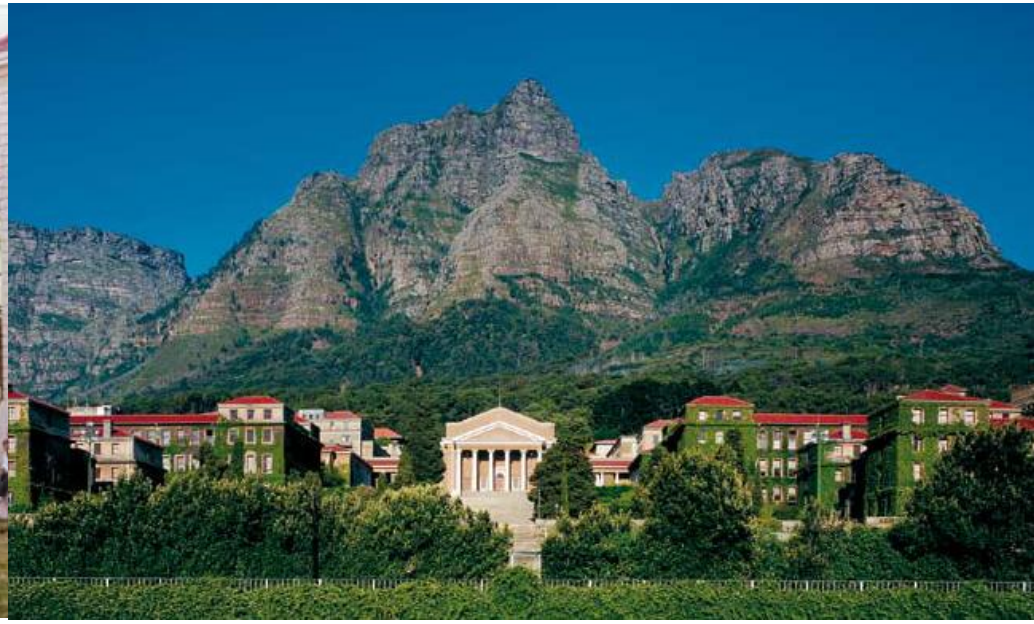
TOP 5 PAPERS MICROBIOLOGY 2010/11



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NATIONAL HEALTH LABORATORY SERVICE

University of Cape Town · iYunivesithi yaseKapa · Universiteit van Kaapstad
SPES BONA

Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study

Timothy R Walsh, Janis Weeks, David M Livermore, Mark A Toleman

Lancet Infect Dis 2011;11:355-62

NDM-1

- Enterobacteriaceae and Acinetobacter
- Plasmids carrying *bla*$_{\text{NDM-1}}$ carry multiple resistance determinants
- Widely disseminated on Indian subcontinent (2006 in retrospective series)
- Europe, North America, Australasia
  - Patients with hospital admission in ISC
  - Some incidental gut colonization

Sample collection

- Channel 4 news employee
- Swabs of seewage water (171) and tap water samples (50) within 12km radius of central Delhi
- Shipped to UK

Procedures

- PCR amplification of *bla*$_{\text{NDM-1}}$ and in-gel DNA hybridization
- Bacterial isolation on selective media – colonies screened by DNA-hybridization and PCR
- Plasmid transfer experiments at 25^{o}C, 30^{o}C and 27^{o}C and plasmid stability

Results

- 2/50 water samples and 51/171 seepage samples positive for *bla*$_{NDM\text{-}1}$
  - Sites close to Connaught Place, Sir Ganga Ram Hospital and Gol Market
- All seepage and water samples positive for Enterobacteriaceae resistant to cefotaxime
- 20 NDM-1 positive strains

	Species	Minimal inhibitory concentration (mg/L)											Typical bla_{NDM-1} antibiogram	Genetic location	Plasmid		
		CTX	CTZ	IMP	MER	ATM	GEN	AMI	TOB	CIP	FOS	TIG	COL			Size	Stability*	Type
From waste seepage																		
B-3-2	*Pseudomonas putida*	64	4	0·5	2	64	0·25	1	0·25	0·125	256	4	0·125	No	Plasmid	ND	No	--
1-19	*Pseudomonas pseudoalcaligenes*	64	64	2	4	32	2	1	4	16	16	2	0·25	No	Plasmid	ND	No	--
3-1	*Escherichia coli*	512	256	16	32	64	8	4	16	32	16	4	0·5	Yes	Plasmid	140 kb	Yes	A/C
21-9	*Pseudomonas oryzihabitans*	16	4	2	2	16	0·25	2	0·25	0·25	4	4	0·25	No	Plasmid	ND	No	--
25-4	*Klebsiella pneumoniae*	512	256	32	128	64	32	64	16	32	256	8	0·25	Yes	Plasmid	140 kb	Yes	--
33-5	*Escherichia coli*	256	256	64	64	64	16	32	64	32	2	0·5	0·125	Yes	Plasmid	140 kb	Yes	A/C
65-4	*Escherichia coli*	256	128	8	64	32	16	2	32	16	16	0·5	0·125	Yes	Plasmid	140 kb	Yes	--
65-5	*Shigella boydii*	512	512	4	16	256	32	16	8	64	2	4	1	Yes	Plasmid	250 kb	Yes	--
72-28	*Sutonella indologenes*	32	4	2	4	32	1	2	0·5	0·25	>1024	8	2	No	Plasmid	--	No	--
79-6	*Pseudomonas pseudoalcaligenes*	128	16	2	4	32	4	2	2	8	16	8	0·25	No	Plasmid	280 kb	Yes	--
107-5	*Aeromonas caviae*	64	32	16	8	8	8	2	8	16	128	8	0·25	Yes	Chromo	--	Yes	--
107-7	*Pseudomonas putida*	64	1	32	4	0·25	16	16	32	16	256	16	0·25	No	Plasmid	250 kb	Yes	--
116-4	*Stenotrophomonas maltophilia*	256	256	128	64	64	32	64	16	64	256	16	0·5	Yes	Plasmid	250 kb	Yes	--
116-14	*Vibrio cholerae*	>256	>256	8	8	2	1	8	2	2	64	0·5	8	Yes	Plasmid and chromo	400 kb	Yes	--
116-17	*Vibrio cholerae*	>256	>256	16	1	2	1	0·5	2	2	64	0·5	8	Yes	Plasmid	170 kb	Yes	A/C
117-4	*Citrobacter freundii*	128	128	64	128	64	32	64	32	32	4	2	0·5	Yes	Plasmid	140 kb	Yes	A/C
From tap water																		
W32-17	*Achromobacter spp*	256	256	4	4	64	32	16	32	32	32	0·5	0·125	No	Plasmid	ND	No	--
W38-14	*Kingella denitrificans*	32	32	4	16	8	8	2	1	4	4	1	0·5	No	Plasmid	ND	No	--
W38-16	*Achromobacter spp*	128	128	4	2	32	32	16	4	16	32	0·5	0·25	No	Plasmid	ND	No	--
W38-17	*Pseudomonas aeruginosa*	256	256	32	32	16	32	64	32	16	256	8	0·5	Yes	Plasmid	ND	No	--

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1-19	*Pseudomonas pseudoalcaligenes*
3-1	*Escherichia coli*
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65-5	*Shigella boydii*	512	512	4	16	256
72-28	*Sutonella indologenes*	32	4	2	4	32
79-6	*Pseudomonas*	128	16	2	4	32

Results

- Plasmids stable in Enterobacteriaceae, not in non-fermenters
- Plasmid transfer successful from all isolates with stable plasmids
  - Transfer frequencies highest at 30^{o}C

Implications

- Widespread environmental dissemination
  - Community acquired infection likely (now described)
  - Massive potential human reservoir
  - Extensive dissemination amongst bacterial species (promiscuous plasmids)
- Plasmid transfer possible/likely in environment
- Need to replicate in other cities on subcontinent and perform carriage studies

Revolutionizing Clinical Microbiology Laboratory Organization in Hospitals with In Situ Point-of-Care

Stephan Cohen-Bacrie, Laetitia Ninove, Antoine Nougairede, Remi Charrel, HerveRichet, Philippe Minodier, Sekene Badiaga, Guilhem Noel, Bernard La Scola, Xavier de Lamballerie, Michel Drancourt, Didier Raoult

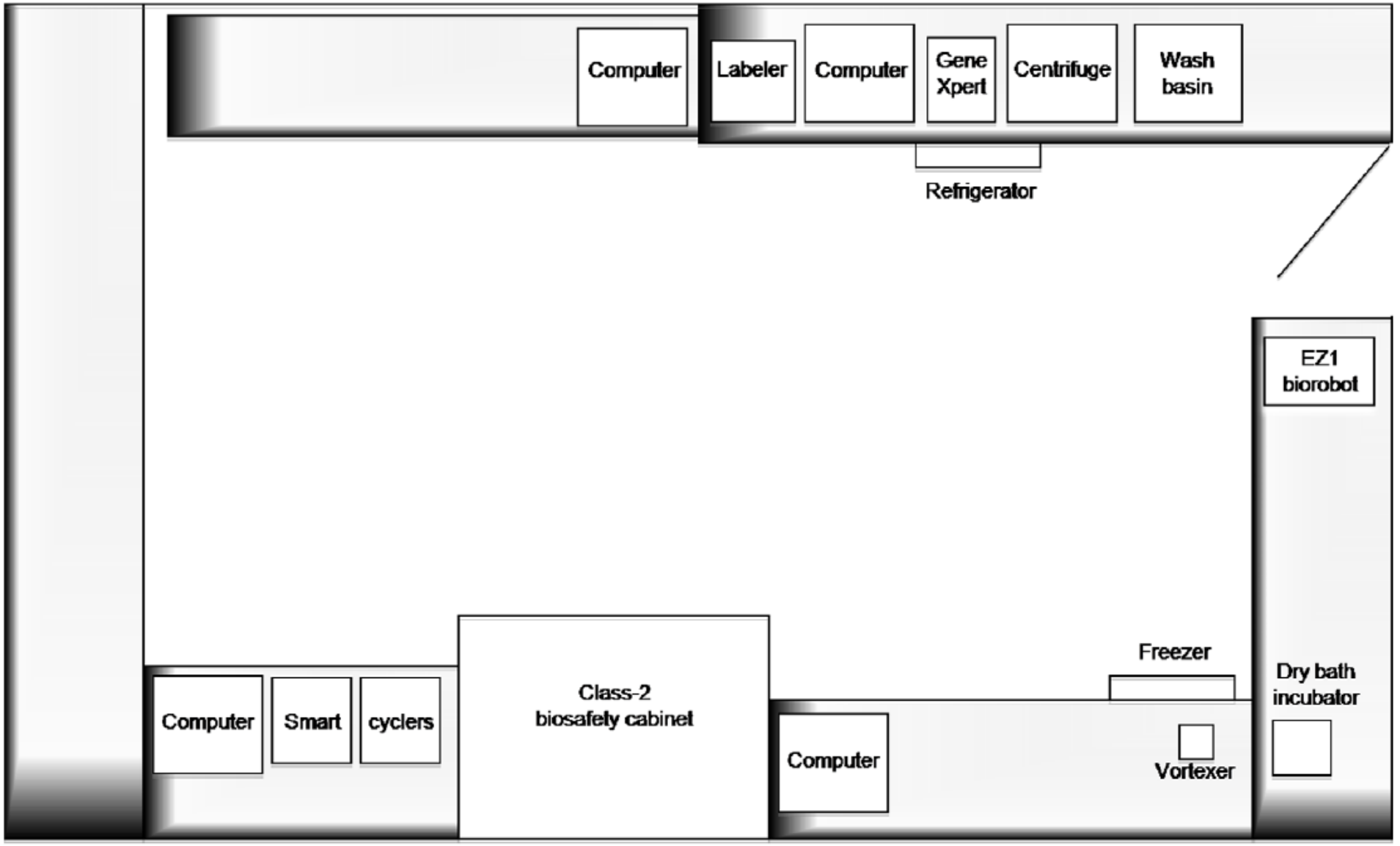
PLoS ONE July 2011; 6: e22403

Quality of care vs. cost containment

- Move to centralization of microbiology laboratories
  - Driven by efficiency and automation
  - Increases turn around time
  - Reduces direct clinical interaction with microbiologists
- Reinforces need for POC testing
  - Decisions re:
    - Hospitalization
    - Isolation
    - Initiation and guidance antimicrobial therapy

Setting and facilities

- Core laboratory facility serving 5 hospitals (3500 beds) in Marseilles
- Pilot POC lab (la Timone) close to core lab
- Remote POC lab in emergency dept of most distant hospital
- Retrospective evaluation of practicality and potential impact over 3 year period


Computer
Labeler
Computer
Gene Xpert
Centrifuge
Wash basin
Refrigerator
EZ1 biorobot
Computer
Smart
cyclers
Class-2 biosafety cabinet
Freezer
Computer
Vortexer
Dry bath incubator

Setting and facilities

- POC lab
  - 24 hours
  - Microbiology residents
  - Repertoire:
    - 13 immunochromatographic tests
    - 2 agglutination assays
    - 3 commercial real-time PCR assays
    - 5 in-house real-time PCR assays
  - Ready to use reaction mixes and controls

Test repertoire

- ICT
  - Influenza, Rotavirus, HIV, RSV, Dengue
  - *S. pneumoniae, L. pneumophila, S. pyogenes, C. difficile, H. pylori, P. falciparum, C. tetani*
  - procalcitonin
- Real-time PCR
  - *B. pertussis, M. pneumoniae, C. burnetti, S. pneumoniae, N. meningitidis, M. pneumoniae, S. agalactiae*
  - Enterovirus, HSV-1/2
- Agglutination
  - EBV, *C. neoformans*

Results

- 51 179 tests over 3 years (8605, 26055, 16519)
- Respiratory testing 78%
  - A/H1N1 outbreak
- Meningitis 4097 tests
- Diarrhoea 4380 tests

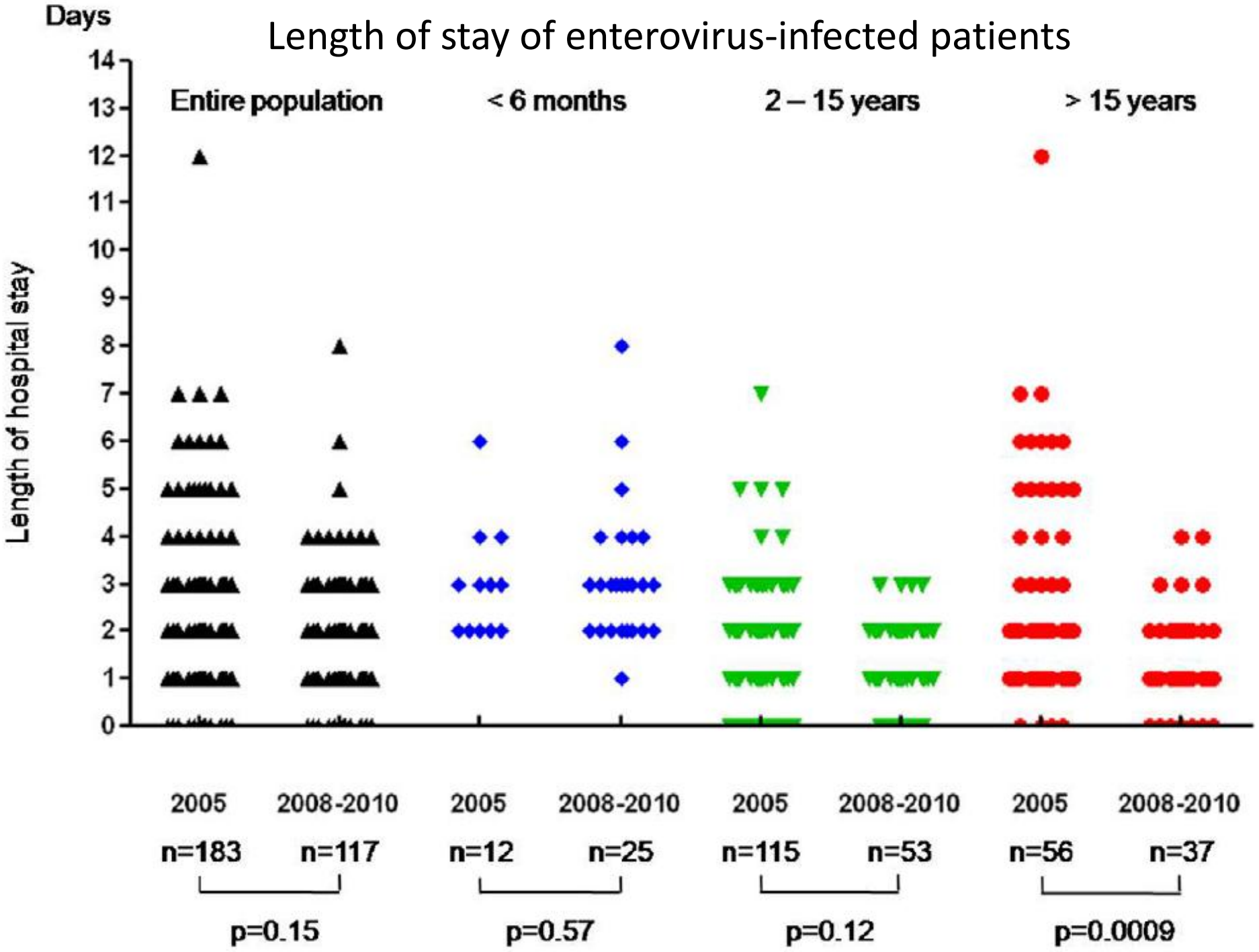
Potential Outcomes

- Isolation of infectious patients
- Avoidance of unnecessary hospitalization
- Avoidance of unnecessary treatment
- Focusing of antimicrobial therapy

Outcome	Test result	n*
Isolation for contagiousness	Positive influenza detection	545
	(A/H1N1)	(335)
	Positive RSV detection	320
	Positive *B. pertussis* detection	14
	Positive rotavirus/adenovirus detection	96
	Positive *C. difficile* detection	7

Avoid unnecessary hospitalization	Positive enterovirus detection	117
Avoid unnecessary treatment	Positive RSV detection	320
	Negative procalcitonin detection	294
	Negative *S. pyogenes* detection	1,827
	Infectious mononucleosis diagnosis	17
	Positive enterovirus detection	117
	Negative *S. agalactiae* detection	763
	Dengue diagnosis	9
	C. tetani antibodies	8
Replace empiric with documented treatment	Positive A/H1N1 influenza detection	335
	Presence of urinary pneumococcal antigens	10
	Presence of urinary *L. pneumophila* antigens	9
	Positive *M. pneumoniae* detection	21
	Bacterial meningitis	13
	HSV meningitis	1
	Malaria	149

Length of stay of enterovirus-infected patients



Lessons

- Large demand
- Limitations
  - Did not measure actual outcomes but predicted
  - Retrospective evaluation
  - No cost-effectiveness analysis

- "..it is possible to make a diagnosis based essentially on a molecular or immunochromatographic approach that produces profound changes in patient care"

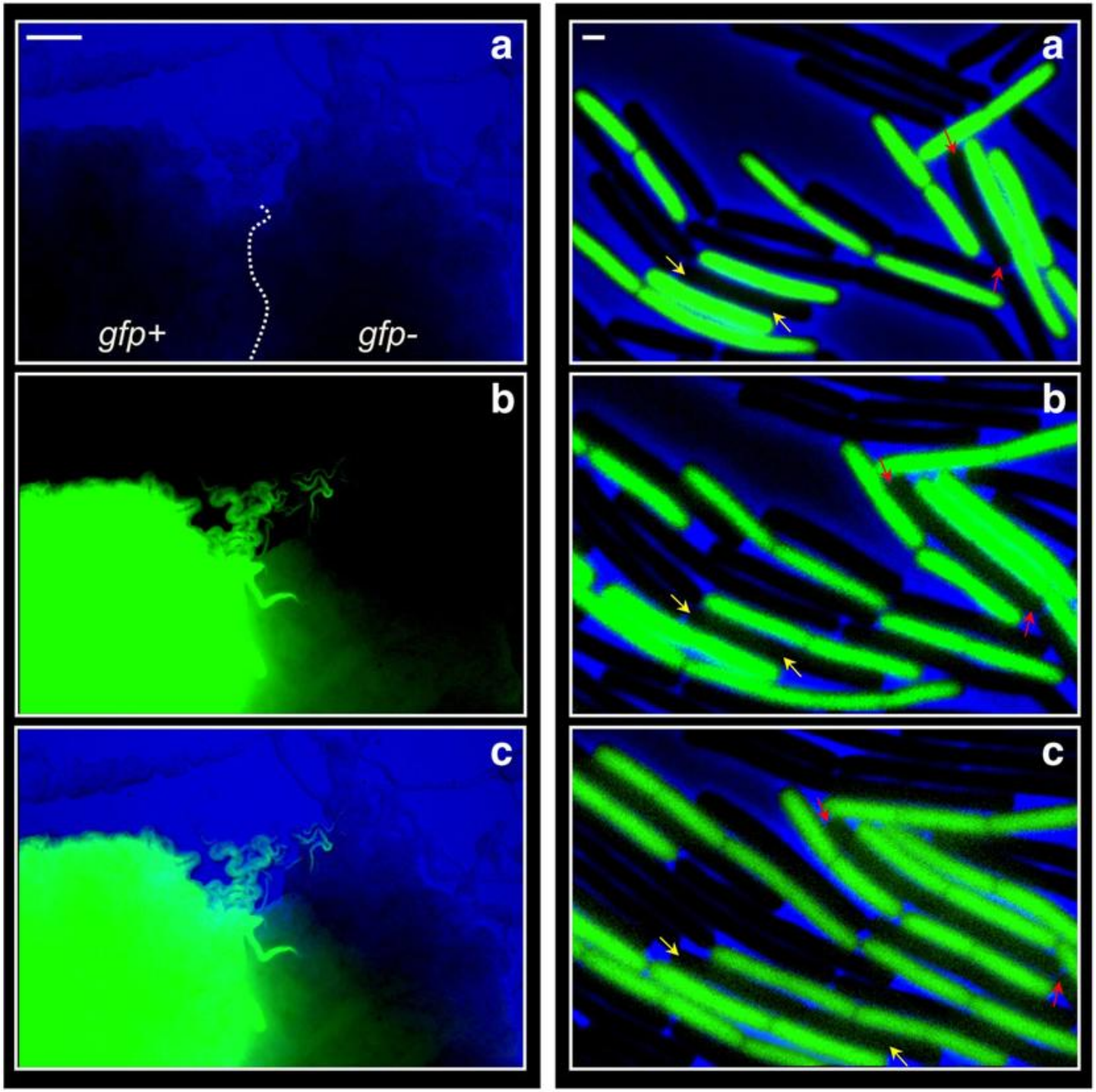
Intercellular Nanotubes Mediate Bacterial Communication

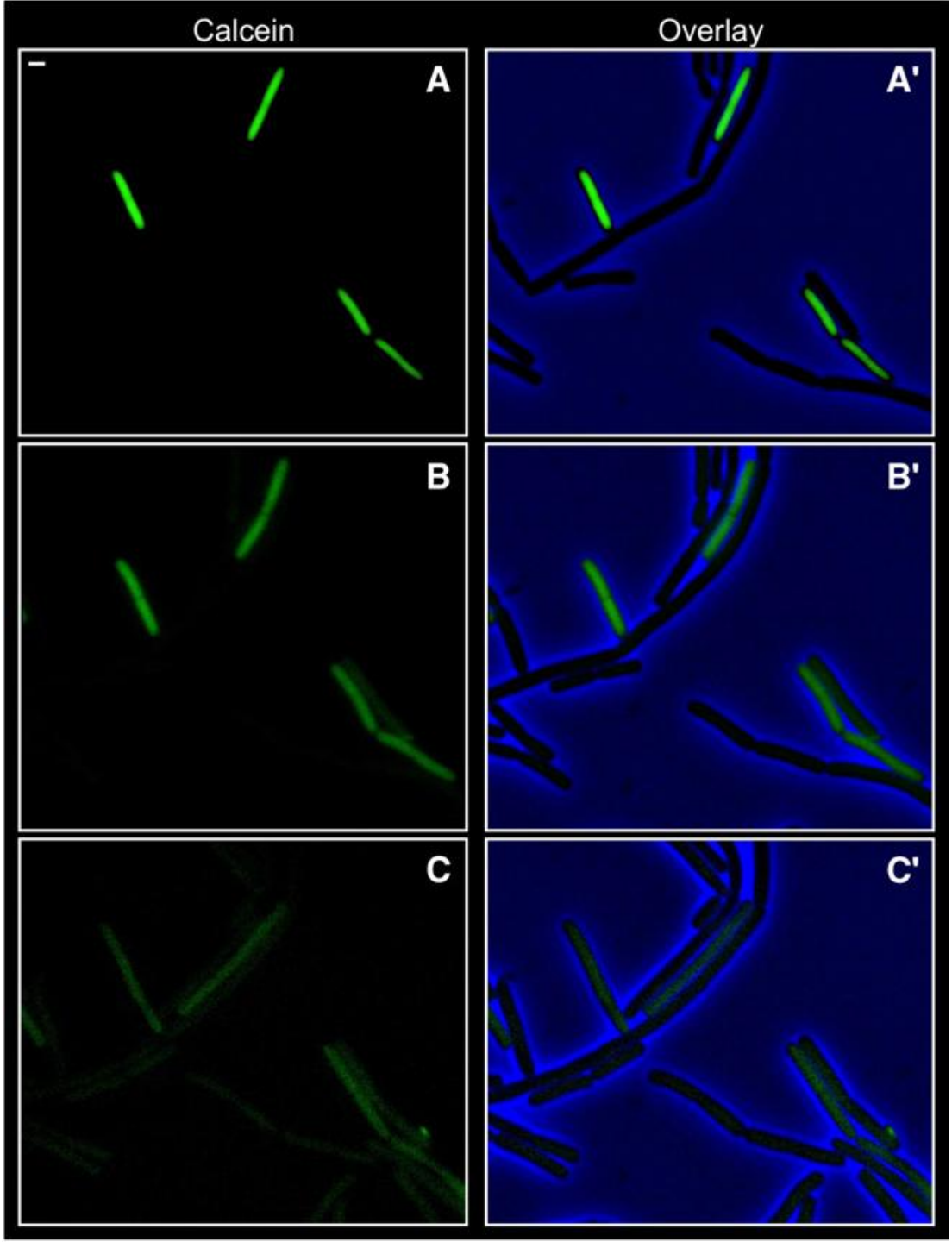
Gyanendra P. Dubey and Sigal Ben-Yehuda

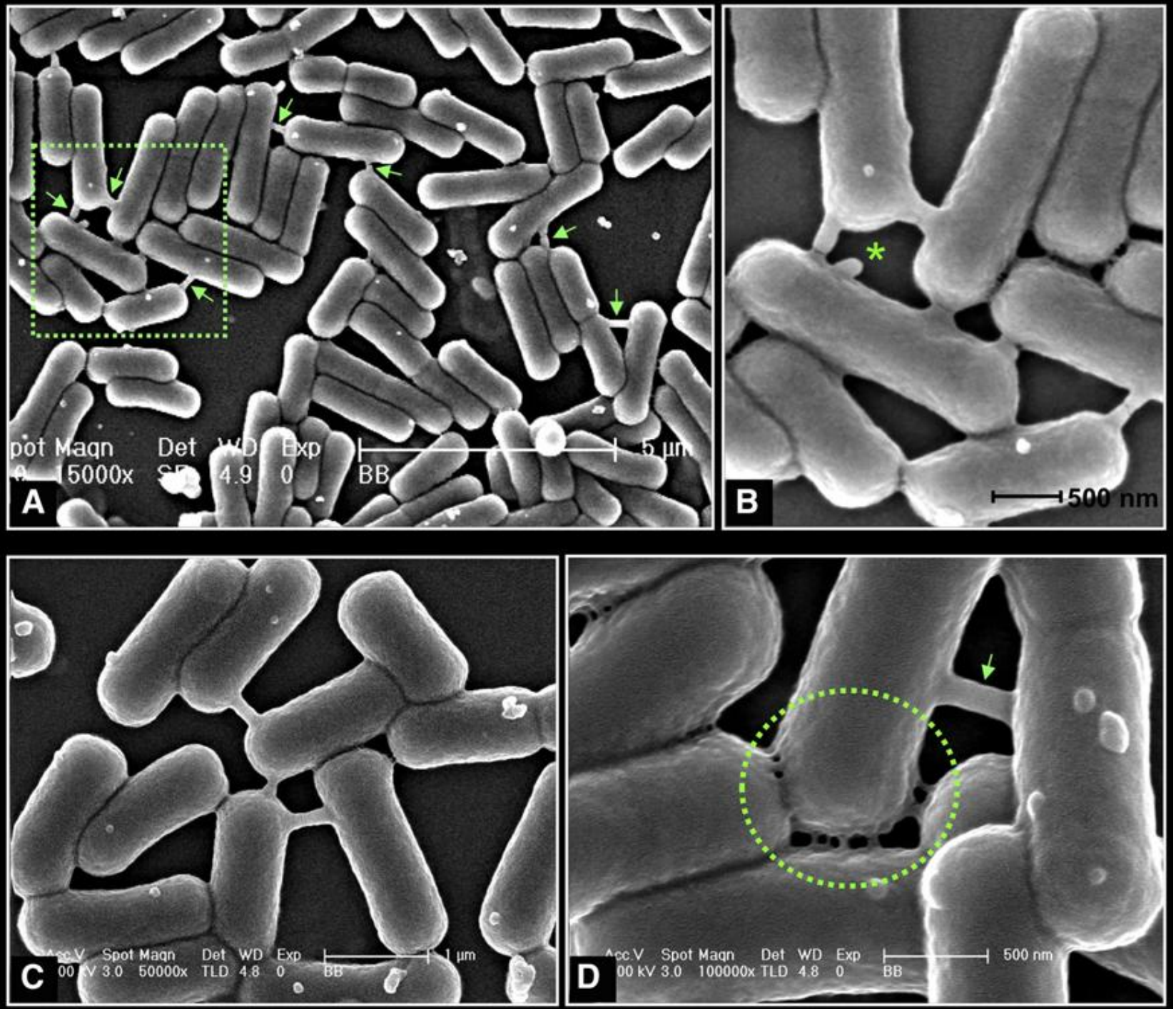
Cell. Feb 2011; 144: 590-600

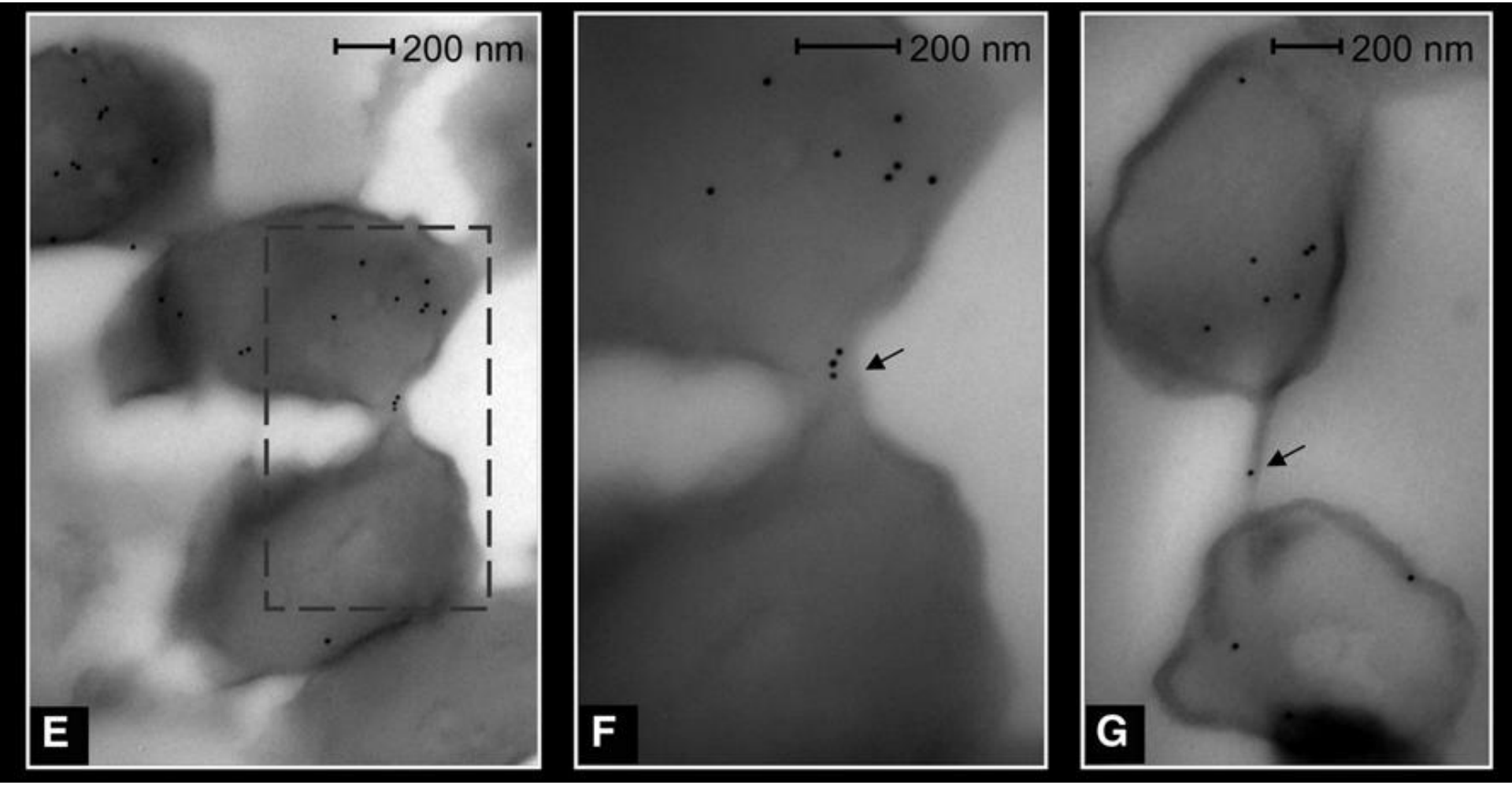
Bacteria are social organisms

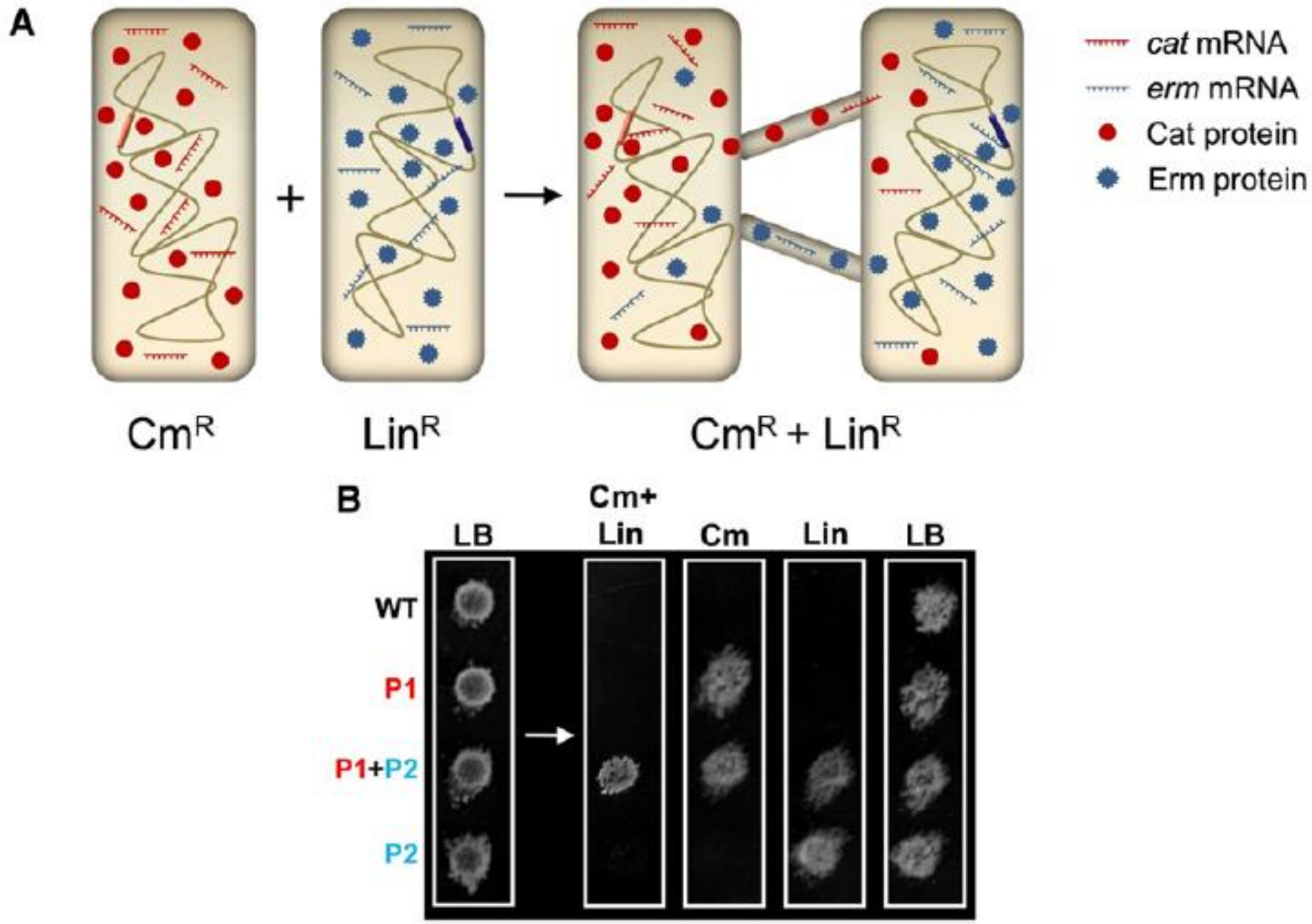
- Social behavior
  - Antibiotic production
  - Virulence factors
  - Competence for DNA uptake
  - Biofilms
- Multicellular behaviour facilitated by
  - Signalling molecules (QS)
  - Extracellular membrane vesicles
  - Conjugation

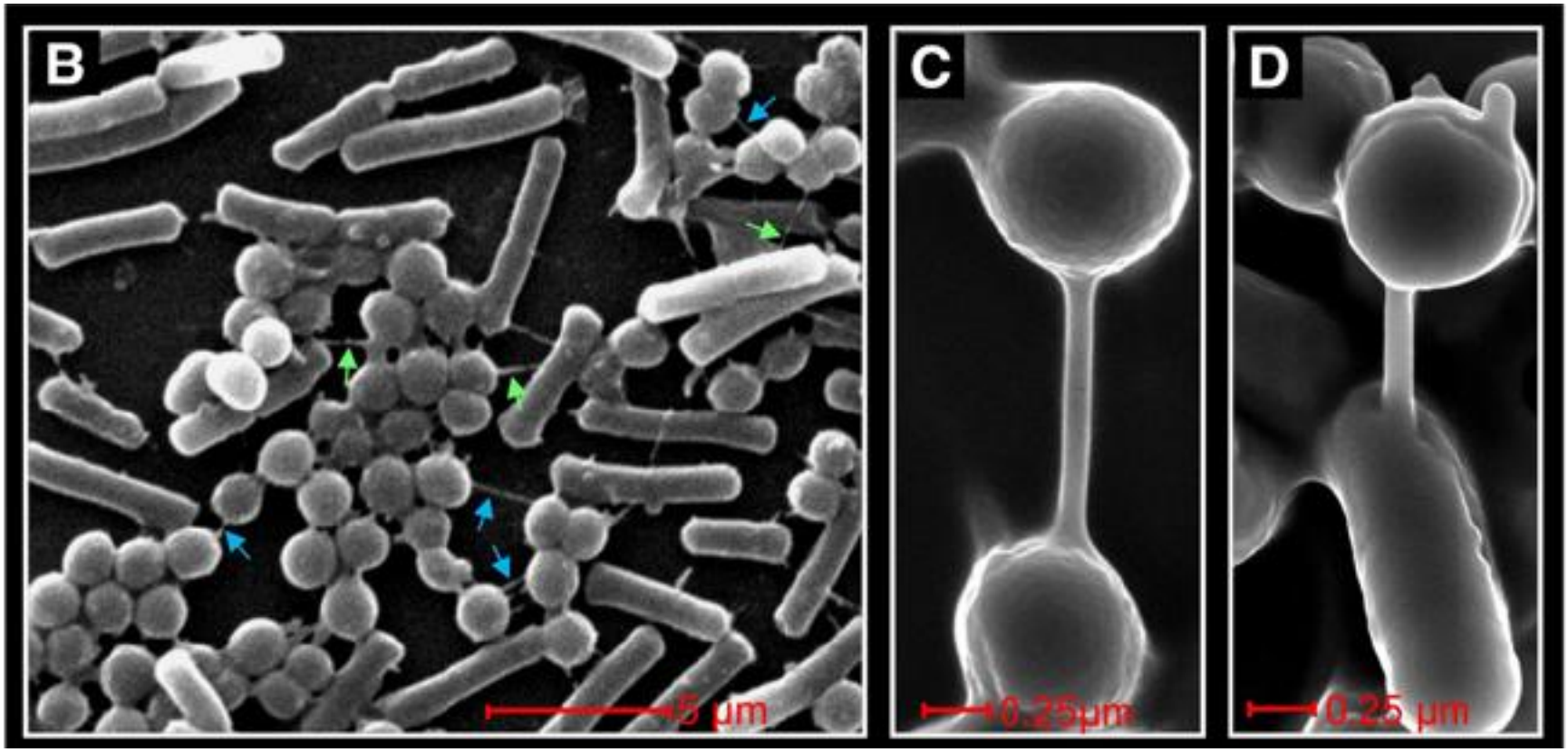

a
gfp+
gfp-
b
c
a
b
c
t0
t30
t60

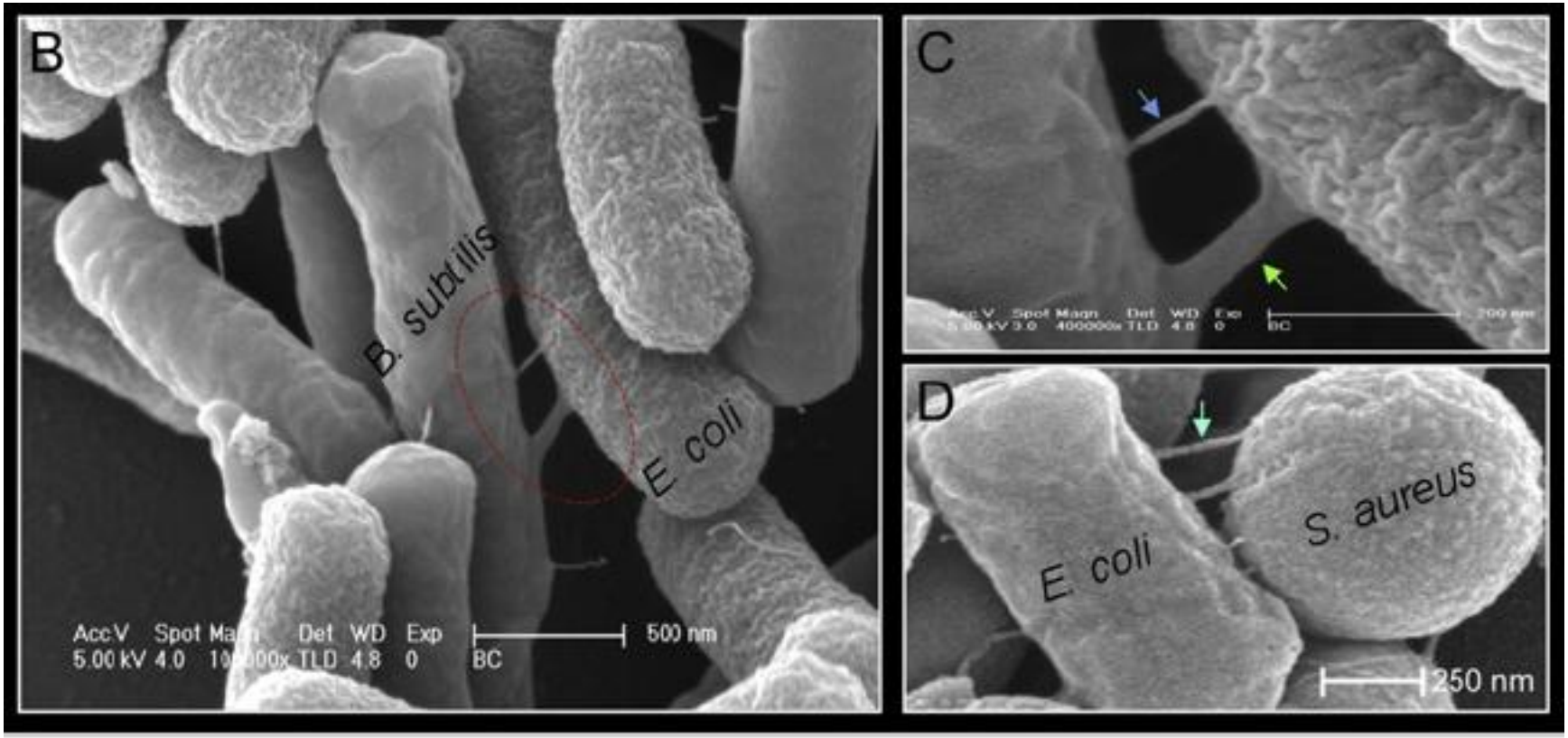

Calcein
Overlay
A
A'
B
B'
C
C'
t0
t15
t30


pot Magn Det WD Exp
5000x 4.9 0 BB
5 µm
A
B
500 nm
C
Acc.V Spot Magn Det WD Exp
00 kV 3.0 50000x TLD 4.8 0 BB
1 µm
D
Acc.V Spot Magn Det WD Exp
00 kV 3.0 100000x TLD 4.8 0 BB
500 nm


200 nm
E
200 nm
F
200 nm
G


A
cat mRNA
erm mRNA
Cat protein
Erm protein
Cm^R
Lin^R
Cm^R + Lin^R
B
LB
Cm+ Lin
Cm
Lin
LB
WT
P1
P1+P2
P2


B
5 µm
C
0.25µm
D
0.25 µm


B
B. subtilis
E coli
Acc.V Spot Det WD Exp
5.00 kV 4.0 TLD 4.8 0 BC
500 nm
C
D
E. coli
S. aureus
250 nm

Implications

- Allows emergence of new phenotypes in bacterial communities
  - Contributes to biofilm formation and resistance to antibiotics
  - Universal interspecies plasmid exchange
  - Commensals may ‘nurture’ pathogenic bacteria
  - Pathogens may transfer virulence factors or determinants to commensals

Ceftriaxone-Resistant *Neisseria gonorrhoeae* , Japan

Makoto Ohnishi, Takeshi Saika, Shinji Hoshina, Kazuhiro Iwasaku, Shu-ichi Nakayama, Haruo Watanabe, and Jo Kitawaki

EID Jan 2011; 17: 148-9

Emergence of untreatable gonorrhoea

- Well documented R to
  - beta-lactams
  - tetracyclines
  - Macrolides
  - fluoroquinolones
- Emerging extended spectrum cefixime resistant organisms in Japan
- Injectable cephalosporins (ceftriaxone) remain active but MIC's increasing

Case report

- 31y commercial sex worker
- Jan 2009
  - Positive NAAT test from throat swab
  - Positive culture from throat swab 2 wk later
  - Rx 1g IV ceftriaxone
- 2 wk later
  - Positive NAAT test on throat swab
  - Further ceftriaxone
- *N. gonorrhoeae* ceftriaxone MIC 2ug/ml (vs. 1 @ 0.5)
  - R to penicillin, cefixime, levofloxacin
  - S to spectinomycin
  - reduced susceptibility to azithromycin (0.5ug/ml)

Further characterization

- MIC agar 2ug/ml; E-test 4ug/ml
- Monte Carlo simulation – free drug time above MIC 6 and 0 hrs respectively
- Unique *penA*$_{H041}$ mosaic allele (PBP2)
- Unlikely to be able to prevent worldwide emergence of R (fluoroquinolones one to two decades)
- Urgent need for new drugs

AAC July 2011; 55: 3538-3545

The Prevalence and Drug Sensitivity of Tuberculosis among Patients Dying in Hospital in KwaZulu-Natal, South Africa: A Postmortem Study

Ted Cohen, Megan Murray, Kristina Wallengren, Gonzalo G. Alvarez, Elizabeth Y. Samuel, Douglas Wilson

PLoS Medicine June 2010, 7: e1000296

No background needed…

- Precise role of TB in adult mortality in SA remains unclear
- Limited autopsy study of patients dying in Edendale hospital Oct 2008 – Aug 2009
  - 860 beds
  - 28% of admissions diagnosed with TB
  - TB 1094/100 000/year in 2006
  - HIV antenatal seroprevalence 39%

Methods

- Study population
  - 20-45y
  - Excluded trauma and obstetric-related deaths
- Data collection
  - Medical records
- Limited autopsy
  - Cricothyroid puncture and lavage
  - Needle biopsies of lung, liver, spleen
  - AFB smear, MGIT culture and DST on solid media
  - HIV test if unknown status
- Review of medical records of eligible but not included decedents

Results

- 240 recruited/997 eligible
- Antiretroviral usage lower amongst those recruited, otherwise similar
- 50% receiving Rx for TB at time of death
  - 61% of these diagnosed during final admission

Study population characteristics

- Female 56%
- Median age 33y
- HIV pos 94%
  - 17% receiving ARV
- Prior hospitalization (6 mth) 15%
- Exposure to MDR 4%
- Recent death in the home 7%
- Failed TB treatment 4%

Results

- On TB treatment at time of death (n=117)
  - Smear pos 39%
  - Culture pos 58%
- Not on TB treatment at time of death (n=119)
  - Smear pos 29%
  - Culture pos 42%
- TB not suspected (n=100)
  - Smear pos 25%
  - Culture pos 42%
  - Pneumonia/LRTI, meningitis, gastroenteritis

Results

- Adjusted odds for positive TB culture
  - Male (2,0; 95% CI 1,1-3,5)
  - On TB treatment (1,8; 95% CI 1,1-3,2)

- Drug resistance
  - 17% of isolates MDR
  - 1 XDR isolate
  - 1 INH monoresistant
  - 1 RIF monoresistant

- Adjusted odds for MDR
  - Current TB treatment (6,7; 95% CI 1,3-32,8)
  - Recent hospitalization not significant

Implications

- TB remains undiagnosed and unsuspected despite high index of suspicion
- High rates of sensitive MTB in patients dying on treatment
  - Diagnosis too late, poor adherence, malabsorption
- Almost 1:6 patients dying during initiation phase of first TB treatment had MDR-TB
  - Primary infection with MDR-TB
  - Community transmission of MDR-TB
- Urgent need for increased testing and early intervention