# Preventing antibiotic resistant interspecies gene transfer on touch surfaces


breaking the chain of infection

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# Hospital-Acquired (Nosocomial) Infections

Hospitals are dangerous places for
hand transmission and cross contamination!

# Rise of the "Superbugs"

70% of HCAI are antibiotic resistant, many broad spectrum

MRSA

VRE

*Clostridium difficile* spores

ESBL e.g. *Acinetobacter baumannii, E. coli, P. aeruginosa*

*Klebsiella pneumoniae* carbapenemase Class A (*KPC*) 1996

New Delhi Metallo-1 beta lactamase Class B *(NDM-1)* 2009

Numerous studies show:

- survive for days/weeks on various surface materials;
- ESBL outbreaks suggest environmental transmission may be important

‘KPC’ Mar 07, 2014 By Andrew Gregory



# Superbug kills 17 people and hundreds have been infected by bacteria highly resistant to antibiotics

Sixteen people have died in the Central Manchester University Hospitals NHS trust area in the past four years – and another died at Wolverhampton's New Cross


Universal Images

Danger: The Klebsiella pneumoniae bacterium



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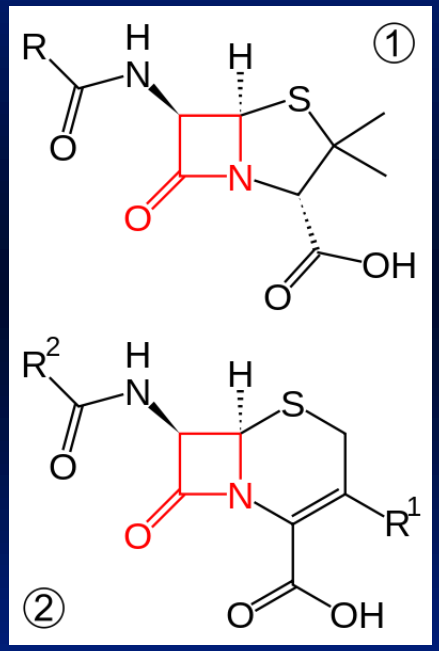
# Evolution of β-lactamase to carbapenamases e.g. *bla*$_{NDM-1}$

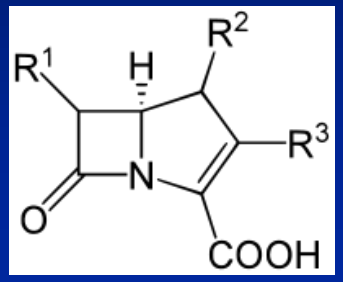
**December 2009**, after unsuccessful treatments in hospitals in New Delhi, a Swedish national was referred back to a Swedish hospital, where it was discovered that he had acquired an antibiotic-resistant bacterial infection during his stay in India; infected with ***Klebsiella pneumoniae*** (Gram-negative 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'.**



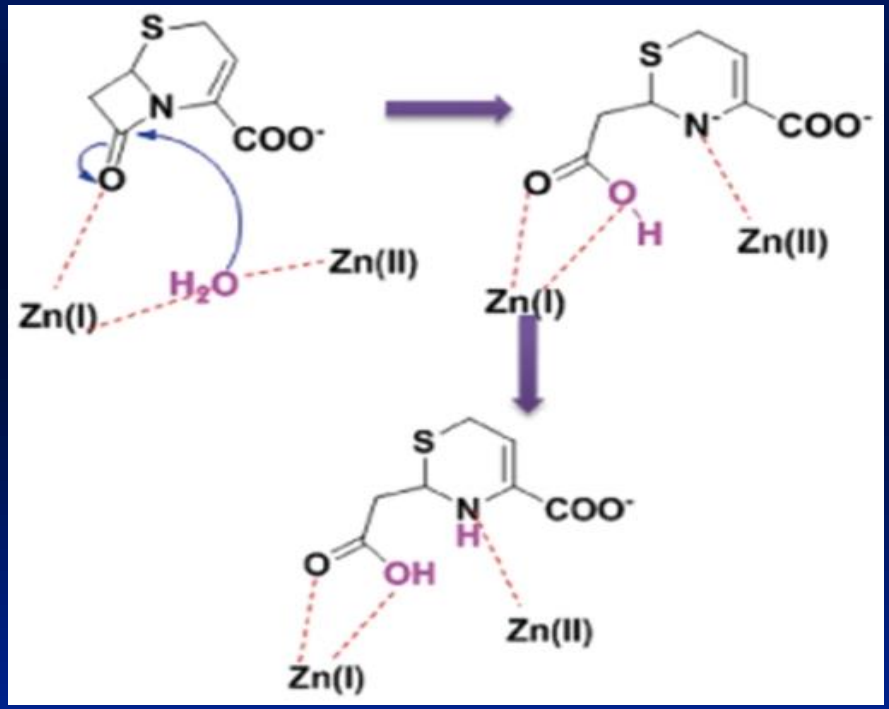

1. Penicillins
   - β-lactamases

2. Cephalosporins
   - BS β-lactamases

3. Cephalosporins
   - ESBL:
   - CTX, OXA, TEM




3. Carbapenems
   - KPC, NDM-1

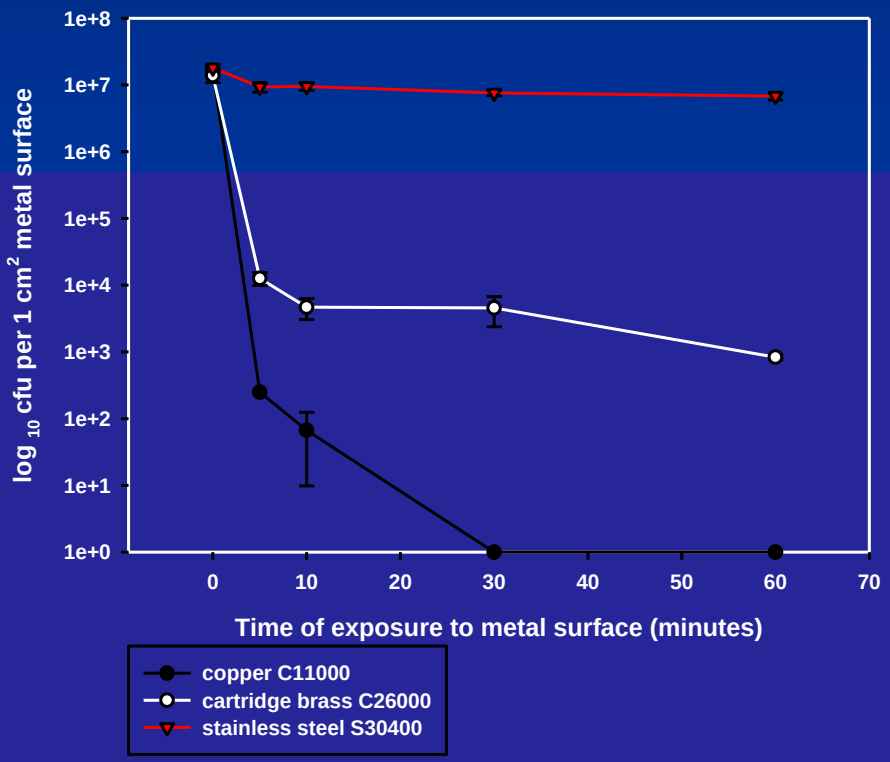



Molecular Basis of NDM-1, a New Antibiotic Resistance Determinant Liang Z, Li L, Wang Y, Chen L, Kong X, Hong Y, Lan L, Zheng M, Guang-Yang C, Liu H, Shen X, Luo C, Li KK, Chen K, Jiang H - PLoS ONE (2011)

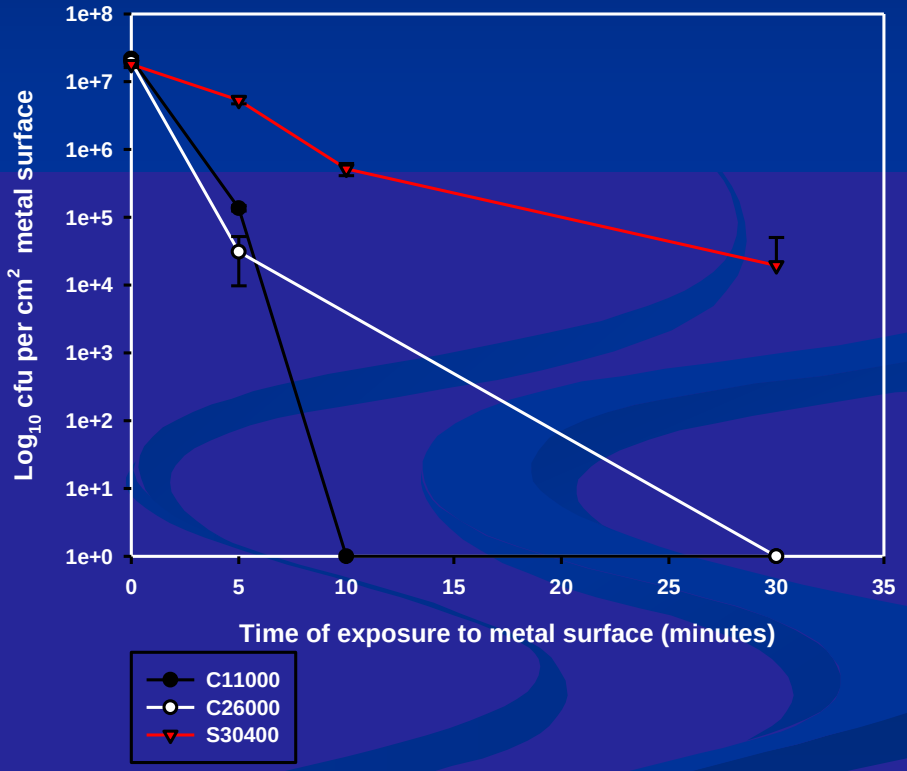
# Dry touch surface model

# Survival of ESBL producing *E. coli bla CTX-M-15* on metal surfaces: 'dry' inoculum

Cells in bacteriological medium (BHIB)



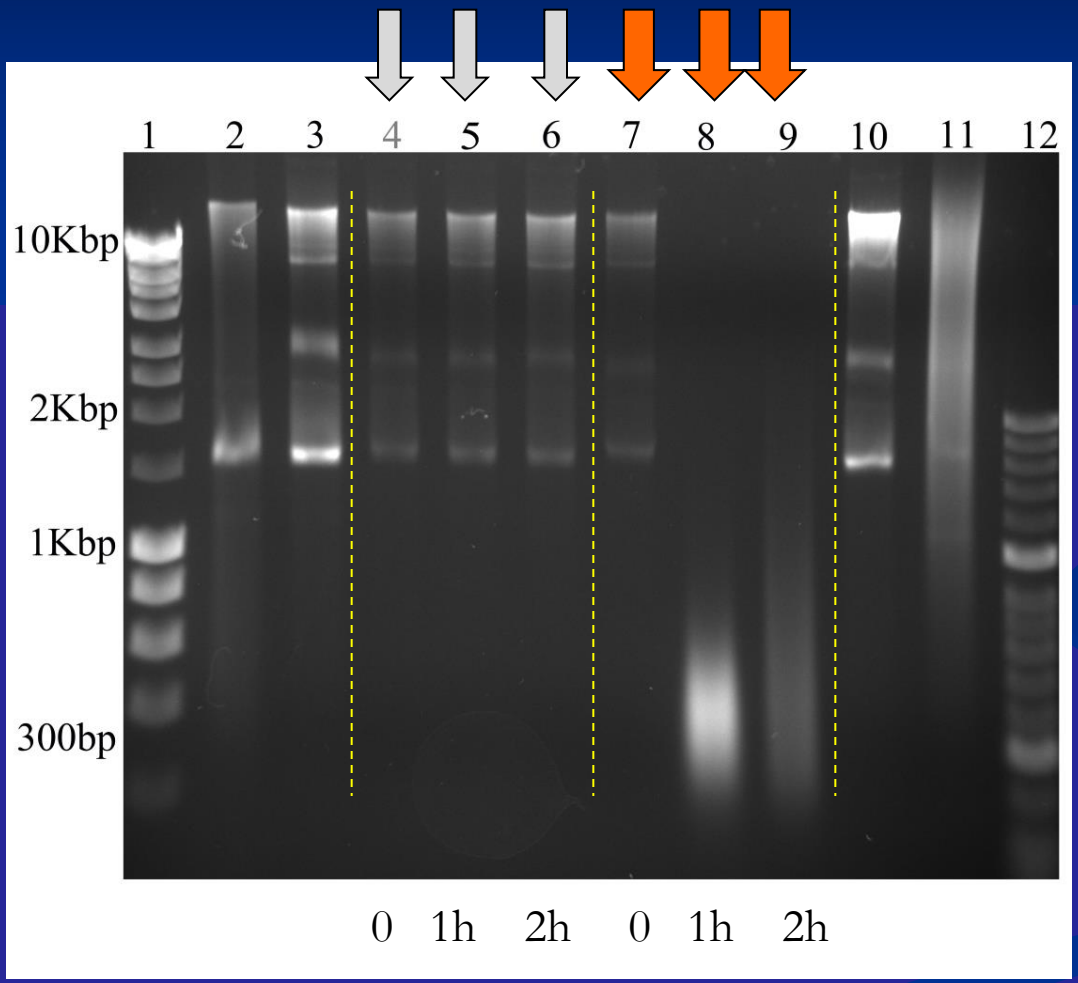

Cells in PBS




Cells in PBS die very quickly on copper and copper alloy surfaces.
As for 'wet' inoculum the death-rate is reduced if cells are inoculated in complex matrix particularly at lower copper concentrations

Warnes *et al*. mBio 3, e00489-12 (2012)

# Destruction of plasmid DNA of *E. coli bla CTX-M-15* following exposure to copper at room temperature




Cells exposed to stainless steel for 0, 60 and 120 minutes (lanes 4, 5,6 respectively) have intact plasmid DNA

Cells exposed to copper surfaces for 0, 60 and 120 minutes (lanes 7, 8, 9) demonstrate progressive denaturation of plasmid DNA over time.

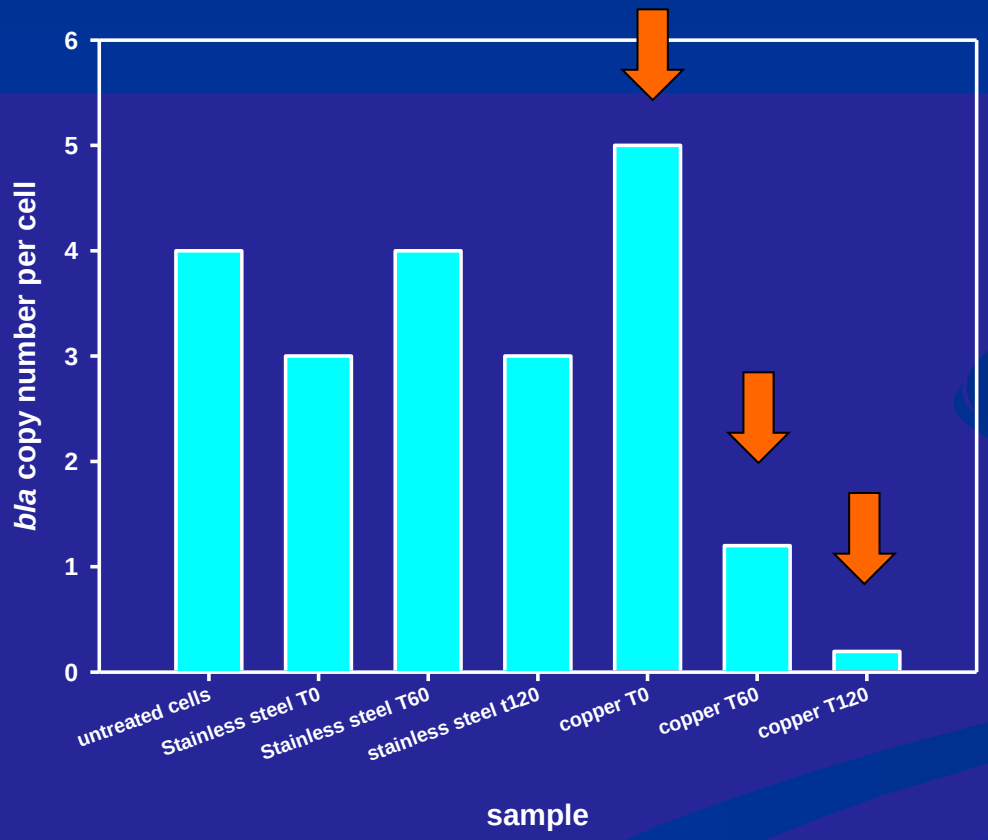
Lanes 10, 3 untreated cells

Lane 11 is heated cells

Warnes *et al*. mBio 3, e00489-12 (2012)

# Direct detection of the *CTX-M-15 bla* gene in the same plasmid preparations using quantitative PCR (qPCR)

**Copy number of beta lactamase gene in antibiotic resistant *E. coli* (untreated cells or those exposed to copper and stainless steel surfaces at room temperature: 'wet' inoculum)**




If the cT values are converted to actual gene copy number per cell it can be seen that copy number has depleted over time when exposed to copper surfaces.

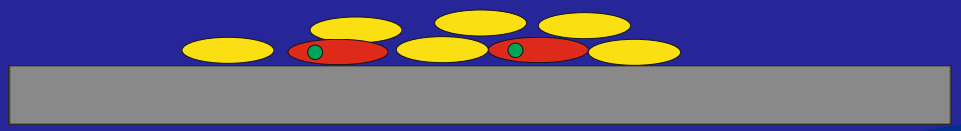
Warnes *et al*. mBio 3, e00489-12 (2012)

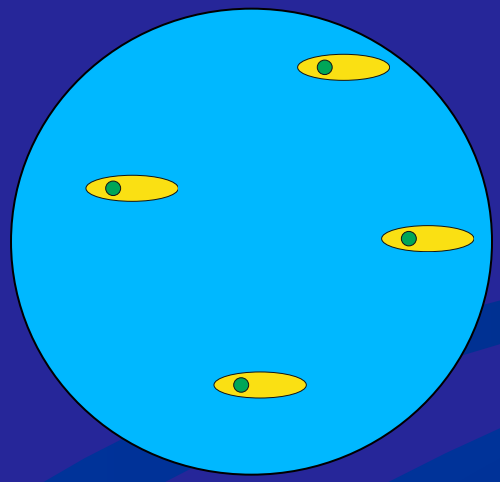
# Can antibiotic resistance genes be transferred by natural conjugation on surfaces?

Pathogen containing antibiotic resistance gene on plasmid (green)
e.g. *K. pneumoniae* NDM-1 and *E. coli* CTX-M-15
DONOR, sensitive to sodium azide

*E. coli* RECIPIENT strain, resistant to sodium azide but sensitive to antibiotic

Bacteria mixed together on surface

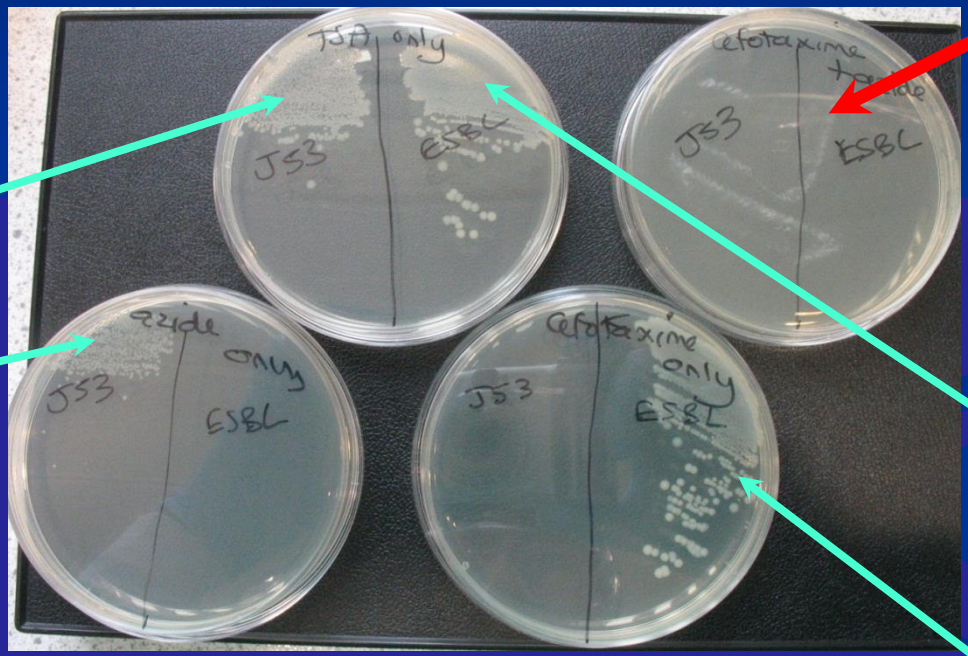




Transconjugants selected for growth on medium containing antibiotic (e.g. cefotaxime, meropenem)
AND sodium azide

# Bacterial cultures checked prior to conjugation experiment

Recipient strain *E. coli* J53 grows on non-selective tryptone soy agar (TSA) and medium containing **sodium azide**

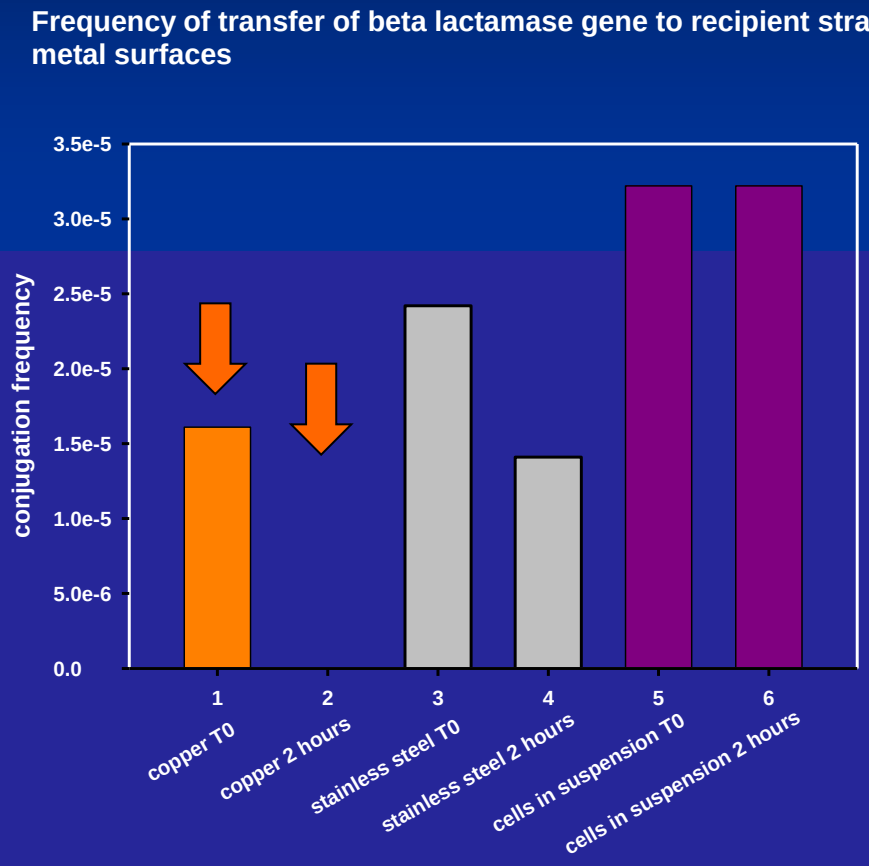


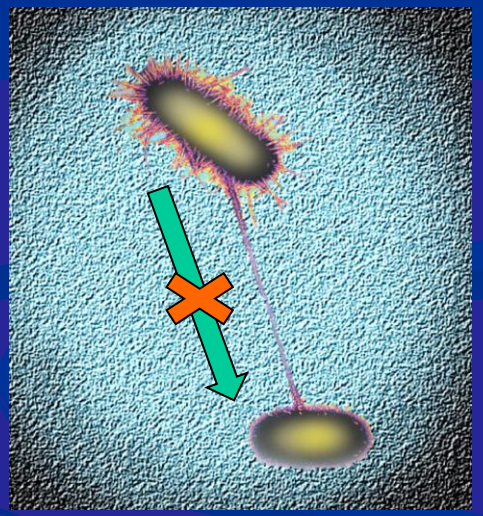

**Neither strain grows on medium containing antibiotic AND sodium azide**

Donor strain grows on TSA and medium containing antibiotic, **cefotaxime**

# Detection of *bla CTX-M-15* in possible transconjugants

(selected by ability to grow on medium containing cephalosporin and sodium azide)



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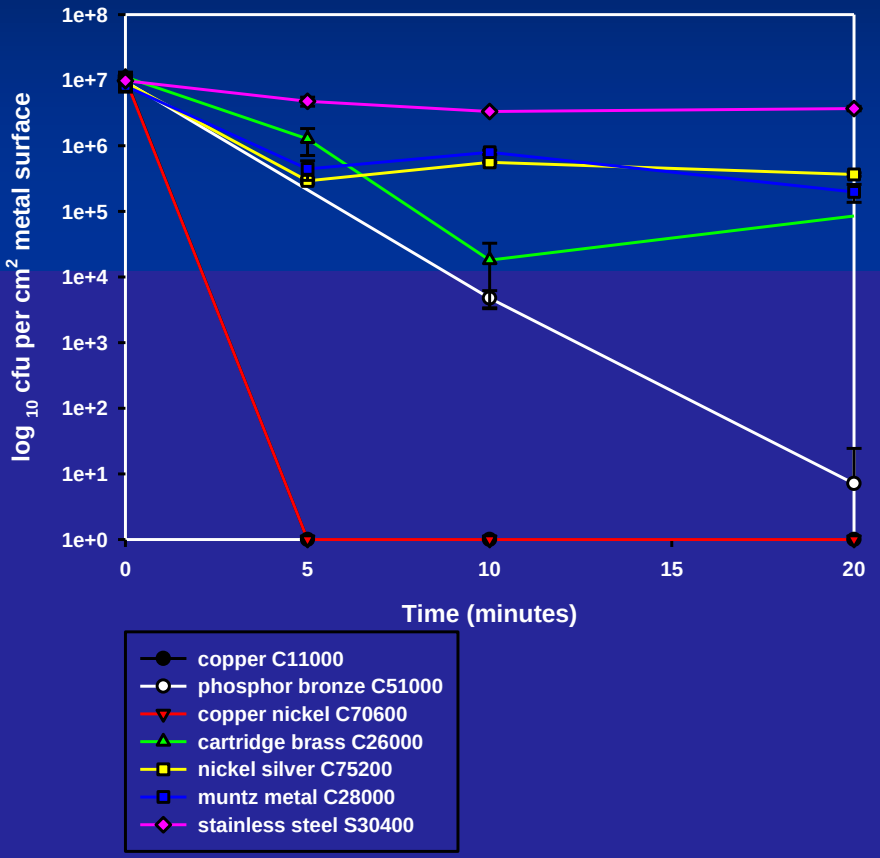
Cu prevents transfer

Conjugation frequency =
no. transconjugants /no. donor cells

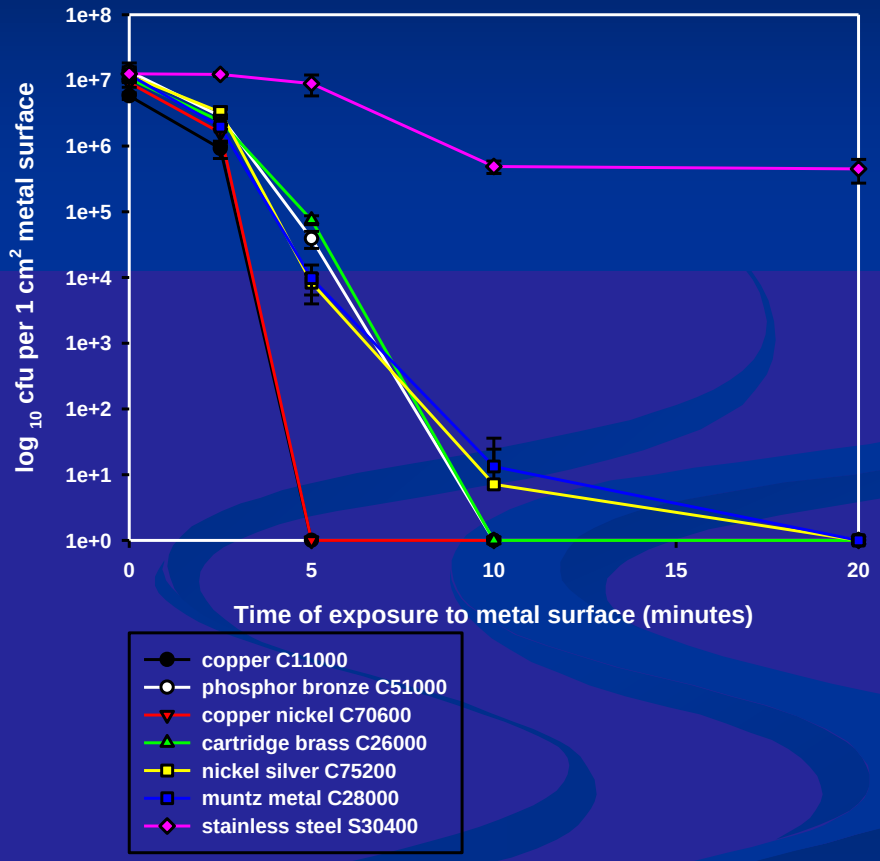
Warnes *et al*. mBio 3, e00489-12 (2012)

# Survival of bla NDM-1 producing *K. pneumoniae* on metal surfaces: 'dry' inoculum

Cells in bacteriological medium (BHIB)

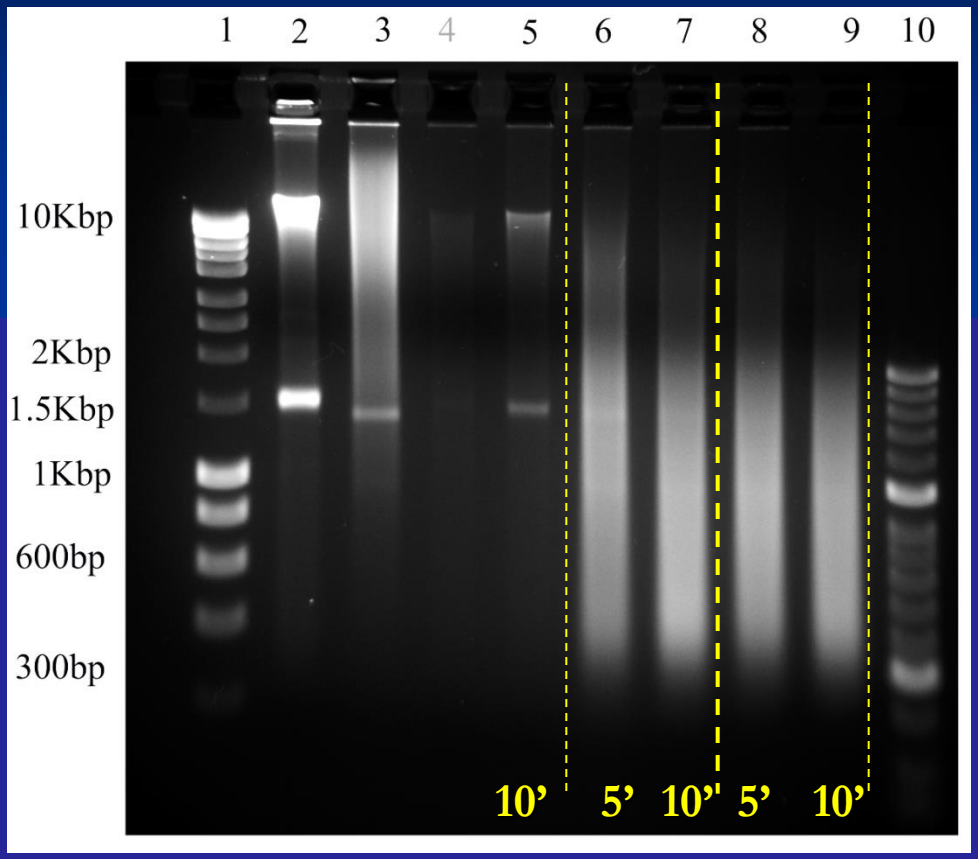



Cells in PBS




Warnes *et al*. mBio 3, e00489-12 (2012)

# Exposure to copper or cartridge brass degrades plasmid DNA of MDR- *Klebsiella pneumoniae* (‘dry’ touch contamination)

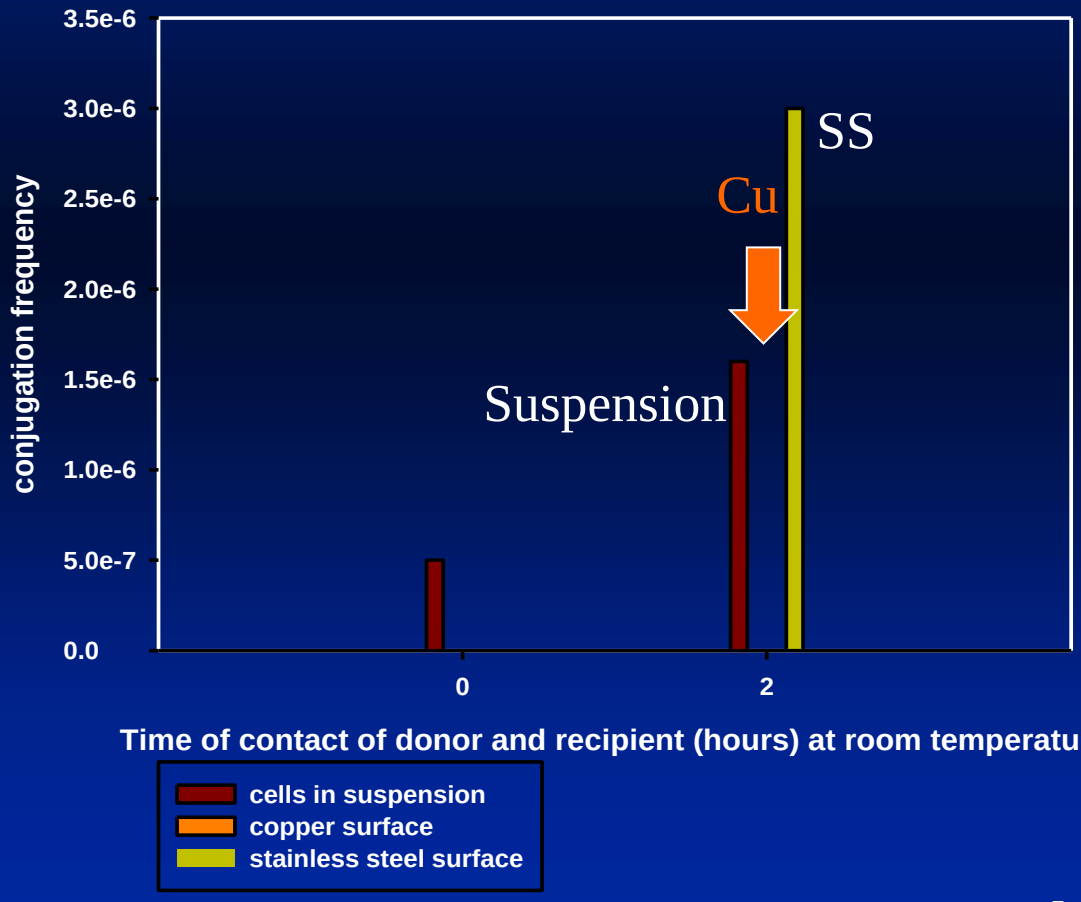


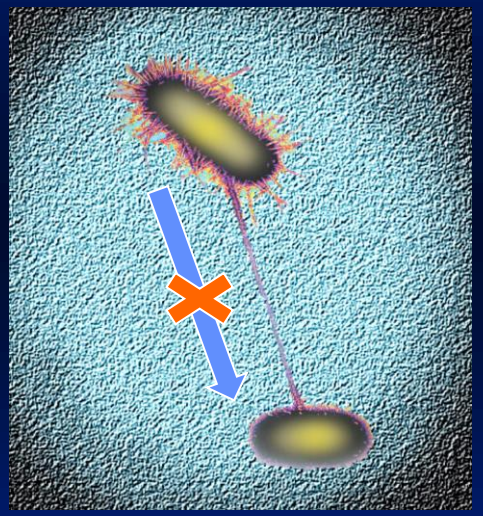

Degradation of *K. pneumoniae* plasmid DNA occurs on copper (lanes 8, 9: 5 and 10 minutes contact respectively) and cartridge brass (lanes 6, 7 :5 and 10 minutes contact) but not on stainless steel (lane 5: 10 minutes). Degraded DNA appears as a ‘smear’ of multi-sized fragments. This can be seen clearly in the small 1.5Kbp plasmid which is evident on untreated, heat-killed and cells exposed to stainless steel for 10 minutes but not on copper or alloy (although faint band can be seen after 5 minutes contact on alloy)

Warnes *et al*. mBio 3, e00489-12 (2012)

# 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.**



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Cu prevents transfer

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# Conclusions

Contact surfaces are hitherto unrecognised reservoir for rapid HGT and emergence of superbugs

Copper alloys kill rapidly, particularly on dry contact

Continuous activity 24/7 through Cu(I)/(II) and ROS

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
- as well as genes responsible for virulence and toxin production.

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

# Acknowledgements


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- Copper Alliance
  - Harold Michels


Cu Copper Alliance