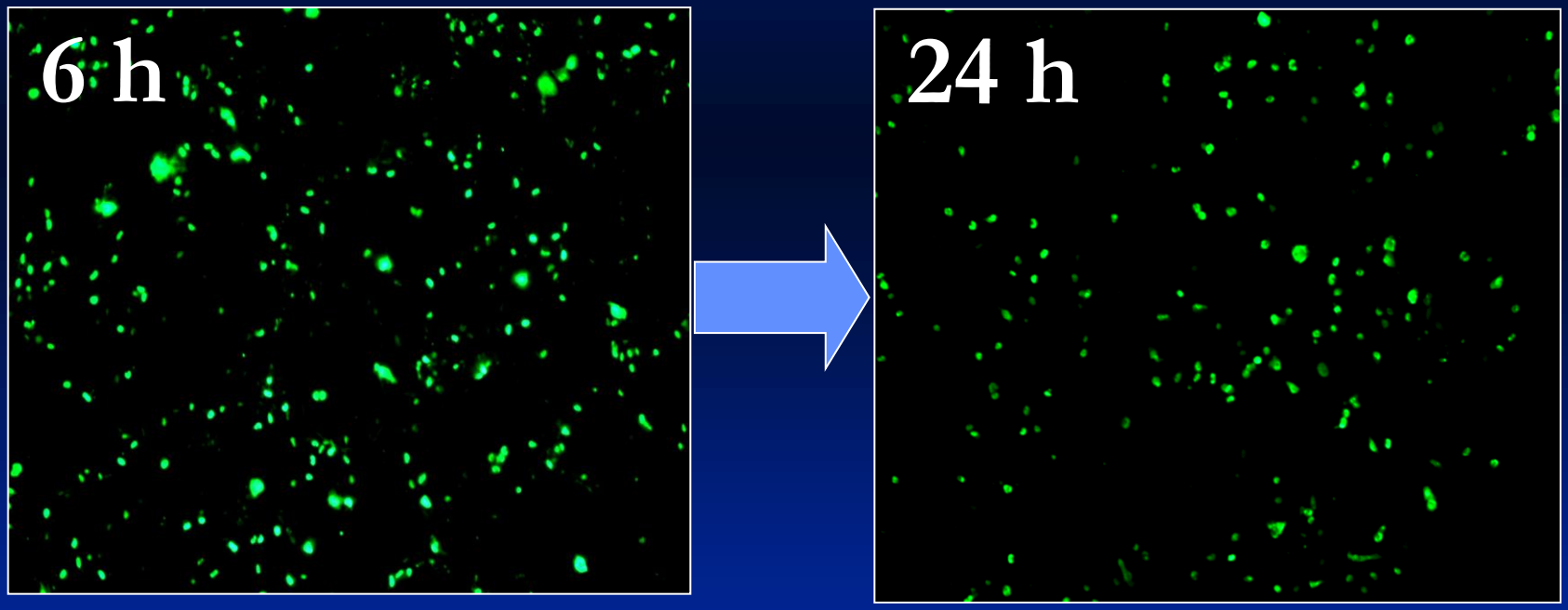


- On average, kills 12000 in UK, 36000 in USA each year
- Survives well on surfaces; easily transferred between hand/surfaces;
- Contaminated hand can contaminate 7 further surfaces

# Influenza A (H1N1) on Stainless Steel



- Viable Influenza A recovered after 6 h and 24 h, as demonstrated by fluorescence from infected cells (orig. $2x10^6$ virus)

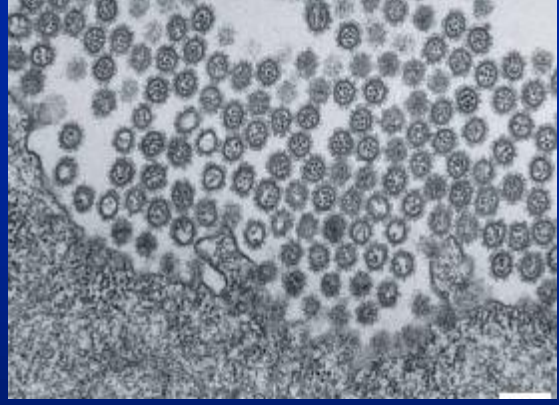



Approx. $10^6$

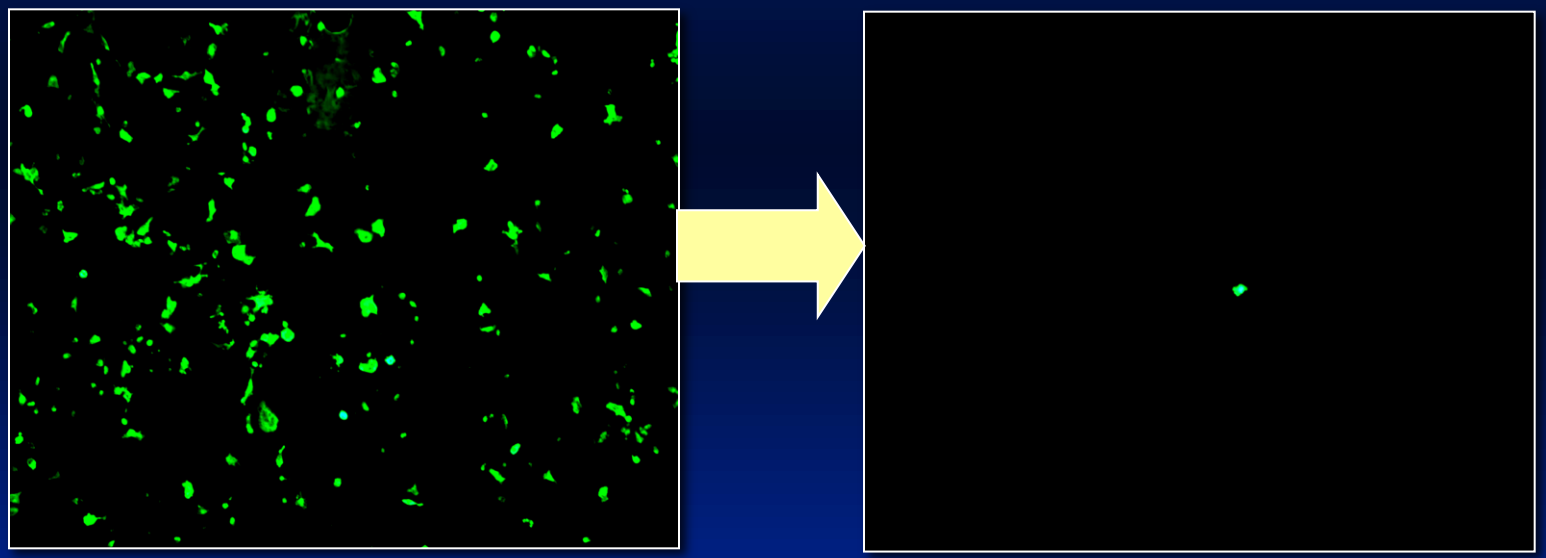
Approx. $5x10^5$

20 µl virus onto coupon, recovered in 5 ml PBS, and 100 µl inoculated to cell culture

# Influenza A (H1N1) on Copper



- 2 x $10^6$ pfu: reduced viability after only 60 minutes exposure on copper, and even greater reduction at 6 h, compared to stainless steel



60 min

**Approx. $5x10^5$ 75% reduction**

6 h

**<$5x10^2$ 4-log reduction**

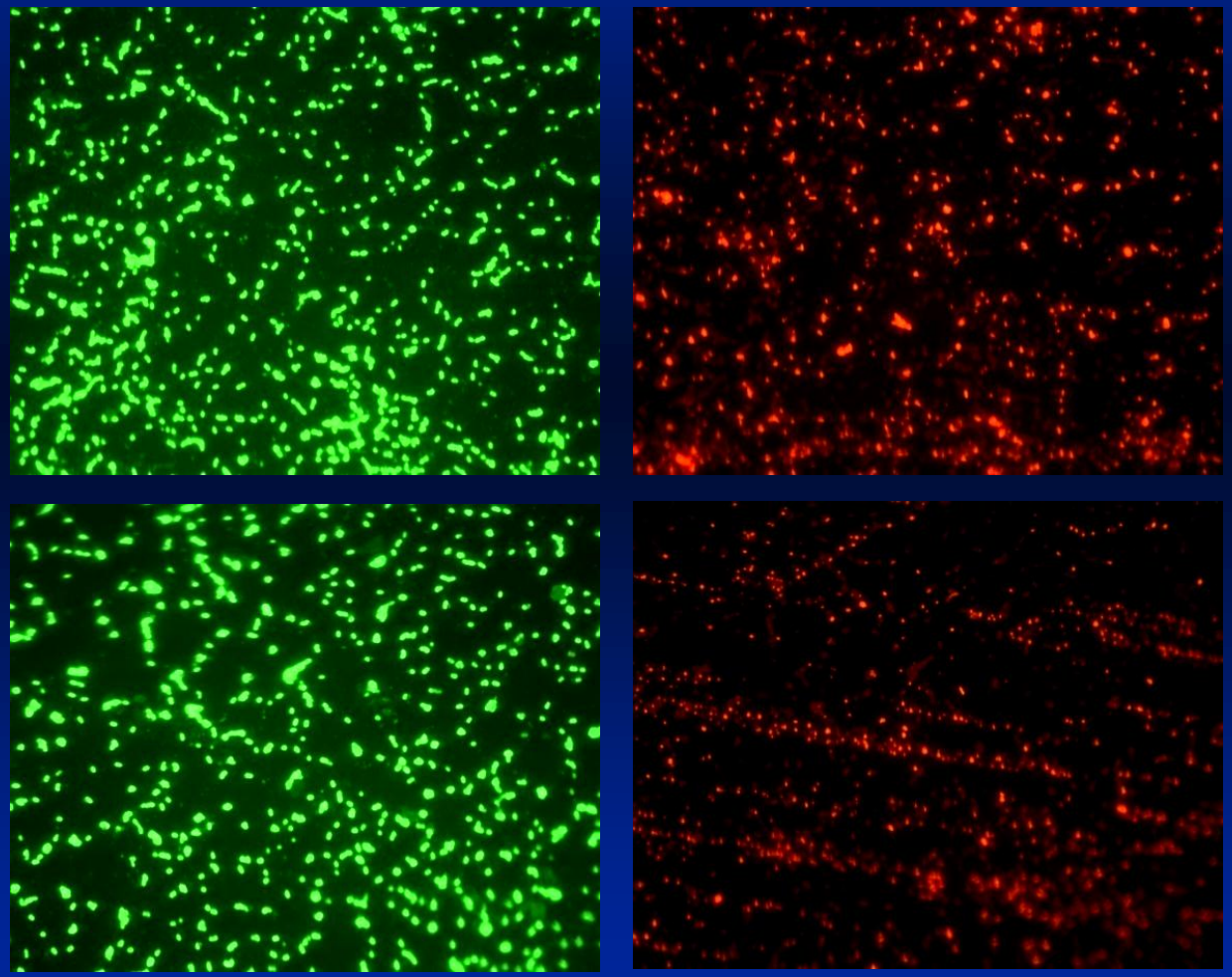
**Control – 20 µl water on copper – no effect on cell viability**

# Dry touch surface model

# VRE DNA content and respiration on stainless steel

**(inoculum $10^6$ cfu per $cm^2$; 4h)**

Dry test simulating hand contact (1 µL inoculum)



*E. faecalis*

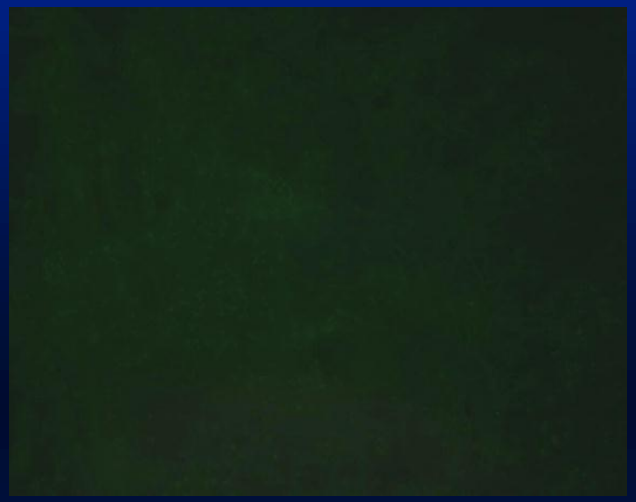
*E. faecium*

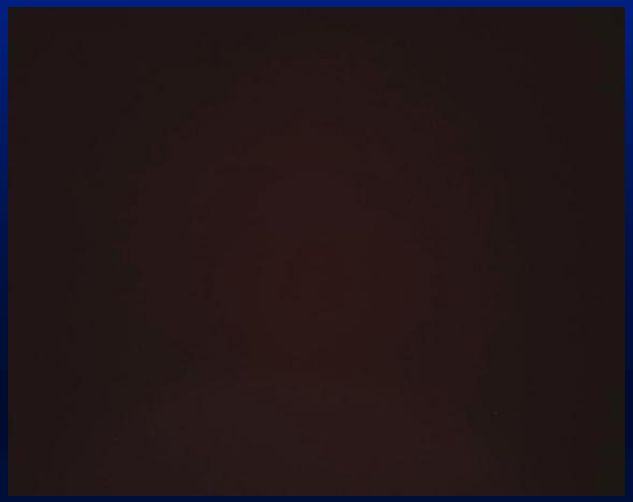
SYTO9

CTC

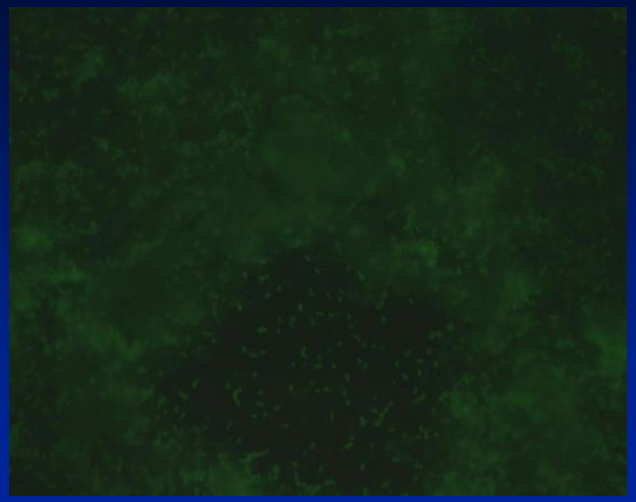
# Destruction of VRE DNA and respiration on copper

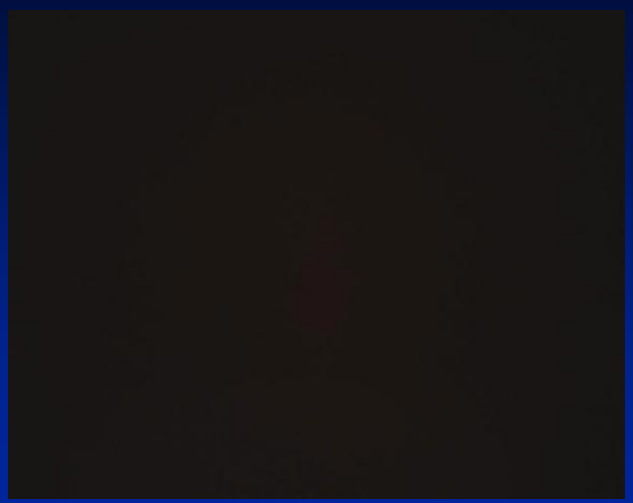
**(inoculum $10^6$ cfu per $cm^2$; 10 min)**





*E. faecalis*



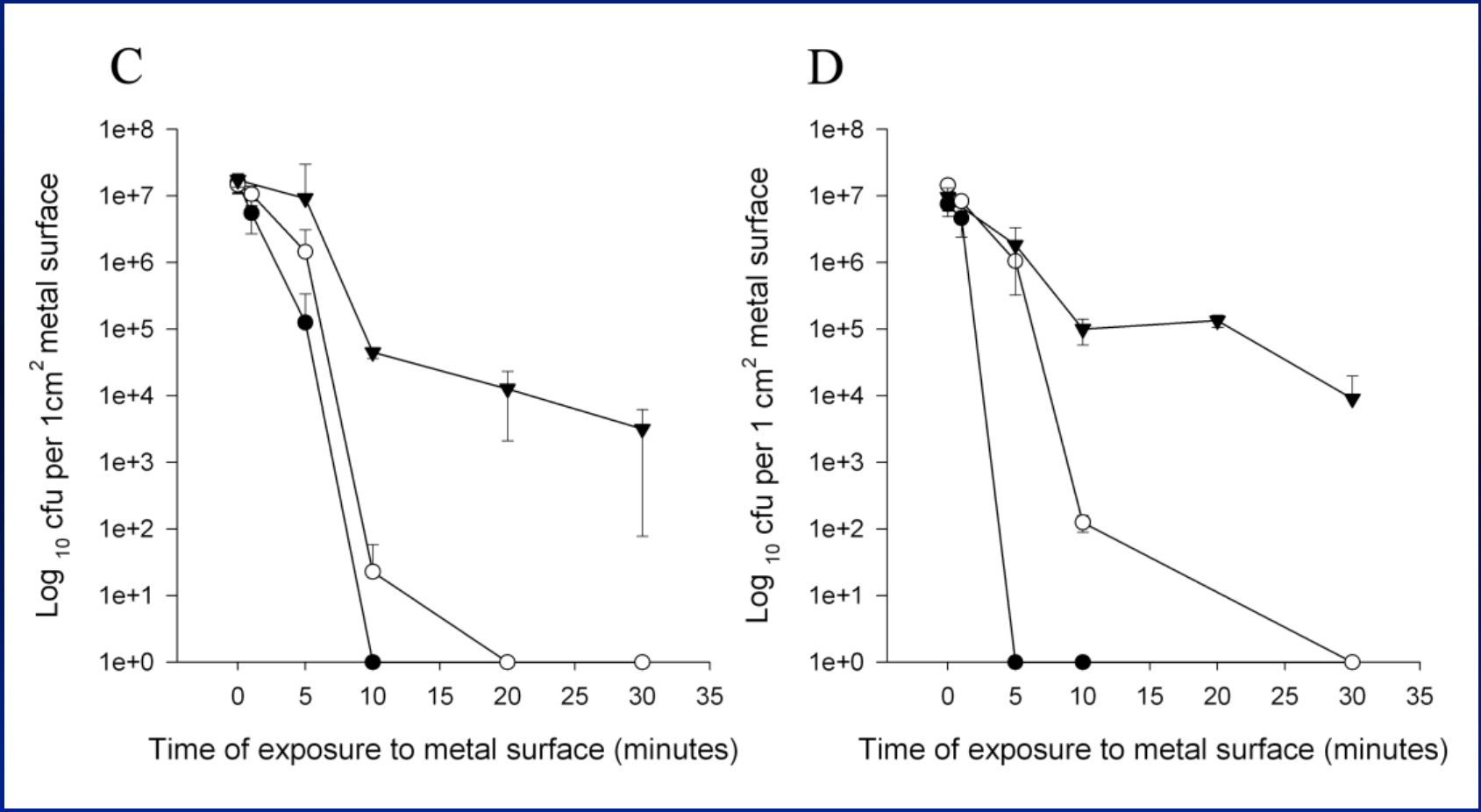


*E. faecium*

SYTO9

CTC

# Rapid death on dry copper and brass surfaces versus stainless steel




*E. coli* O157

*Salmonella* Typhimurium

# Copper disrupts membrane electrical potential in Gram negatives – 10 min exposure

FIGURE 4

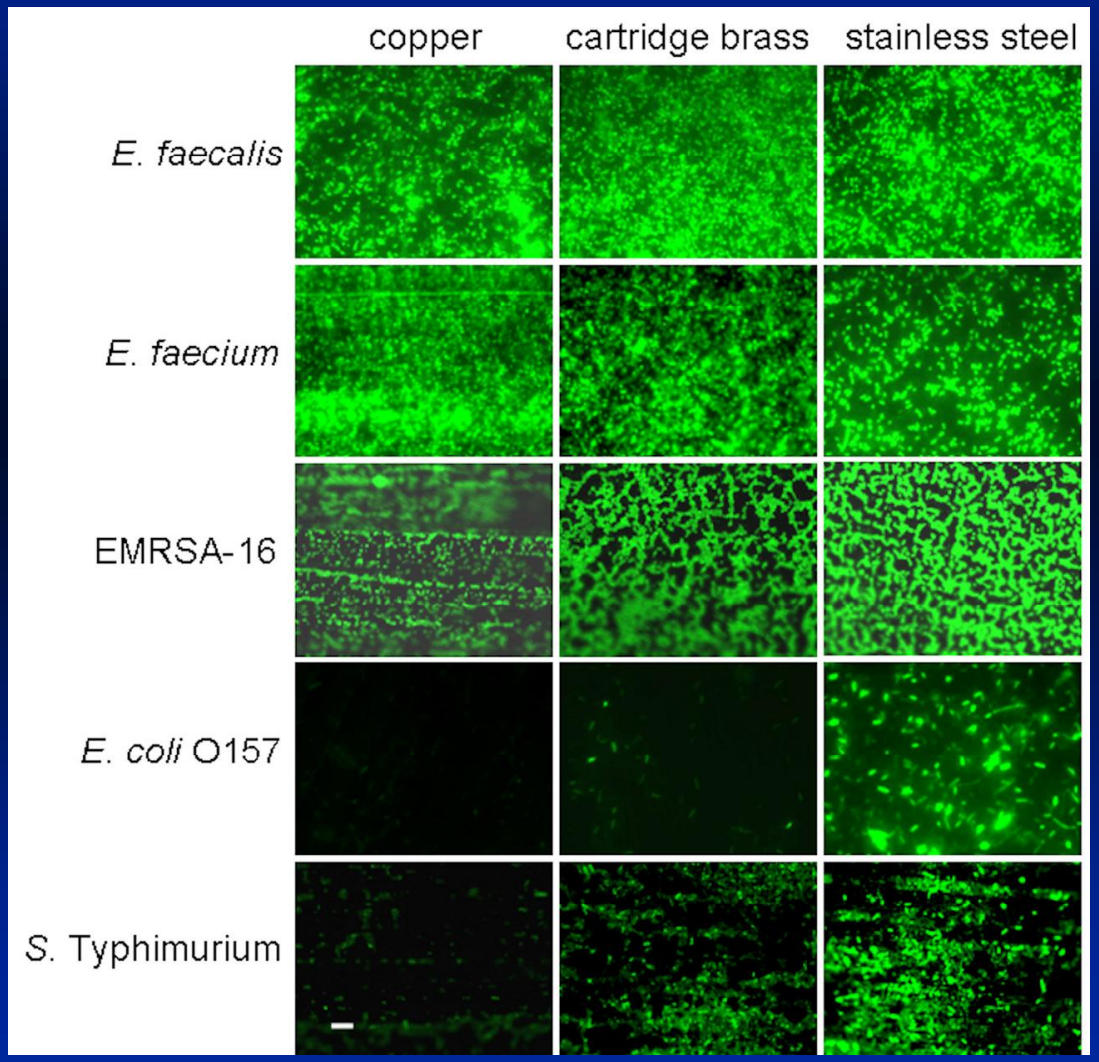
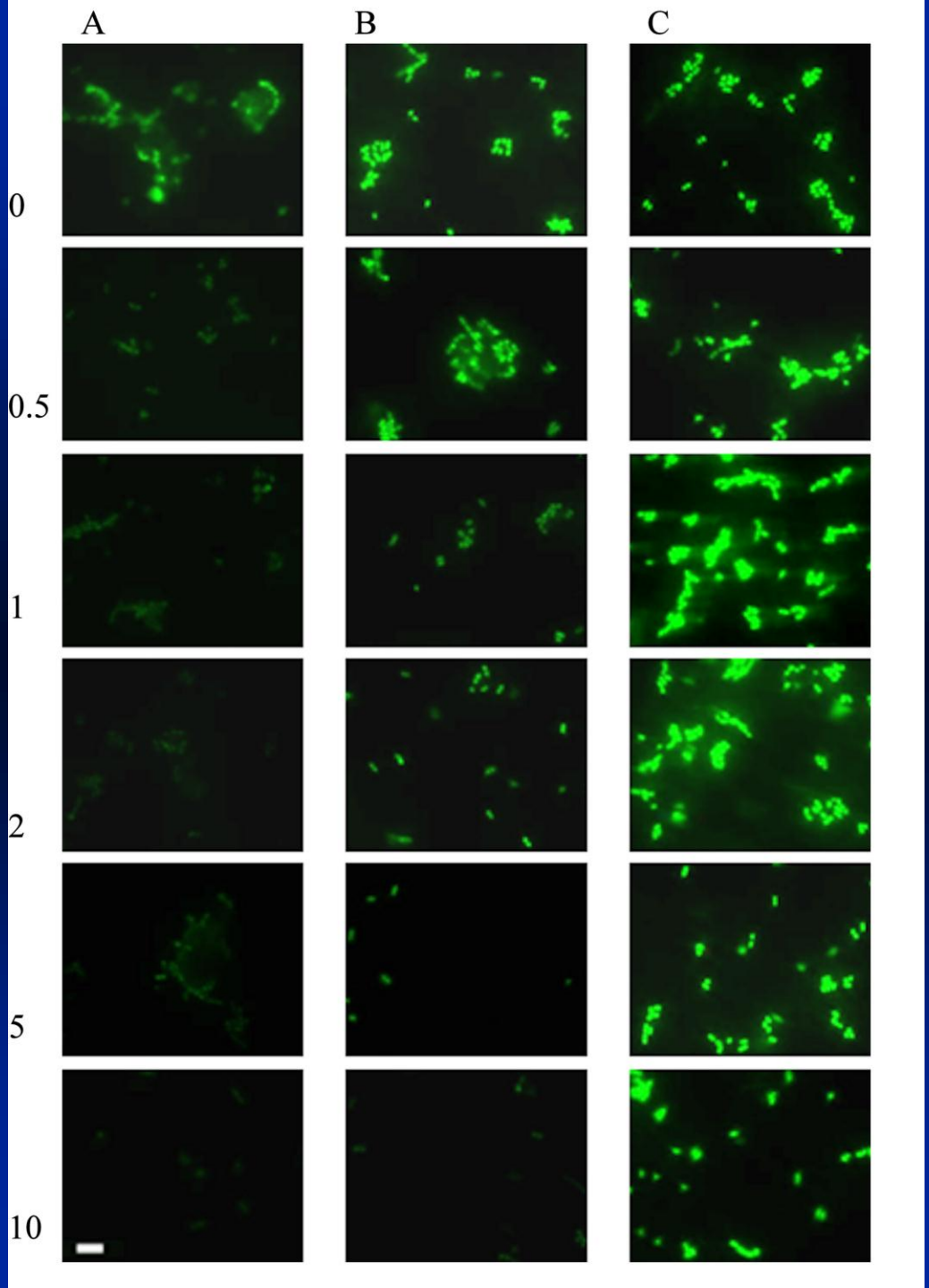


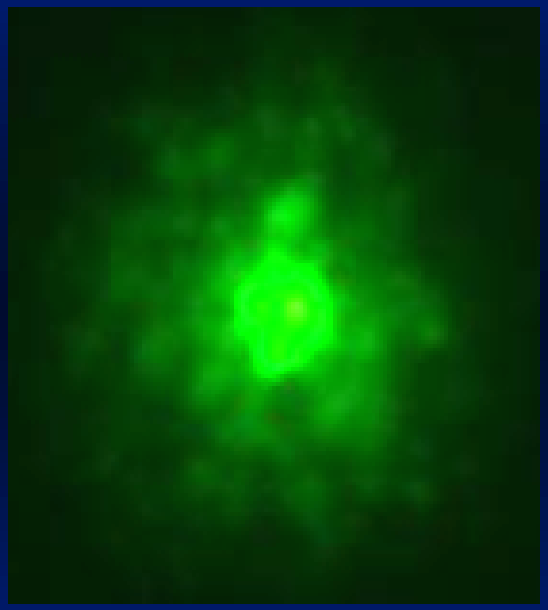

FIGURE 5

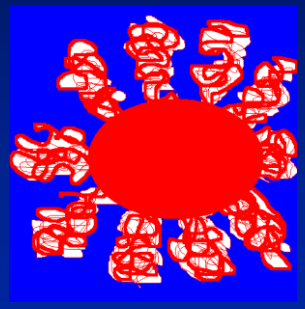
Rhodamine 123 uptake

Rapid breakdown of bacterial DNA on copper (A) and alloy (C26000) (B) surfaces compared to stainless steel (C) occurs as part of the killing mechanism in VRE.



Analysis of genomic DNA of bacteria exposed at room temperature to copper or stainless steel



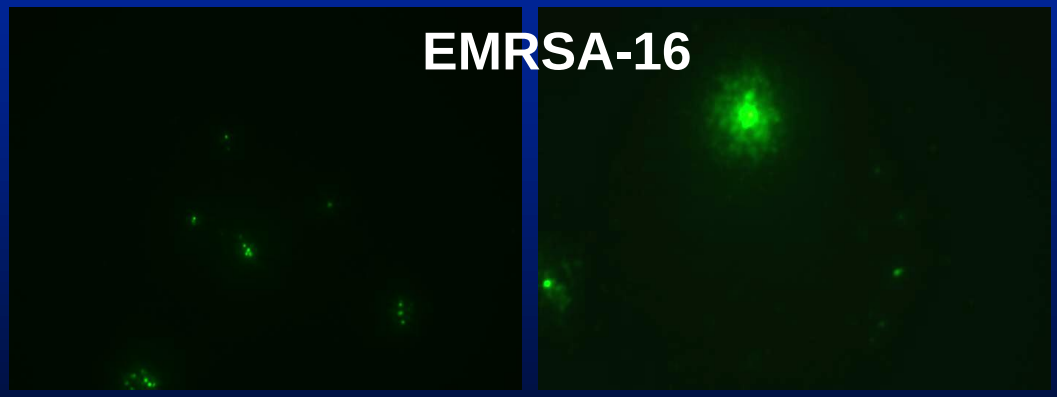


**Untreated EMRSA-16**

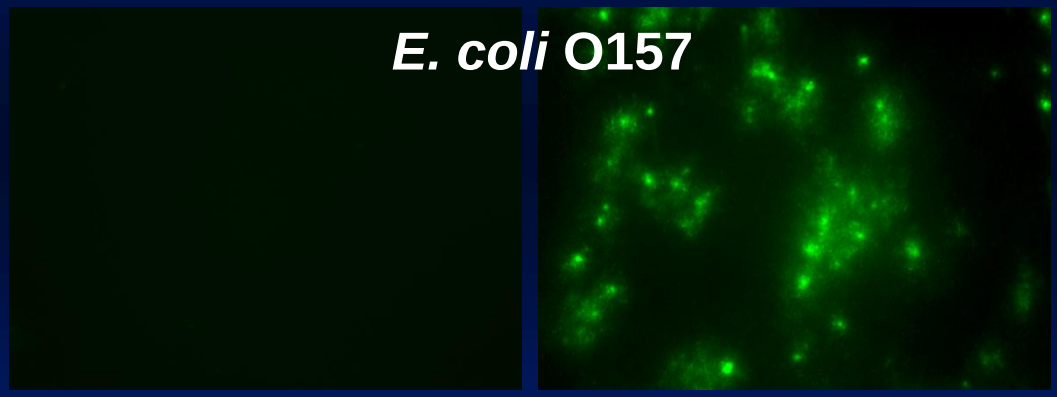
**Exposed to copper 10min**

**stainless steel 2h**

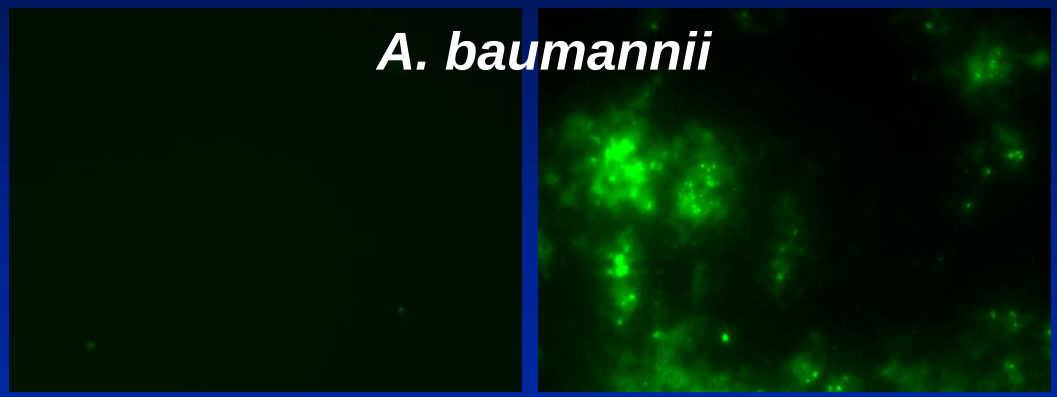
**EMRSA-16**



***E. coli* O157**



***A. baumannii***



# MRSA Genomic analysis

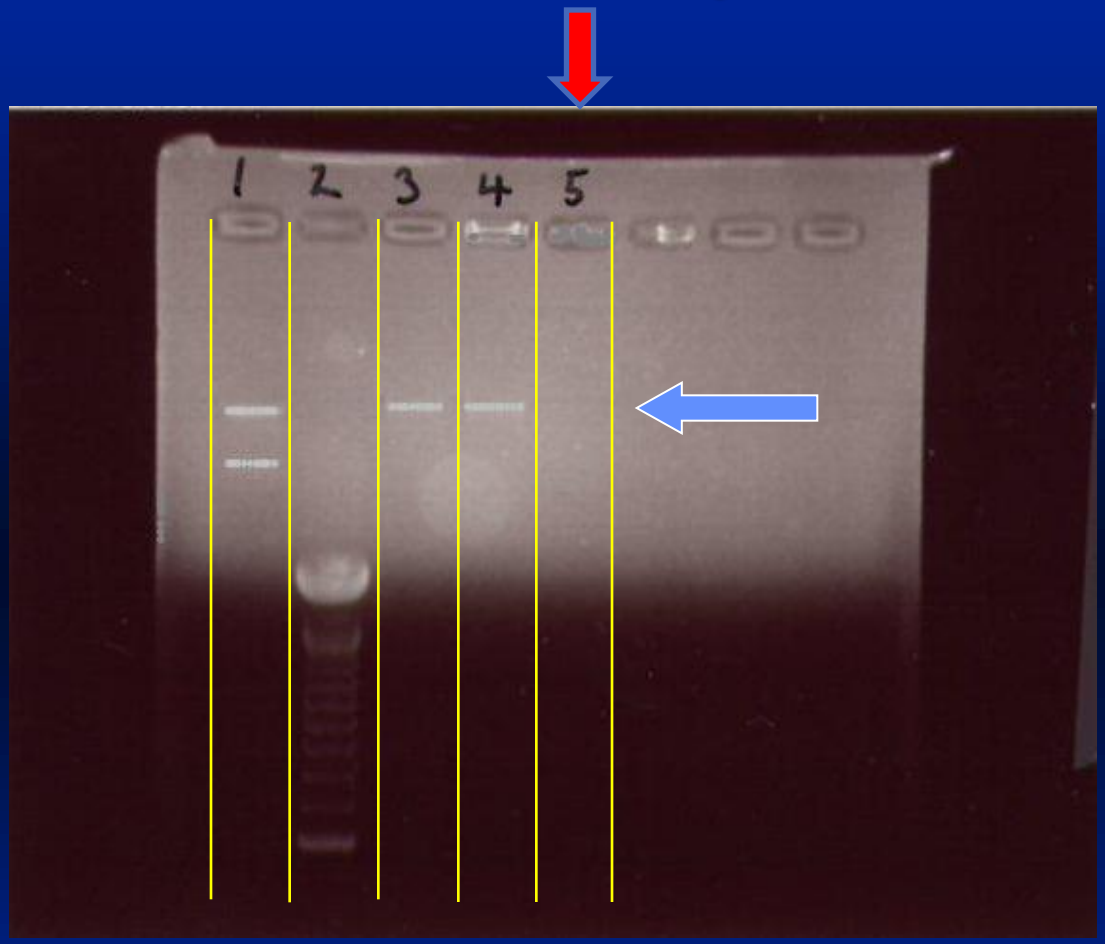
Lane 1: DNA ladder

Lane 2: 100 bp ladder

Lane 3: MRSA culture

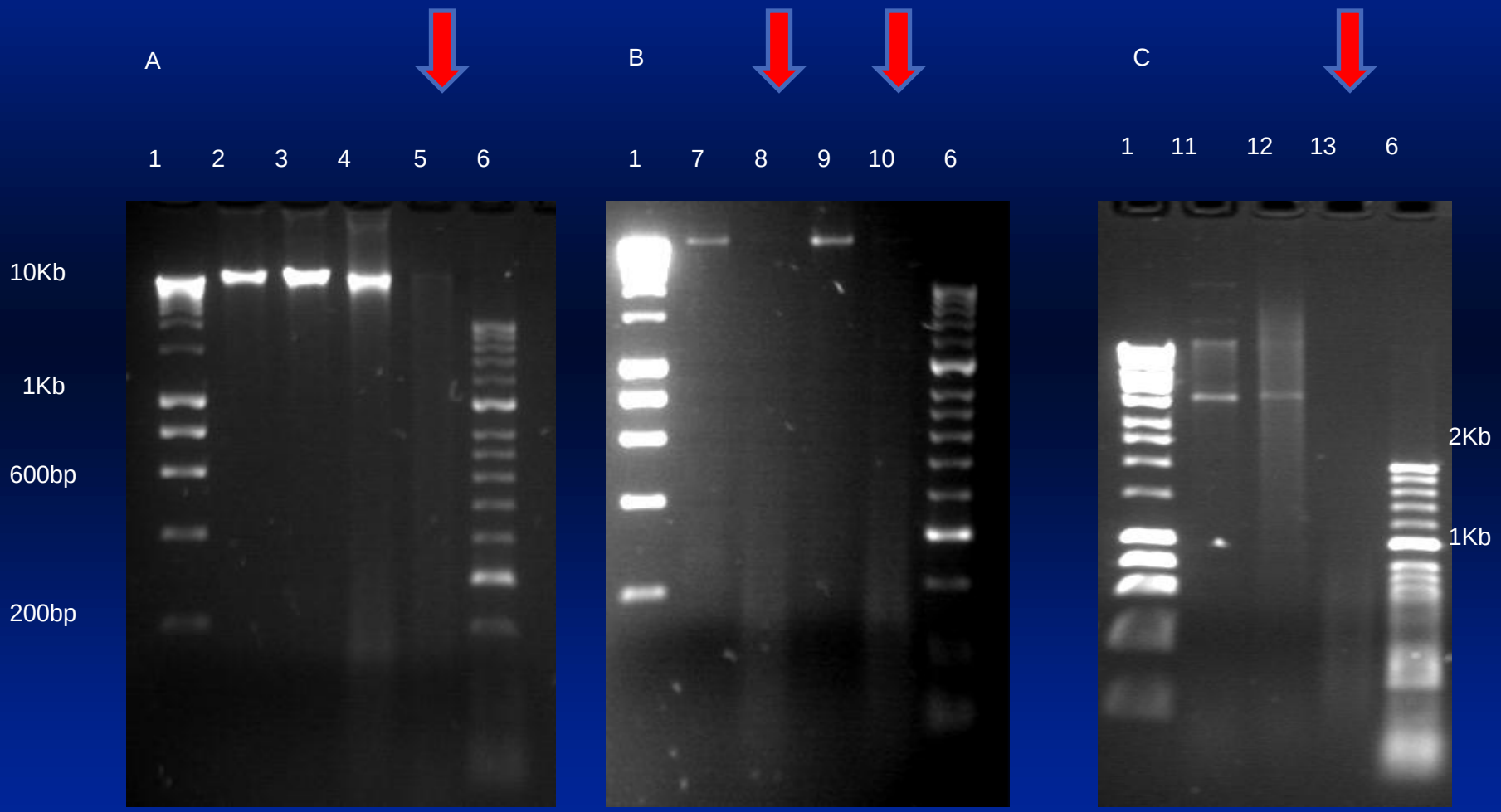
Lane 4: MRSA S30400

Lane 5: MRSA C11000




- Sigma Genelute genomic DNA kit extracts fragments of the DNA up to 50 kbps.
- No genomic DNA recovered from copper

# Genomic and plasmid DNA degradation on copper v stainless steel surfaces



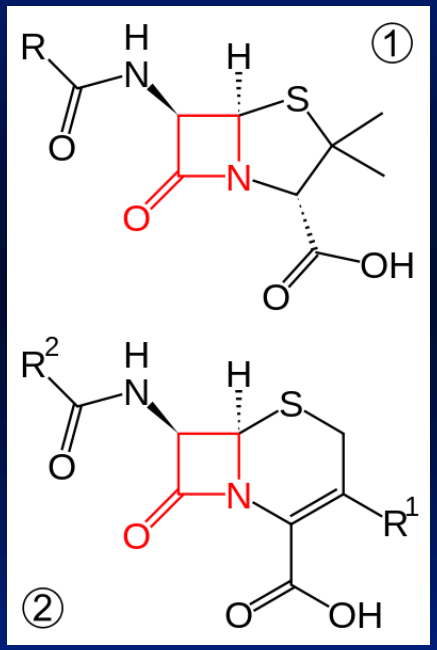

*E. faecium*
12202

*E. faecium/faecalis*
clinical

*E. faecium*
12202 plasmid

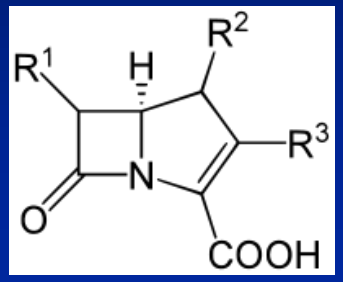
# Carbapenamases and *bla*$_{NDM-1}$

**December 2009**, after unsuccessful treatments in hospitals in New Delhi, a Swedish national was referred back to a Swedish hospital, where it was discovered that he had acquired an antibiotic-resistant bacterial infection during his stay in India; infected with ***Klebsiella pneumoniae*** (Gram-negative bacterium found in the normal flora of the mouth, skin, and intestines) infection.
NDM-1 gene now found in **India, Pakistan, Bangladesh, Australia, Canada, the Netherlands, United States, UK.**
**Carbapenamases hydrolyse carbapenems** called **'antibiotics of last resort'.**

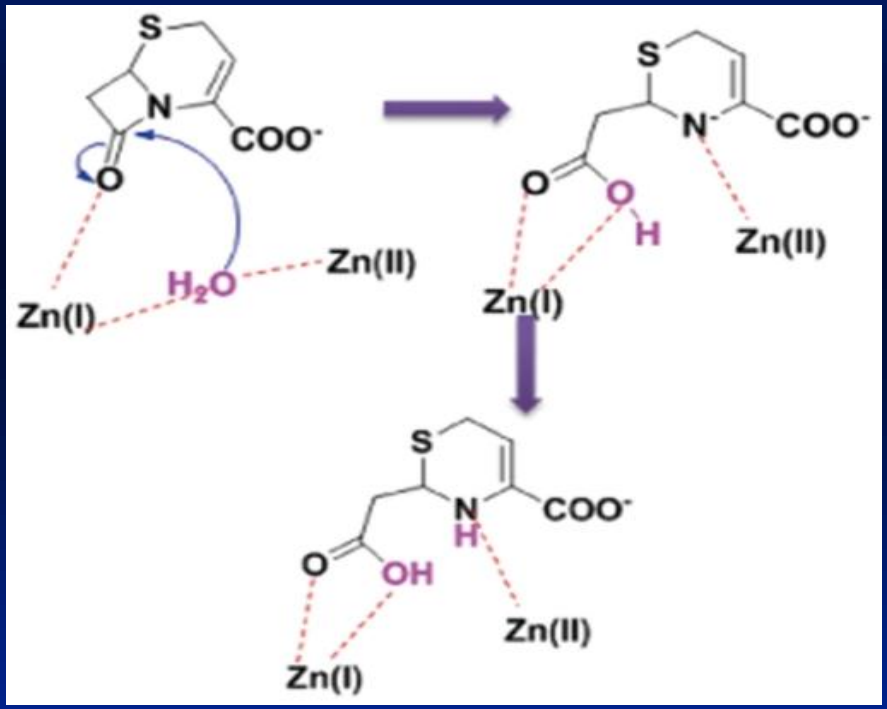



Penicillins

Cephalosporins




Carbapenems



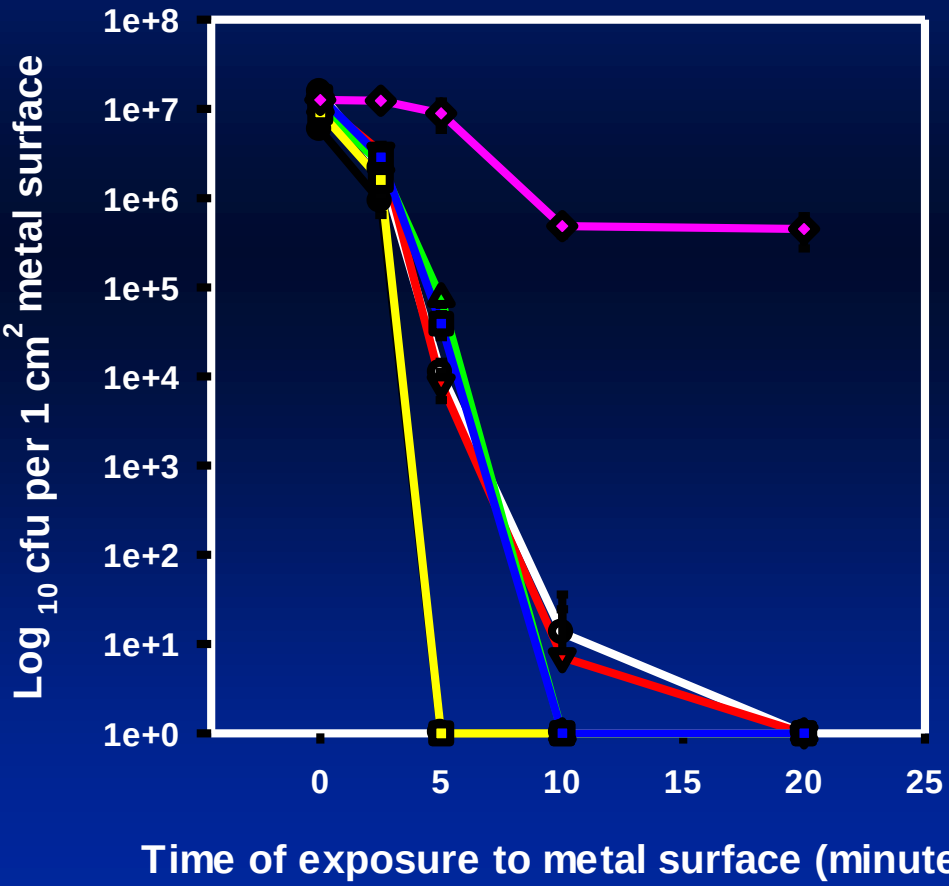

Molecular Basis of NDM-1, a New Antibiotic Resistance Determinant
Liang Z, Li L, Wang Y, Chen L, Kong X, Hong Y, Lan L, Zheng M, Guang-Yang C, Liu H, Shen X, Luo C, Li KK, Chen K, Jiang H - PLoS ONE (2011)

# Rapid death of *K. pneumoniae* encoding $bla_{NDM-1}$ on copper and alloy surfaces: dry inoculum

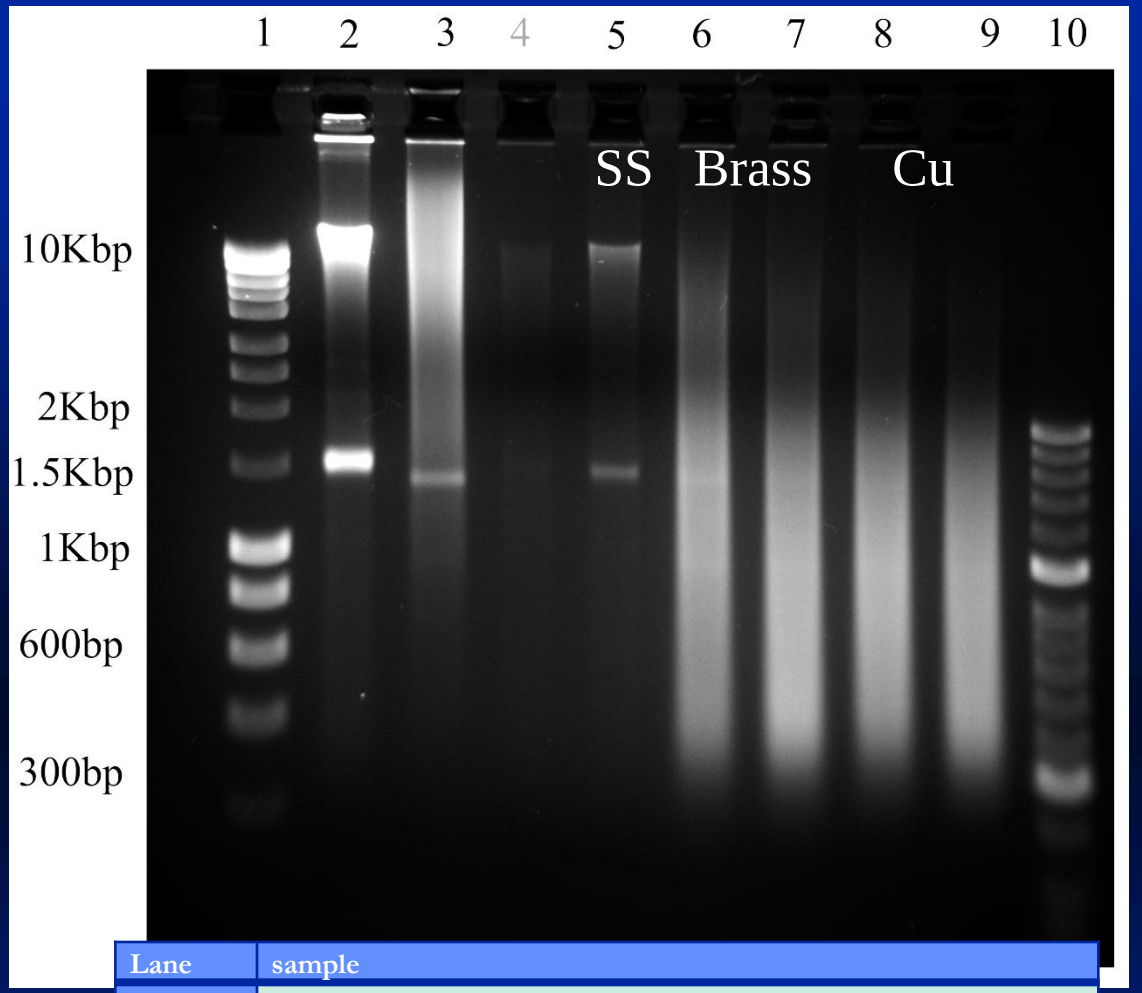
**Survival of *Klebsiella pneumoniae* NCTC 13443 (NDM-1) metal surfaces at room temperature**
**'dry' inoculum**
**120811**

Complete kill within 5 minutes occurs on copper and copper nickel ;

10 minutes for phosphor bronze and cartridge brass and 20 minutes for nickel silver and muntz metal.



# Exposure to copper or cartridge brass degrades plasmid DNA of MDR-*Klebsiella pneumoniae*

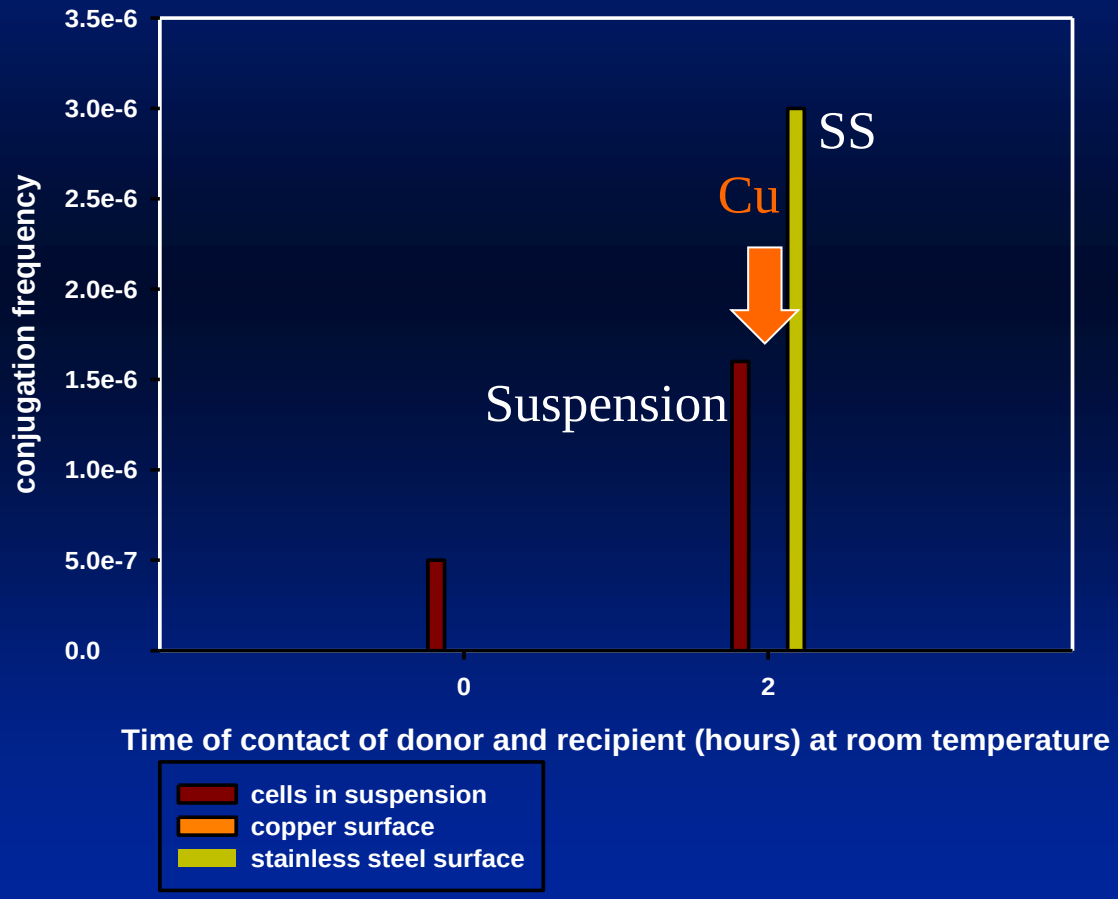


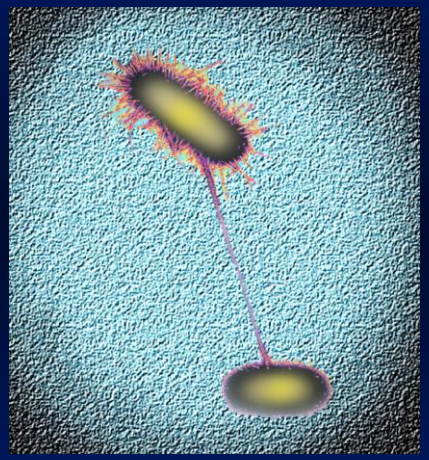

| Lane | sample |
|---|---|
| 1 | Hyperladder I |
| 2 | Untreated cells |
| 3 | Heat killed cells |
| 4 | Cells exposed to stainless steel 5' –poor DNA yield in this sample? |
| 5 | Cells exposed to stainless steel 10' |
| 6 | Cells exposed to cartridge brass 5' |
| 7 | Cells exposed to cartridge brass 10' |
| 8 | Cells exposed to copper 5' |
| 9 | Cells exposed to copper 10' |
| 10 | Hyperladder II |

Degradation of *K. pneumoniae* plasmid DNA occurs on copper (lanes 8, 9) and cartridge brass (lanes 6, 7) but not on stainless steel (lane 5). Degraded DNA appears as a 'smear' of multi-sized fragments.

# Horizontal transfer of *K. pneumoniae bla* $_{\text{NDM-1}}$ occurs in suspension and on stainless steel surfaces

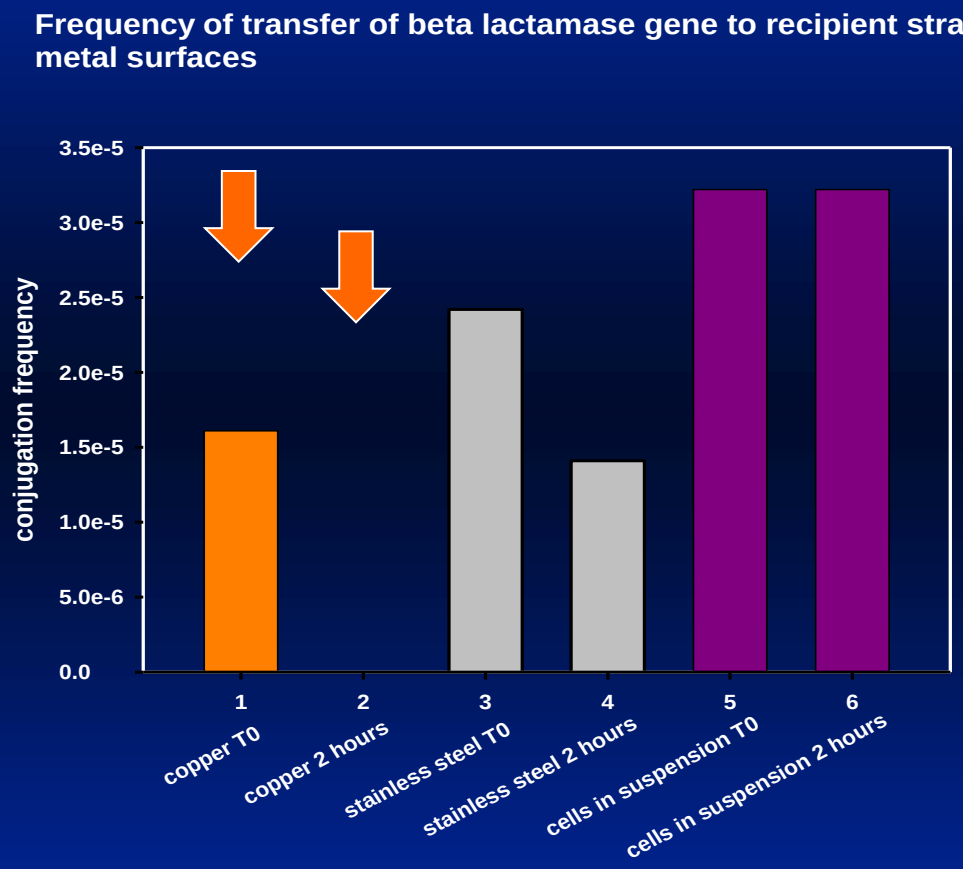
**Frequency of transfer of *bla* $_{\text{NDM-1}}$ to recipient cells on surfaces or in suspension.**



www.flickr.com

# Horizontal transfer of *E. coli* *bla CTX-M-15* occurs in suspension and on stainless steel surfaces




Conjugation frequency =
no. transconjugants /no. donor cells

# Possible Mechanisms for Solid Copper's Antimicrobial Effect

1) $H_2O_2 + O_2^- \rightarrow O_2 + OH^- + OH^\cdot$

2) Cu (II) + $O_2^-$ → Cu (I) + $O_2$

3) Cu (I) + $H_2O_2$ → Cu (II) + $O_2$ + $OH^-$ + $OH^\cdot$

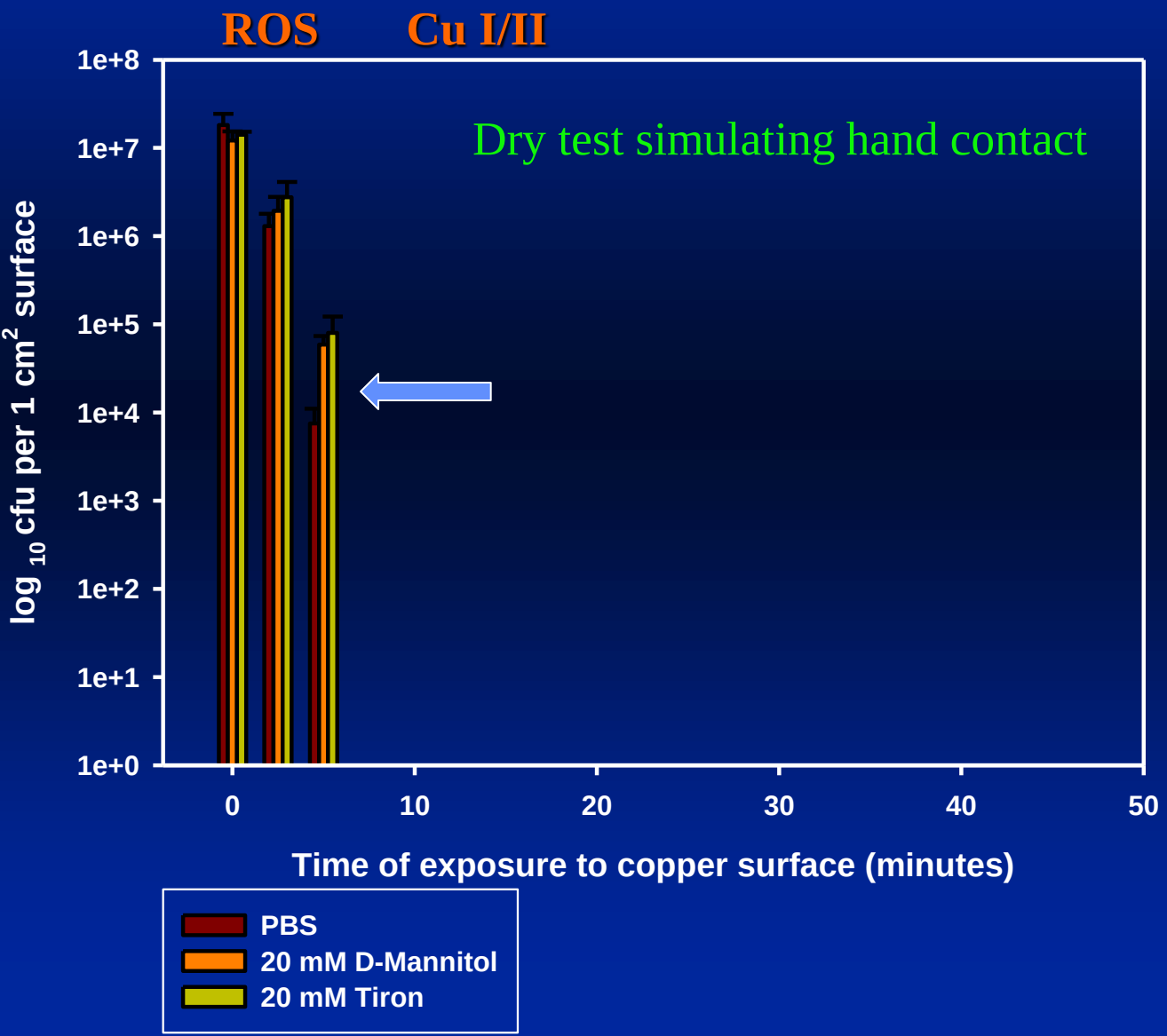
- Copper redox cycling (Fenton-like reactions) generates reactive oxygen species e.g. hydroxyl radicals, superoxide
- from reactions with dihydrogen peroxide (by product of electron transport chain of bacteria and also generated at copper surface in aqueous systems)

- Rate constant for Fe(II) Fenton reaction is 76 $M^{-1}.sec^{-1}$ (Walling, 1975)
- 60x faster for Cu(I) 4700 $M^{-1}.sec^{-1}$ (Halliwell and Gutteridge, 1990)

# VRE *E. faecalis*: Rapid Assay

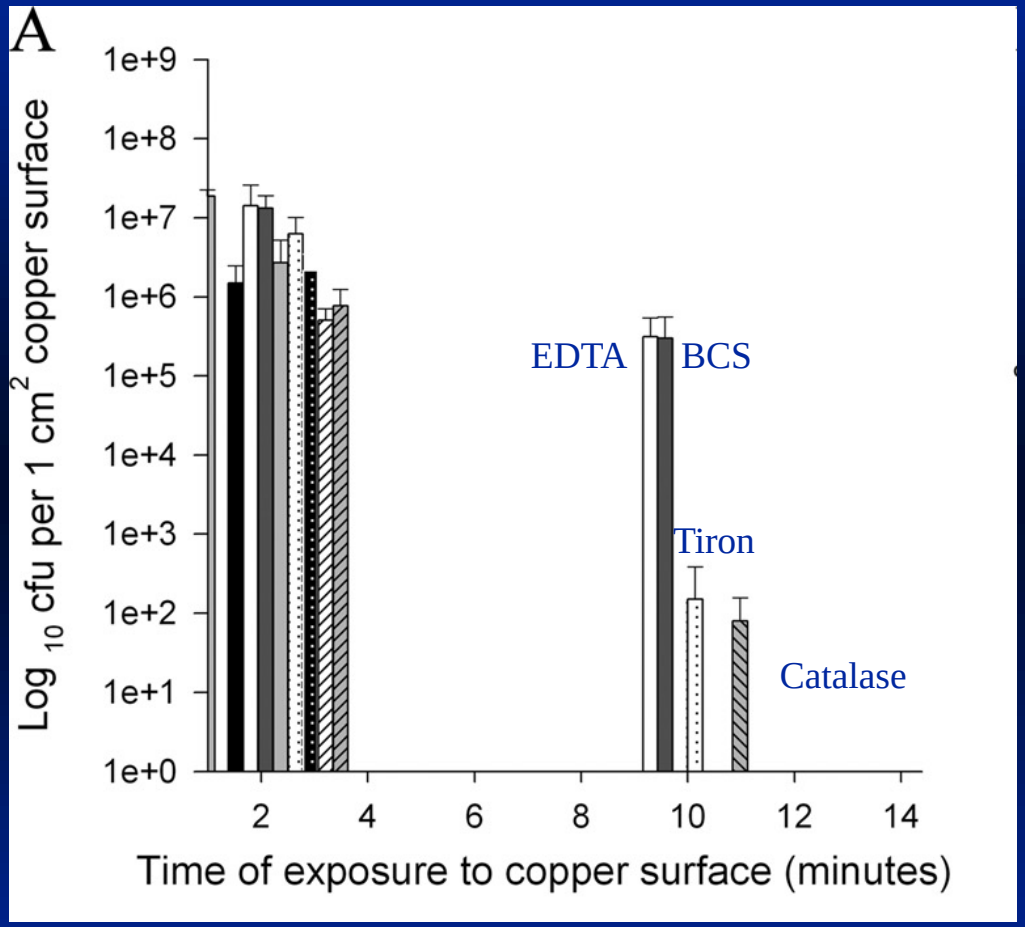
- Reduced inoculum volume, rapid drying time (<5 minutes)
- D-Mannitol and Tiron significantly protective up to 5 minutes
- The Cu(I)/(II) overwhelms cell

**Survival of *E. faecalis* ATCC 51299 on copper surfaces**
**1 microlitre inoculum**
**100709**



# VRE *E. faecalis* rapid death on dry copper and protection from ROS

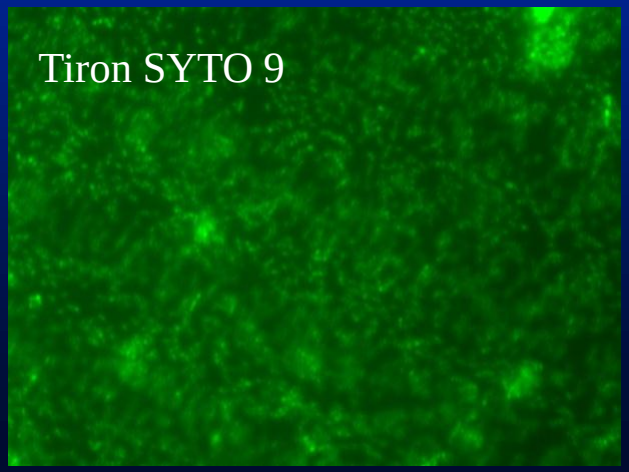
Protection from later stage Cu(I)/(II) kill

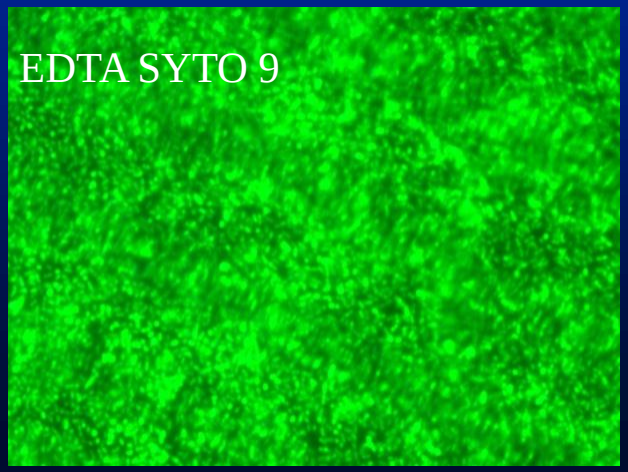


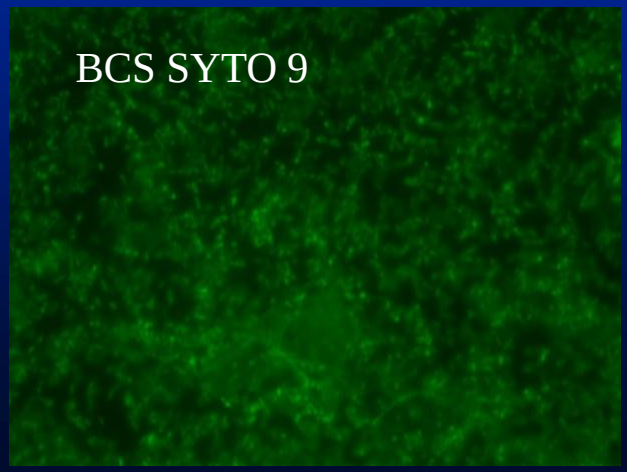

Approx $10^7$ CFU in 1 µl inoculated onto 1-$cm^2$ coupons in PBS (black bars) or PBS with EDTA (white bars), BCS (dark gray bars), D-mannitol (light gray bars), Tiron (white spotted bars), SOD (black spotted bars), catalase (white diagonal striped bars), and sucrose (gray diagonal striped bars) at 22° C (Warnes and Keevil, AEM 2011)

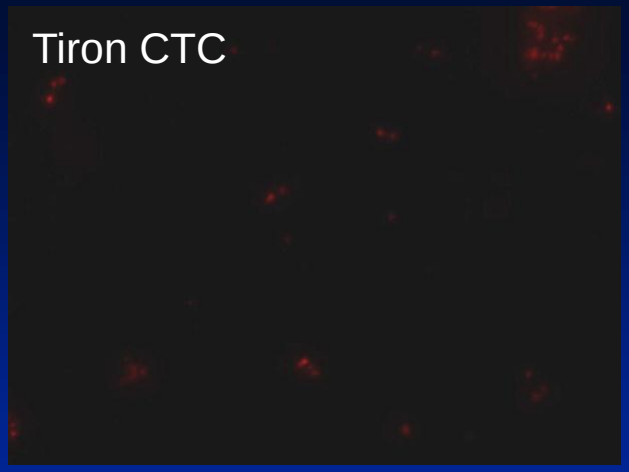
# *E. faecalis* : DNA & Respiration Damage

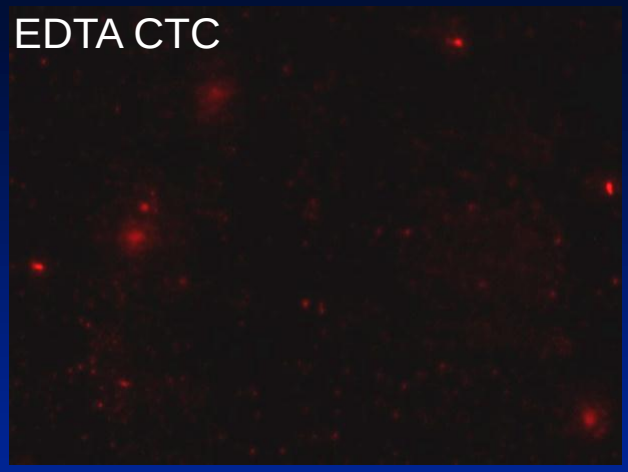
## 10 min contact with copper

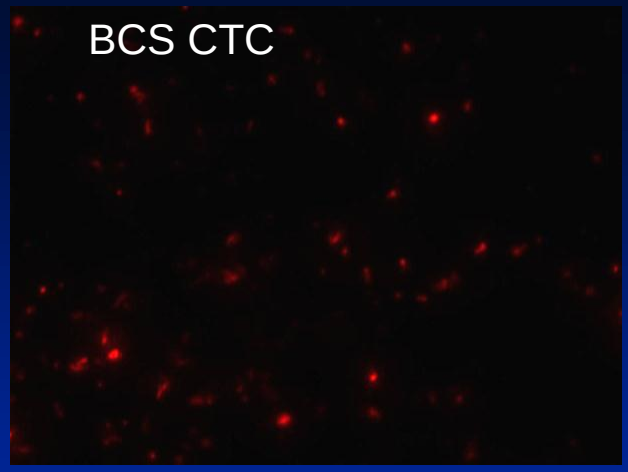


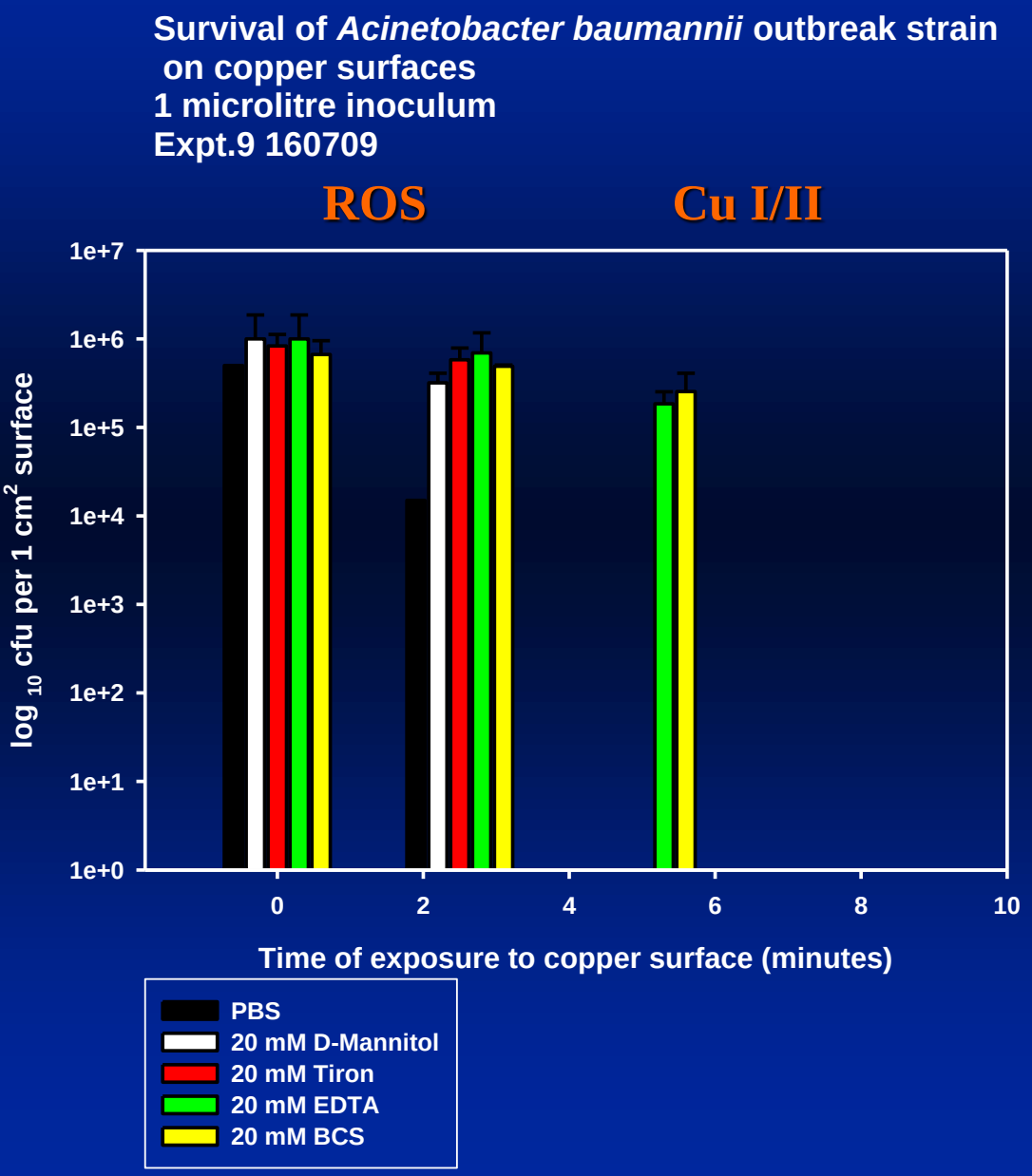




**Superoxide** **Cu(II)** **Cu(I)**

# *A. baumannii* : Rapid Assay on copper

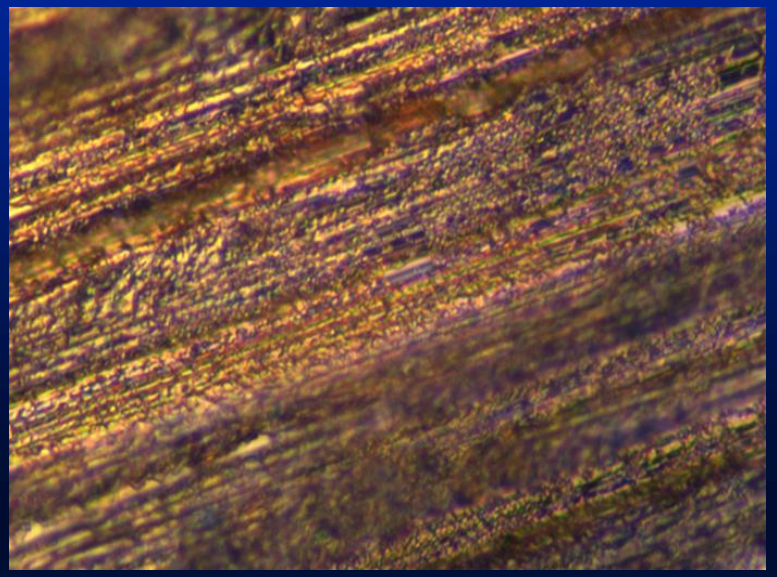
Dry test simulating hand contact

- Significant short term protection with D- Mannitol and Tiron (up to 2.5 minutes contact )
- Chelator protection continues and is still detected for EDTA at 30 minutes

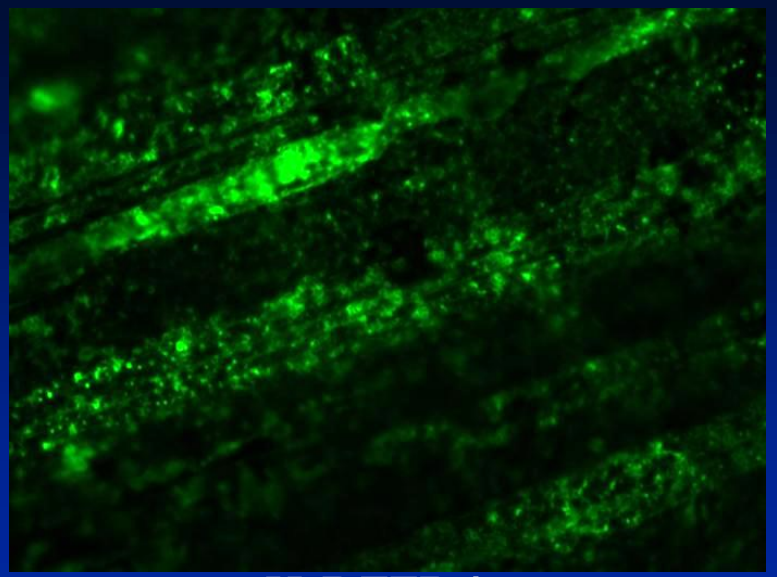


# *ROS generation by A. baumannii* on copper surfaces

- EDIC image reveals the location of bacterial cells on the ridges of the copper coupon.



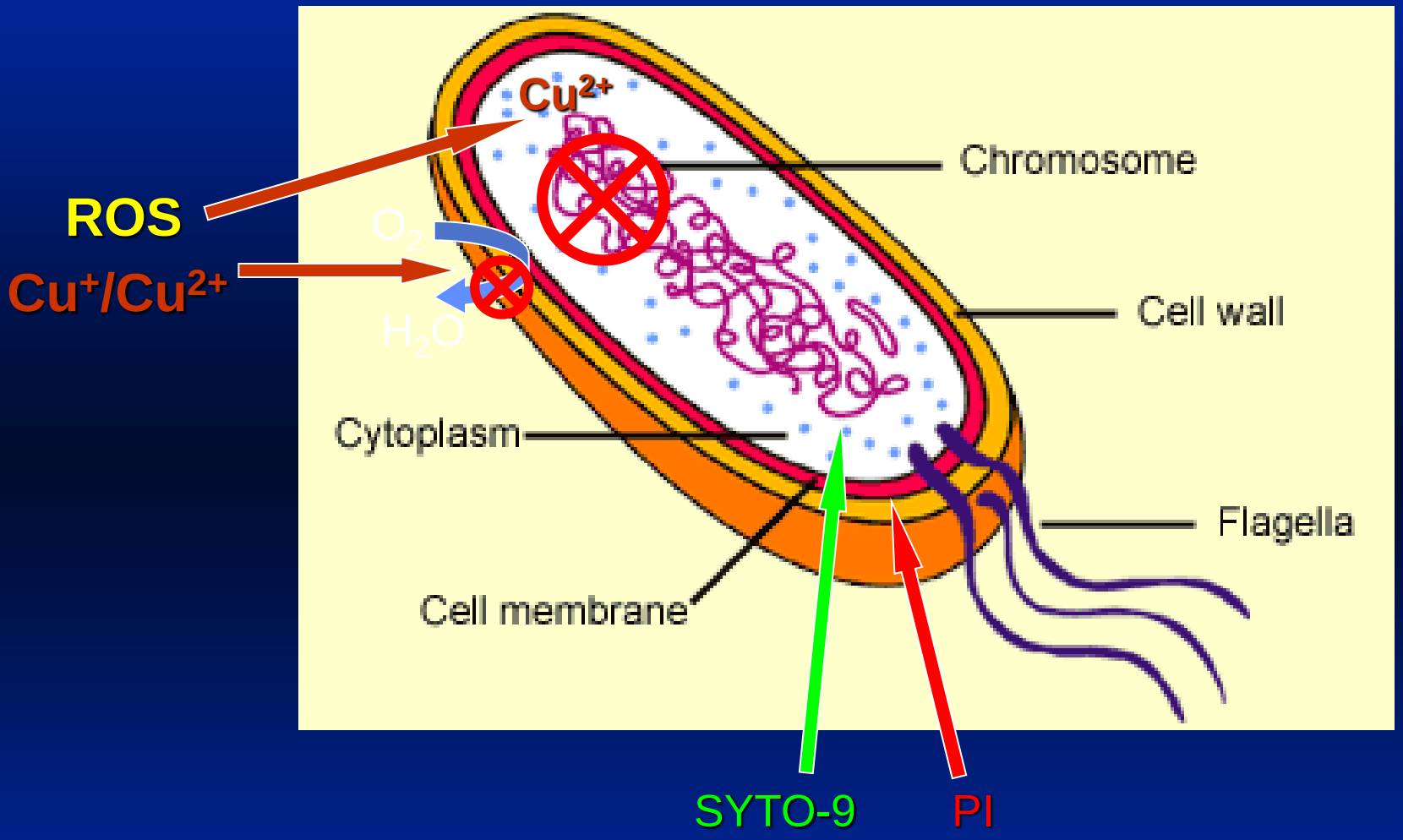
- After 15 minutes contact ROS-positive staining cells detected in the surface ridges = Metabolic Suicide



- No staining on stainless steel

$H_2$DFFDA

# SITES OF COPPER AND/OR ROS INHIBITION




**Cu(I)/(II) important when dry/wet; ROS also important when dry; Gram-positive membrane compromised**

# Antimicrobial Copper Effects on Bacteria

| | Gram-positives | Gram-negatives |
|---|---|---|
| ROS generation | Rapid | Rapid |
| Death | Rapid | Rapid |
| Respiration inhibition | Rapid | Rapid |
| Membrane electrical potential inhibition | Slow | Rapid |
| Cytoplasmic Membrane integrity* | Slow | Rapid |
| Outer Membrane integrity* | n/a | Rapid |
| Plasmid DNA destruction | Rapid | Rapid |
| Genomic DNA destruction | Rapid | Slower |

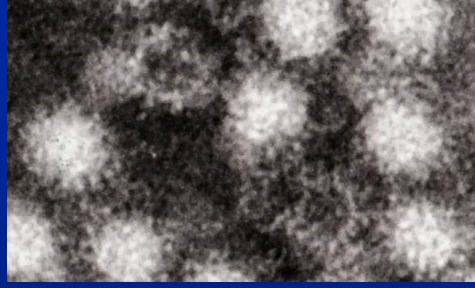
*probably due to long-chain unsaturated fatty acid peroxidation (Hong et al., AEM 2012)

**Multi-factorial killing mechanisms**

# Norovirus: the most common cause of viral gastroenteritis worldwide

- Norovirus causes > 267 million infections worldwide (23 million in US)
- Disease highly infectious, resistant to cleaning agents, persists on environmental surfaces, spreads rapidly and contracted by:
  - person-to-person contact
  - ingestion contaminated food e.g. shellfish, water
  - touching contaminated (faeces, aerosolised vomitus) surfaces
- Outbreaks especially in closed environments e.g. cruise ships and care facilities
- Large reservoir because of prolonged shedding complicated by asymptomatic carriage
- Low level contamination of environment is a transmission risk because of low infectious dose.
- Usually self limiting but can be serious in children, elderly and immunosuppressed and malnourished and no vaccine or specific chemotherapy available. Linked to Crohn's disease and necrotising enterocolitis in neonates.
- Economic burden: Outbreaks estimated costs UK costs NHS 2002-3 £184 million and $2 billion/year in US. Endemic costs in US $500 million/ year

# Norovirus infectivity assay following exposure to metal surfaces



- Testing underway
- Early results positive

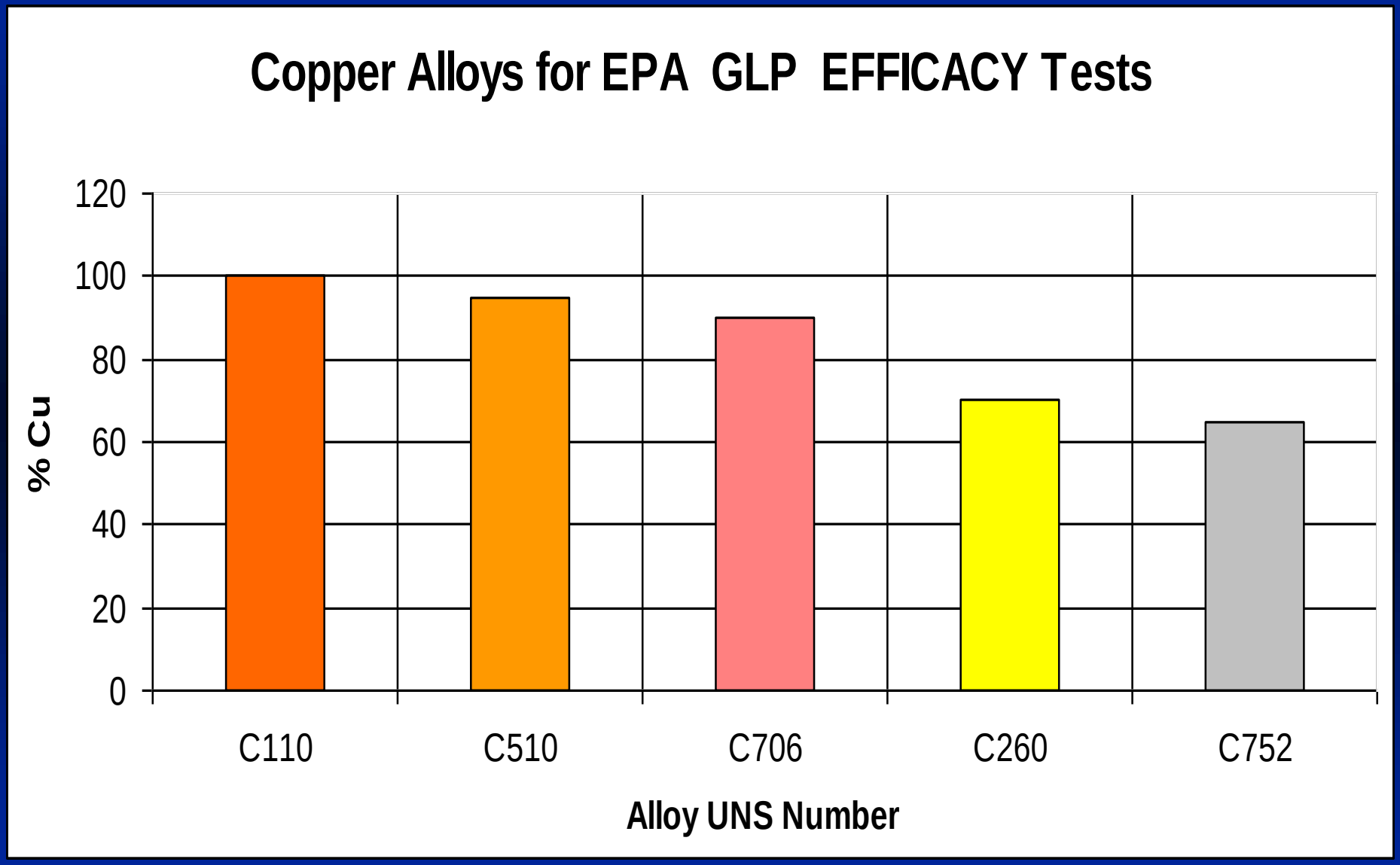
# Conclusions

- Copper alloys kill rapidly, particularly on dry contact
- Bacteria and spores, viruses, yeasts, fungi
- Continuous activity 24/7 through Cu(I)/(II) and ROS

- Combination of effective cleaning regimes and contact surfaces containing copper could be invaluable to prevent spread of viable pathogens.

- Rapid destruction of genomic and plasmid nucleic acid could:
  - prevent mutational resistance developing
  - help reduce the spread of antibiotic resistance genes to receptive and potentially more virulent organisms (Keevil, IFIC, Friday 2pm)
  - as well as genes responsible for virulence and toxin production.

# EPA Antimicrobial Efficacy GLP Tests




>350 copper alloys approved, unlike silver

# INTERNATIONAL WARD TRIALS















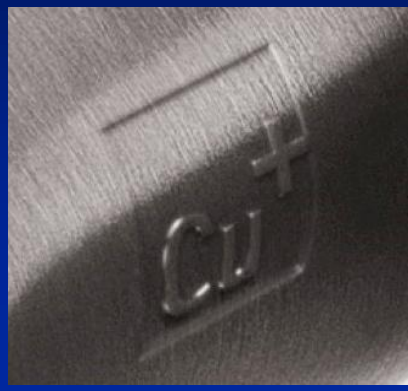


# Back to the Future

- Many international trials in hospital wards worldwide shown >90% reduction in bioburden on copper alloy surfaces

- Infection control – next speaker

- Future - >350 Cu alloys now registered with US EPA with an antimicrobial claim – ready to deploy in healthcare, public buildings, public transportation etc



How many times a day are contact surfaces cleaned?

How frequently do people wash hands?

Dissemination of respiratory and faecal pathogens

# Acknowledgements


- Sarah Warnes, Sandra Wilks
- Jonathan Noyce, Louise Weaver, Emma Goode

- Harold Michels (CDA)


- International Copper Association