

A close-up photograph of a petri dish containing a bacterial culture. The agar is a light green color, and there are several distinct, elongated, and somewhat irregular bacterial colonies visible. The colonies have a slightly raised, textured appearance. The petri dish is dark, and the background is a dark, textured surface.

Wounds: The ID/Microbiology Perspective

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CCDHB
2018

Contents

- The lab perspective
- Colonisation vs infection
- Sampling of wounds
- Common bugs and which antibiotics to use
- Biofilms
- Antibiotic resistance/stewardship
- Cases

A sample arrives in the lab...

Step 1.

- **Microscopy**

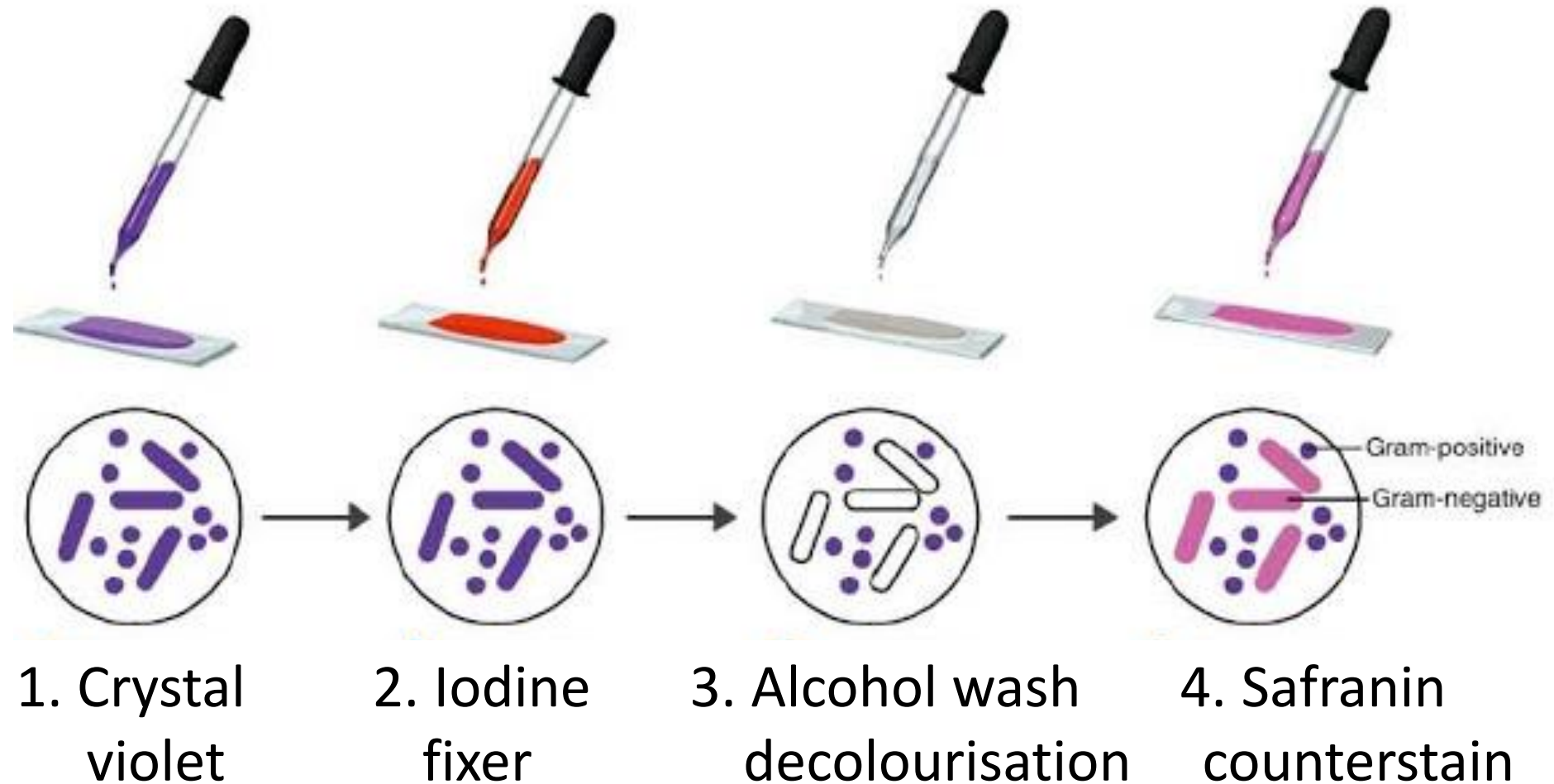
- Part of sample is put onto a glass slide

- **Gram stain** (over 100 years old and still going strong)





Gram Stain Method

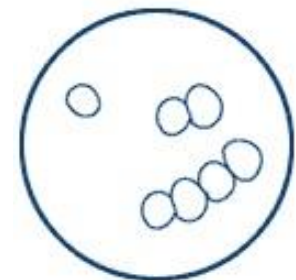






Gram Stain

- “First step” in identification of bacteria
- Differentiates bacteria by cell wall properties
- Divided into “**Gram Positive**” and “**Gram Negative**”
- **Shape** of bacteria also identified:
 - Round shaped → cocci
 - Rod shaped → bacilli
- Can be performed rapidly
- Used to guide initial antibiotic choice



Cocci



Bacilli

Gram stain



```
graph TD; A[Gram stain] --> B[Thick cell wall]; A --> C[Thin cell wall]; B --> D[Purple]; D --> E[Gram "positive"];
```

The diagram is a flowchart illustrating the results of a Gram stain. It starts with a central title 'Gram stain' at the top. From this title, two lines branch out to two separate columns. The left column, represented by dark purple boxes, describes Gram-positive bacteria: 'Thick cell wall', 'Purple', and 'Gram "positive"'. The right column, represented by pink boxes, describes Gram-negative bacteria: 'Thin cell wall', 'Pink', and 'Gram "negative"'. The boxes are connected by vertical lines, and the two columns are connected at the top by a horizontal line branching from the main title.

Thick cell wall

Thin cell wall

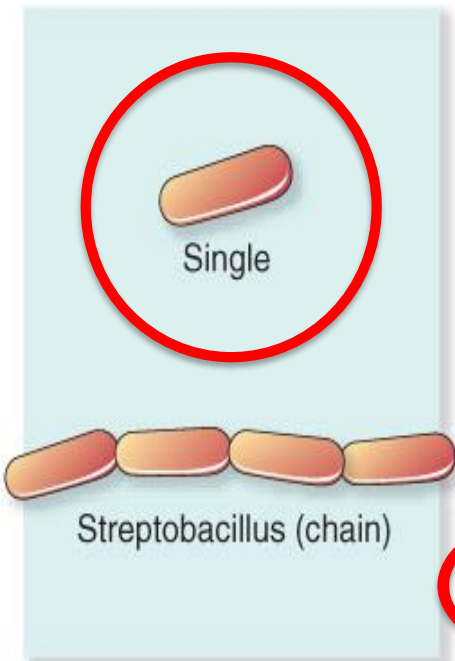
Purple

Pink

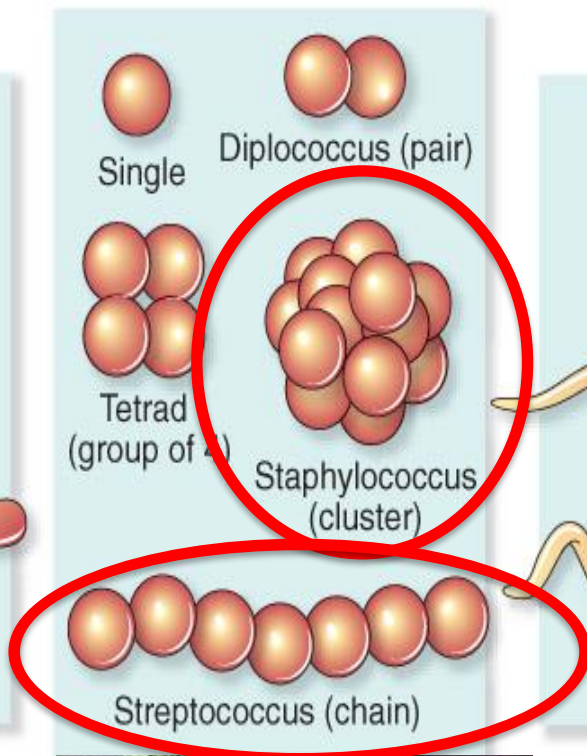
Gram "positive"

Gram "negative"

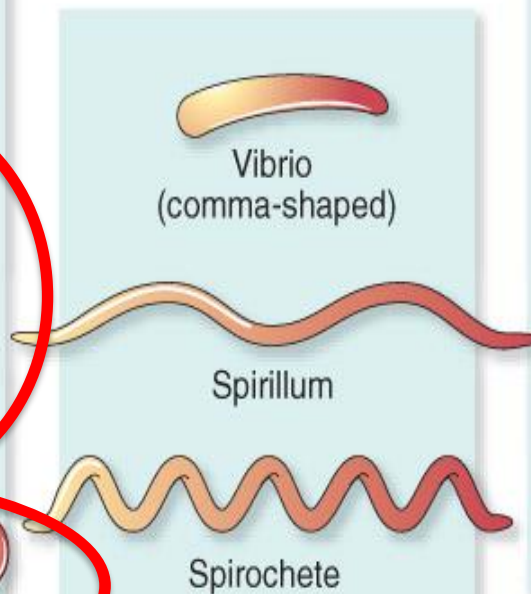
Bacillus (rod)



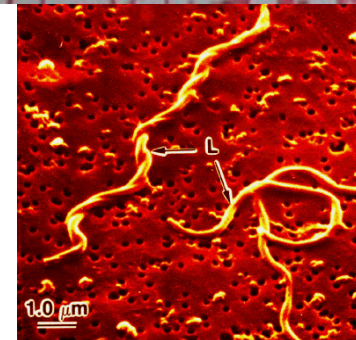
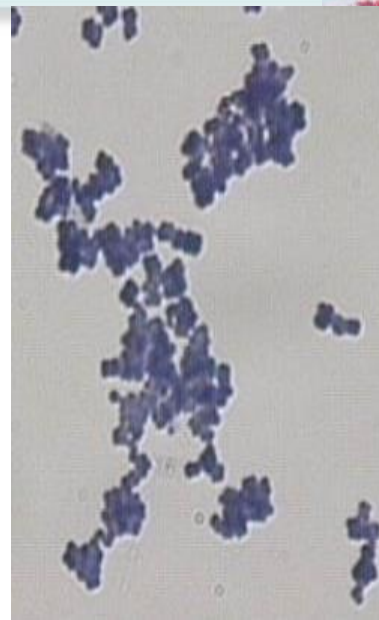
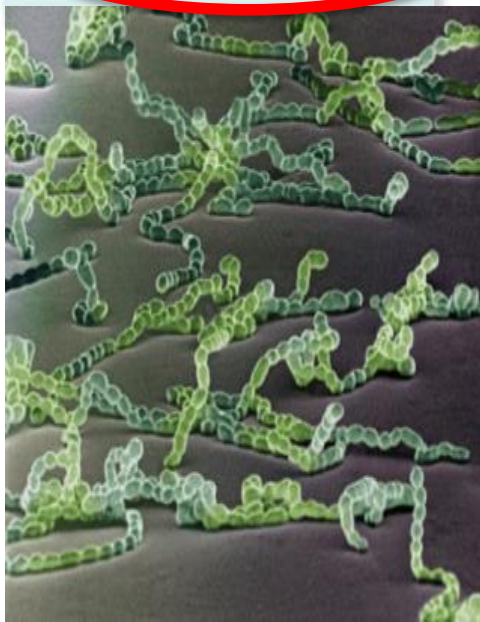
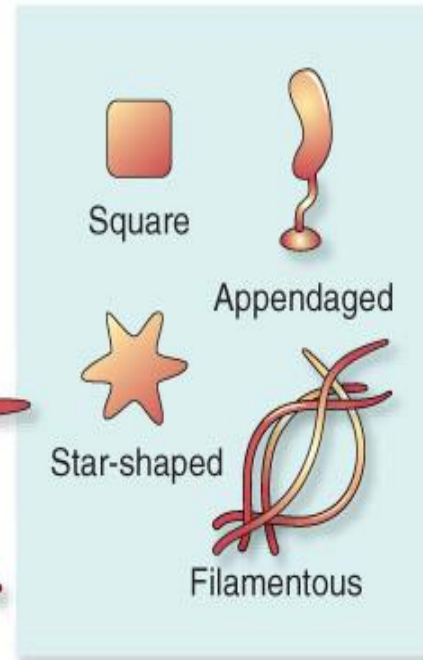
Coccus (sphere)



Spiral



Other shapes



Step 1.

- **Microscopy**
 - **Gram stain** (over 100 years old and still going strong)
- Microscopy can give initial clue as to bugs causing infection



Pus swab

Site Right Mastoid- Post Auricular

Gram stain

Large number of leucocytes

Large number of Gram positive cocci

Culture

Heavy growth of *Staphylococcus aureus*

MRSA - non multi resistant

Penicillin Resistant

Flucloxacillin	Resistant
----------------	-----------

Erythromycin	Susceptible
--------------	-------------

Doxycycline	Susceptible
-------------	-------------

Co-trimoxazole	Susceptible
----------------	-------------

This organism is resistant to flucloxacillin and cephalosporins.

Alternatives include vancomycin, cotrimoxazole and doxycycline.

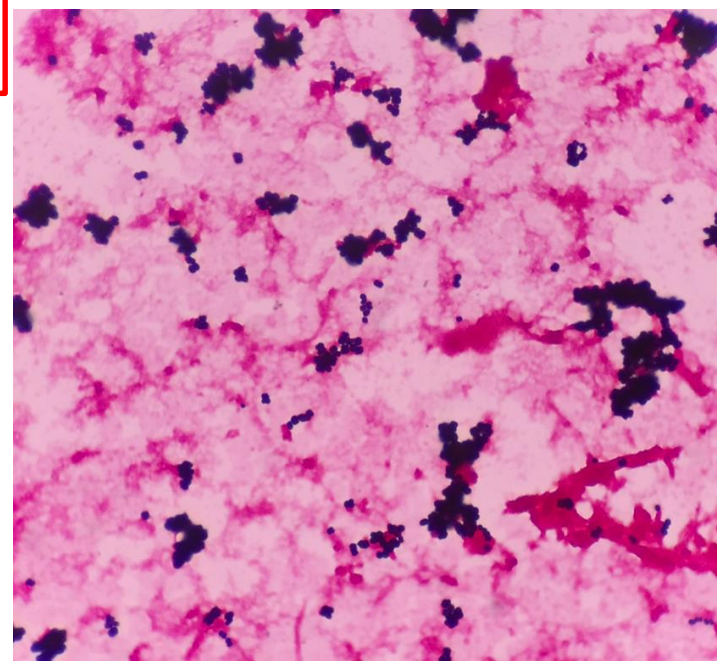
Difficult infections, especially those causing sepsis or prosthetic infections should be discussed with ID or micro. Vancomycin therapy should be approved by a ID or Micro.

Heavy growth of *Streptococcus agalactiae*

Erythromycin	Susceptible
--------------	-------------

Penicillin is the treatment of choice for non-allergic patients.

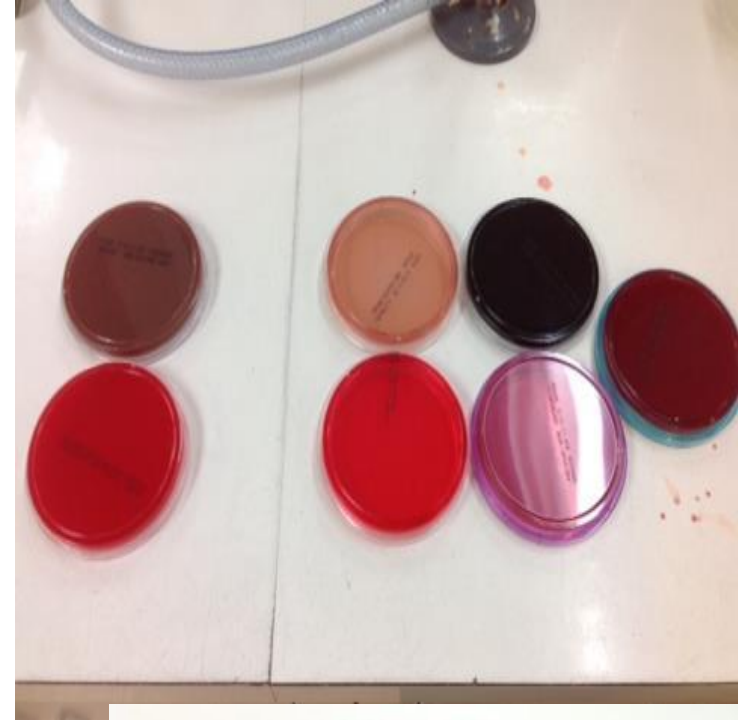
Cephalosporins and amoxicillin are reliably effective. Clindamycin is only indicated for betalactam allergy and sepsis syndrome.



Step 2.

- **Culture**

- Sample put onto different types of growth media
- Incubated to allow bugs to grow
- Usually takes 24-48h for growth
- Bugs that are grown are identified and reported



Pus swab

Site Right Mastoid- Post Auricular

Gram stain

- Large number of leucocytes
- Large number of Gram positive cocci

Culture

Heavy growth of *Staphylococcus aureus*
MRSA - non multi resistant

Penicillin	Resistant
Flucloxacillin	Resistant
Erythromycin	Susceptible
Doxycycline	Susceptible
Co-trimoxazole	Susceptible

This organism is resistant to flucloxacillin and cephalosporins. Alternatives include vancomycin, cotrimoxazole and doxycycline. Difficult infections, especially those causing sepsis or prosthetic infections should be discussed with ID or micro. Vancomycin therapy should be approved by a ID or Micro.

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Step 3.

- **Susceptibility testing**
 - Significant organisms selected for testing
 - Tested against common antibiotics
 - Grow the organism in the presence of different antibiotic discs
 - Measure amount of growth around antibiotic disc to determine susceptibility/resistance
 - Note: designed for 'planktonic' bacteria, doesn't work well for bacteria in biofilms – more to come on this



Pus swab

Site Right Mastoid- Post Auricular

Gram stain

Large number of leucocytes

Large number of Gram positive cocci

Culture

Heavy growth of *Staphylococcus aureus*

MRSA - non multi resistant

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Heavy growth of *Streptococcus agalactiae*

Erythromycin	Susceptible
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Step 4.

Pus swab

Site

Right Mastoid- Post Auricular

Gram stain

Large number of leucocytes

Large number of Gram positive cocci

Culture

Heavy growth of *Staphylococcus aureus*

MRSA - non multi resistant

Penicillin

Resistant

Flucloxacillin

Resistant

Erythromycin

Susceptible

Doxycycline

Susceptible

Co-trimoxazole

Susceptible

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Heavy growth of *Streptococcus agalactiae*

Erythromycin

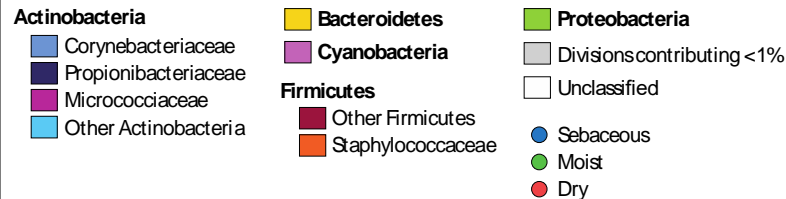
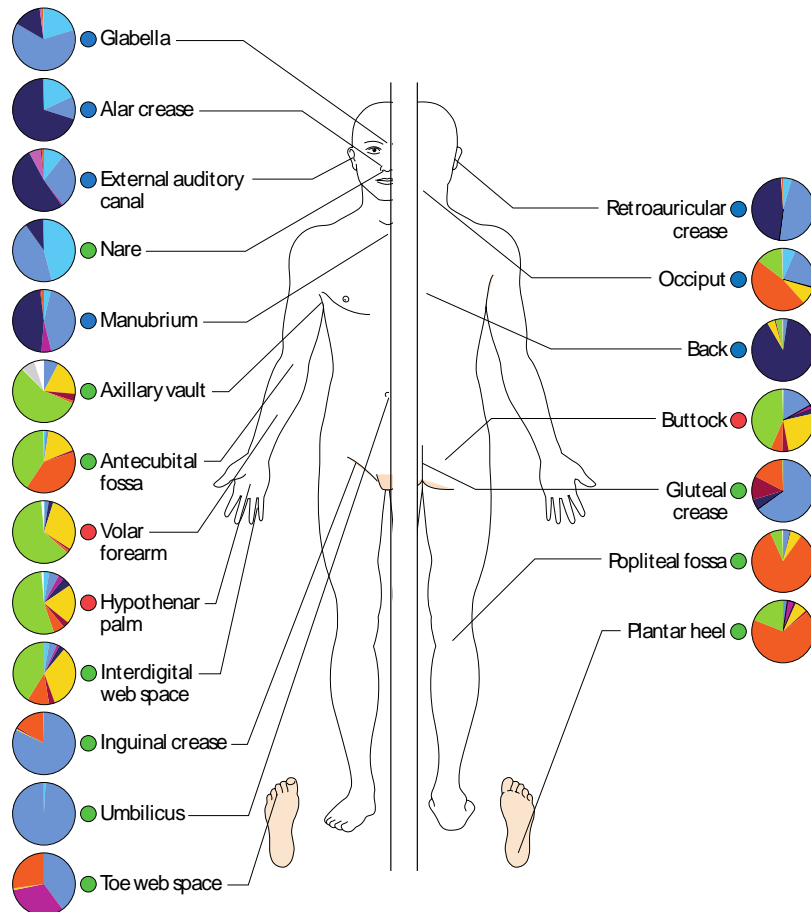
Susceptible

Penicillin is the treatment of choice for non-allergic patients. Cephalosporins and amoxicillin are reliably effective. Clindamycin is only indicated for betalactam allergy and sepsis syndrome.

• Result reporting

- Microbiologist reviews the results and clinical information
- Decides how to report the results onto the clinical information system
- May add interpretive comment

The problem with wound samples...



Surface Sampling:

- Emphasis on semi-quantitative & qualitative microbiology
- Provides a comprehensive profile of the wound microflora
- Often considered to provide misleading results
- The bioburden and microbial diversity is greatest in this area
- Therefore control of micro-organisms in this area is critical to minimize the opportunity for dissemination into deeper tissue, and also minimize the opportunity for cross-contamination

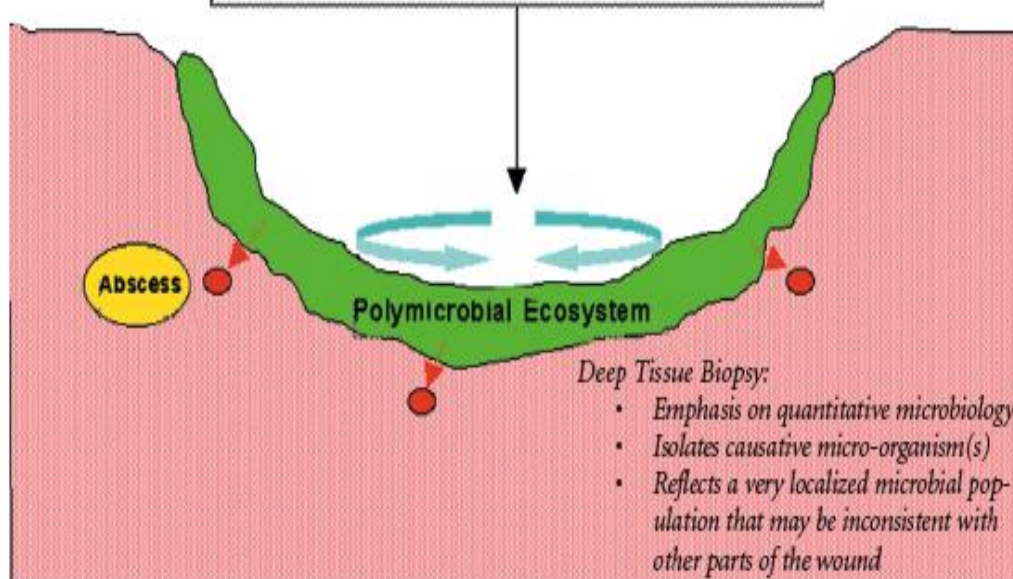


Figure 2

Key aspects associated with superficial and deep tissue sampling. © 2003 ConvaTec.
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Interpretation

- We know there are lots of bugs there anyway
- A WS cannot tell you the difference between infection and colonisation

Results

Tissue Culture

Tissue

Site Right Foot Ulcer

Gram stain

Small number of Gram negative bacilli

Culture

Heavy growth of *Escherichia coli*

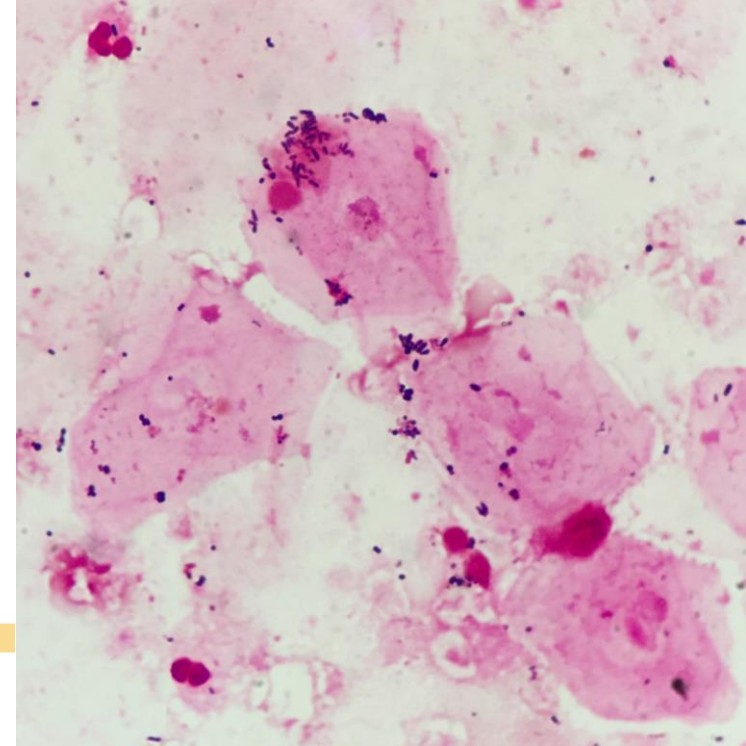
This organism was present as the predominant isolate in a mixed growth

Moderate growth of *Pseudomonas aeruginosa*

Ciprofloxacin Resistant

The growth of Gram negative rods including *Pseudomonas* is to be expected from moist wounds and ulcers. Best management is by good wound care. Antibiotic therapy is not routinely recommended.

- Why would we want to sample from a wound, and when should we do it?



Ulcers and Wounds

- **All** wounds are contaminated with bacteria
 - Only sample wounds/ulcers if there is *clinical evidence of infection*
 - Sampling from non-infected wounds/ulcers leads to unnecessary antibiotic treatment (Drs/nurses find it hard to ignore results)



Table 2. Infectious Diseases Society of America and International Working Group on the Diabetic Foot Classifications of Diabetic Foot Infection

Clinical Manifestation of Infection

No symptoms or signs of infection

Infection present, as defined by the presence of at least 2 of the following items:

- Local swelling or induration
- Erythema
- Local tenderness or pain
- Local warmth
- Purulent discharge (thick, opaque to white or sanguineous secretion)

Local infection involving only the skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). If erythema, must be >0.5 cm to ≤ 2 cm around the ulcer.

Exclude other causes of an inflammatory response of the skin (eg, trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, venous stasis).

- Clinical Infectious Diseases 2012;54(12):132-173

Sampling

- If we suspect an ulcer or wound is infected, we are **NOT** interested in the organisms on the surface of the wound, but rather the bacteria deep down **in the tissues**
- The best samples are actually:
 1. Tissue / biopsy / aspirate
 2. Wound fluid/pus
 3. Swab – properly collected
- The more of the contaminating surface bugs we can avoid the better

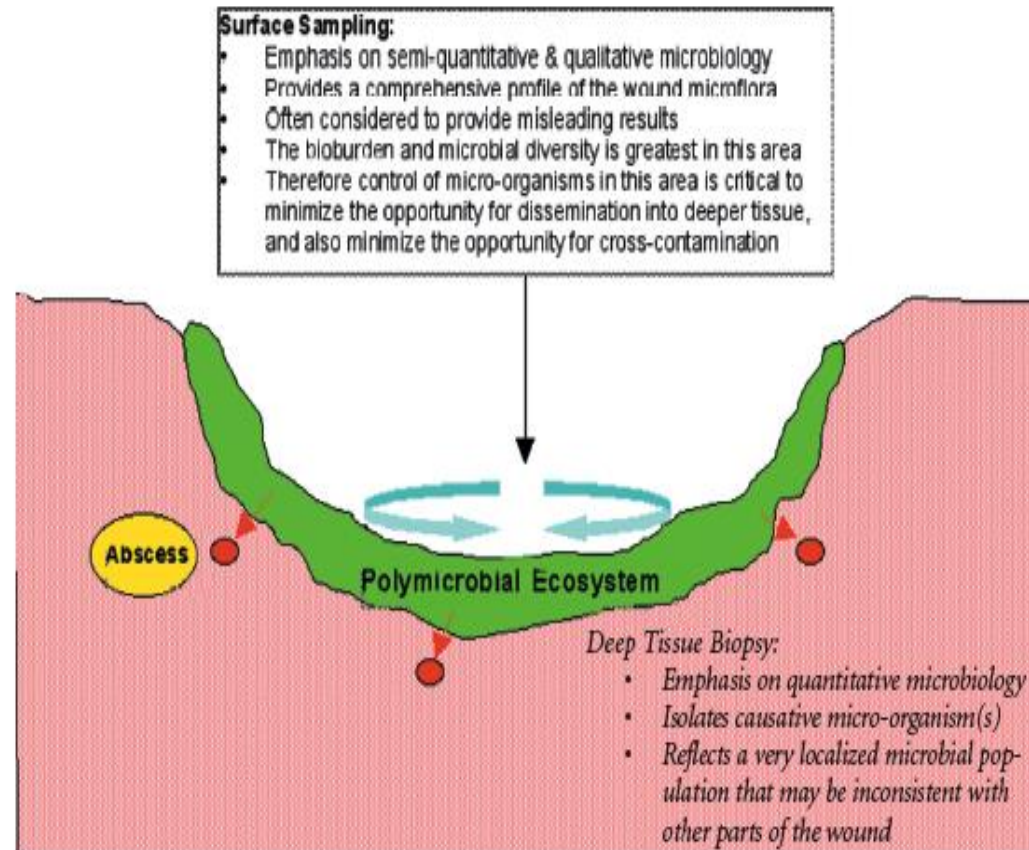


Figure 2

Key aspects associated with superficial and deep tissue sampling. © 2003 ConvaTec.
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Table 5. Recommendations for Collection of Specimens for Culture From Diabetic Foot Wounds

Do

- Obtain an appropriate specimen for culture from almost all infected wounds
- Cleanse and debride the wound before obtaining specimen(s) for culture
- Obtain a tissue specimen for culture by scraping with a sterile scalpel or dermal curette (curettage) or biopsy from the base of a debrided ulcer
- Aspirate any purulent secretions using a sterile needle and syringe
- Promptly send specimens, in a sterile container or appropriate transport media, for aerobic and anaerobic culture (and Gram stain, if possible)

Do not

- Culture a clinically uninfected lesion, unless for specific epidemiological purposes
- Obtain a specimen for culture without first cleansing or debriding the wound
- Obtain a specimen for culture by swabbing the wound or wound drainage

How to take a wound swab

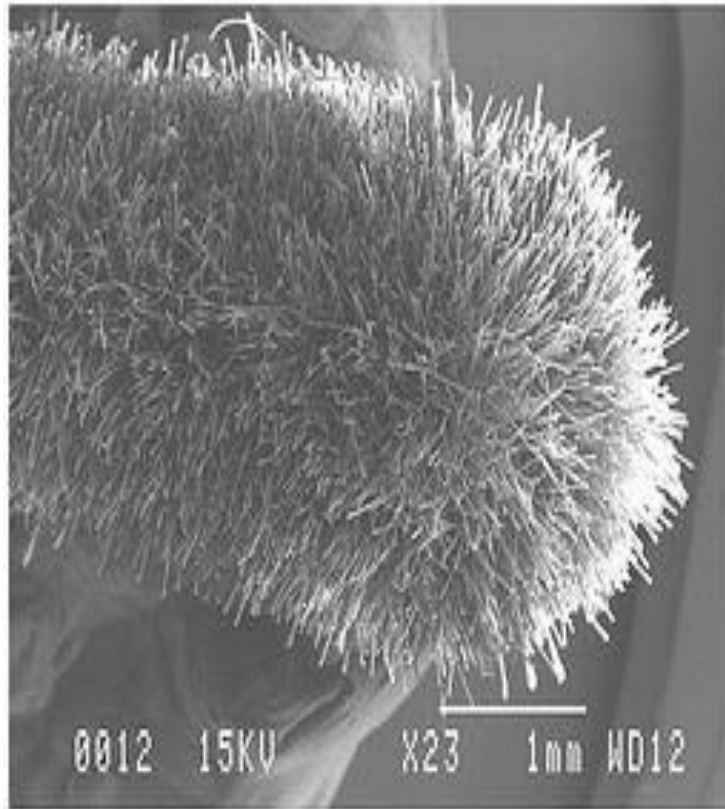
1. Clean the surface of the wound of debris with sterile saline
2. Then express **fresh** pus if possible, and aspirate with needle/syringe
 - a. If there's pus, send us the pus! (much better yield than a swab)
3. Avoid the peri-wound areas (edges) when swabbing
4. The “Levine” technique may be better than “zig-zag” technique
 - Rotate the wound swab over a 1 cm² area of the **central** wound over an area of **viable tissue** (not necrotic), with sufficient pressure to express fluid from within the wound tissue

IDSA Guidelines recommend: Recommendations for collection of specimens for culture from
diabetic foot wounds

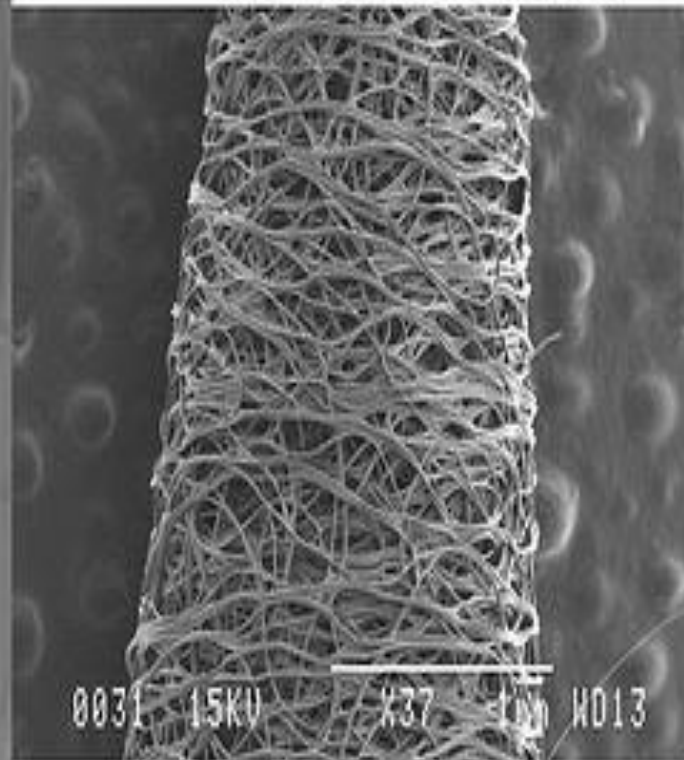
<http://www.nursesfornurses.com.au/admin/uploads/WoundSwabTips1.pdf>

Why don't we like swabs?

New flocked swab under electron microscope

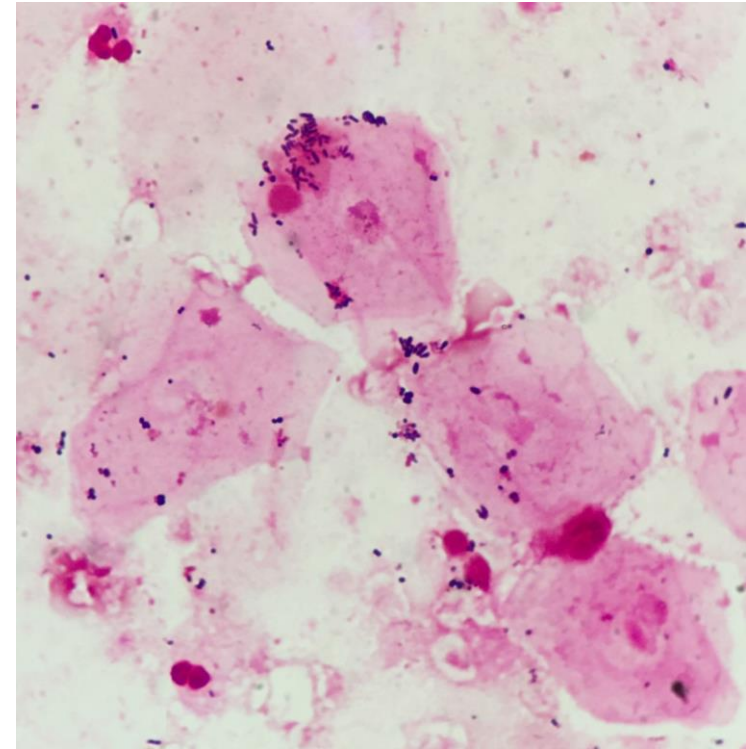


Traditional swab under EM



Vac Wound Swabs

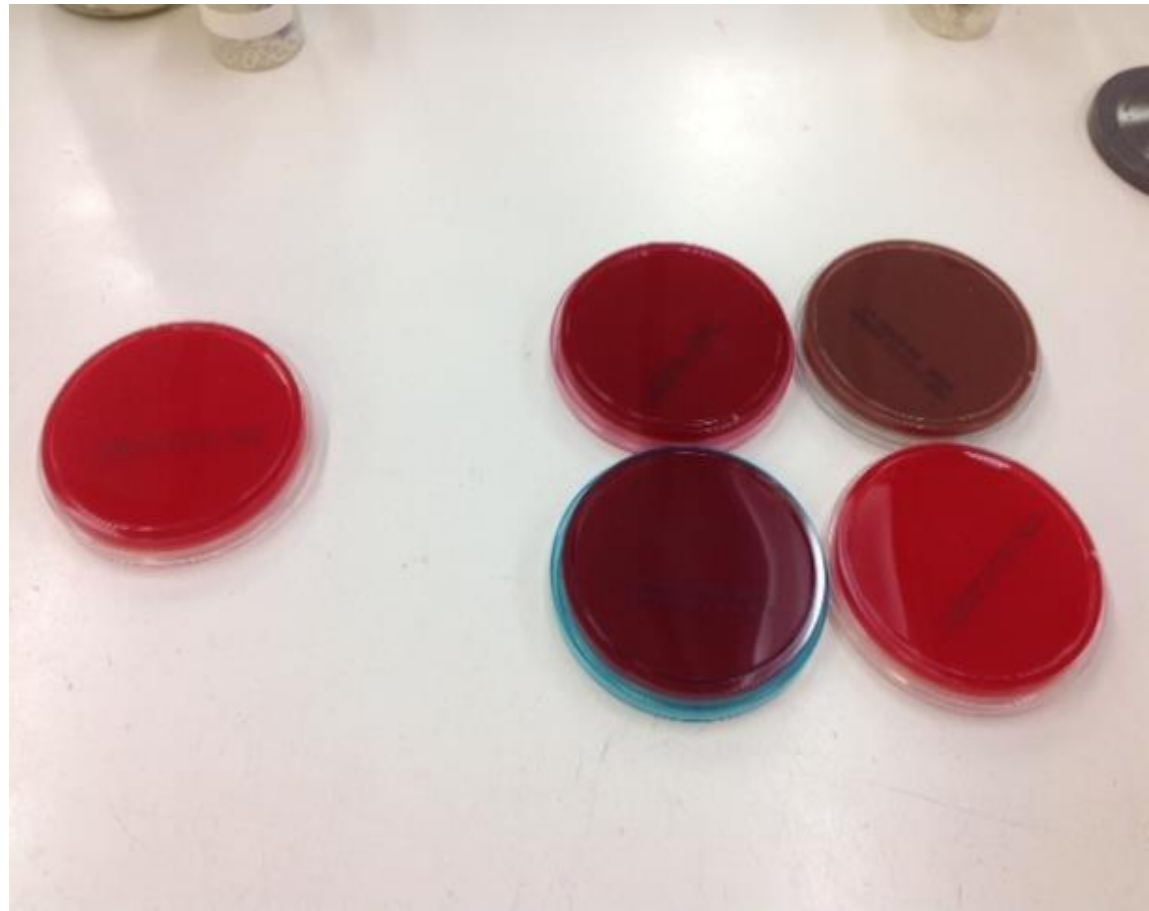
- We find these even more difficult to interpret!
- Tend to get overgrown with gram negative organisms, or others that are resistant to the antibiotics the patient is on
- We don't recommend taking swabs from these wounds (certainly not routinely)



Clinical Details

- “wound swab” vs “facial cellulitis”

- Very important!
- The lab knows nothing about your patient
- Affects all stages of processing
 - Whether a Gram stain is done
 - Type of culture plates
 - Which antibiotics are tested
 - How the sample is reported onto the system



Relevant Clinical Details for Wound Samples

- Animal/human bites (*Pasteurella*, *Capnocytophaga*, *Eikenella*, anaerobes etc)
- Travel (e.g. Pacific Islands -> *C.diphtheriae*)
- Environmental exposure (e.g. water -> *Aeromonas*, *Vibrio*, MVA -> *Bacillus cereus*, other environmental org.s, Soil/plant matter contamination -> fungi/other environmental bacteria)
- Burns (Gram negative organisms, Yeasts, other fungi)
- Immunosuppression/neutropenia (Gram negatives, fungi)
- Antibiotic allergies

- Because wound swabs are often heavily contaminated with colonising organisms making them difficult to interpret, we often ignore the results
- Treatment generally aimed at most common organisms expected in a given scenario...

Results

Swabs

SWAB OF LEFT TOE AMPUTATION SITE

GRAM STAIN

Small numbers of leucocytes seen.
Small numbers of gram positive cocci
Small numbers of gram negative bacilli

CULTURE

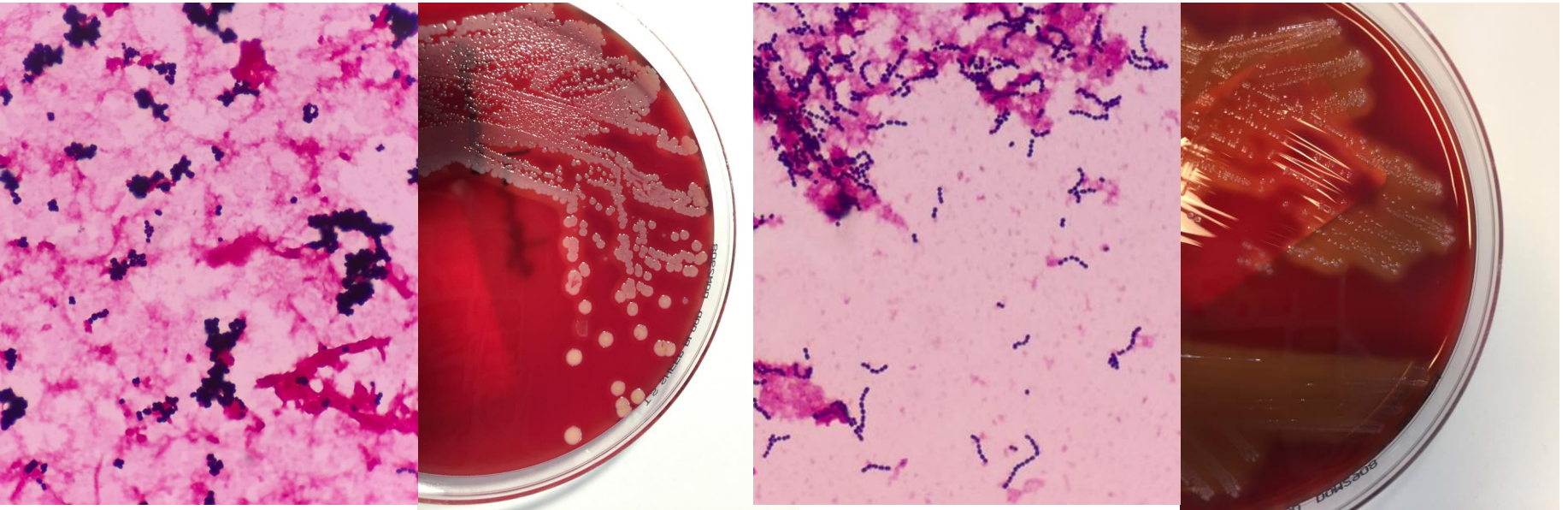
Moderate growth of *Pseudomonas aeruginosa*

SUSCEPTIBILITIES

Results to follow

THE USUAL SUSPECTS

The Main Offenders



- *Staphylococcus aureus*
- Beta haemolytic streps (e.g. *S.pyogenes*/Grp A strep)
- Abx:
 - Flucloxacillin
 - Mild penicillin allergy: cefalexin

Organism group	No. strains	Routinely Reported antibiotics											Second line or restricted antibiotics						Combinations					Augmentin and Gentamicin				
		Amoxycillin	Amoxycillin-clavulanate	Amoxycillin-clavulanate: cystitis breakpoint	Cephalexin	Cefuroxime	Co-trimoxazole	Erythromycin	Flucloxacillin	Gentamicin	Nitrofurantoin	IV Penicillin	Doxycycline	Trimethoprim	Amikacin	Ceftazidime	Ceftriaxone	Clindamycin	Ciprofloxacin	Meropenem	Piperacillin-tazobactam	Vancomycin	Flucloxacillin + Clindamycin		Flucloxacillin + Gentamicin	Amoxicillin + Gentamicin	Amoxicillin + Gentamicin + Metronidazole	Cefuroxime + Metronidazole
Swabs, sputum																												
<i>Pseudomonas aeruginosa</i>	424	IR	IR		IR	IR	IR	IR	IR	92					93	94	IR		93	94	92	IR		92	92	92	IR	
<i>Haemophilus influenzae</i>	603	72	94			100	71									100	100		100			IR						
<i>Klebsiella</i> spp all	650	IR	82		72	79	86			95					99	89	82		92	97	78	IR		95	95	95	79	
<i>Enterobacter</i> spp	328	IR	0			IR	89			98					98		62		98	99	60	IR					IR	
<i>Campylobacter</i> spp	430							98											72			IR		NA	NA	NA	NA	
<i>Staphylococcus aureus</i>	6837	15	87		87	87	98	88	87	99		15	97					90	94			100	97				100	
Methicillin-resistant <i>Staphylococcus aureus</i>	891	IR					98		IR	94		IR	96				IR	78	79			100	71				IR	
<i>Streptococcus pyogenes</i>	603	100			100	100		97	100			100			100		98	IR			100	100					100	
Group B strep	521	100			100	100		83				100			100		100	84	IR		100	100					100	
<i>Streptococcus pneumoniae</i> - meningitis breakpoint	127											80					100											
<i>Streptococcus pneumoniae</i> - noninvasive breakpoint	127	96					81	82				96	77				100											
Urine isolates																												
<i>Escherichia coli</i> from urinary sources	3284	46	77	95	95	87	72			88	98	IR		73	97		91		89	100	91	IR						
<i>Klebsiella</i> spp from urine	408	IR	85	95	76	86	84			96	83	IR		84	98		84		90			80	IR					
Bacteraemia																												
<i>all major pathogens associated with sepsis</i>	710	42	78			81				75							91			99	89			75	92	96	84	95
GNB	488	31	68			72				84							87			100	83			84	85	92	76	94
GPC major pathogens	343	57	92			92			89	55							77			81	90		97	86	96	96	92	96
<i>Escherichia coli</i>	306	49	78		76	89	72			92					93		95		89	99.8	91	IR	IR	96	96	96	93	92
<i>Klebsiella</i> spp	37	IR	97		87	95	97			97					100		97		100	100	95	IR	IR	97	97	97	95	97
<i>Staphylococcus aureus</i>	95	23			90	90	98	93	90	100		23	100				NR	NR	93	98		100	97	100			90	90
Coagulase -ve staphylococci	160		33		33		79	47	33	86		9	86						71	100		99	63	86	9	9	63	63

* one patient with CRE

Notes:

The testing results do not necessarily relate to treatment choice. For example gentamicin has activity against *S. aureus*, but it is not regarded as front line therapy.

Campylobacter and pneumococcal numbers include community and hospital isolates

There were only 2 episodes where *S. pneumoniae* was cultured from CSF: the numbers are too small to interpret.

MRSA



- Abx options:
 - Co-trimoxazole
 - Doxycycline
 - Clindamycin



- Wound swab useful if MRSA detected

Organism group	No. strains	Routinely Reported antibiotics											Second line or restricted antibiotics							Combinations									
		Amoxycillin	Amoxycillin-clavulanate	Amoxycillin-clavulanate: cystitis breakpoint	Cephalexin	Cefuroxime	Co-trimoxazole	Erythromycin	Flucloxacillin	Gentamicin	Nitrofurantoin	IV Penicillin	Doxycycline	Trimethoprim	Amikacin	Ceftazidime	Ceftriaxone	Clindamycin	Ciprofloxacin	Meropenem	Piperacillin-tazobactam	Vancomycin	Flucloxacillin + Clindamycin	Flucloxacillin + Gentamicin	Amoxicillin + Gentamicin	Amoxicillin + Gentamicin + Metronidazole	Cefuroxime + Metronidazole	Augmentin and Gentamicin	
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Bacteraemia																													
More common in Maori or Pacific Island																													
all major pathogens associated with sepsis	710	42	78			81				73						91				99	89				75	92	96	84	95
GNB	488	31	68			72				84						87				100	83				84	85	92	76	94
GPC major pathogens	343	57	92			92			89	55						77				81	90			97	86	96	96	92	96
<i>Escherichia coli</i>	306	49	78		76	89	72			92					93	95		89	99.8	91	IR		IR	96	96	96	93	92	
<i>Klebsiella</i> spp	37	IR	97		87	95	97			97					100	97		100	100	95	IR		IR	97	97	97	95	97	
<i>Staphylococcus aureus</i>	95	23			90	90	98	93	90	100		23	100			NR	NR	93	98			100	97	100			90	90	
Coagulase -ve staphylococci	160		33		33		79	47	33	86		9	86					71	100			99	63	86	9	9	63	63	

* one patient with CRE

Notes:

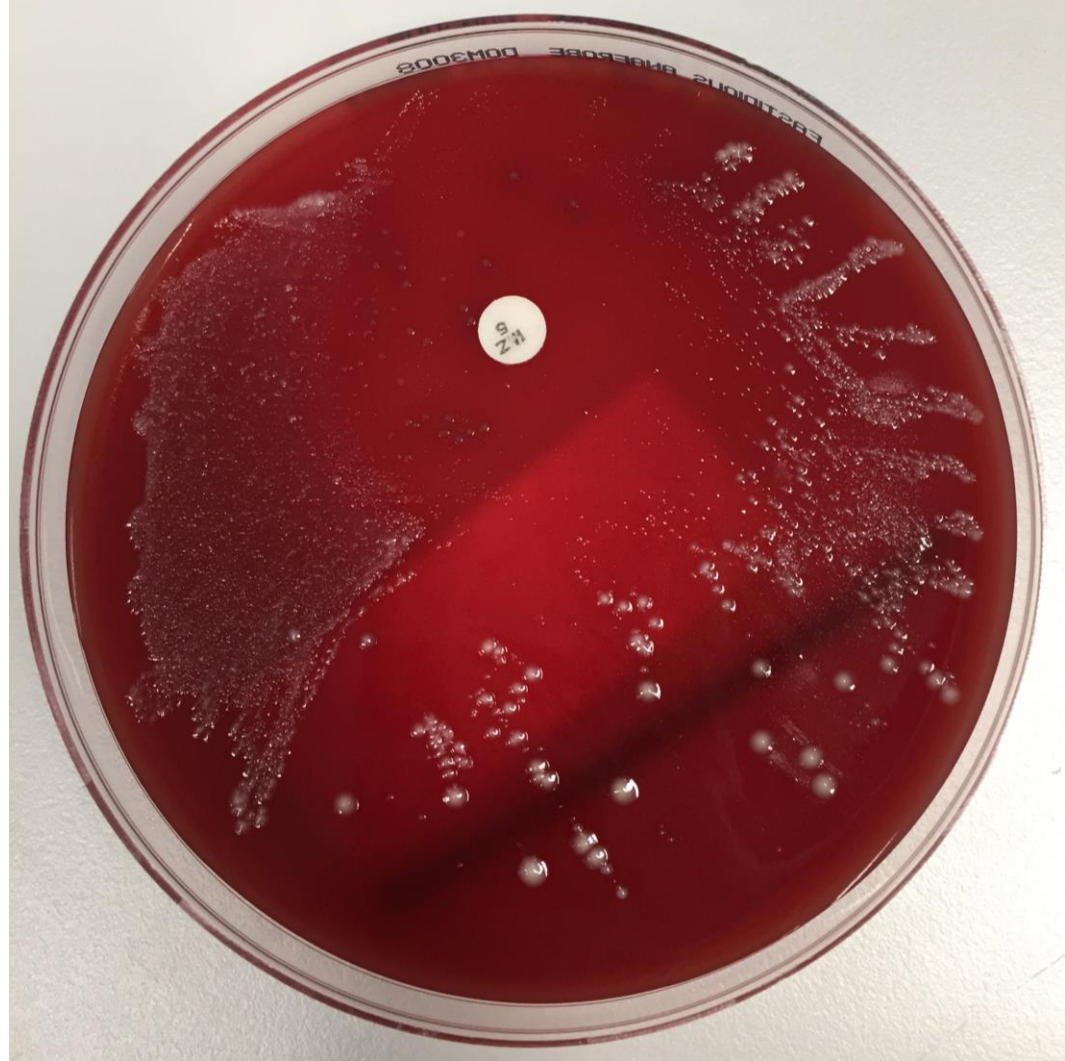
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There were only 2 episodes where *S. pneumoniae* was cultured from CSF: the numbers are too small to interpret.

Diabetics

- *Staph aureus*
- Beta-haemolytic streptococci
- Anaerobes:
 - Augmentin (good staph/strep cover)
 - Metronidazole (no staph/strep cover)



Other Organisms

- Gram negative organisms e.g. *Pseudomonas*, Enterobacteriaceae e.g. *E.coli*, *Klebsiella*
 - Not primary skin/soft tissue pathogens
 - Don't tend to target these organisms with Abx treatment unless failing + found on culture, or certain situations:
 - Burns
 - Immunocompromised or debilitated patients
 - Other specific situations e.g. wounds sustained in water, flooding victims, bites etc

A Common Question...

- Swab of wound grows heavy growth of *Pseudomonas aeruginosa*
- What should we treat with?



Pseudomonas aeruginosa

- Opportunistic pathogen
- Environmental/aquatic organism
 - Lives everywhere, including hospital taps
 - Spa pool folliculitis
- Readily forms biofilm e.g. in taps
- Naturally resistant to many antibiotics
 - Selected out in patients receiving antibiotics
- Unsurprisingly, finds its way into chronic ulcers/wounds!



Pseudomonas aeruginosa

- Naturally resistant to many antibiotics
 - Presents treatment difficulties
 - Only one option for oral treatment: ciprofloxacin
 - Side effects (FDA Boxed Warning)
 - Resistance develops easily



WARNING: SERIOUS ADVERSE REACTIONS INCLUDING TENDINITIS, TENDON RUPTURE, PERIPHERAL NEUROPATHY, CENTRAL NERVOUS SYSTEM EFFECTS AND EXACERBATION OF MYASTHENIA GRAVIS

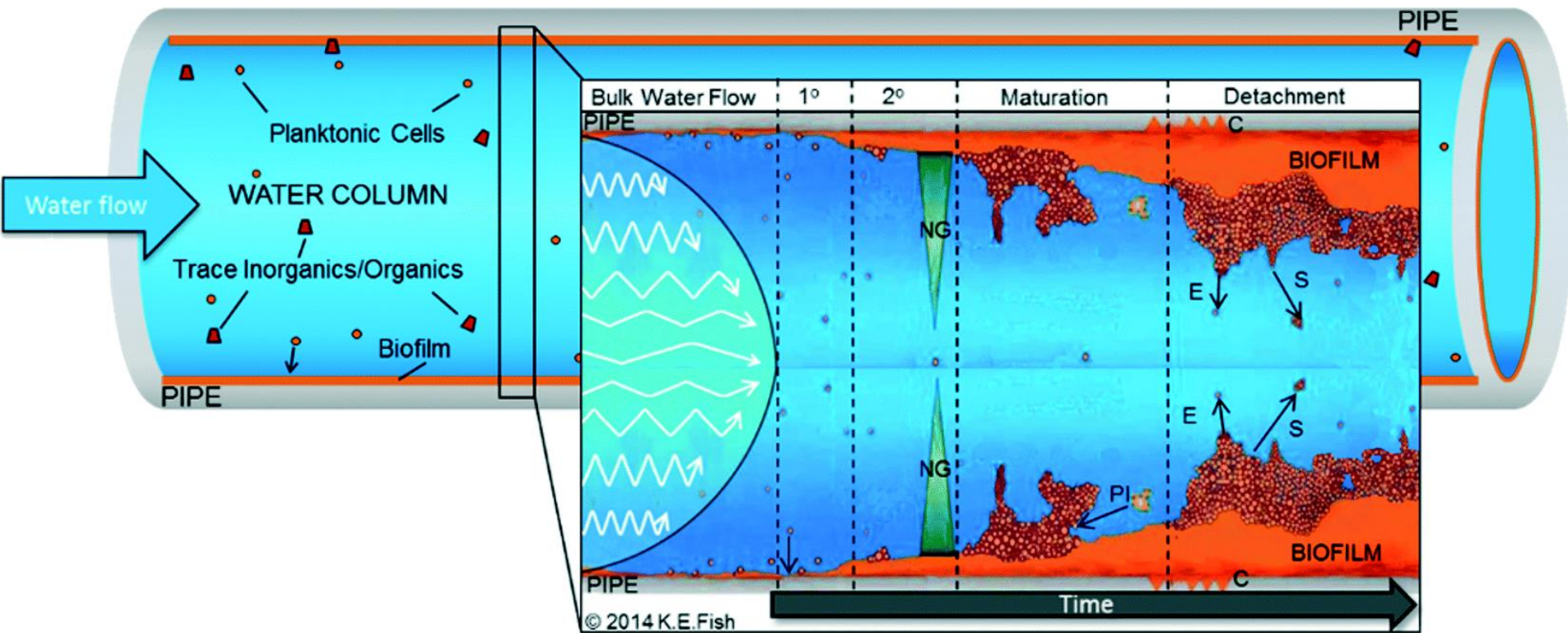
- Fluoroquinolones, including CIPRO*, have been associated with disabling and potentially irreversible serious adverse reactions that have occurred together [see Warnings and Precautions (5.1)] including:
 - Tendinitis and tendon rupture [see Warnings and Precautions (5.2)]
 - Peripheral neuropathy [see Warnings and Precautions (5.3)]
 - Central nervous system effects [see Warnings and Precautions (5.4)]
- Discontinue CIPRO immediately and avoid the use of fluoroquinolones, including CIPRO, in patients who experience any of these serious adverse reactions [see Warnings and Precautions (5.1)]. Fluoroquinolones, including CIPRO, may exacerbate muscle weakness in patients with myasthenia gravis. Avoid CIPRO in patients with known history of myasthenia gravis [see Warnings and Precautions (5.5)].
- Because fluoroquinolones, including CIPRO, have been associated with serious adverse reactions [see Warnings and Precautions (5.1–5.15)], reserve CIPRO for use in patients who have no alternative treatment options for the following indications:
 - Acute exacerbation of chronic bronchitis [see Indications and Usage (1.10)]
 - Acute uncomplicated cystitis [see Indications and Usage (1.11)]
 - Acute sinusitis [see Indications and Usage (1.12)]

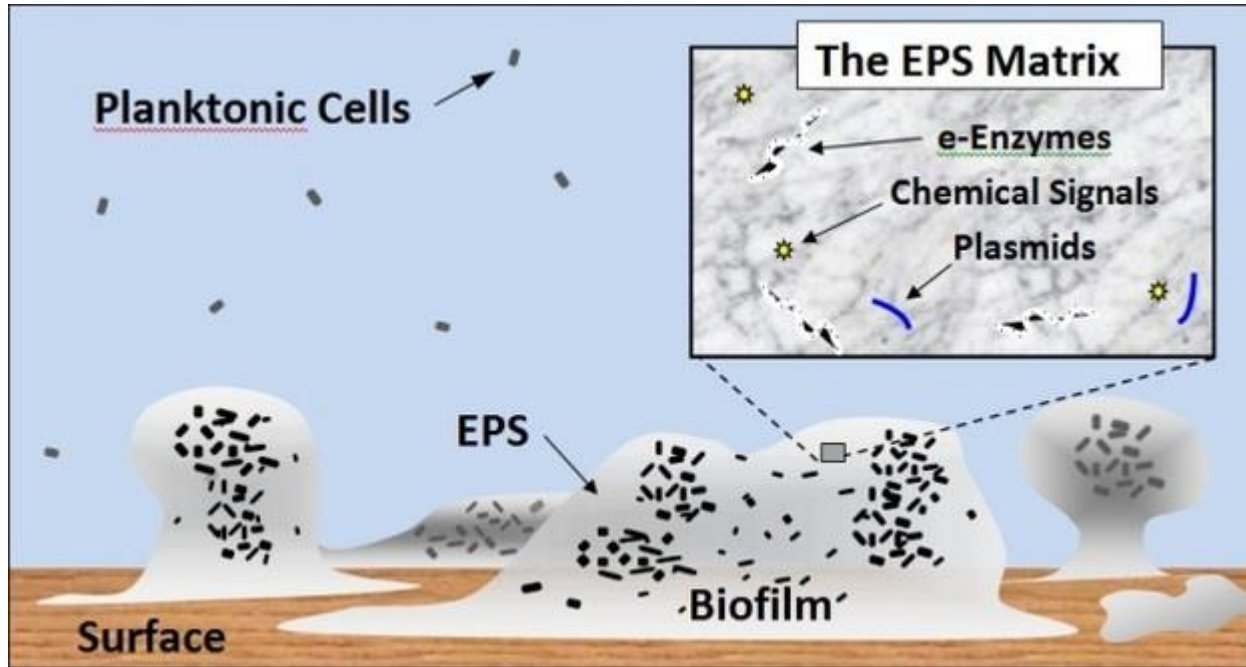
A Common Question...

- Swab of wound grows heavy growth of *Pseudomonas aeruginosa*
- What should we treat with?

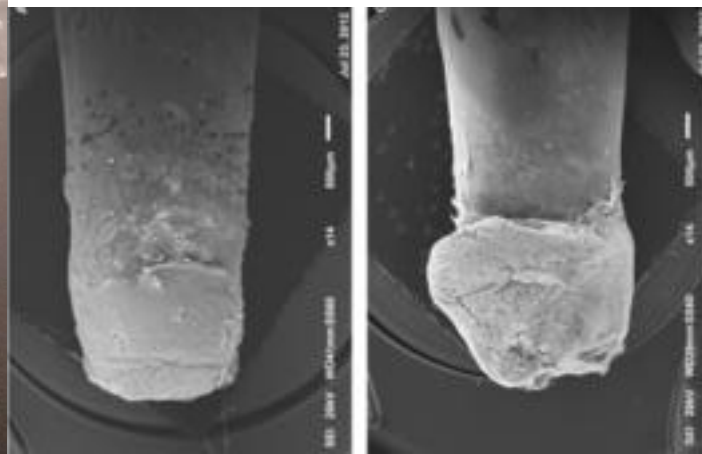
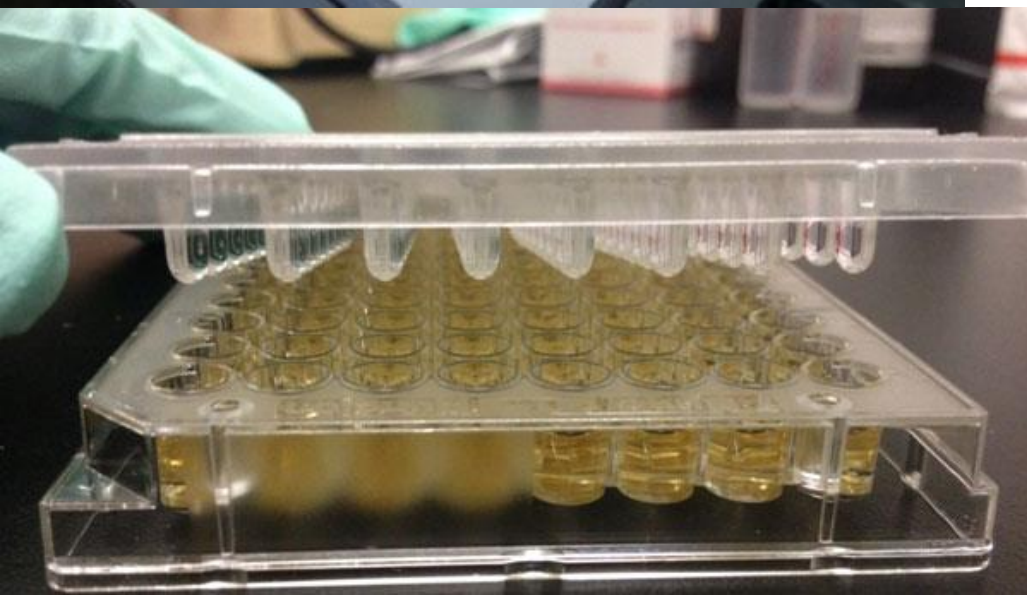


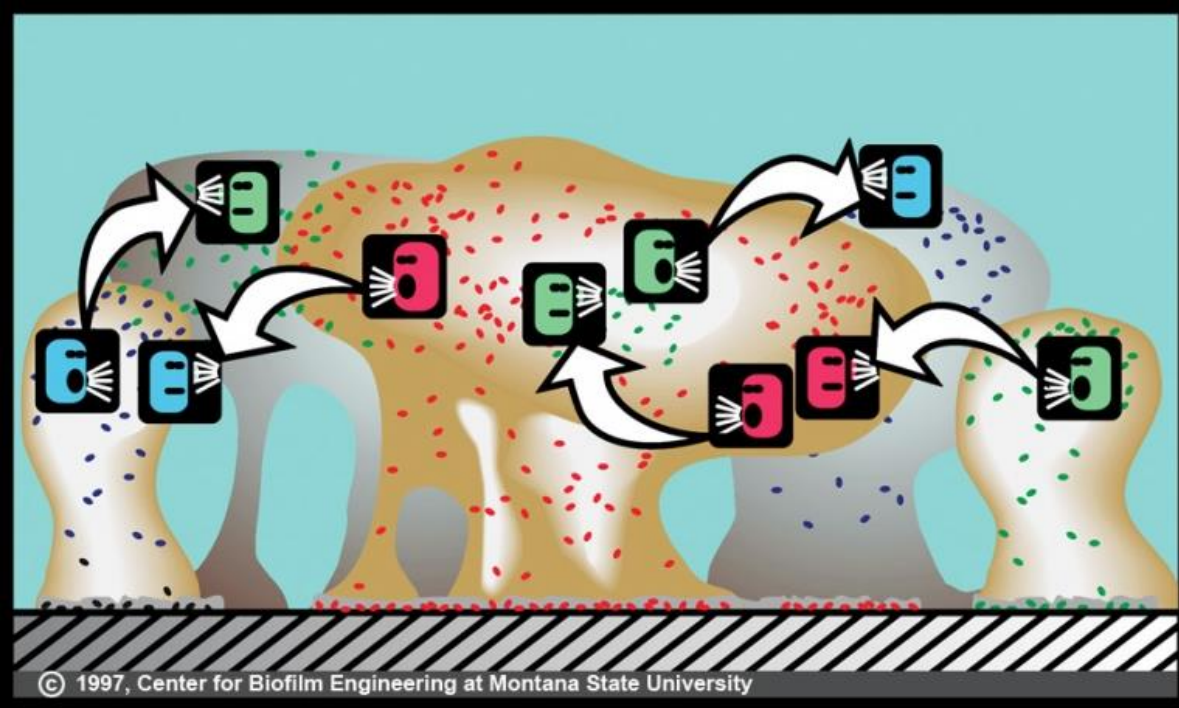
BIOFILMS





- Complex community of bacteria bound in matrix
 - Protects them from environmental and other stresses
 - Interact in complex ways, bugs behave very differently in biofilm
- Biofilm bacteria 50-1000x more tolerant to ABx:
 - Abx can't penetrate the matrix very well
 - Bugs share resistance enzymes and genes
 - Bacteria less metabolically active
 - Adverse conditions e.g. low pH
 - Lab susceptibility testing designed for planktonic bacteria, NOT biofilms





- Antibiotics not effective at eradicating biofilms
 - Poor blood supply in chronic wounds (Abx don't get in)
 - Also more likely to generate resistance
- **Need to physically remove the biofilm**
 - Debridement/wound care



ANTIBIOTIC RESISTANCE & STEWARDSHIP

THE NEW ZEALAND MEDICAL JOURNAL

Journal of the New Zealand Medical Association



Rising antimicrobial resistance: a strong reason to reduce excessive antimicrobial consumption in New Zealand

Mark G Thomas, Alesha J Smith, Murray Tilyard

Figure 3. Annual per capita consumption of antimicrobials, by community-based patients in New Zealand, measured in DDDs/1000 population/day, between 2005 and 2012

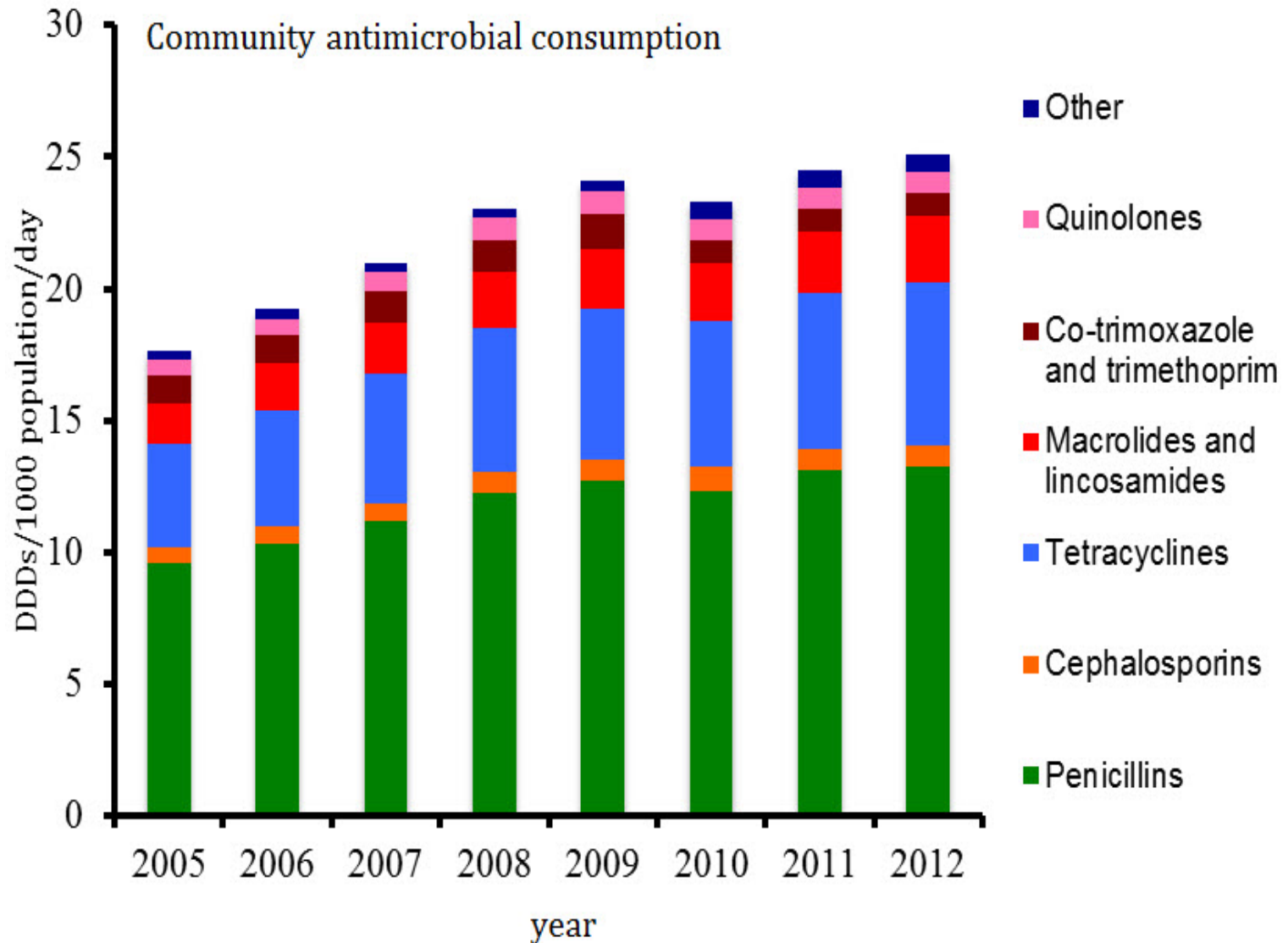
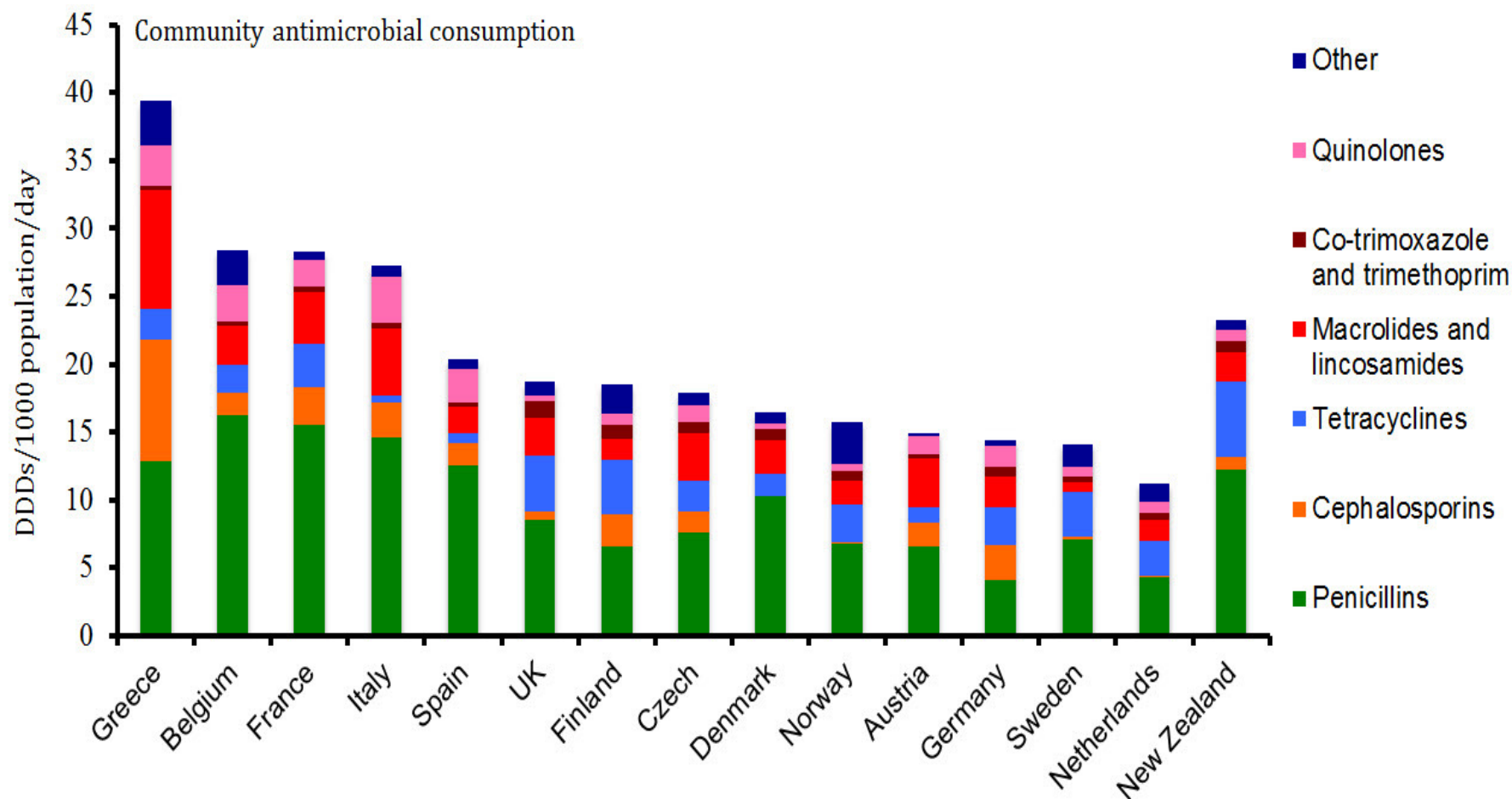
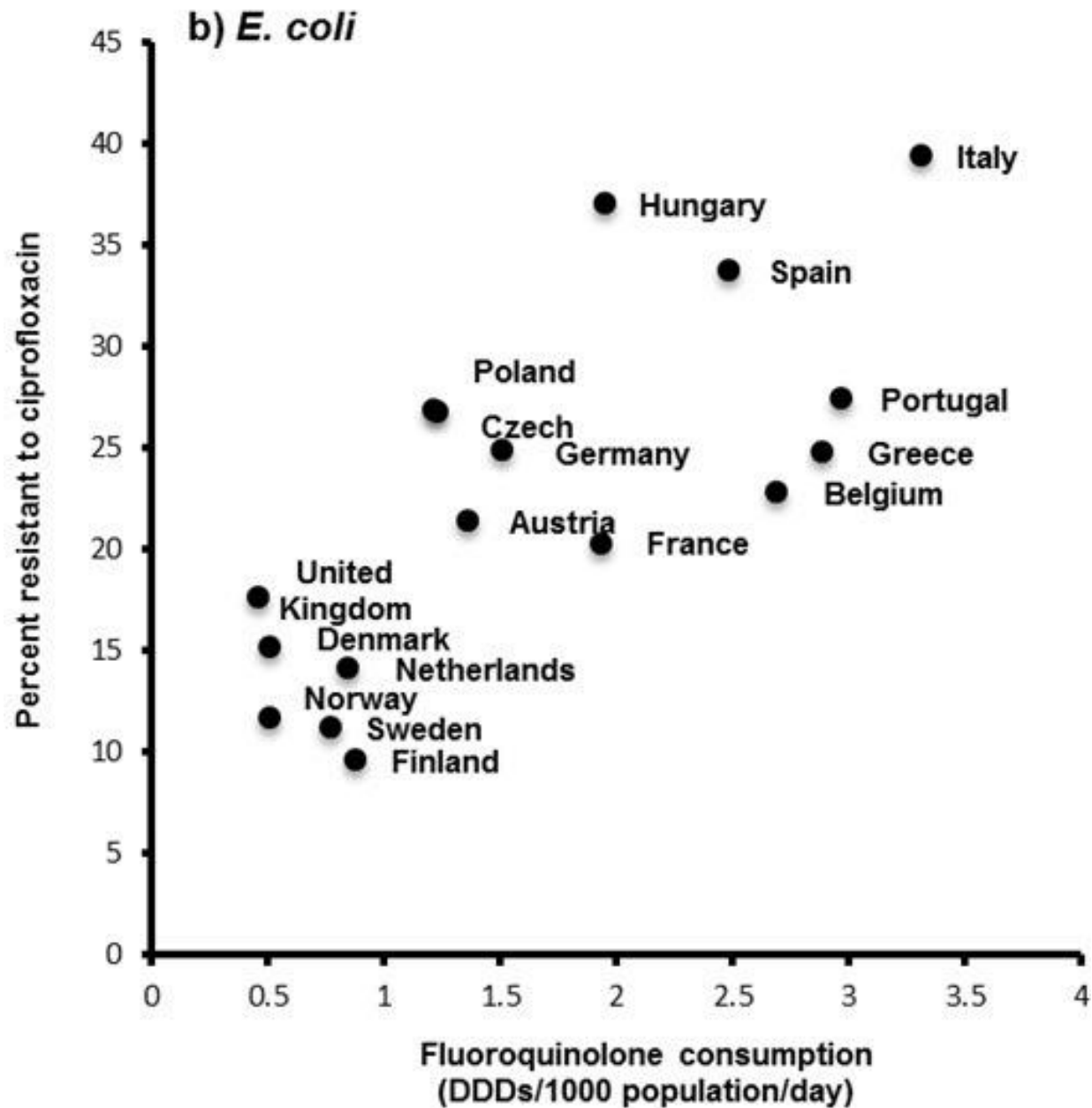
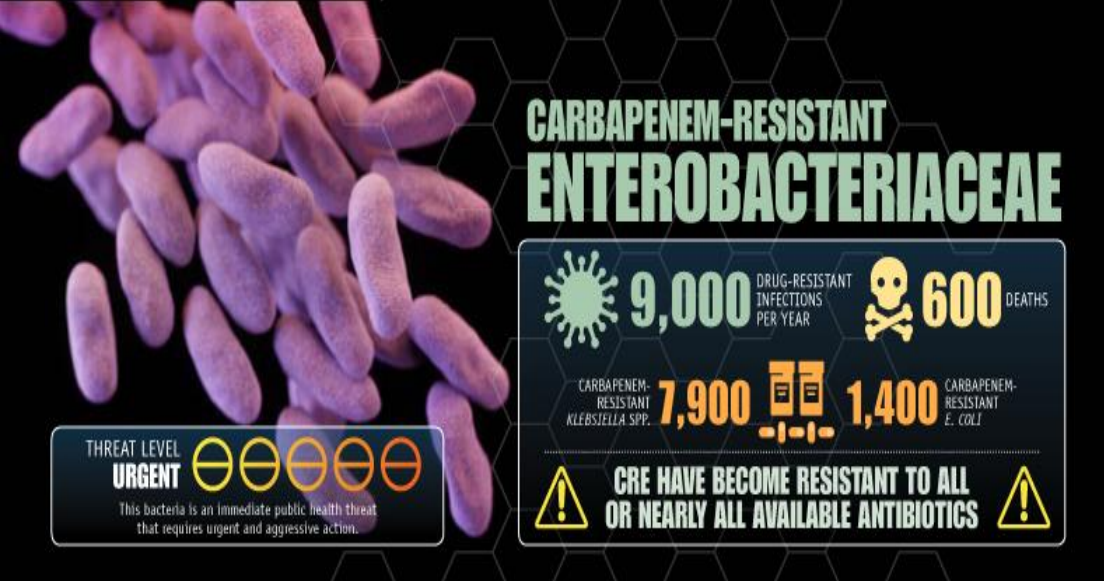


Figure 4. Annual per capita consumption of antimicrobials by community-based patients, in various European countries¹⁰ and in New Zealand, during 2010, measured in DDDs/1000 population/day







Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations

Antimicrobial resistance

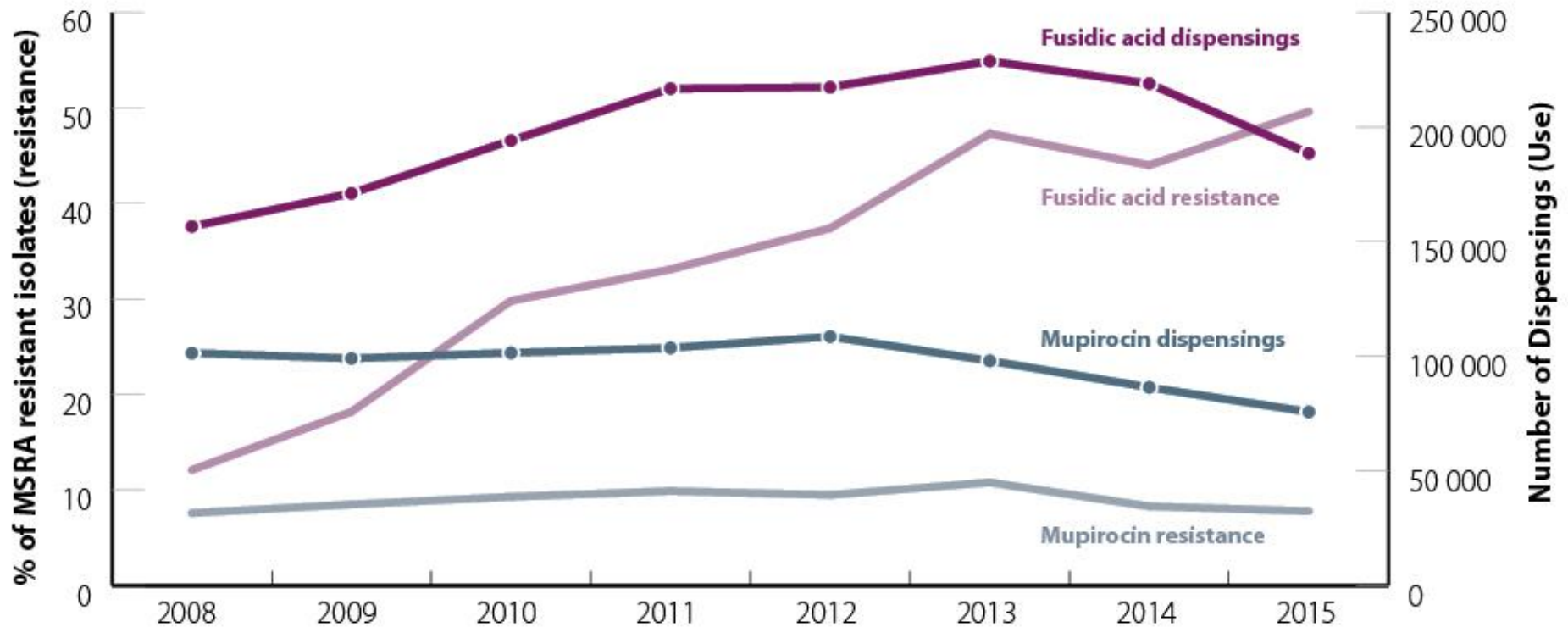
United Nations high-level meeting on antimicrobial resistance

Antimicrobial resistance summit to shape the international agenda



The Review on Antimicrobial Resistance
Chaired by Jim O'Neill
December 2014

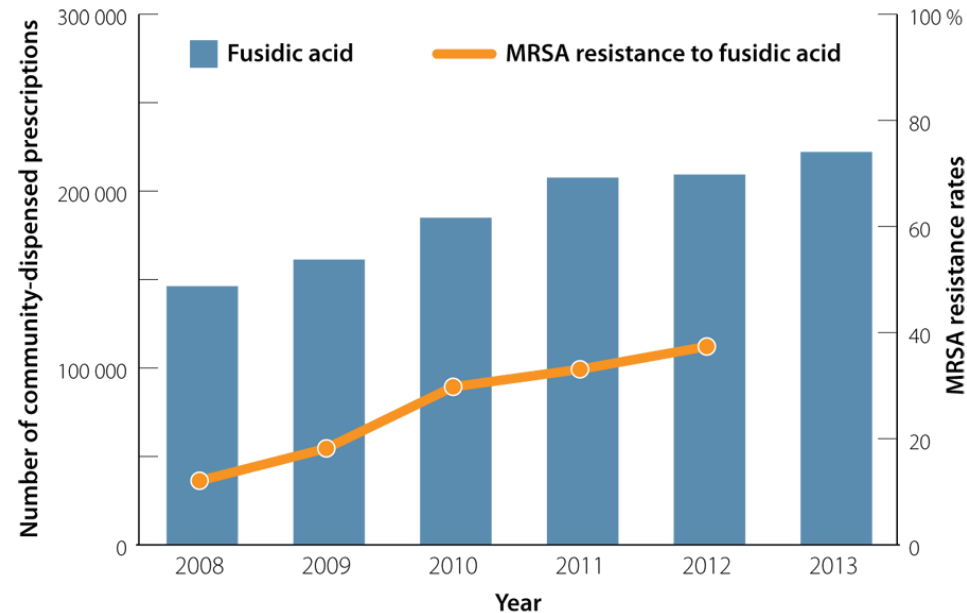
Topical Antibiotics



- NZ has very high fusidic acid resistance
 - BPAC guidelines suggesting use
- Gene for fusidic acid resistance carried with gene for common NZ MRSA clone (AK3)

Topical Antimicrobials

- Topical antibiotics not recommended in general (esp. if also available systemically)
- Preference for other antimicrobial/antiseptic agents if required to reduce colonisation
 - Less likely to drive resistance to useful antibiotics



How Can We Help?

- Good wound care to decrease the risk of infection
- Good clinical assessment of whether wounds infected
 - Don't give antibiotics for colonisation!
- Avoid topical antibiotics – use of topical antiseptics/antimicrobials if required

How Can We Help?

- Don't send samples for micro unless there is clinical evidence of infection
 - Sampling is often the first step along the path to an antibiotic prescription
 - We know we will grow bugs – someone will want to treat them if we show this on a lab report
- Appropriate, timely narrow spectrum Abx treatment for most, if clinical infection

Take Home Messages

- All wounds have bugs in them whether they're infected or not – they will grow if sample is taken!
- We should only take samples if there is clinical evidence of infection
 - They don't tell us if treatment is required or not, but act as a guide to what the best antibiotic might be
- Pus or tissue is much better than a swab
- Antibiotics don't work against biofilms – need physical removal
- *Staph aureus* and beta-haemolytic streptococci are the most important bugs
 - We try not to treat *Pseudomonas* and other gram negatives with antibiotics if possible

Thanks for your attention

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