

Microbiology Nuts & Bolts: Clinical Scenarios for Critical Care Pharmacists

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Aims & Objectives

- To consider some of the difficulties that arise in treating "simple" infections
- To discuss the role of microbiology samples in the diagnosis of infection
- To understand how antibiotic allergies challenge treatment options in an era of increasing antibiotic resistance
- To recognise how "antimicrobial stewardship" is nothing new...! (controversial?)

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Antimicrobial stewardship

The use of the right antibiotic at the right dose, route and duration, for the right infection at the right time to improve patient care whilst reducing antibiotic resistance



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Case 1 - Mary

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Mary

- 70 years old
- Presents with fever & shortness of breath
- On examination
 - Temperature 38.5 °C, B.P. 140/85
 - Decreased air-entry at the right base
- Diagnosed with community acquired pneumonia
- CURB-65 score 3
- Commenced on IV Co-amoxiclav PLUS Clarithromycin
- Transferred to Critical Care Unit

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How do we diagnose CAP?

British Thoracic Society Guidelines for Community Acquired Pneumonia (CAP)

- Cough PLUS one other respiratory tract symptom
 - Shortness of breath
 - Purulent sputum
 - Chest pain
- New focal chest signs
 - Reduced expansion
 - Bronchial breathing
 - Dull percussion
 - ↑ Vocal resonance
- Systemic symptoms
 - Fever, sweats, shivers, aches & pains
- No other explanation

- Exacerbation of COPD
 - Shortness of breath
 - Purulent sputum
 - ↑ Amount of sputum



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- Full history and examination
- Bloods
 - FBC, CRP, U&Es
 - Blood Cultures
- Urine
 - Point-of-care +/- laboratory
- Sputum
- Chest X-ray

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How to interpret a sputum result?

- Appearance
 - Mucoid, Salivary, Purulent, Blood Stained...
- Microscopy
 - Gram's stain, Ziehl Nielsen (ZN) stain...
- Culture
 - Is the organism consistent with the clinical picture?

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Appearance of sputum

- Salivary
 - Spit not phlegm, risk of contamination
- Mucoid
 - Upper respiratory tract specimen, no evidence of inflammation
 - Beware neutropaenic patients
- Purulent
 - Pus, indicates inflammation not infection
- Blood stained
 - May indicate infection but not pathognomic

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Causes of Respiratory Infections

Community Acquired Pneumonia

- Viruses:
 - RSV
 - Influenza
 - Parainfluenza
 - Adenovirus
- Bacteria:
 - S. pneumoniae
 - H. influenzae
 - S. aureus
 - M. pneumoniae
 - C. pneumoniae
 - L. pneumophila
 - P. aeruginosa (if COPD)
 - M. tuberculosis

Exacerbation of COPD

- Viruses:
 - RSV
 - Rhinovirus
 - Influenza
 - Parainfluenza
 - Adenovirus
- Bacteria:
 - S. pneumoniae
 - H. influenzae
 - S. aureus
 - M. catarrhalis

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Culture: classification of bacteria

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    graph TD
      GS[Gram's Stain] --> P[Positive]
      GS --> N[Negative]
      GS --> NSU[No Stain Uptake]
      P --> PC[Cocci]
      P --> PB[Bacilli]
      N --> NC[Cocci]
      N --> NB[Bacilli]
      NSU --> AFB[Acid Fast Bacilli]
      NSU --> NCult[Non-culturable]
    
```

Causes of pneumonia usually originate in the upper respiratory tract

Classification of Gram-positive cocci

GRAM POSITIVE (These colours)	
COCCUS	BACILLUS
Anaerobe	Anaerobe
<i>Phycothreptococcus</i> sp.	<i>Clostridium</i> sp.
	<i>Propionibacterium</i> sp.
	<i>Actinomyces</i> sp.
Facultative anaerobe	Facultative anaerobe
Clumps	<i>Listeria</i> sp.
<i>Staphylococcus</i> sp.	<i>Corynebacteria</i> sp.
<i>Micrococcus</i> sp.	<i>Spirillum</i> sp.
<i>Rothia</i> sp.	<i>Lactobacillus</i> sp.
Chains	<i>Rhodococcus</i> sp.
<i>Streptococcus</i> sp.	
<i>Enterococcus</i> sp.	
<i>Microthapsa</i> sp.	
<i>Levonicostoc</i> sp.	
	Aerobe
	<i>Acetaria</i> sp.
	<i>Bacillus</i> sp.

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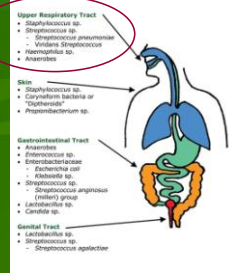
Culture: how is sputum processed?

- Plated to mixture of selective and non-selective agar depending on clinical details
 - E.g. Cystic Fibrosis = B. cepacia agar
- Incubated for 48 hours before reporting
- Sensitivities take a further 24-48 hours
- Total time 48-96 hours after receipt.



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Community Normal Flora

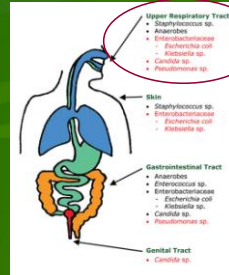


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What happens in Hospital?

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Hospital Normal Flora



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Factors Affecting Normal Flora

- Exposure to antibiotics provides a selective pressure
 - e.g. previous antibiotics for CAP
- Increased antimicrobial resistant organisms in the environment
 - e.g. *Pseudomonas* in intensive care units
- Easily transmissible organisms
 - e.g. *Staphylococcus aureus*
- Immunosuppressants
 - e.g. steroids, chemotherapy, tracheostomy tubes etc

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Why Co-amox & Clari?

Community Acquired Pneumonia

- Viruses:
 - RSV
 - Influenza
 - Parainfluenza
 - Adenovirus
- Bacteria:
 - S. pneumoniae*
 - H. influenzae*
 - S. aureus*
 - M. pneumoniae*
 - C. pneumoniae*
 - L. pneumophila*
 - P. aeruginosa* (if COPD)
 - M. tuberculosis*

Exacerbation of COPD

- Viruses:
 - RSV
 - Rhinovirus
 - Influenza
 - Parainfluenza
 - Adenovirus
- Bacteria:
 - S. pneumoniae*
 - H. influenzae*
 - S. aureus*
 - M. catarrhalis*

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How do you choose an antibiotic?

- Is the antibiotic active against the common micro-organisms?
- Do I need a bactericidal antibiotic rather than bacteriostatic?
- Does the antibiotic get into the site of infection in adequate amounts?
- How much antibiotic do I need to give?
- What route do I need to use to give the antibiotic?
- Are there any cautions or contraindications?
- What monitoring is required?

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Mild to moderate CAP

- Amoxicillin
- Clarithromycin
- Levofloxacin
- Doxycycline
- Cefradine?

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Severe CAP

- Amoxicillin PLUS Clarithromycin
- Co-amoxiclav PLUS Clarithromycin
- Benzylpenicillin PLUS Levofloxacin
- Teicoplanin PLUS Levofloxacin
- Cefuroxime PLUS Clarithromycin?

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Caution β -Lactam Allergy

- Beta-lactam antibiotics
 - Penicillins, Cephalosporins, Carbapenems
- Reactions
 - Rash, facial swelling, shortness of breath, Steven-Johnson Reaction, anaphylaxis
 - NOT diarrhoea and vomiting!
- Incidence Penicillin allergy
 - Rash 5% population (1 in 20)
 - Severe Reaction 0.05% population (1 in 2,000)
 - Cross reaction (risk of severe reaction if rash with Penicillin)
 - Penicillin to Cephalosporin 5% (1 in 40,000)
 - Penicillin to Carbapenem 0.5% (1 in 400,000)
 - Cross reaction (risk of severe reaction if severe reaction to Penicillin)
 - Penicillin to Cephalosporin 5% (1 in 20)
 - Penicillin to Carbapenem 0.5% (1 in 200)

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Myasthenia gravis

- Myasthenia gravis is a neuromuscular disease caused by antibodies blocking acetylcholine receptors at the neuromuscular junction leading to muscle weakness and fatigue
- Made worse by drugs that further inhibit acetylcholine receptors
- Myasthenic crisis = progressive difficulty with breathing and protecting the airway can be fatal
- Contraindicated antibiotics
 - Aminoglycosides
 - Fluoroquinolones
 - Macrolides
 - (Polymyxins)

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Mild to moderate CAP

	Amoxicillin	Clarithromycin	Levofloxacin	Doxycycline
Beta-lactam allergy	X	✓	✓	✓
Myasthenia gravis	✓	X	X	✓
Beta-lactam allergy PLUS myasthenia gravis	X	X	X	✓

Warning: Doxycycline sensitivity unpredictable

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Severe CAP

	Amoxicillin OR Co-amoxiclav	Clarithromycin	Levofloxacin	Teicoplanin
Beta-lactam allergy	X	✓	✓	✓
Myasthenia gravis	✓	X	X	✓
Beta-lactam allergy PLUS myasthenia gravis	X	X	X	✓

Warning: Teicoplanin only Gram-positives

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Severe CAP

	Amoxicillin OR Co-amoxiclav	Clarithromycin	Levofloxacin	Teicoplanin
Pseudomonas	X	X	✓	X
Pseudomonas PLUS Beta-lactam allergy PLUS myasthenia gravis	X	X	X	X

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Severe CAP Solution?

	Teicoplanin	Aztreonam	Doxycycline
Activity	Gram-positive	Gram-negative	Non-culturable
Beta-lactam allergy PLUS myasthenia gravis	✓	✓	✓
Pseudomonas PLUS Beta-lactam allergy PLUS myasthenia gravis	X	✓	X

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Mary

- Not allergic to beta-lactams
- Does not have myasthenia gravis
- Urine antigen for Streptococcus pneumoniae is positive
- What is the treatment of choice?
- Changed to Benzylpenicillin for total of 7 days

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Case 2 - Betty

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Betty

- 87 years old
- Presents with confusion, fever & shortness of breath
- On examination
 - Temperature 37.5 °C
 - Abdominal pain
- Diagnosed with probably UTI and started on Trimethoprim
- Mid-stream urine (MSU) sent to the laboratory
 - How do you take a proper MSU?

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How to interpret a urine result?

- Urine dipstick
 - Poor PPV, Good NPV
- Microscopy
 - White blood cells, red blood cells, epithelial cells
- Culture result
 - Is the organism consistent with the clinical picture?

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Microscopy of urine

- White blood cells
 - $>100 \times 10^6/L$ definitely significant
 - $>10 \times 10^6/L$ significant if properly taken MSU (rare!)
- Red Blood Cells
 - Poor correlation with UTI, used by urologist and renal physicians
- Epithelial cells
 - Indicator of contact with, and therefore contamination from, the perineum

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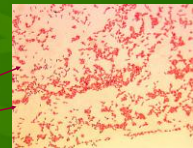
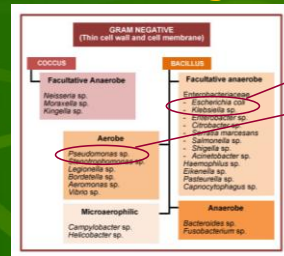


Culture: classification of bacteria



Causes of UTI usually originate in the gastrointestinal tract

Bacterial Identification: Gram-negative bacilli



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Culture: how is urine processed?

- Day 1 Automated Microscopy
 - If values not significant reported as negative
 - If values significant or specific patient group cultured with direct sensitivities
- Day 2
 - Reported with identification and sensitivities
- Patient groups always cultured
 - Cancer and haematology
 - Pregnant
 - Urology
 - Children < 5 years old



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Betty

- Next day pharmacist notes Betty is also on Methotrexate for psoriasis and alerts ward doctors
- Why worry about Methotrexate?
- What other commonly used antibiotic should not be used?
- The doctors change Betty to 2nd line Nitrofurantoin

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Betty

- Over next 24 hours
 - Worsening confusion and agitation
 - Nausea and vomiting
- As condition deteriorating end-of-life care started
- All medication stopped

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Betty

- 24 hours later
 - Much improved
 - Still slightly confused
 - Nausea and vomiting settled
- Taken off end-of-life care
- As still hypotensive she was transferred to Critical Care for closer monitoring
- What happened? Why did she deteriorate and then improve?

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Antibiotic dosing in renal failure

- Many antibiotics require dose reduction in renal failure
- eGFR is not an accurate predictor of renal function
- Use Cockcroft Gault equation
 - Actual body weight or Ideal Body Weight (IBW) if weight > 20% above IBW
 - Also use IBW for patients with oedema & ascites

The following antibiotics require dose modification in the presence of renal impairment (Severe impairment (CrCl < 30ml/min))

Antibiotic	Adjusted oral	Amoxicillin
Amikacin	Amoxicillin	Amphotericin (IV) / Fungizone
Gentamicin (once-daily regimen)	Azithromycin	Ceftriaxone
Mercaptopurine	Benzylpenicillin	Erythromycin
Metronidazole	Cefuroxime	Fluconazole
Sildenafil	Cefuroxime	Mefloquine
Tacrolimus	Ciprofloxacin	Rifampicin
Tetracycline	Clarithromycin	
Tobramycin	Co-trimoxazole	
Vancomycin	Ethambutol	
	Sulphonamides	
	PIP-IV	
	Trimethoprim	

Male: $CrCl = \frac{1.23 \times (140 - \text{age in years}) \times \text{weight in kg}^{\frac{74}{70}}}{\text{Serum creatinine } (\mu\text{mol/L})}$

Female: $CrCl = \frac{1.04 \times (140 - \text{age in years}) \times \text{weight in kg}^{\frac{74}{70}}}{\text{Serum creatinine } (\mu\text{mol/L})}$

How might weight effect Betty's GFR (ml/min)

Female, Age 87, Creatinine 75

Weight (kg)	eGFR	Calculated GFR	Variance
45	63	33	-30
50	63	37	-26
55	63	40	-23
60	63	44	-19
65	63	47	-16
70	63	51	-12
75	63	55	-8
80	63	59	-4

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Back to Betty...

- 55kg, Creatinine 75
- Calculated GFR = 40 ml/min
- Not only will Nitrofurantoin not work but she will get toxicity!
- Review of MSU result
 - Microscopy >100 x10⁶/L WBC, no epithelial cells
 - Culture E. coli ESBL positive
 - Resistant to Amoxicillin, Co-amoxiclav, Trimethoprim, Cephadrine, Ciprofloxacin
 - Sensitive to Nitrofurantoin
- What is an ESBL?

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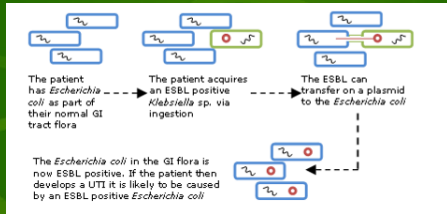
Caution: Extended Spectrum Beta-lactamase

- Enzyme excreted into periplasmic space which inactivates antimicrobials by cleaving the beta-lactam bond.
- Cause resistance to almost all beta-lactams including 3rd-generation cephalosporins
- Associated with multiple antibiotic resistances
- Can be chromosome, plasmid or transposon encoded
- Can be constitutive or inducible
- Ideally patients with ESBLs should be managed in side-rooms with contact precautions

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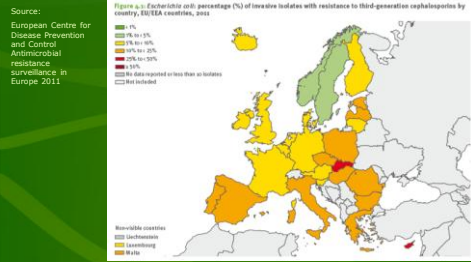


Transfer of antibiotic resistance



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Caution: Extended Spectrum Beta-lactamase



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Caution: Extended Spectrum Beta-lactamase

- Carbapenems are the treatment of choice
- Some advocate Beta-lactamase inhibitor combinations (BLI) e.g. Co-amoxiclav, Piptazobactam
 - Insufficient evidence
 - Systematic review & metanalysis JAC 2012; 67: 2793-2803
 - Carbapenems > non-BLI
 - BLI not < carbapenems
 - BLI not > non BLI
 - How can BLI = carbapenems?!
- Personally use carbapenems for serious infections caused by ESBL positive bacteria

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But what about carbapenemes?

- Carbapenems are the broadest spectrum antibiotics available
 - Ertapenem
 - Meropenem
 - Imipenem
 - Doripenem
- Carbapenemases are Beta-lactamase enzymes which hydrolyse carbapenems
- Confer resistance to ALL Beta-lactam antibiotics
- Often transferable on mobile genetic element e.g. plasmid

Public Health England

Patient Safety Alert

NHS England

Stage Two: Resources

Addressing rising trends and outbreaks in carbapenemase-producing Enterobacteriaceae

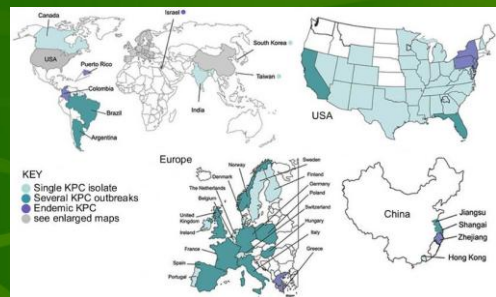
6th March 2014

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- The "Big Five":
 - *Klebsiella pneumoniae* carbapenemase (KPC)
 - Verona integron-encoded metallo-beta-lactamase (VIM & IMP)
 - New Delhi metallo-beta-lactamase (NDM)
 - Oxacillin Carbapenemases (OXA)
- Should be considered in all patients transferred to UK from abroad
- Recent guidance supports screening and infection control precautions for these patients

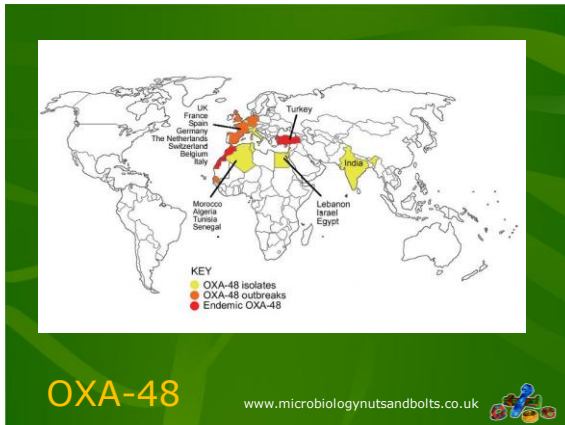
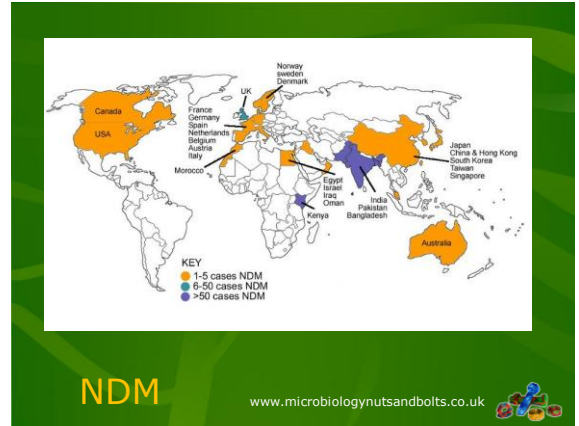
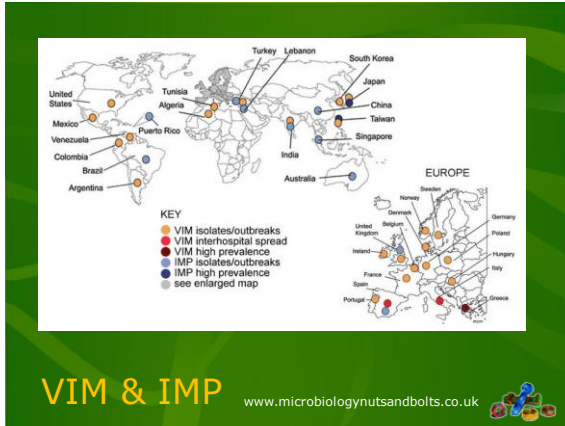


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KPC

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- Investigation
 - Difficult
 - No perfect single method for detecting
 - Treatment
 - Colistin PLUS carbapenem
 - Colistin PLUS Tigecycline
 - Colistin PLUS aminoglycoside (very nephrotoxic)
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Why worry?

- If antibiotic choice inappropriate mortality increases 7% per hour!

Figure 1. Cumulative effective antimicrobial isolation following onset of sepsis shock associated hypotension and associated survival. The x-axis represents time from following the commencement of sepsis shock associated hypotension. Shaded bars represent the fraction of patients surviving to hospital discharge in effective therapy initiated within the given time interval. The grey bars represent the cumulative fraction of patients having received effective antimicrobials at any given time point.

Kumar, Crit Care Med 2006; 34; 1589-96

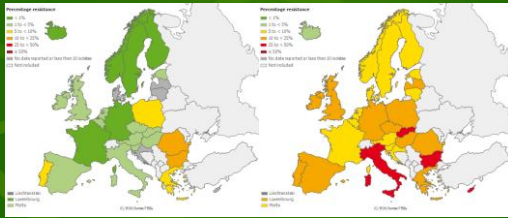
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- ### Why worry?
- Overreliance on single classes of antibiotics is a selective pressure that drives resistance
 - There are no new antibiotics for Gram-negative bacteria in the pipeline
 - We are approaching the Post-antibiotic era (only 100 years after the first antimicrobial was discovered – Salvarsan for syphilis 1911)
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ESBLs in Europe

2002

2012

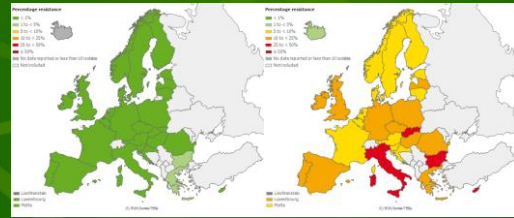


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Carbapenemases?

2012

2024?



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Betty

- Seen on Critical Care by the Microbiologist who recommended IV Meropenem
- Betty was given 7 days of IV Meropenem for presumed pyelonephritis (secondary to failure to appropriately treat her simple UTI)
- She made a full recovery and went home
- Warning – Betty is now known to be colonised with a Antibiotic-resistant E. coli so her future UTIs are likely to be resistant as well (it is part of her normal flora!)

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Case 3 - Mark

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Mark

- 19 year old university student
- Admitted with severe headache, fever and confusion
- On examination
 - Temperature 40°C, B.P. 110/80 mmHg
 - Blanching rash
- He undergoes a lumbar puncture then starts antibiotics
- What is the most likely diagnosis?
- What antibiotic(s) should be given?

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- Lumbar Puncture
 - RBC 1st 162 x10⁶/L
 - RBC 3rd 36 x10⁶/L
 - WBC 1420 x10⁶/L
 - 90% Polymorphs
 - 10% Lymphocytes
 - No organisms seen
 - Protein 7.80 g/L
 - Glucose <0.4 mmol/L (Peripheral Glucose 4.0 mmol/L)

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How to interpret a CSF result?

- Appearance
 - Clear & Colourless, blood-stained, yellow, turbid...
- Microscopy
 - RBC, WBC, Differential WBC, Gram stain...
- Culture
 - Is the organism consistent with the clinical picture?

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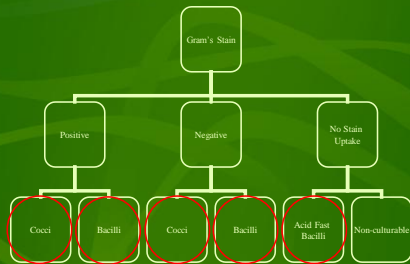
Appearance of Cerebrospinal Fluid

- Clear & Colourless
 - Pure CSF
- Blood-stained
 - Traumatic tap or acute intracranial bleed
- Yellow
 - Possible xanthochromia or patient on drug causing discolouration e.g. rifampicin
- Turbid
 - Purulent or packed full of bacteria!

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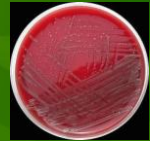
Culture: classification of bacteria



Causes of meningitis usually originate in the upper respiratory tract

Culture: how is CSF processed?

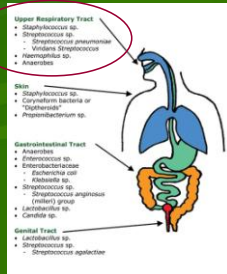
- Urgent specimen
 - Need to call to tell microbiology it is coming
 - Should be processed within 2 hours
 - High-risk for laboratory staff
- Microscopy
- Culture 24-48 hours
- Identification and antibiotic sensitivities further 24-48 hours
- PCR for *N. meningitidis* and *S. pneumoniae* if had antibiotics already



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Community Normal Flora



Also
Streptococcus pneumoniae
Neisseria meningitidis
Haemophilus influenzae

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Mark

- Rash becomes non-blanching & purpuric
- Capillary refill time >5s, un-recordable blood pressure
- Mark transferred to Critical Care
- What is the diagnosis?
- What other investigations should be done?
- What antibiotic could be given now?



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Meningitis

	Ceftriaxone Cefotaxime PLUS Amoxicillin	β -lactam allergy Chloramphenicol	Meropenem	β -lactam allergy Co-trimoxazole (Septrin)
Neisseria meningitidis	✓	✓	✓	✓
Streptococcus pneumoniae	✓	✓	✓	✓
Haemophilus influenzae	✓	✓	✓	✓
Listeria monocytogenes	✗	✗	✓	✓

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Mark

- Aggressive resuscitation
- IV Benzylpenicillin for 7 days
- Notified to Public Health
 - University contacts given antibiotic prophylaxis
- Mark made a full recovery and was discharged home 2 weeks later.

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Caution: Prophylaxis & Infection Control

- Organised and co-ordinated by Public Health
- Contact tracing household contacts
- Oropharyngeal decolonisation
 - Adults - Ciprofloxacin
 - Children - Rifampicin
 - Pregnancy - IM Ceftriaxone
- Infection Control
 - Isolate patient
 - Personal Protective Equipment (PPE)
 - Gloves and aprons
 - Face mask if manipulating airway
 - If splashed in face consider antibiotics



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Conclusion

- In order to practice good antimicrobial stewardship you need to know:
 - How to diagnose infections
 - How to interpret microbiology results
 - The common causes of different infections
 - The spectrum of activity of different antimicrobials
 - The tissue penetration of different antimicrobials
 - The cautions and contraindications of different antimicrobials
 - How long to treat different infections for
- It's not just for Antimicrobial Pharmacists!

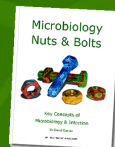
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Further reading:

- Microbiology Nuts & Bolts by Dr David Garner
- www.microbiologynutsandbolts.co.uk
- Facebook page for Microbiology Nuts & Bolts



Available to buy on amazon.co.uk

www.microbiologynutsandbolts.co.uk



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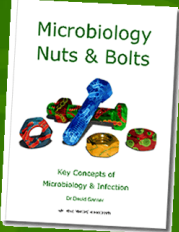


- **Royal College of Pathologists**
 - A well-written book...concise, well set out and easy to use. It contains a wealth of useful information and is a valuable resource
- **Royal College of Physicians**
 - This book delivers a uniquely relevant and accessible take on microbiology and does an excellent job of bridging the gap between the dry lists of pathogens learnt at medical school and the clinical reality of infection.
- **British Society for Antimicrobial Chemotherapy**
 - This book provides an impressively broad coverage of microbiology in theory and practice and I can see uses for it for students, junior doctors and general practitioners
- **Royal Pharmaceutical Society**
 - Pocket guide to all things infection related packs a vast amount of information into a small space, and would be a useful back-up or portable revision aid for any pharmacist dealing with infection
- **Institute of Biomedical Science**
 - A comprehensive yet concise book that would be useful to any healthcare professional managing patients with infections
- **Hospital Infection Society**
 - A very good pocket guide covering the basics of microbiology... It forms a good base of knowledge for specialist trainees

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Any Questions?



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