ESBLs - Where Are We Now?
Dr. Fong Chiew
A Webber Training Teleclass

Perspectives from a Clinical/Medical Microbiologist Working in a Routine Laboratory

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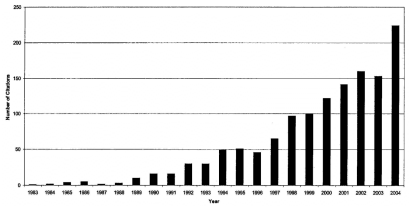
Society of Infectious Diseases of Singapore
American Society for Microbiology
Singapore Society of Pathology

Slide 2

Literature Search on PubMed

Paterson DL & Bonomo RA *Clin Micro Rev* 2005:
Explosion of knowledge on ESBL (upto October 2005)



Slide 3

The Woes of a Medical/Clinical Microbiologist

- Last 2 years: > 400 articles on ESBL
- For the more enthusiastic – search 'pathogens' yield > 48480 articles from:
  http://www.ncbi.nlm.nih.gov/sites/entrez

Slide 4

Outline of Lecture

I. Historical perspectives
II. Update of most recent ESBLs
III. Epidemiology of spread of ESBL
IV. High index of suspicion – when to apply it
V. Laboratory detection
VI. Multi-faceted approach to manage ESBL

Slide 5

Historical Perspectives (I-1)

The Evolution of ß-lactamases:
'Lamentations' of Sanders CC & Sanders WE in *Clin Infect Dis* 1992

- >50% ampicillin resistance in *E. coli*
- One of the early reports of resistance to third generation cephalosporins

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Hosted by Jane Barnett jane@webbertraining.com
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Classification of ß-Lactamases (I-2)

Ambler – Structure Group

Ambler RP. The structure of ß-lactamases. *Philos Trans R Soc Lond B Biol Sci* 1980;289:321-31

Bush – Functional Group

Antimicrob Agents Chemother 1989 March; 33(3): 264–270

Slide 7

Comparison of Ambler and Bush classifications (I-3)

http://upetd.up.ac.za/thesis/available/etd-11042005-080132/unrestricted/01chapter1.pdf

Beta-lactamase classification schemes

Bush Group	Sub group	Ambler Molecular Class	Characteristics
1		C	Mainly chromosomal-located in Gram-negative bacteria but may be plasmid mediated. Confer resistance to beta-lactams (except carbapenems). Not inhibited by clavulanate.
2		A.D	Most enzymes inhibited by clavulanate (unless otherwise stated).
	2a	A	Penicillinases (narrow hydrolysis spectrum) conferring resistance to penicillins.
	2b	A	Broad-spectrum penicillinases TEM-1 & SHV-1 primarily from Gram-negative bacteria.
	2be	A	Extended-spectrum beta-lactamases conferring resistance to oxyimino-cephalosporins and monobactams.
	2br	A	Inhibitor resistant beta-lactamases (mostly TEM-types and to a lesser extent SHV-derived enzymes).
	2c	A	Carbenicillinases.
	2d	D	Oxacillinases. modestly inhibited by clavulanate.
	2e	A	Cephalosporinases inhibited by clavulanate.
	2f	A	Serine active site carbapenemases. inhibited by clavulanate.
3	3a. b. c	B	Metallo-beta-lactamases conferring resistance to beta-lactams (except monobactams). not inhibited by clavulanic acid.
4			Miscellaneous unsequenced beta-lactamases that do not conform to other groups.

Slide 8

Definition of ESBL (I-4)

- ESBL is acronym for Extended-Spectrum ß-Lactamases
- First reported in 1983 (Knothe H et al, *Infect* 1983)
- Characteristics of ESBL:
- Class A by Ambler or Group 2be by Bush classifications
- Typically, enzymes are plasmid-mediated derived from older ß-lactamases of TEM and SHV
- In early 2000s, CTX-M derived ß-lactamases are included

Slide 9

What is it in 2007? (I-5)

- Plasmid-mediated AmpC ß-lactamases, PER, Toho, etc?
- Chromosomal AmpC in *K. pneumoniae* & *E. coli*?
- Integron-mediated ß-lactamases with multidrug resistances?:

Machado E et al; *AAC* 2007
Poirel L et al; *AAC* 2006

Slide 10

Renaming ESBL? (I-6)

- Use clinical approach to depict multiplicity of enzymes in the same isolate?
- Change to **Expanded Spectrum ß-Lactamases** or **X-Spectrum ß-Lactamases?**
- Useful read on controversies about ESBL and AmpC ß-lactamases – see Thomson KS, *EID* 2001

Slide 11

Update of Most Recent ESBLs (II-1)

Reference

http://www.lahey.org/studies/

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Update of Most Recent ESBLs (II-2)

- Important not to restrict singular enzyme per pathogen
- Rather, multiple enzymes can be found within the same pathogen
- Co-resistances to other antimicrobial groups compound the complexity (e.g. fluoroquinolones, trimethoprim, gentamicin, carbapenem)

The pathogens conduct their own conjugation and transformation experiments (without human funding)

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Epidemiology of ESBL (III-1)

Paterson DL and Bonomo RA *Clin Microbiol Rev* 2005

- Europe
- North America
- South and Central America
- Africa
- Asia

Slide 14

Epidemiology of ESBL (III-2)

Europe

France – early 1990s 25-35% nosocomial *K. pneumoniae*
- Year 2000, 30.2% *Enterobacter aerogenes*

SENTRY 1997 and 1998 - 25 European hospitals (ICU and non-ICU)
21% *K. pneumoniae*

North America

NNIS 1998-2002 - 6.1% *K. pneumoniae* from 110 ICUs
- 10% of ICU >25%
- Non-ICU 5.7% *K. pneumoniae*
- Outpatients 1.8% and 0.4%

Between 2003-2004 – emergence of CTX-M

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Epidemiology of ESBL (III-3)

South & Central America

1989 - CTX-M linked to *Salmonella enterica* spread to many parts of the continent. Did the method for detection included CTX in addition to CAZ?

ICUs (Brazil, Colombia, Venezuela) - 30-60% of klebsiellae

Africa

36.1% of *K. pneumoniae* from a single South African hospital

CTX-M found in Kenya reported in 2001

Australia

SENTRY 1998-1999 Overall 5% in hospitals

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Epidemiology of ESBL (III-4)

Asia

- SENTRY 19898-1999
  30.7% *K. pneumoniae* and 24.5% *E. coli*.
- Teaching hospital in Beijing reported in 2002 27%
  (both *K. pneumoniae* and *E. coli*) from blood cultures
- Zhejiang Province (China)
  34% *E. coli* and 38.3% *K. pneumoniae*
- A Japanese hospital in 1998-1999 25% *K. pneumoniae*
- Reports of CTX-M from 2001:
  India, China, Japan, Korea, Taiwan and spreading

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Epidemiology of ESBL (III-5)

Subsequent to 2005:

- Increasing reports of plasmid-mediated AmpC in *E. coli* and *K. pneumoniae*
- Co-existence of ESBL, AmpCs and other ß-lactamases in the same isolate

Slide 18

High Index of Suspicion in Healthcare Settings – when to apply it (IV-1)

- A) Endogenous – enemies from within
- B) Exogenous – enemies from without
- C) Level of infection control
- D) Level of antimicrobial utilisation

Others, e.g., antimicrobials & food production

Slide 19

Endogenous: The Gastrointestinal Tract (IV-2)

- Within the 7 feet or so of intestinal tract in humans, genetic exchange can occur between pathogens and/or microbes?
- For e.g. AmpCs from *Enterobacter* spp (& others) can pass onto *E. coli* and *K. pneumoniae* and vice versa?

[The same events occur in food animals?]

Slide 20

Exacerbation of Genetic Exchange (IV-3)

- Multiple courses of antimicrobials
- Indwelling catheters
- Multiple premorbid conditions

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B. Exogenous – Enemies from Without (ie in the vicinity) (IV-4)

- Long hospital stay
- Residing in long term care facilities
- Patient in the next cubicle or someone transferred from elsewhere who harbours ESBL (hospital, centre, country)

Slide 22

C. Level of infection control (IV-5)

Is this a recent implementation?

For a long time, MRSA was the only pathogen deserving strict hand hygiene & not GNR

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D. Level of Antimicrobial Utilisation (IV-6)

What are the significance and relevance of selective pressure?

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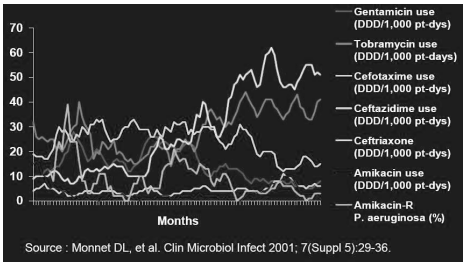
Is There an Association Between Cephalosporins Usage and Their Ccorresponding Resistances? (IV-8)

- How much cephalosporins are used and what are these?
- If cefotaxime (and or ceftriaxone) are used in far greater excess over ceftazidime, then CTX (and or 'CEF') predominates?

Slide 25

Monnet D 'How Antimicrobials Drive Resistance'
ASA 2007 see http://www.asainc.net.au/news/ (IV-9)



Source : Monnet DL, et al. Clin Microbiol Infect 2001; 7(Suppl 5):29-36.

Slide 26

V. Laboratory Detection (V-1)

Historical perspectives

1988

Jarlier effect – CTX with Augmentin (Jarlier V et al *Rev Infect Dis* 1988)

1990

NCCLS– ceftazidime zone <15mm Kirby Bauer Method for screening

1994

Synergy testing with ceftazidime (Sader HS et al *Diagn Microbiol Infect Dis* 1994)

Slide 27

Laboratory Detection (V-2)

1996

Etest with ceftazidime and clavulanate was recommended (Cormican MG et al *JCM*)

1996

>50% ESBL *E. coli* and 29% of ESBL *K. pneumoniae* were resistant to cefoxitin and 10% of non-ESBL *E.coli* and *K. pneumoniae* also resistant to cefoxitin

(Jacoby GA & Han P *JCM*)

2001

Cefpodoxime recommended for screening *Clin Microbiol Rev* 2001

Slide 28

Current Modern Methods (V-3)

- CLSI – Clinical Laboratory and Standards Institute
- ARMRL - Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, London
- EUCAST- European Society of Clinical Microbiology & Infectious Diseases
- Commercial methods – Etest, BD Phoenix, Vitek, Neo-tabs & others

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Law of Serendipity (V-4)

in association with:

Otago Diagnostic Laboratories (ODL)
Method for the detection of
ß-lactamases in *Enterobacteriaceae*

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Observations and Questions from Staff of ODL in Dunedin Hospital, NZ (V-5)

- Routine laboratory struggles with ESBL detection: clinical isolates do not conform to behaviour of research isolates
- Jarlier effect appears obsolete for the detection of ESBL
- Reference laboratories e.g. ESR Wellington (NZ) uses it (Jarlier effect)
- Double Disk Test for ESBL from Mount Sinai Hospital, Toronto, Canada looks plausible

Slide 31

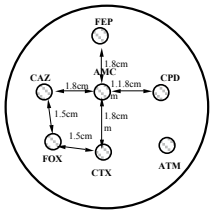
TRICKS OR TREATS

Presented at 2004 NZ National ASID Meeting
Refer *Pathology* Oct 2005;37(5):371-7

expanded spectrum β-lactamases images

Slide 32

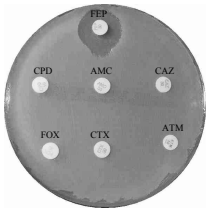
Improved Template for Placement of Antimicrobial Discs (V-6)



AMC: Augmentin 20/10 µg
CAZ: Ceftazidime 30 µg
CTX: Cefotaxime 30 µg
ATM: Aztrenam 30 µg
CPD: Cefpodoxime 10 µg
FOX:Cefoxitin 30 µg
FEP: Cefepime 30 µg

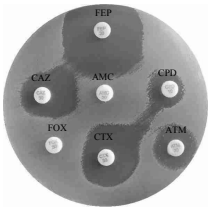
Slide 33

Reference Strain *E. cloacae* ARL04/111 Demonstrates derepressed AmpC and ESBL (V-7)



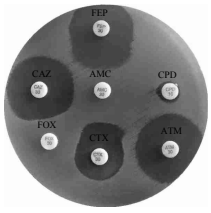
Slide 34

Reference Strain *E. cloacae* ARL04/173 Demonstrates Inducible AmpC and ESBL (V-8)



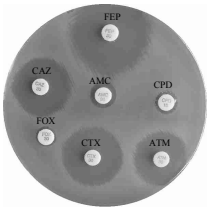
Slide 35

Clinical Isolate *E. cloacae* Demonstrates Inducible AmpC and CTX-M (V-9)



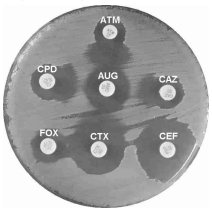
Slide 36

Clinical Isolate *E. coli* Demonstrates AmpC-like ß-Lactamase (note resistance to cefoxitin) (V-10)



Slide 37

ATCC *K. pneumoniae* 700603 Reference Strain for ESBL: note cefoxitin resistance (V-11)



NB: Older version of ODL Method was used

Slide 38

Real vs Apparent (V-12)

Is Epidemiology of ESBL directed by laboratory methods?

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VI. Multi-faceted Approach to Manage ESBL / XSBL (VI-1)

A) Ascertain clinical significance of isolate obtained
B) Reduce unnecessary antibiotic utilisation (& reduce unnecessary adverse effects too)
C) Determine third generation cephalosporins usage
D) Pharmacokinetics and Pharmacodynamics
E) Infection control
F) Funding
G) Closer collaboration between research and clinical laboratories
H) Investments by manufacturer on education
I) Manage antimicrobials in food production

Slide 40

A. Ascertain Clinical Significance of Isolate Obtained (VI-2)

Is it a contaminant, colonizer or true invasive pathogen?

Slide 41

Overdiagnosis of Infections (VI-3)

Bruce et al; *J Hosp Infect* 2001 Oct;49(2):99-108. Review
The quality of measurement of surgical wound infection as the basis for monitoring: a systematic review

Ehrenkranz NJ et al; *Infect Control Hosp Epidemiol* 1995 Dec;16(12):712-6
An apparent excess of operative site infections: analyses to evaluate false-positive diagnoses

Taylor G et al; *Am J Infect Control* 1990 Oct;18(5):295-9
Effect of surgeon's diagnosis on surgical wound infectionrates

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A comparison of infection rate using different methods of assessment for surveillance of total hip replacement surgical site infections (VI-4)
Yoke-Fong Chiew & Jean-Claude Theis ANZ J Surg 2007
46th ICAAC, Session 074/Surgical site infection: Prophylaxis, Prevention and Prevalence

Outcomes of Comparison of 4 Approaches to the Diagnosis of SSI

Risk index	No. of patients studied	Case No. and type of infection according to risk index, day of diagnosis (post-op day), microorganisms isolated[a]	Description of infections according to Case No[a]	Diagnosis of infection according to ASEPSIS score[b] (≤ 20 >)	Diagnosis of infection according to presence of pus cells[c]	Assessment by medical microbiologist
0	114	5 cases of SI: (1) 7th POD = no specimen (2) 8th POD = *E. faecalis*, no WBC (3) 14th POD = *S.aureus*, no WBC (4) 28th POD = *S.aureus*, moderate WBC (5) 30th POD = no specimen	(1) Stitch abscess (2) Erythema of wound (3) Erythema of wound (4) Purulent wound (5) Erythema of wound	(1) Uninfected (≤20) (2) Infected (>20) (3) Infected (>20) (4) Infected (>20) (5) Uninfected (≤20)	(1) Unknown (2) Uninfected (3) Uninfected (4) Infected (5) Unknown	(1) Uninfected (2) Uninfected, possible colonizers. (3) Uninfected, possible colonizers (4) Infected (5) Uninfected
1	80	1 case of SI: (6) 3rd POD = no specimen 2 cases of DI: (7) 1st POD = *Streptococcus spp* from enrichment broth, no WBC (joint tissue) (8) 10th POD = *Enterococcus spp*, moderate WBC	(6) Serous discharge (7) Erythema of wound (8) Purulent wound with discharge	(6) Uninfected (≤20) (7) Infected (>20) (8) Infected (>20)	(6) Unknown (7) Uninfected (8) Infected	6) Uninfected (7) Uninfected, possible contaminant. (8) Infected
2	12	1 case of SI: (9) 7th POD = *Staphylococcus* spp + diphtheroids, no WBC	(9) Erythema of wound	(9) Uninfected (≤20)	(9) Uninfected	(9) Uninfected, possible colonizers.
Total number of infected patients			9	5	2 (3 unknown)	2

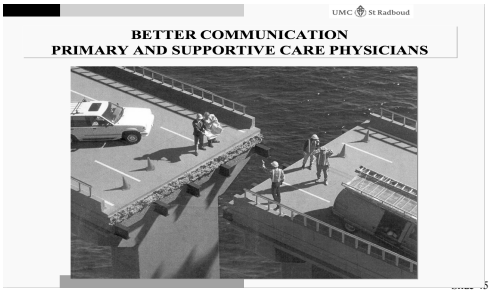
Interpretation of Laboratory Results (VI-5)

Close clinician-laboratory interaction to minimise over-diagnosis and miss-diagnosis of infections

Slide 44

Metaphorically Speaking…..(VI-6)

Prof Ben de Pauw's presentation at ISAAR 2007



B. Reduce Unnecessary Antibiotic Utilisation To Decrease Selective Pressure & <u>Reduce Unnecessary Adverse Effects Too</u> (VI-7)

- Mazzeo F et al, *Pharmacol Res* 2005
  Hospital-based intensive monitoring of antibiotic-induced adverse events in a university hospital.
- Sanford Guide to Antimicrobial Therapy: http://www.sanfordguide.com/
- John Hopkins Antibiotic Guide: http://hopkins-abxguide.org/

Slide 46

C. Determine Third Cephalosporins Usage and Frequency of ESBLs (VI-8)

K Urbanek, M Kolar, Y Loveckova, J Strojil, and L Santava

Influence of third-generation cephalosporin utilization on the occurrence of ESBL-positive *Klebsiella pneumoniae* strains

J Clin Pharm Ther 1 Aug 2007 32(4): p. 403.
http://highwire.stanford.edu/cgi/medline/pmid;17635342

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D. Choice of Antimicrobials for Treatment (VI-9)

A Carbapenem?

What are the Pharmacokinetics and Pharmacodynamics?

Slide 48

D. Pharmacokinetics and Pharmacodynamics (VI-10)

Lessons learnt and
Questions arising from
46th ICAAC Workshops
San Francisco, USA

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Questions/Problems about PK/PD approach to prevent emergence of resistance (VI-11)

- Polymicrobial infection
- Subtherapeutic dosing in the presence of biofilm formation arising from indwelling catheters
- Choice of parameters? – for e.g.:C_{max}/MIC, AUC/MIC, T>MIC
- MIC is not sufficient to evaluate the PK/PD relationships of antimicrobial agents.
- PK/PD analysis based on MIC alone can be misleading.
- Protein binding and tissue distribution are important pharmacokinetic parameters that need to be considered
- Variance of PK in population?
- What is the correct PD index target (static, -1 log, -2 log drop@24h?, 48h? 5d end point?)

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Questions/Problems about PK/PD approach to prevent emergence of resistance (VI-12)

- Variability in the PD target size ie inoculum.
- Variance of PD for different micro-organisms groups?
- What is the prediction in chronic infection (bone; abscess formation)
- There are variations in methods and definitions of indices as well as uncertainty about errors.
- What about combination antimicrobial therapies – synergy, antagonism, additional effects?
- What about drug interactions with non-antimicrobial agents?
- MICs may be lower or higher for different regions?
- Is PK/PD different for neutropenics and non-neutropenics?

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Nevertheless……(VI-13)

PK/PD is vital to prevent:

- Subtherapeutic dosing which leads to emergence of resistance
- Overdosing which leads to toxicity

Slide 52

E. Infection Control (VI-14)

Plays a vital role in centres where ESBL rates are low but becomes desperate when rates >50%

Slide 53

ESBL and Infection Control (VI-15)

- Gould IM et al *J Hosp Infect* 1996;33:249-62
  http://www.documents.hps.scot.nhs.uk/ewr/supp/0450ESBLsupplement.pdf
  'Grampian outbreak'
- Conterno LO et al *J Hosp Infect* 2007
  Impact and cost of infection control measures to reduce nosocomial transmission of extended-spectrum beta-lactamase-producing organisms in a non-outbreak setting
- Mammina C et al *Am J Infect Contr* 2007
  Surveillance of multidrug-resistant gram-negative bacilli in a neonatal intensive care unit: prominent role of cross transmission
- Muratani T et al *Int J Antimicrob Agents* 2006
  Emergence and prevalence of beta-lactamase-producing *Klebsiella pneumoniae* resistant to cephems in Japan
- Ben-Ami R et al *Clin Infect Dis* 2006
  Influx of extended-spectrum beta-lactamase-producing enterobacteriaceae into the hospital.

Slide 54

F. Funding – for what? (VI-16)

- For e.g. inadequate staffing and inadequate training?
- And for all of the mentioned VI (A-I)

Slide 55

G. Closer Collaboration Between Research and Clinical Laboratories (VI-17)

Clinical isolates are more complex than the research ones and they evolve faster too in clinical settings

Slide 56

H. Contributions by Manufacturer Towards Combating Antimicrobial Resistance e.g. ESBL (VI-18)

Priority (amongst others) given to education concerning: -

- antimicrobial resistance
- how to sustain shelf-lives of antimicrobials
- benefits to individual patient care
- consequential profit gains

Slide 57

I. Antimicrobial Usage in Food Production (VI-19)

Direct effects of selective pressure are more urgent/important on human pathogens?

For e.g. Rapid spread of *Staphylococcus aureus* with reduced susceptibilities to vancocmycin widely reported in Europe due to prescriptions in healthecare - despite withdrawal of avoparcin in food production?

Van Griethuysen A et al JCM 2003; 41:2487-91; Reverdy ME et al 2001; Nonhoff C et al 2005; Mallaval FO et al 2004; Heym B et al 2002; Bernard L et al 2004; Garnier F et al 2006; Lecaillon E et al 2002

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Nevertheless, Indirect Selective Pressure Is Eminent & Imminent (VI-20)

- Mayrhofers et al *Microb Drug Resist* 2006
- Bengtsson B & Wierup M al *Anim Biotechnol* 2006
- Chen J et al *Appl Environ Microbiol* 2007
- Slorach SA *Rev Sci Tech* 2006
- Wagenaar JA et al *Rev Sci Tech* 2006
- Fluckey WM et al *J Food Prot* 2007
- Many others

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What Approach Should We Adopt to Address Antimicrobial Resistance? (VI-21)

- Gentle approach
- Forceful approach

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ESBLs - Where Are We Now?
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Gentle Historical Approach (VI-22)

French Philosopher Blaise Pascal said:

"No one is strong unless he or she bears within their character antithesis strongly marked"

In the arena where we watch humans pit their wits against the ingenuity of microbes, some resemblance to this philosophy may be observed. The brilliance and tenacity of the human mind that I shall summarily call 'Thesis' are in constant battle with the counterbacks from microbes. 'Anti-thesis' in the form of Human **Arrogance or Despair** may well tip the balance in favour of the counterattacks. Our 'Thesis' must be held in tension with other virtues like **Humility or Hope** accordingly – where strength is to be found. And by balancing these virtues, we then become more fully developed and stronger people.

Taken from MD Thesis 'The Epidemiology and Laboratory Detection of Resistant Enterococci' carried out by Dr Yoke-Fong Chiew at the National University of Singapore

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Modern More Forceful Approach At The ISAAR 2007 (VI-23)

Dr Keryn Christiansen (Royal Perth Hospital, Australia) on 'Managing Antibiotic Policies'

Dr Wing Hong Seto (Queen Mary Hospital, HK) on 'Immediate Concurrent Feedback'

Dr Walter R Wilson on 'Pathogens vs Humans', some e.g.s: Similaries: Both are diversified competitors

Differences: United (Pathogens) vs Divided (Humans)

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Nobel Prizes and Antimicrobials

- Nobel prize awarded to discovery of penicillin
- Nobel prize to be awarded for capping antimicrobial resistances or miraculously reversing the trends for some of them?

What are involved?

Processes and team efforts?

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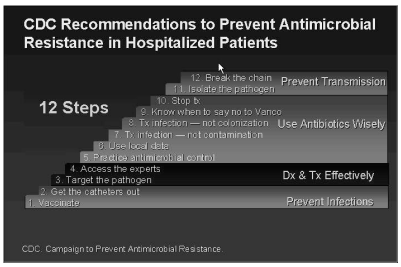
Process and Team Efforts Outlined By CDC Campaign on Combating Antimicrobial Resistances in Healthcare Settings



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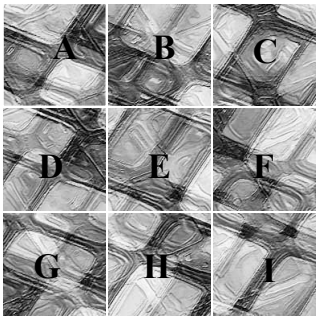
Solution VI (A-I) to Enigma of ESBL



PERSEVERE!

Don't Give Up

Have a Good Day

Contact:
yfchiew@hotmail.com

For more beautiful jigsaw images by Mr Stephen Linhart, refer - http://www.stephen.com/enigma/enigma.html

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The Next South Pacific Teleclass - October 10, 2007

Hosted by Jane Barnett jane@webbertraining.com
www.webbertraining.com