

CONSENSUS GUIDELINES FOR THE MANAGEMENT OF INFECTIONS BY ESBL-PRODUCING BACTERIA

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**Committee Members of
Consensus Guidelines For The Management of Infections
by
ESBL-Producing Bacteria**

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Chapter 1: Beta-lactamases and ESBLs (Extended Spectrum Beta-lactamases)

Introduction

Betalactamases are a group of enzymes capable of hydrolysing the 4-membered betalactam ring of betalactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems). They are the most common and most important mechanism of resistance to betalactam antibiotics. Up to the year 2001 some 340 discrete betalactamases have been identified.¹ It is important to know the types of betalactamases produced by various clinical pathogens as this has an impact on the selection of antimicrobial agents.

Classification of betalactamases

There have been a number of schemes for the classification of betalactamases. The most often used scheme is that which was developed by Bush, Jacoby and Medeiros.² This scheme, which is probably the most recent and complete, attempts to combine the elements of previous schemes and to correlate this with molecular structure. Under this scheme, betalactamases are divided into 4 groups. An earlier scheme proposed by Ambler³ is also frequently used. These two schemes are shown in Table I.

Table I: Classification of betalactamases

Ambler Class	Bush Group	Characteristics of betalactamases	Number of enzymes
C	1	Often chromosomal enzymes in gram-negatives but some are plasmid-coded. Not inhibited by clavulanic acid.	51
A	2a	Staphylococcal and enterococcal penicillinases	23
	2b	Broad spectrum betalactamases including TEM-1 and SHV-1, mainly occurring in gram-negatives	16
	2be	Extended spectrum betalactamases (ESBL)	119
	2br	Inhibitor-resistant TEM (IRT) betalactamases	24
	2c	Carbenicillin-hydrolysing enzymes	19
	2d	Cloxacillin (oxacillin) hydrolysing enzymes	31
	2e	Cephalosporinases inhibited by clavulanic acid	20
	2f	Carbapenem-hydrolysing enzyme inhibited by clavulanic acid	4
B	3	Metallo-enzymes that hydrolyse carbapenems and other betalactams except monobactams. Not inhibited by clavulanic acid	24
D	4	Miscellaneous enzymes that do not fit into other groups	9

Group I (Ambler Class C) betalactamases (also known as AmpC enzymes) are intrinsically resistant to betalactamase inhibitors and are mostly coded for by chromosomal genes.^{4, 5} When chromosomally coded the enzymes are inducible; i.e. the level of production increases many fold when the bacteria is exposed to certain betalactam antibiotics. Betalactam antibiotics differ in their potential to induce betalactamase production. Group I enzymes are mainly found in *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp. and *Pseudomonas aeruginosa*. Some of these chromosomal enzymes have also moved onto plasmids in clinical strains of *Escherichia coli* (*E.coli*) and *Klebsiella* spp.⁶ Bacteria producing Group I betalactamases are resistant to betalactam/betalactamase inhibitor combinations, penicillins, cephamycins and 1st, 2nd and 3rd generation cephalosporins. They remain susceptible to cefepime and the carbapenems.⁷

Group 2 (Ambler Class A) enzymes⁸ are plasmid mediated. The genes encoding for the enzyme reside on plasmids and since plasmids are readily transferable from one bacterial cell to another, resistance due to this enzyme can be easily spread. The original Group 2 enzymes are inhibited by the betalactamase inhibitors like clavulanic acid, sulbactam and tazobactam. Group 2 enzymes include the commonly encountered TEM enzymes and SHV enzymes. TEM-1 was discovered in 1965 in Enterobacteriaceae but has since spread to *Haemophilus* spp., *Neisseria* spp. and *Vibrio* spp.. SHV-1 was discovered in 1979 and commonly occurs in *Klebsiella* spp..

Ampicillin and 1st generation cephalosporins are hydrolysed by early Group 2 enzymes but 2nd and 3rd generation cephalosporins were stable. The early Group 2 enzymes have unfortunately mutated to forms of enzymes that are capable of destroying monobactams and third generation cephalosporins (**the extended spectrum betalactamases or ESBLs**)⁸ and enzymes that are resistant to betalactamase inhibitors (the inhibitor-resistant TEM betalactamases or IRTs).⁹

Group 3 (Ambler Class B) enzymes are metallo-enzymes that are capable of destroying carbapenems.¹⁰ These enzymes are found in certain bacteria including *Stenotrophomonas maltophilia*, *Bacteroides fragilis* and *Pseudomonas aeruginosa*. **Group 4 betalactamases** are infrequently encountered.

ESBLs (Extended Spectrum Betalactamases)

These enzymes emerged soon after the introduction of extended spectrum cephalosporins and were first reported in Europe in the early 80s.^{11,12,13} They are now found all over the world. The ESBLs are, as mentioned previously, mutant forms of TEM-1, TEM-2 and SHV-1 enzymes. The ESBLs often differ from the original enzymes by only one to a few changes in their amino acid sequences.

Chapter 2: EPIDEMIOLOGY OF ESBL-PRODUCING ORGANISMS IN MALAYSIA

Not until the mid-1990's, formal surveillance studies on the prevalence of ESBL-producing gram-negative bacteria (GNB) had been done in Malaysia. Epidemiology data had hitherto been based on reports from single centres involving selected patient groups^{1,2} or indirectly obtained from evaluation studies of certain antibiotics.³

In 1994, Cheong *et al* reported on resistance patterns of 36343 bacteria isolated from six general hospitals between 1991 to 1992. Ceftazidime resistance in *E.coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa* were 5.5%, 16.6% and 6.8% respectively. Testing for ESBL production was not performed.⁴

Important epidemiological data is also available from yearly audits done in the Institute of Medical Research. In a 1995 study, multiply drug resistant *E.coli* and *K. pneumoniae* were tested for ESBL production using the E-test. ESBL productions in these two bacterial species were 19% and 27% respectively.⁵

ESBLs are quite commonly encountered in the Asia-Pacific region (Table 2). Malaysia and Singapore seem to have a particular problem with ESBL producing *Klebsiella* spp..

Table 2: Prevalence of ESBL-organisms in some Asian countries.

Country	<i>Klebsiella</i> spp.	<i>E. coli</i>
Japan ⁶	5.0%	8.1%
Taiwan ⁷	21.7%	16.7%
Philippines ⁸	31.3%	13.3%
Malaysia/Singapore ⁹	38.0%	5.6%
Indonesia ¹⁰	33.3%	23.0%

In conclusion, accurate figures for the prevalence of ESBL-producing GNB in Malaysia are not available. Estimated prevalence of ESBL *E.coli* is between 7-19% whilst in *Klebsiella* spp. is 27 - 38%.

Chapter 3: LABORATORY METHODS FOR THE DETECTION OF ESBL-PRODUCING ENTEROBACTERIACEAE

Methods for the laboratory detection of ESBLs are based on recommendations from the National Committee for Clinical Laboratory Standards (NCCLS) and the Canadian External Quality Assessment Advisory Group for Antibiotic Resistance. However, there have been minor variations from these guidelines to suit the operations of laboratories in our setting.

What organisms to test?

Klebsiella pneumoniae and *E. coli* are most frequently associated with ESBL production.¹

ESBL producing isolates of *Enterobacter aerogenes* and *E. cloacae*, *Serratia marcescens*, *Morganella morganii* and *Citrobacter freundii* have been detected but appears to be relatively rare and ESBL detection methods established for *Klebsiella pneumoniae* and *E. coli* have not been shown to be valid for other ESBL producing bacteria.

When to test?

All clinically significant isolates of *E. coli* or *K. pneumoniae* should be tested against beta lactam drugs either using a disc diffusion method or the minimum inhibition concentration (MIC) method (as advocated by the revised NCCLS interpretive criteria). Any decrease in the zone sizes or MIC less than 2mg/L for the 3^d generation cephalosporins should be used as a criterion to test for ESBLs.²

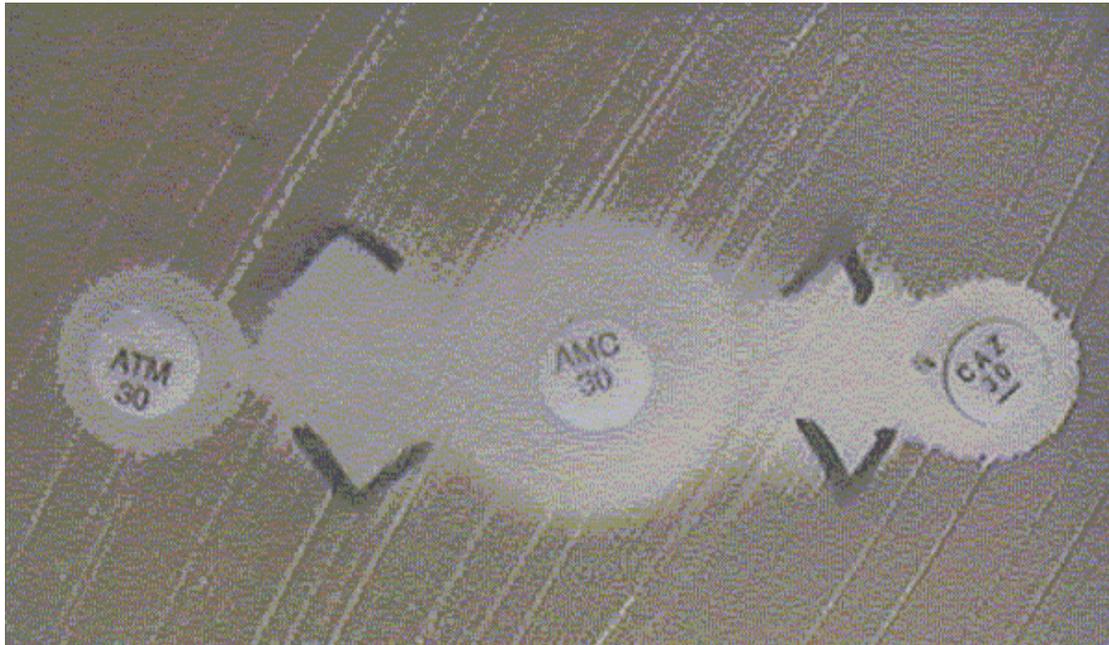
ESBL SCREENING METHODS

1). **Standard disc diffusion method** – *in-vitro* sensitivity testing using established NCCLS procedure is carried out with ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), aztreonam (30µg) and cefpodoxime (10µg). Zone diameters are read using the revised NCCLS document² as shown in Table 3. Any zone diameter within the “grey zone” must be considered as a probable ESBL producing strain requiring phenotypic confirmatory testing.

Table 3: MIC and Inhibition Zone Criteria for the Detection of ESBLs in <i>K. pneumoniae</i> and <i>E. Coli</i>					
		Zone diameter for susceptible strains	Zone diameter for possible ESBL-producing strains	MIC for susceptible strains	MIC for possible ESBL-producing strains
Aztreonam	30 mg	≥ 22 mm	≤ 27 mm	≤ 8 mg/L	≥ 2 mg/L
Cefotaxime	30 mg	≥ 23 mm	≤ 27 mm	≤ 8 mg/L	≥ 2 mg/L
Cefpodoxime	10 mg	≥ 21 mm	≤ 22 mm	≤ 8 mg/L	≥ 2 mg/L
Ceftazidime	30 mg	≥ 18 mm	≤ 22 mm	≤ 8 mg/L	≥ 2 mg/L
Ceftriaxone	30 mg	≥ 21 mm	≤ 25 mm	≤ 8 mg/L	≥ 2 mg/L

* adapted from NCCLS document M100-S88

2). **Double disc synergy / Disk approximation method** – this method uses multiple target disc with clavulanic acid disc; or a single cefpodoxime disc with clavulanic acid discs. Mueller–Hinton agar plate is inoculated with a suspension (adjusted to 0.5 McFarland turbidity standard that has been vortexed) made from an overnight agar plate of the test strain. Disc containing the standard ceftazidime (30ug), ceftriaxone (30µg), aztreonam (30µg) or cefpodoxime (10µg) are placed 15mm to 20mm (edge to edge) from an amoxicillin-clavulanic acid disc. Plates are then incubated overnight at 35°C. Enhancement of zone of inhibition is indicative of presence of an ESBL (Figure 1).



Zone enhancement
Figure. 1

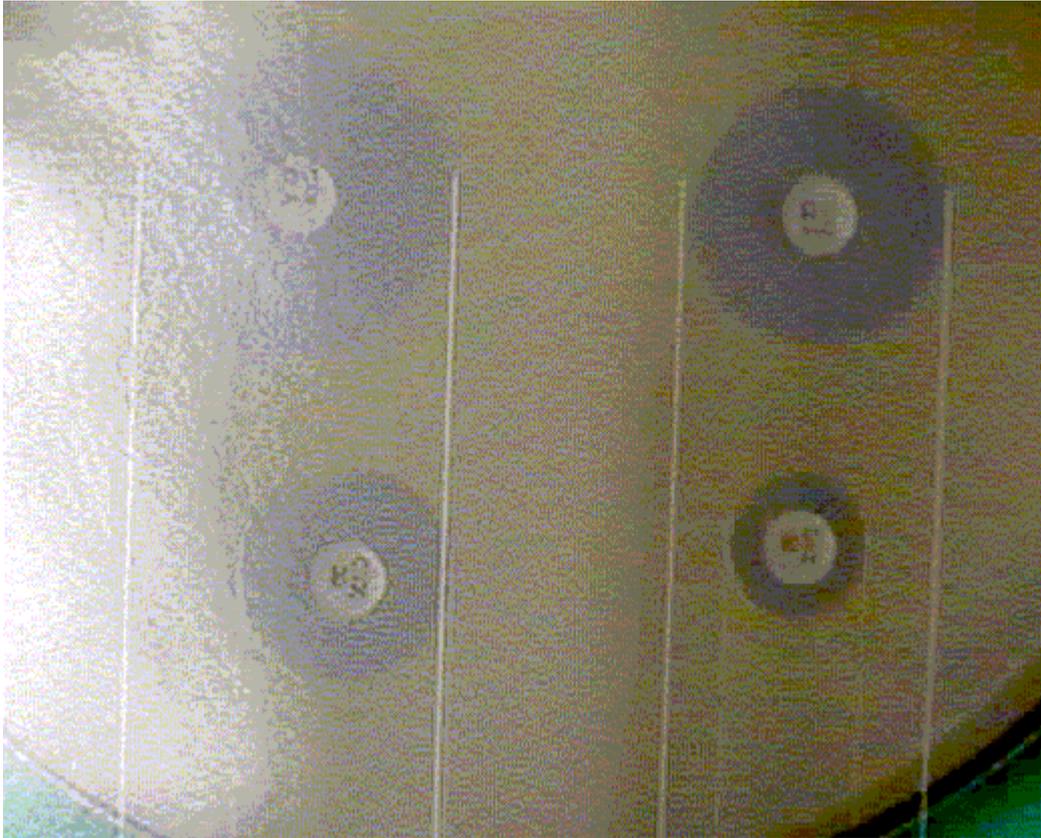
It is vital to place disc at the precise distance as recommended; proper storage of antibiotic disc, bringing discs to room temperature together with regular performance of quality control (QC) on the antibiotic disc are critical to the sensitivity of the disc approximation test.³

PHENOTYPIC CONFIRMATORY METHODS

Disc diffusion method

Ceftazidime (30µg) versus ceftazidime/clavulanic (30/10µg) and cefotaxime (30µg) versus (cefotaxime/clavulanic acid (30/10µg) are placed onto a Muller-Hinton agar plate lawned with the test organism and incubated as described above. Regardless of the zone diameters, a ≥ 5 mm increase in a zone diameter for an antimicrobial agent tested in combination with clavulanic acid versus its zone size when tested alone, indicates probable ESBL production (Figure 2).

Note: The above discs (BBL Sensi-Disc) are available locally.

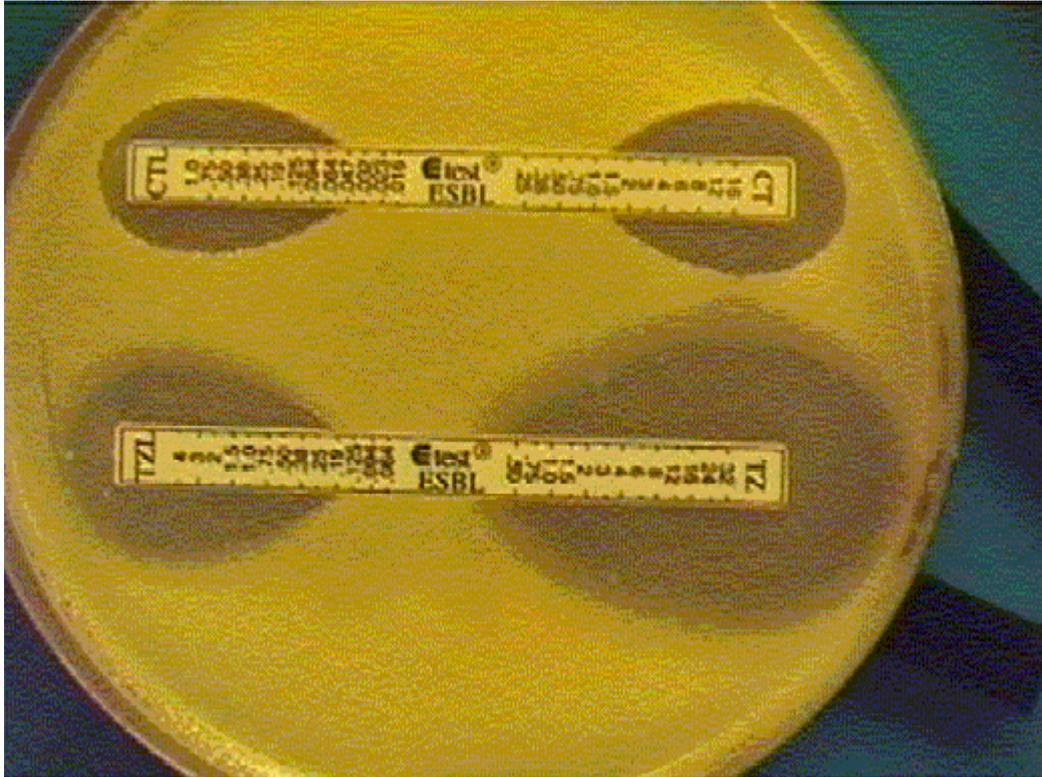


Confirmed ESBL-producing *Klebsiella pneumoniae* (A ≥ 5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone)

Figure. 2

MIC method – ESBL E-test strips (AB Biodisk, Sweden)

Two E-test combination strips e.g. ceftazidime/ceftazidime-clavulanic acid and cefotaxime/cefotaxime-clavulanate are employed to perform the phenotypic confirmatory testing. These strips are inoculated on the surface of the agar plate and incubated overnight. Any reduction of $\geq 3 \log_2$ (doubling) dilution is considered as positive (Figure 3). Note: Not all ESBL producing strains are specific for ceftazidime, strains with other substrate specificities may not be detected with the ceftazidime/clavulanic acid strip alone hence cefotaxime is also used.



MIC ratio of Ceftazidime vs. Ceftazidime/clavulanate acid >8 indicates ESBLs
Figure. 3

SCREENING FOR ESBL-PRODUCING ORGANISMES FROM CARRIAGE SITES

In event of an outbreak, patients may be screened for ESBL producing organisms. Selective media screening for ESBL producing Enterobacteriaceae is by using MacConkey containing ceftazidime 4 mg/L. Any lactose-fermenting colonies growing on the above selective media will be confirmed as ESBL producing by using double disc technique (Figure 1).

QUALITY CONTROL STRAIN RECOMMENDED

Klebsiella pneumoniae ATCC 700603, is used as a control for ESBL tests.
Klebsiella pneumoniae ATCC 700603 diameter range are as follows:

Cefpodoxime (10µg)	6-9mm
Ceftazidime (30µg)	10-18mm
Cefotaxime (30µg)	17-25mm
Ceftriaxone (30µg)	16-24mm
Aztreonam (30µg)	9-17mm

Note: Other quality control strains as recommended by NCCLS must be used to carry out the routine quality control on antibiotic discs.

REPORTING

Patients' report must state that the isolate is a suspected or proven ESBL producer. ESBL production may predict therapeutic failure with beta-lactam antibiotics.

Chapter 4: INFECTIONS ASSOCIATED WITH ESBL-PRODUCING BACTERIA

Infections caused by ESBL-producing bacteria can be subdivided according to the various organs/systems as follows:

1. Urinary tract infection
2. Bacteraemia
 - primary or secondary
3. Respiratory tract infection
 - nosocomial pneumonia
 - ventilator associated pneumonia
4. Gastrointestinal tract infection
 - intra-abdominal abscess
 - peritonitis
 - cholangitis
5. Skin and soft tissue infection
6. Catheter or device related infection
7. Sinusitis
8. Neurosurgical meningitis
 - related to ventricular drainage catheters

All references pertaining to the above infections are at level III.

Studies on patients infected or colonised by ESBL producing bacteria have been shown to share several common factors (Table 4). The presence of these risk factors should alert the attending physician to the possibility of an ESBL-related infection and the appropriate measures need to be taken.

Table 4: Risk factors for infection or colonisation with ESBL producing organisms	
1.	Device related ^{2,4,5} <ul style="list-style-type: none"> • Arterial catheters • Central venous catheters • Urinary tract catheters • Gastrostomy or jejunostomy tube • Umbilical catheters
2.	Surgical related ⁶ <ul style="list-style-type: none"> • Abdominal surgery • Emergency laparotomy
3.	Antibiotic exposure ^{2,5} <ul style="list-style-type: none"> • 3rd generation cephalosporins (especially ceftazidime) • Fluoroquinolones • Trimetoprim-sulfamethoxazole
4.	Previous nursing home residence ⁴
5.	Prolonged duration of hospital or ICU stay ⁴ Longer stay is associated with more severe underlying disease, with invasive procedures and with antibiotic administration
6.	Severity of illness (APACHE III Score) ⁴

Chapter 5 (I): OVERVIEW OF TREATMENT OF INFECTIONS DUE TO ESBL-PRODUCING ORGANISMS

Infections due to ESBL-producing organisms present a major therapeutic dilemma as the choice of antibiotics is extremely limited. Due to the broad-spectrum of the beta-lactamases produced by these organisms, ESBL producing Enterobacteriaceae are typically resistant to beta-lactam antibiotics including broad-spectrum cephalosporins, aztreonam, and extended-spectrum penicillins. Furthermore antibiotics such as trimethoprim-sulfamethoxazole and aminoglycosides especially gentamicin are often co-transferred on a resistance plasmid, resulting in multiple drug resistance.

Infections with ESBL-producing organisms are usually hospital acquired and may include urinary tract infections, peritonitis, cholangitis, intra-abdominal abscesses, ventilator-associated pneumonia and central-line associated bacteraemia. Although these organisms may cause a multitude of nosocomial infections, it is also important to distinguish between colonisation and significant infection prior to commencing antibiotic therapy as ESBL-producing organisms have a propensity to colonise the upper respiratory tract and skin of seriously ill patients.

Despite increased recognition of serious infections due to ESBL-producing organisms, there have been no randomised controlled trials on therapy of such infections nor are such studies likely to be performed in the near future. Therefore recommendations for optimal therapy are based on *in vitro* effectiveness of antimicrobial agents, case series and prospective observational studies.

[a] Cephalosporins

One of the major problems with cephalosporins and ESBL producers is detecting *in vitro* resistance. These organisms may appear susceptible at standard inoculum of 10^5 but at higher inoculums of 10^7 or 10^8 they have elevated MICs, indicating resistance. This inoculum effect is seen with 3rd generation cephalosporins such as ceftazidime, cefotaxime and ceftriaxone.

Clinical outcome is poor when 3rd generation cephalosporins are used to treat ESBL-producing organisms even in the presence of apparent susceptibility. 3rd generation cephalosporins should therefore not be used to treat serious infections with ESBL-producing organisms. Eventhough cefepime exhibits more stability to hydrolysis by ESBLs than the 3rd generation cephalosporins, a positive clinical outcome from treatment with this antibiotic has not been established. If cefepime is considered for treatment, MIC for these organisms should be determined. Like the 3rd generation cephalosporins, MICs for cefepime rise substantially when the inoculum of infecting organisms rises.

[b] b-lactam/b-lactamase inhibitor combinations

These antibiotics are also subject to rising MICs as the inoculum rises. In addition hyperproduction of β -lactamases or the combination of β -lactamases and porin loss can also lead to reduction in activity of β -lactam/ β -lactamase inhibitor combinations. In limited reports the mortality rates from infections due to ESBL producing organisms treated with β -lactam/ β -lactamase inhibitor combinations have been in excess of 50%.

[c] Aminoglycosides

Many of the ESBL producers are already gentamicin resistant due to co-transfer of aminoglycoside resistance on a resistance plasmid. Amikacin resistance may also be more common in ESBL-producing isolates.

[c] Fluoroquinolones

The fluoroquinolones may be used in the treatment of less severe infections due to ESBL-producing organisms e.g. urinary tract infections. Several reports have indicated a rise in *in vitro* resistance to fluoroquinolones in isolates, which are ESBL producers. The newer fluoroquinolones are unlikely to confer added benefits.

[d] Carbapenems

This class of drugs should be regarded as the drugs of choice based on *in vitro* susceptibility studies and clinical experience. The carbapenems are highly stable to betalactamase hydrolysis. Clinical observational studies have shown that the mortality rates in patients with ESBL-producing bacteraemia treated with the carbapenems are lower than if treated with other antibiotic combinations. There is no evidence that a combination therapy with an aminoglycoside is superior to monotherapy. Widespread use of carbapenem may however lead to emergence of carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Vancomycin resistant enterococci*.

Indications for treatment

1. Bacteraemia

- Treatment of choice : Carbapenem
- Second-line treatment : Fluoroquinolones

2. Nosocomial pneumonia

The diagnosis of nosocomial pneumonia can be problematic. The isolation of an ESBL-producing organism from a sputum sample or an endotracheal aspirate does not necessarily indicate that it is the cause of the pneumonia. In the absence of clinical signs such as fever, signs of consolidation or radiological changes, a positive culture may indicate colonisation and not require any antibiotic therapy.

In nosocomial pneumonia due to an ESBL-producing organism

- Treatment of choice : Carbapenem
- Second-line treatment : Fluoroquinolone

3. Intra-abdominal infection

- Treatment of choice : Carbapenem
- Second-line treatment : Fluoroquinolone

4. Urinary tract infection

Isolation of an ESBL-producing organism from catheter specimen urine in the absence of clinical symptoms or signs may also indicate colonisation and not warrant therapy. In the presence of symptoms

- Treatment of choice : Fluoroquinolone

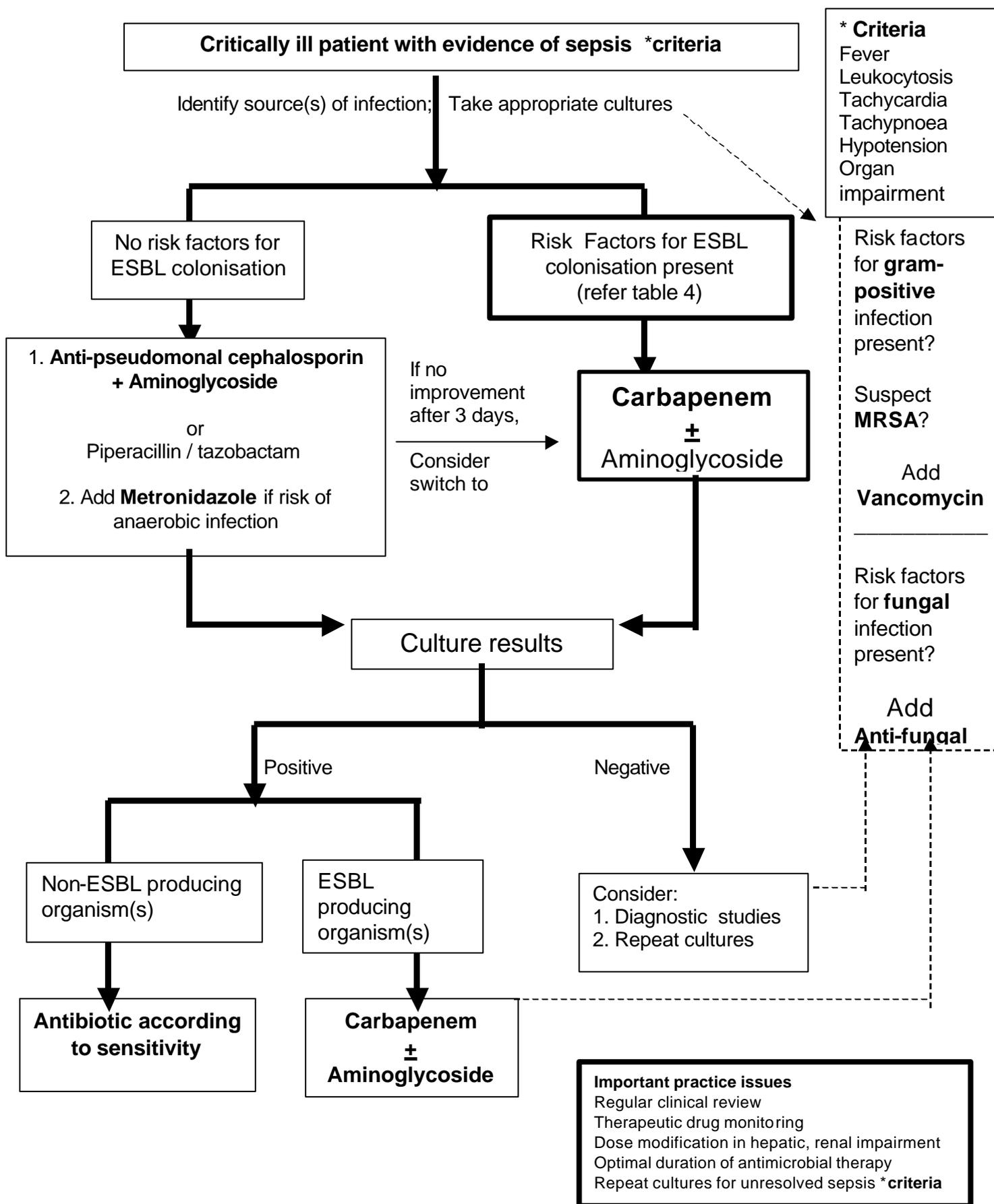
Chapter 5 (II): MANAGEMENT OF INFECTIONS BY ESBL-PRODUCING ORGANISMS IN THE INTENSIVE CARE UNIT (ICU)

Multiresistant infections in ICU patients are a major cause of morbidity, increased length of ICU admission and mortality. Several studies and surveillance programs indicate increasing rates of nosocomial infection, with the highest rates in ICU, from Methicillin resistant *Staphylococcus aureus* (MRSA), multiresistant *Pseudomonas aeruginosa*, *Acinetobacter* spp. and ESBL-producing *Enterobacteriaceae* (*Klebsiella pneumoniae* and *E. coli*).

Rates of ICU infection with ESBL-producing *Klebsiella pneumoniae* have ranged from 11-59%. In a multi-centre, prospective, observational study, Paterson *et al.* reported that 15% of 216 consecutive cases of *Klebsiella pneumoniae* bacteraemia were due to ESBL-producing organisms. 84% of ESBL-*Klebsiella pneumoniae* were hospital-acquired, of which 44% were acquired in the ICU. Sources of ESBL-*Klebsiella pneumoniae* included intravascular catheter infection (34%), pneumonia (28%) and intra-abdominal infection (19%). In the same study it was also noted that infections due to ESBL-*Klebsiella pneumoniae* were associated with higher mortality (46%) compared to those due to non-ESBL-*Klebsiella pneumoniae* (34%). Empiric therapy with antibiotics to which the ESBL-*Klebsiella pneumoniae* was resistant resulted in the highest mortality (75%), compared to the 28% death rate with antibiotics to which ESBL-*Klebsiella pneumoniae* was sensitive. The lowest mortality rate was seen when imipenem was used as empirical therapy (23% vs. 42% when other active antibiotics were used).

Based on current evidence we recommend that a critically ill patient with a high possibility of ESBL-*Klebsiella pneumoniae* infection should be treated empirically, following appropriate diagnostic cultures, with a carbapenem. Imipenem and meropenem are equally efficacious in their activity against ESBL-*Klebsiella pneumoniae*. The addition of an aminoglycoside can also be considered if clinically indicated. If a non-ESBL gram-negative infection is suspected, empiric therapy should consist of either an anti-pseudomonal cephalosporin in combination with an aminoglycoside or piperacillin-tazobactam. Metronidazole can be added to this regimen if there is risk of anaerobic infection. Other causes of infection, such as from MRSA or fungi should be diagnosed and treated accordingly.

If culture results confirm ESBL-bacterial infection, treatment should proceed with a carbapenem with aminoglycoside. If the infection is due to non-ESBL gram-negative bacteria, antibiotics should be adjusted according to the known sensitivities. Negative culture results in the presence of unresolved criteria of sepsis should prompt further diagnostic interventions and repeat cultures. It is also important to review the patient's clinical status regularly, achieve therapeutic drug levels by appropriate assays, modify drug doses in the presence of hepatic and renal impairment, stop antimicrobial therapy at the appropriate time, and institute further diagnostic interventions including repeat cultures for unresolved criteria of sepsis.



MANAGEMENT OF INFECTIONS CAUSED BY ESBL-PRODUCING ORGANISMS IN NEONATES

1) Epidemiology

The bacterial pathogens causing neonatal sepsis include both gram-positive organisms such as Group B *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. and gram-negative organisms such as *Klebsiella pneumoniae* and *E. coli*. In a recent Nosocomial Prevalence and Resistance Survey (NPRS) conducted from 1997 to 1998 involving three neonatal units in Malaysia, *E.coli* (29.4%), *Klebsiella* spp. (28.5%), *Pseudomonas* spp. (14.9%) and *Acinetobacter* spp. (12.1%) were the most common gram-negative organisms isolated ⁽¹⁾. Rates of ESBL production among *Klebsiella pneumoniae* and *E.coli* in neonates vary between countries, ranging from 11.8% -100%.^{1,2,3}

Infections caused by ESBL-producing organisms in neonates are usually hospital-acquired and these may involve the bloodstream, lungs, central nervous system and urine.^{4,5,6,7} NPRS data from 1997-1998 showed urine (31.8%), blood (22.7%) and respiratory tract(18.2%) were the most common sites where ESBL-producing organisms were isolated from neonates.¹

2) Management

No randomized controlled trials on therapy of infections in neonates caused by ESBL-producing organisms have so far been conducted. Therefore, recommendations for optimal therapy of these infections are based on *in vitro* antimicrobial susceptibility studies and small case series or observational studies.

Limited case series and observational studies involving neonates have documented the clinical efficacy of carbapenems in the treatment of infections caused by both ESBL and non-ESBL producing gram-negative bacteria.^{6,8,9,10} Although data is limited due to lack of clinical trials and there is good *in vitro* activity, a carbapenem such as imipenem or meropenem is recommended for treating ESBL infections in the neonate. Meropenem is preferred when these infections involve the central nervous system due to its lower seizurogenic potential.

Although there is *in vitro* activity of quinolones against ESBL-producing isolates causing infections in neonates, there is a lack of clinical data to guide treatment. Sporadic case reports suggest clinical efficacy with ciprofloxacin for nosocomial sepsis and meningitis.^{11,12,13} Ten out of 12 cases of nosocomial meningitis in babies receiving ciprofloxacin at dosages between 10-60 mg/kg/day were cured.¹² Pharmacokinetics of ciprofloxacin in intravenous dosages ranging from 4-40 mg/kg/day revealed adequate serum peak and cerebrospinal fluid (CSF) concentrations.¹³ Side effects were limited to dental dyschromia. In nine neonates given ciprofloxacin at 20mg/kg/day for multiresistant bacterial infections, no osteoarticular problems or joint deformities were observed during follow-up for 42 months.¹⁴ Ciprofloxacin should only be considered for therapy when there are no other alternative antibiotics that are active *in vitro* or there are severe adverse reactions with carbapenems.

Chapter 5 (IV): MANAGEMENT OF ESBL-PRODUCING BACTERIAL INFECTIONS IN NEUTROPENIC PATIENTS

Neutropenic patients are at high risk for various infections even if cultures of clinical specimens are not positive. Between 48% and 60 % of neutropenic patients who become febrile have an established or occult infection, and about 16% of patients with neutrophils counts of $< 100/\text{mm}^3$ have bacteraemia. Bacteremia is most frequently due to aerobic gram-positive cocci or aerobic gram-negative bacilli.¹

Gram negative bacteraemia is a leading cause of death in febrile neutropenic patients, accounting up to ~ 30% of deaths by infections in some series.² Several antibiotics regimens have been recommended by the Infectious Diseases Society of America for empiric treatment of fever in neutropenic patients. Among the regimens are combination of an antipseudomonal β -lactam and an aminoglycoside.¹ Hence, many cancer patients, especially those with leukemia who experience several episodes of neutropenia with fever during the typical year, received repeated and prolonged course of antibiotics. As a consequence, some specific susceptibility problems related to these changes are due to mainly the emergence of resistant enteric gram-negative bacteria.³

CLINICAL MANIFESTATIONS

Empirical administration of broad spectrum antibiotics is necessary for febrile neutropenia patients because the currently available diagnostic tests are not sufficiently rapid, sensitive, or specific for identifying or excluding the microbial cause of a febrile episode.¹

There have not been many reports on ESBL infection especially in the adult population. Most reports were on the paediatric patients. These reports in paediatric oncology patients showed relatively high incidence of ESBL producing bacteraemia; about 50% - 56% of all *Klebsiella pneumoniae* blood isolates and about 18% of *E.coli* isolates were inferred to be ESBL producers. The infections were deemed to be nosocomial in origin.^{5,6} In Ariffin *et. al.*, the risk factors of ESBL infection (again in blood specimens) were mainly related to the duration of hospital stay (2 weeks or longer) and prior use of third generation cephalosporin within 2 weeks of presentation.⁵ Similar findings were documented in Johnson *et. al.* where he compared the neutropenic and non-neutropenic adult patients with β -lactam resistant *Enterobacter* bacteraemia. The results showed that fewer non-neutropenic patients were infected with the ESBL producing *Enterobacter*. He attributed the findings to prior hospitalisation as well as prior β -lactam antibiotic exposure.⁷

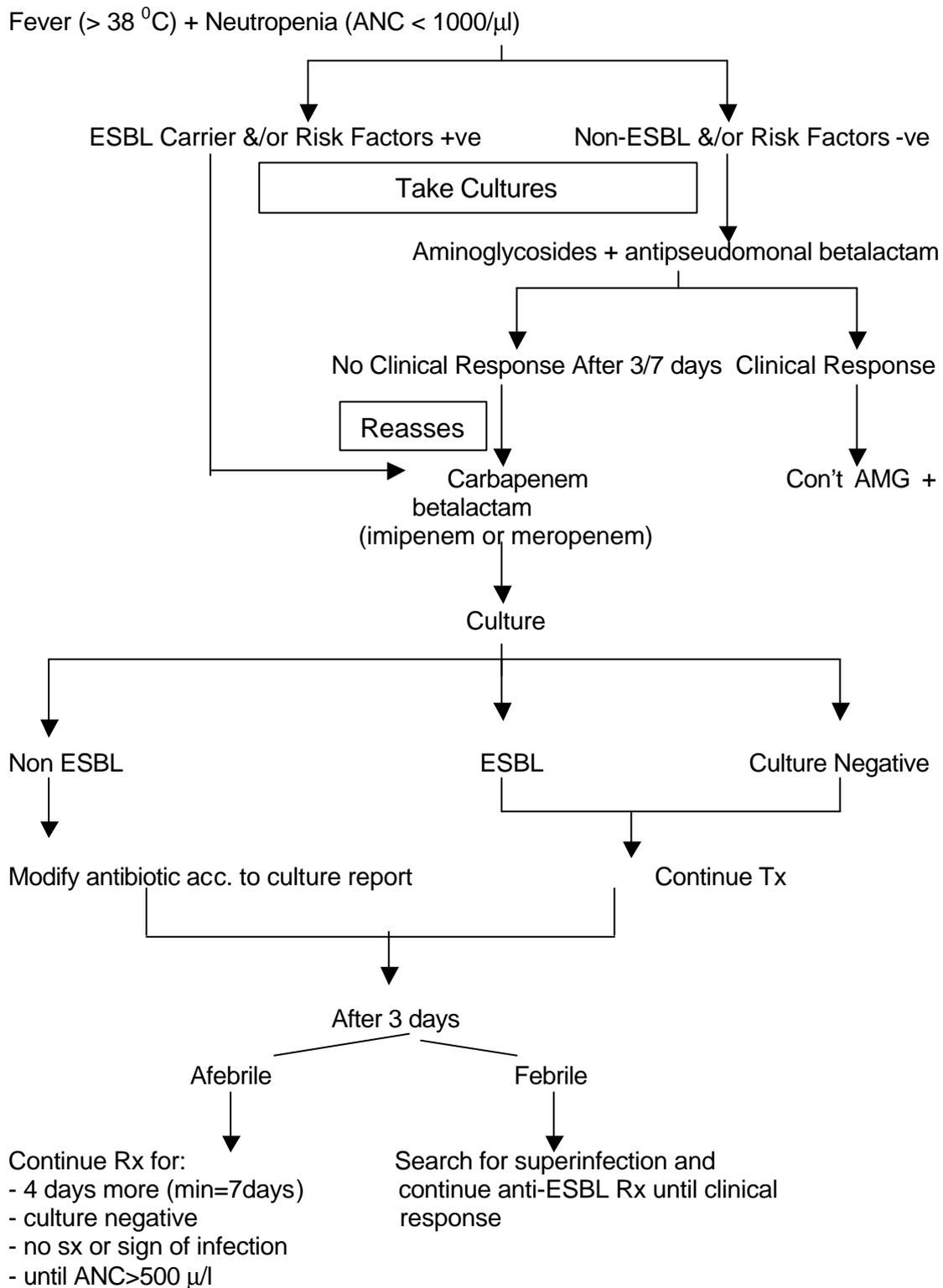
The mortality due to resistant organisms shows a higher trend in neutropenic patients but due to the relatively small population studied, it was not statistically significant.⁷ However, in Ariffin *et. al.*, the mortality was higher in patients with ESBL producing *Klebsiella pneumoniae*.⁵

Therefore, in a hospital or ward with high incidence of ESBL-producing bacteria, if symptomatic improvement is not seen during empirical treatment with a combination of an antipseudomonal β -lactam and an aminoglycoside, a change of antibiotic treatment regimen to include agents that are active against ESBL producers, e.g. a carbapenem, should be promptly considered.⁶

No randomised controlled trials have ever been performed to guide optimal treatment⁸ for infection caused by ESBL organisms. However, *in vitro* studies and observational studies strongly suggest that carbapenems should be regarded as drugs of choice for serious infections due to ESBL-producing organisms.

We recommend that screening for ESBL colonisers and identification of risk factors for ESBL colonisation/infection to be performed upon admission for all patients prior to administration of cytotoxic chemotherapy. The optimum duration of therapy has not been confirmed up to the present time. Similarly, the cut off time to justify change of antibiotics presumably due to antibiotic failure has not been defined. The alternative antibiotics that can be used if a carbapenem to combat the infection has not been studied. To date, there has been no randomized controlled trial performed that has addressed these several very important issues pertaining to the treatment of infection caused by ESBL producing organisms.

Figure 5: Algorithm for the management of adult neutropenic patients with infections caused by ESBL-producing organisms



(Clinical response includes : afebrile, stable vital signs, no new clinical signs)

Chapter 6: INFECTION CONTROL MEASURES IN MANAGING INFECTIONS CAUSED BY ESBL-PRODUCING BACTERIA

Surveillance

Laboratory-based surveillance^{1,2} should be conducted on a continuous basis to detect ESBL producing gram-negative bacteria among patients who have had cultures obtained for clinical reasons. If ESBL gram-negative bacteria are isolated from any sample, the ward should be informed promptly. Known ESBL cases at the time of readmission are identified via ESBL labels or a 'flag' be put in the facility's computer database that is accessible. Screening of patients in high risk units (ICUs and oncology wards)^{1,2} is optional after discussion with the microbiologist.

Preventing Cross-Infection

All the protocols that address preventing cross-infection^{1,3,4,5} emphasize that some degree of physical separation of patients infected with resistant gram-negative bacteria will decrease the risk of transmission of the resistant organisms. Therefore isolation precautions are recommended for both colonised and infected patients.

Controlling Antimicrobial Pressure

Antimicrobial committee in the hospital will provide guidelines for improving use of antimicrobial agents in hospital,^{2,6} thus minimizing the pressure for emergence of resistant bacteria among patients.

A. Procedures for patients infected / colonised by ESBL Producing Organisms

1. Tagging records/patients' cards
Attach ESBL identification label to medical records/ x-ray envelopes
2. Education
Develop a system to ensure that hospital patients, personnel, and visitors are educated about the use of precautions and their responsibility for adhering to them.
3. Contact Precautions
 - 3.1 If possible, attend to the patient **last** after dealing with all non-infected patients.
 - 3.2 Wash hands thoroughly with antiseptic (eg. Chlorhexidine or an alcohol rub) and dry **before** and **after** contact with the patient.
 - 3.3 If a nearby wash-basin is not available, preferably the patient should have a dedicated alcohol-chlorhexidine (Hibisol) container next to the bed.
 - 3.4 Wear gloves and aprons when handling or nursing the patient.
 - 3.5 Dispose of all clinical wastes in a colour-coded bag for incineration.
 - 3.7 Do not move between patients without decontaminating the hands, without removing protective clothing or with dirty equipment.

- 3.8 Keep all essential items of patient care in the cubicle with the patient. Alternatively, if separate equipment is not available, decontaminate reliably before using it on another patient

4. Additional Nursing Procedures

- 4.1 Clothes
Change all night clothes **daily** after body wash. Other disposable garments should be changed preferably daily.
- 4.2 Linen
Change all bed linen **daily**. Send to laundry in appropriate bags for "infected" linen.
- 4.3 Bedpan and urinals
 - 4.3.1 Provide a dedicated bedpan (if possible) for ESBL stool carriers.
 - 4.3.2 Heat-treat bedpans and urinals at 80°C by using bedpan disinfectant.
 - 4.3.3 Do not soak bedpans or urinals in disinfectant.
 - 4.3.4 Ensure that bedpans, urinals and bowls are stored clean, inverted and dry.
- 4.4 Urinary catheters
 - 4.4.1 Ensure that an aseptic procedure is used for insertion of catheters.
 - 4.4.2 Do not catheterize patients repeatedly.
 - 4.4.3 Empty the urinary drainage bag by the tap and wear disposable gloves while doing so. Do not break the circuit and reconnect.
 - 4.4.4 Use a separate jug or container for each patient when empty urinary drainage bags.

5. Transfer/transport to other hospital departments

Limit the moment and transport of patients to essential purpose only. If transfer is necessary, the receiving unit should be informed to ensure that precautions are maintained to minimize the risk of cross-contamination. Early discharge if possible, provided no medical contra-indication.

B. Control of an outbreak

In an event of an outbreak:

- Form Action Group: Infection Control Team -linked nurse, ward sister and patient's doctor.

- Review of: - Ward procedures, Ingested NG feeds

- Disinfectants & moist equipment that come in contact with one or more patients

- Inspect: Bedpan washer, medical equipment, gel & liquid, treatment room facilities

- Identify further asymptomatic colonized/ infected patients :
 - Screen other patients if possible, otherwise all patients in the affected unit are assumed to be colonised.

- Review the current nursing arrangement Hand hygiene practices

- Assess facilities available for isolation
 - e.g. adequate supply of disposable gloves, plastic aprons

- Consider need of restricting new admission

- Review of antibiotic policy of the affected wards

- ICT meet regularly with the ward staff to review the outbreak and results of screening

Chapter 7 : STRATEGIES FOR PREVENTION OF EMERGENCE OF ESBL-PRODUCING ORGANISMS

Several lines of evidence suggest that there is a causal association between antimicrobial usage in hospitals and antimicrobial resistance.^{1,2,3} These observations include :

- Antimicrobial resistance is more prevalent in nosocomial strains than in those from community-acquired infections.
- Patients infected with resistant strains are more likely than controls to have received prior antimicrobials
- Hospital areas that have the highest prevalence of resistance also have the highest rates of antibiotic use
- Changes in antimicrobial usage are paralleled by changes in the prevalence of resistance

Is there a link between specific antibiotic use and emergence of ESBLs ?

There is some evidence for this which includes :

- The extensive use of extended-spectrum penicillins and cephalosporins has correlated with the emergence of a variety of broad-spectrum beta-lactamases with different affinity to the various beta-lactams.^{4,5}
- In hospitals, betalactamase producing bacteria encounter simultaneous or consecutive selective pressure with different beta-lactam molecules and it is likely that ESBL variants in hospital isolates result from fluctuating selective pressure with several beta-lactams rather than with a single antibiotic.⁶ So it may be important to review the overall use of all beta-lactam antibiotics.
- Patients infected with ESBL producers often share heavy prior use of 3rd generation cephalosporins.⁷ Thus the empirical use of 3rd generation cephalosporins should be reviewed.

Strategies for the control of emergence of ESBL-producing organisms

Strategies for the control of emergence of antimicrobial resistance in general apply to ESBL-producing organisms.

These include ^{2,3}

- Optimal use of all antimicrobials in general. This will decrease resistance towards first and second-line antibiotics; thus decreasing the need to use more broad-spectrum antibiotics.
- Specific for ESBLs, selective removal, control, or restriction of antimicrobials or classes of antimicrobials e.g 3rd generation cephalosporins and making available alternatives.

- Rotational or cyclic antimicrobial use to reduce resistance to certain antibiotics is another strategy. However, further evidence is required from clinical trials.

Several reports have stated a reduction in occurrence of ESBL-producing organisms by replacing 3rd generation cephalosporins for widespread empirical use, either through formal restriction of availability or by education and increasing availability of alternatives.^{7,8,9} However, replacing the extended-spectrum β -lactams with imipenem or piperacillin-tazobactam may reduce the occurrence of ESBL-producing organisms but resulted with increasing resistance in *Pseudomonas aeruginosa* to imipenem or piperacillin-tazobactam. Thus, formulary changes will have to be made after careful considerations and their effects monitored.

Chapter 8: FUTURE DIRECTIONS

The prevalence of ESBL-producing organisms in Malaysian hospitals appear to be on the uptrend.^{1,2,3,4} Since ESBL-producing organisms often acquire additional mechanisms of resistance they can become resistant to many classes of antibiotics thus posing a formidable clinical challenge to clinicians.

It is far too simplistic to think of ESBL-producing organisms as a single entity. As shown in the classification of betalactamases there are now at least 119 ESBLs identified.⁵ These different ESBLs may have different substrate profiles hence different antibiotic susceptibility patterns. There is a need to better characterise the organisms such that a more precise identification of the enzyme involved can be obtained. Such studies require molecular methods and research projects on the characterisation of ESBLs in Malaysia should be undertaken.

At the moment the carbapenems appear to be the most reliable betalactam antibiotics to use for the treatment of ESBL-producing organisms. The currently available carbapenems in Malaysia are imipenem and meropenem but several new carbapenems are currently undergoing clinical trials.

There is a dearth of new anti-gram negative antimicrobials although some new fluoroquinolones and carbapenems (including oral agents) are in the pipeline. There has also been some developments in antimicrobial peptide compounds e.g. defensins.⁸ Attempts are also being made to develop new compounds through studying microbial genomes, identifying potential targets and synthesizing molecules that will attack these targets. Other strategies include chemical modifications of current compounds, developing potentiators of known antibiotics and even inhibitors of bacterial genes.⁹ Such compounds hold promise but it is highly unlikely that a new agent for ESBL-producing organisms will be available for general clinical use in the immediate future.

ESBL-producing organisms are carried in the gut and studies have suggested that the carriage even after discharge from hospitals can be prolonged. As colonisation precedes infection, is there a role for elimination of gut carriage? Selective decontamination of the digestive tract (SDD) using non-absorbable antibiotics like polymyxin has been used to eliminate gut carriage of aerobic gram-negative bacilli.¹⁰ However SDD is an expensive option and not without its drawbacks.

Strategies are also being developed to intervene directly at the process of sepsis, severe sepsis and septic shock.¹¹ A wide range of strategies with therapeutic potential (antiendotoxins, anticytokines, anticoagulants, NOS inhibitors, pentoxifylline, etc). Unfortunately none as yet has been shown to be effective.

There is an urgent need to improve infection control measures in Malaysian hospitals to reduce cross-infection of these resistant organisms. Education and behavior modification are the cornerstones of improving compliance with infection control measures. The possibility of using high-technology equipment e.g. sensors to warn of non-compliance with handwashing is also being studied.

In conclusion, treatment options for ESBL-producing organisms will remain limited in the near future. There is a need to better characterise strains to allow for maximum use of existing agents. There is a need to better define strategies to prevent emergence and more studies in this area are clearly required. Finally there is also a need to improve on infection control methods.

REFERENCES.

Chapter 1: Introduction to Betalactamases and ESBL.

1. Bush K. New beta-lactamases in gram-negative bacteria : diversity and impact on selection of antimicrobial therapy. *Clinical Infectious Diseases*. 32 : 1085-9, 2001 Apr
2. Bush K. Jacoby GA. Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents & Chemotherapy*. 39(6):1211-33, 1995 Jun
3. Ambler RP. Coulson AF. Frere JM. Ghuysen JM. Joris B. Forsman M. Levesque RC. Tiraby G. Waley SG. A standard numbering scheme for the class A beta-lactamases [letter]. *Biochemical Journal*. 276 (Pt 1):269-70, 1991
4. Minami S. Yotsuji A. Inoue M. Mitsunashi S. Induction of beta-lactamase by various beta-lactam antibiotics in *Enterobacter cloacae*. *Antimicrobial Agents & Chemotherapy*. 18(3):382-5, 1980 Sep.
5. Shannon K. Phillips I. The effects on beta-lactam susceptibility of phenotypic induction and genotypic derepression of beta-lactamase synthesis. *Journal of Antimicrobial Chemotherapy*. 18 Suppl E:15-22, 1986 Dec.
6. Sanders CC. Sanders WE Jr. beta-Lactam resistance in gram-negative bacteria: global trends and clinical impact. *Clinical Infectious Diseases*. 15(5):824-39, 1992 Nov
7. Sanders WE Jr. Tenney JH. Kessler RE. Efficacy of cefepime in the treatment of infections due to multiply resistant *Enterobacter* species. *Clinical Infectious Diseases*. 23(3):454-61, 1996 Sep.
8. Thomson KS. Prevan AM. Sanders CC. Novel plasmid-mediated beta-lactamases in enterobacteriaceae: emerging problems for new beta-lactam antibiotics. *Current Clinical Topics in Infectious Diseases*. 16:151-63,1996.
9. Sirot D. Chanal C. Henquell C. Labia R. Sirot J. Cluzel R. Clinical isolates of *Escherichia coli* producing multiple TEM mutants resistant to beta-lactamase inhibitors. *Journal of Antimicrobial Chemotherapy*. 33(6):1117-26, 1994 June
10. Livermore DM. Bacterial resistance to carbapenems. [Review] [80 refs] *Advances in Experimental Medicine & Biology*. 390:25-47, 1995.

Chapter 2: Epidemiology.

1. Ariffin H , Parasakthi N, Mahfuzah M , Arasu A , Ariffin WA , Chan LL , Lin HP . Ceftazidime-resistant *Klebsiella pneumoniae* bloodstream infection in children with febrile neutropenia . *Int J Infect Dis* 1999 ; 4 : 21 - 25
2. Parasakthi N , Vadivelu J , Ariffin H, Iyer L , Selvi P , Arasu A . Epidemiology and molecular characterisation of nosocomially-transmitted multi-resistant *Klebsiella pneumoniae* . *Int J Infect Dis* 2000 ; 4 : 123 - 8
3. Malaysia/Singapore Antimicrobial Resistance Study Group. Biedenbach et al. In vitro-evaluation of cefepime and other broad-spectrum beta-lactams for isolates in Malaysia and Singapore medical centres. *Diagn Microbiol Infect Dis* 1999 ; 35 : 277 - 83
4. Cheong YM, Lim VKE, Jegathesan M, Suleiman AB.(1994) Antimicrobial resistance in 6 Malaysian General Hospitals. *Med J Malaysia* 49 : 317-26
5. Rahizan I. *Int J Med Research* 1998 ; 2 : 93 – 5
6. Lewis MT. Biedenbach DJ. Jones RN. In vitro evaluation of cefepime and other broad-spectrum beta-lactams against bacteria from Indonesian medical centers. The Indonesia Antimicrobial Resistance Study Group. *Diagnostic Microbiology & Infectious Disease*. 35(4):285-90, 1999

7. Biedenbach DJ , Johnson DM , Jones RN . In vitro evaluation of cefepime and other broad-spectrum beta-lactams in Taiwan medical centres. *Diagnostic Microbiology & Infectious Disease* 1999 ; 35(4) : 299 - 305
8. Johnson DM , Biedenbach DJ , Jones RN . In vitro evaluation of broad-spectrum beta-lactams in the Philippines medical centres : role of fourth-generation cephalosporins. *Diagnostic Microbiology & Infectious Disease*. 1999 ; 35(4): 291- 7
9. Biedenbach DJ. Lewis MT. Jones RN. In vitro evaluation of cefepime and other broad-spectrum beta-lactams for isolates in Malaysia and Singapore medical centers. The Malaysia/Singapore Antimicrobial Resistance Study Group. *Diagnostic Microbiology & Infectious Disease*. 35(4):277-83, 1999 Dec
10. Lewis MT. Biedenbach DJ. Jones RN. In vitro evaluation of cefepime and other broad-spectrum beta-lactams against bacteria from Indonesian medical centers. The Indonesia Antimicrobial Resistance Study Group. *Diagnostic Microbiology & Infectious Disease*. 35(4):285-90, 1999 Dec

Chapter 3.Laboratory identification and guidelines on antibiotic susceptibility testing.

- 1). Coudron PE, Moland ES, Sanders CG. Occurrence and Detection of Extended Spectrum beta-lactamases in Members of the Family Enterobacteriaceae at a Veterans Medical Centre: Seek and You May Find. *J Clin Microbiology* 1997;35:2593-2597
- 2). NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; Eighth Informational Supplement. NCCLS document M100-s8. NCCLS, Wayne, PA 1998
- 3). Moland ES, Thompson KS. Extended-spectrum beta-lactamases of Enterobacteriaceae. *J Antimicrob Chemother* 1

Chapter 4 : Infections associated with ESBL-producing bacteria

1. Coudron PE, Moland ES, Sanders CC. Occurrence and detection of ESBL in members of the Enterobacteriaceae at a Veterans Medical Center :Seek and you may find. *J Clin Microbiology* 1997; 35: 2593-7
2. Wiener J, Quinn JP, Bradford PA. Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. *JAMA* 1999; 281:517- 23
3. Emery CI, Weymouth LA . Detection and clinical significance of ESBL in a tertiary care medical centre *Journal of Clinical Microbiology* 1997; 35: 2061-2067
4. Shiappa DA, Hayden MK, Matuhek MG. Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* blood stream infection: a case control and molecular epidemiologic investigation . *J Infect Dis* 1996; 174: 529- 36
5. De Champs C, Rouby D, Guelon D . A case control study of infections caused by *Klebsiella pneumoniae* strains producing CTX-1(TEM-3) beta lactamases *J Hosp Infect* 1991;18 : 5-13

Chapter 5 : Overview of treatment of infections due to ESBL-producing organisms

1. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). *Clin Microbiol Infect* 2000;6:460-463

2. Paterson JE. Problems in Gram-negative resistance:extended-spectrum beta-lactamases. In *Emerging Pathogens: Implications for the Future*.Ed Moellering RC.Pharmaceutical 2000: 33-50
3. Paterson DL, Yu VL. Extended-spectrum beta-lactamases a call for improved detection and control. *Clin Infect Dis* 1999;29:1419-22
4. Karas JA, Pillay DG, Muckart D, Sturm AW. Treatment failure due to extended-spectrum beta-lactamases. *J Antimicrob Chemother.*1996;37:203-204

[b] Intensive Care Unit

1. Rahal K, Wang F, Schindler J, Rowe B, Cookson B, Huovinen P, Marton A, Lalitha M K, Semina N, Kronvall G, Guzman M. Reports on surveillance of antimicrobial resistance in individual countries. *Clin Infect Dis* 1997; **24(Suppl)**: S169-S175. [III]
2. Jones R N. Summation: Beta-lactam resistance. Surveillance in the Asia-Western Pacific Region. *Diagn Microbiol Infect Dis* 1999; **35**: 333-338. [III]
3. Lee K, Lee H S, Jang S J, Park A J, Lee M H, Song W K, Chong Y, Members of Korean Nationwide Surveillance of Antimicrobial Resistance Group. Antimicrobial resistance surveillance of bacteria in 1999 in Korea with a special reference to resistance of enterococci to vancomycin and gram-negative bacilli to third generation cephalosporin, imipenem and fluoroquinolone. *J Korean Med Sci.* 2001; **16**: 262-70. [III]
4. Arakawa Y, Ike Y, Nagasawa M, Shibata N, Doi Y, Shibayama K, Yagi T, Kurata T. Trends in antimicrobial-drug resistance in Japan. *Emerg. Infect. Dis* 2000; **6(6)**: 72-75.
5. Ariffin H, Navaratnam P, Mohamed M, Arasu A, Abdullah W A, Chan L L, Lin H P. Ceftazidime-resistant *Klebsiella pneumoniae* bloodstream infection in children with febrile neutropenia. *Int J Infect Dis* 1999; **4**: 21-25.
6. Paterson D L, Mulazimoglu L, Casellas J M, Ko W C, Goossens H, von Gottberg A, Mohapatra S, Trenholme G M, Klugman K P, McCormack J G, Yu V L. Epidemiology of ciprofloxacin resistance and its relationship to extended spectrum beta lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000; **30**: 473-478.
7. Paterson D L. Recommendation for treatment of severe infections caused by enterobacteriaceae producing extended-spectrum-beta-lactamases (ESBLs). *Clin Microbiol Infect.* 2000 (Sep); **6(9)**: 460-463.
8. Jones R N. Detection of emerging resistance patterns within longitudinal surveillance systems: data sensitivity and microbial susceptibility. MYSTIC Advisory Board. Meropenem Yearly Susceptibility Test Information Collection. *J Antimicrob Chemother.* 2000 (Sep); **46 (Suppl T2)**: 1-8.
9. Hospital Infections Program , National Centre for Infectious Diseases , Centres for Disease Control. Intensive Care Antimicrobial resistance Epidemiology (ICARE) surveillance report . *Am J Infection Control* 1999;27:279-284

[c] Neonatal patients

1. Nosocomial Prevalence and Resistance Survey 1997-1998. (AHCPR Level III)
2. Pillay T, Pillay DG, Adhikari M, Sturm AW. Piperacillin/tazobactam in the treatment of *Klebsiella pneumoniae* infections in neonates. *Am J Perinatol* 1998;15:47-51. (AHCPR Level III)

3. Wojsyk-Banaszak I, Szumala-Kakol A, Szczapa J, Gadzinowski J. Epidemiology of multiresistant *Klebsiella pneumoniae* infections in neonates. *Clin Microbiol Infect* 2000;6:460-63. (AHCPR Level III)
4. Roilides E, Kyriakides G, Kaditsoglou I, Farmaki E, Venzon D, Katsaveli A et al. Septicemia due to multiresistant *Klebsiella pneumoniae* in a neonatal unit: A case-control study. *Am J Perinatol* 2000;17:35-39. (AHCPR Level III)
5. Royle R, Halasz S, Eagles G, Gilbert G, Dalton D, Jelfs P et al. Outbreak of extended spectrum β lactamase producing *Klebsiella pneumoniae* in a neonatal unit. *Arch Dis Child Fetal Neonatal Ed* 1999;80:F64-F68. (AHCPR Level III)
6. Godula-Stuglik U, Mikusz G. Clinical efficacy of meropenem in the management of severe nosocomial infections in neonates. *J Antimicrob Chemother* 1999;44(Suppl):63-4, Abs P106. (AHCPR Level III)
7. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs). *Clin Microbiol Infect* 2000;6:460-3. (AHCPR Level IV)
8. Koksall N, Hacimustafaoglu M, Bagci S, Celebi S. Meropenem in neonatal severe infections due to a multiresistant gram-negative bacteria. *Indian J Pediatr* 2001;68:15-9. (AHCPR Level III)
9. Oral R, Akisu M, Kultursay N, Vardar F, Tansug N. Neonatal *Klebsiella pneumoniae* sepsis and imipenem/cilastatin. *Indian J Pediatr* 1998;65:121-9. (AHCPR Level IIa)
10. Boswald M, Dobig C, Kandler C, Kruger C, Scharf J, Soergel F et al. Pharmacokinetics and clinical evaluation of serious infections in premature and newborn infants under therapy with imipenem/cilastatin. *Infection* 1999;27:299-304. (AHCPR Level III)
11. Khaneja M, Naprawa J, Kumar A, Piecuch S. Successful treatment of late-onset infection due to resistant *Klebsiella pneumoniae* in an extremely low birth weight infant using ciprofloxacin. *J Perinatol* 1999;19:311-4. (AHCPR Level III)
12. Krcmery V, Filka J, Uher J, Kurak H, Sagat T, Tuharsky J et al. Ciprofloxacin in treatment of nosocomial meningitis in neonates and in infants: report of 12 cases and review. *Diagn Microbiol Infect Dis* 1999;35:75-80. (AHCPR Level III)
13. van den Oever HL, Versteegh FG, Thewessen EA, van den Anker JN, Mouton JW, Neijens HJ. Ciprofloxacin in preterm neonates: case report and review of the literature. *Eur J Pediatr* 1998;157:843-5. (AHCPR Level III)
14. Gurpinar AN, Balkan E, Kilic N, Kiristioglu I, Dogruyol H. The effects of a fluoroquinolone on the growth and development of infants. *J Int Med Res* 1997;25:302-6. (AHCPR Level III)

[d] Neutropenic patients

1. Hughes WT, Armstrong D, Bodey G, et al. 1997 Guidelines for The Use of Antimicrobial Agents in Neutropenic Patients with Unexplained fever. *Clin Infect Dis* 1997; 25:551-73 **[IV]**
2. Levin AS, Schimpff SC, Gaw RG Jr, Young Rc. Hematologic Malignancies and other marrow failure states: progress in the management of complicating infections. *Sem Hematol* 1974;11:141-202 **[IV]**
3. Ramphal R, Guclap R, Rotstein C, Cimino M, Oblon D. Clinical Experience with Single Agent and Combination Regimens in the Management of Infection in the Febrile Neutropenic Patient. *Am J Med.* 1996;100 (suppl 6A) 83S-89S **[Ib]**

4. Fraimow HS, Abrutyn E. Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanism, and clinical management. *Infect Dis Clin North Am* 1995;9:497-530 [IV]
5. Ariffin H, Paraksathi N, Mohamed M, Arasu A, Abdullah WA, Chan LL, Lin HP. Ceftazidime-Resistant *Klebsiella pneumoniae* Bloodstream Infection in Children with Febrile Neutropenia. *Int J Infect Dis.* 1999;4:21-25 [III]
6. Siu LK, Lu PL, Hsueh PR, Lin FM, Chang SC, Luh KT, Ho M, Lee CY. Bacteremia Due to Extended-Spectrum β -Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in a Pediatric Oncology Ward: Clinical Features and Identification of Different Plasmids Carrying both SHV-5 and TEM-1 Genes . *J Clin Microbiology* 1999;37(12): 4020-4027 [III]
7. Johson MP, Ramphal R. β -Lactam-Resistant *Enterobacter* Bacteremia in Febrile Neutropenic Patients Receiving Monotherapy. *J Infect Dis* 1990;162:981-983 [III]
8. Paterson DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum β -lactamases(ESBLs). *Clin Microbiol Infect* 2000;6:460-3. (AHCPR Level IV)

Adult neutropenic patients

1. Urban, C. An overview on Extended-Spectrum Beta-Lactamases. *Guest Editorial.* 3-5 [Ia].
2. Katsanis, GP., Spargo, J., Ferraro, MJ., Sutton, L., and GA. Jacoby. Detection of *Klebsiella pneumoniae* and *Escherichia coli* strains producing extended-spectrum beta-lactamases. *J. Clin. Microbiol.* 1994; **32**: 691-696[Ia]
3. Meyer, KS., Urbaan, C., Eagan, J., Berger, BJ., and JJ. Rahal. Nosocomial outbreak of *Klebsiella* infection resistant to late generation cephalosporins. *Annals Intern. Med.* 1993; **119**: 353-358 [Ia].
4. Naumovski, L., Quinn, JP., Miyashiro, D., Patel, M., Bush, K., Singer, SB., et al. Outbreak of ceftazidime resistance due to a novel extended-spectrum beta-lactamase in isolates from cancer patients. *Antimicrob. Agents Chemother.* 1992; **36**: 1991-1996 [Ia].
5. Paterson, DL. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). *Clin. Microbiol. Infect.* 2000; **6(9)**: 460-463 [Ib].
6. Essack. SY. Treatment options for extended-spectrum beta-lactamase-producers. *FEMS Microbiol. Lett.* 2000; **190(2)**: 181-184 [Ib].
7. Sirot. D. Extended-spectrum plasmid-mediated beta-lactamases. *J. Antimicrob. Chemother.* 1995; **36 Suppl A**: 19-34 [Ib].
8. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, Mohapatra S, Trenholme GM, Klugman KP, McCormack JG, Yu VL. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin. Infect.* 2000; **30(3)**: 473-478 [Ib].
9. Vedel, G.A., Belaouaj, A., Gilly, L., Labia, R., Philippon, A., Nevot, P., and G. Paul. Clinical isolates of *Escherichia coli* producing TRI beta-lactamase: novel TEM enzymes conferring resistance to beta-lactamase inhibitors. *J. Antimicrob. Chemothe.* 1992; **30**: 449-462 [Ia].
10. Zhou, XY., Bordon, F., Sirot, D., Kitzis, MD., and L. Gutmann. Emergence of clinical isolates of *Escherichia coli* producing TEM-1 derivatives or an OXA-1 beta-lactamase conferring resistance to beta-lactamase inhibitors. *Antimicrob. Agents Chemother.* 1994; **38**: 1085-1089 [Ia].

11. Urban, C., Mariano, N, Mosinka-Snipas, K., Jaiswal, A., Spinucci, C., and JJ. Rahal. In vitro evaluation of antimicrobial agents against ceftazidime-resistant *Klebsiella pneumoniae*. 34th ICAAC, Orlando, FL., 1994; Abstract #E57 [IIa].
12. Brook, I. Inoculum effect. *Rev. Infect. Dis.* 1989; **2**: 361-368 [IIb].
13. Ito, H., Arakawa, Y. Oshuka, S., Wacharo, TR., Kato, N., and M. Ohta. Plasmid-mediated dissemination of the metallo-beta-lactamase gene BLA_{imp} among clinically isolated strains of *Serratia marcescens*. *Antimicrob Agents Chemother* 1995; **39**: 824-829 [IIb].
14. Watanabe, M., Iyobe, S., Inoue, M., and S. Mitsuhashi. Transferable imipenem resistance in *pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991; **35**: 447-451 [IIb].
15. Martinez-Martinez L, Pascual A, Suarez AI, et al. Resistance to carbapenems in porin-deficient *Klebsiella pneumoniae* (Kp) mediated by plasmid-encoded AmpC β -lactamases. In: Abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 28-October 1, 1997; Toronto, Ontario. Abstract C-94 [IIa].
16. Jan E. Patterson, MD. Extended-spectrum β -lactamases. Successful interventions for gram-negative resistance-therapy, control, and prevention. Emerging pathogens in infectious disease. *A hospital practise special report*. 22-27 [Ib].
17. Bingen EH, Desjardins P, Arlet G, et al. Molecular epidemiology of plasmid spread among extended broad-spectrum β -lactamase-producing *Klebsiella pneumoniae* isolates in a pediatric hospital. *J Clin Microbiol* 1993;**31(2)**: 179-184 [IIa].
18. Gori A, Espinasse F, Deplano A, et al. Comparison of pulsed-field gel electrophoresis and randomly amplified DNA polymorphism analysis for typing extended-spectrum- β -lactamase-producing *Klebsiella pneumoniae*. *J Clin Microbiol* 1996; **34(10)**: 2448-2453 [IIa].
19. Monnet DL, Biddle JW, Edwardds JR, et al. Evidence of interhospital transmission of extended-spectrum β -lactam-resistant *Klebsiella pneumoniae* in the United States, 1986 to 1993. *Infect Control Hosp Epidemiol* 1997; **18(7)**: 494-498 [Ia].
20. Rice LB, Eckstein EC, DeVente J, et al. Ceftazidime-resistant *Klebsiella pneumoniae* isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. *Clin Infect Dis* 1996; **23(1)**: 118-124 [IIa].
21. Bradford PA, Cherubin CE, Idemyor V, et al. Multiply resistant *Klebsiella pneumoniae* strains from two Chicago hospitals: identification of the extended-spectrum TEM-12 and TEM-10 ceftazidime-hydrolyzing β -lactamases in a single isolate. *Antimicrob Agents Chemother* 1994; **38(4)**: 761-766 [Ib].
22. Jacoby GA. Epidemiology of extended-spectrum β -lactamases. *Clin Infect Dis* 1998; **27(1)**: 76-80 [Ib].
23. Kenneth S. Meyer, MD; Carl Urban, PHD; Janet A. Eagan, BS, RN; Barbara J. Berger, MD; and James J. Rahal, MD. 1993. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation Cephalosporins. *Ann Intern Med*. **119**: 353-358 [Ib].
24. Roussel-Delvallez M, Sirot D, Berrouana Y, et al. Bactericidal effects of β -lactams and amikacin alone or in association against *Klebsiella pneumoniae* producing extended-spectrum β -lactamases. *J Antimicrob Chemother* 1995; **36(1)**: 241-246.

Chapter 6 : Infection control measures.

1. Weinstein RA, Hayden MK. Multiply drug resistant pathogens: Epidemiology and control. In Bennett JV, Brachman PS (eds): Hospital Infections. Philadelphia: Lippincott, Williams & Wilkins, 1998;215-236.

2. Goldmann DA, Weinstein RA, Wenzel RP, et al. Strategies to prevent and control the emergence and spread of antimicrobial resistant microorganisms in hospitals. A challenge to hospital leadership. *JAMA* 1996;275:234-240.
3. Tolzis P, Blumer JL. Antibiotic-resistant gram-negative bacteria in the critical care setting. *Pediatr Clin North Am* 1995;42:687-702.
4. Society for Healthcare Epidemiology of America and Infectious Disease Society of America Joint Committee on the Prevention of Antimicrobial Resistance: Guidelines for the prevention of antimicrobial resistance in hospitals. *Infect Control Hosp Epidemiol* 1997;18:275-291.
5. Garner JS. The Hospital Infection Control Practices Advisory Committee: Guideline for isolation precautions in hospitals. *Infect Control Hosp Epidemiol* 1996;17:53-80.
6. McGowan JE, Jr. Do intensive hospital antibiotic control programs prevent the spread of antibiotic resistant? *Infect Control Hosp Epidemiol* 1994;5:478-483.

Chapter 7 : Strategies for the prevention of emergence of ESBL-producing organisms.

1. McGowan JE Jr. Antibiotic resistance in hospitals organisms and its relation to antibiotic use. *Rev Infect Dis* 1983; 5: 1033-48.
2. Weber DJ, Raasch R; Rutala WA. Nosocomial Infections in the ICU: the growing problem of antibiotic resistant pathogens. *Chest* 1999; 115(suppl): 34S-41S
3. Society for healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: Guidelines for the Prevention of Antimicrobial Resistance in Hospitals. Shales DM, Gerding DN, John Jr JF, et al. *Clin Infect Dis* 1997; 25: 584-99.
4. K Bush, The evolution of beta lactamases. *Ciba Found Symp* 1997; 207:152-63.
5. Antimicrobial resistance in Enterobacteriaceae in Brooklyn: epidemiology and relation to antibiotic useage pattern, Saurina G, Quale JM, Manikal VM, Oydna E, Landman D. *J Antimicrob Chemother* 2000; 45: 895-8.
6. Blazquez J, Morosini MI, Negri MC, Baquero F. Selection of naturally occurring extended-spectrum TEM beta-lactamase variants by fluctuating beta-lactam pressure. *Antimicrob Agents Chemother* 2000; 44:2182-4.
7. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, Mohapatra S, Trenholme GM, Klugman KP, McCormack JG, Yu VL. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin. Infect.* 2000; 30(3): 473-478 [lb].
8. Rice LB, Eckstein EC, DeVente J, et al. Ceftazidime-resistant *Klebsiella pneumoniae* isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. *Clin infect Dis* 1996; 23(1): 118-124 [IIa].
9. Rahal JJ, Urban C, Horn D, Freeman K, Segal-Maurer S, Maurer J, Mariano N, Marks S, Burns JM, Dominick D, Lim M. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella* *JAMA*. 280(14):1233-7, 1998

Chapter 8 : Future directions.

1. Ariffin H , Parasakthi N, Mahfuzah M , Arasu A , Ariffin WA , Chan LL , Lin HP . Ceftazidime-resistant *Klebsiella pneumoniae* bloodstream infection in children with febrile neutropenia . *Int J Infect Dis* 1999 ; 4 : 21 - 25
2. Parasakthi N , Vadivelu J , Ariffin H , Iyer L , Selvi P , Arasu A . Epidemiology and molecular characterisation of nosocomially-transmitted multi-resistant *Klebsiella pneumoniae* . *Int J Infect Dis* 2000 ; 4 : 123 - 8
3. 3. Malaysia/Singapore Antimicrobial Resistance Study Group. Biedenbach et al. In vitro-evaluation of cefepime and other broad-spectrum beta-lactams for isolates in Malaysia and Singapore medical centres. *Diagn Microbiol Infect Dis* 1999 ; 35 : 277 - 83

4. Cheong YM, Lim VKE, Jegathesan M, Suleiman AB.(1994) Antimicrobial resistance in 6 Malaysian General Hospitals. *Med J Malaysia* 49 : 317-26
5. Bush K. (2001) New beta-lactamases in gram-negative bacteria : diversity and impact on selection of antimicrobial therapy. *Clinical Infectious Diseases*. 32 : 1085-9
6. Alarcon T. de la Obra P. Lopez-Hernandez S. de las Cuevas C. Lopez-Brea M. [In vitro activity of piperacillin-tazobactam against *Klebsiella pneumoniae* clinical isolates, producers or not of extended spectrum beta-lactamases]. [Spanish] *Revista Espanola de Quimioterapia*. 12(3):229-33, 1999
7. Elkhaili H. Kamili N. Linger L. Leveque D. Pompei D. Monteil H. Jehl F. In vitro time-kill curves of cefepime and ceftazidime combined with amikacin, gentamicin or ciprofloxacin against *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase. *Chemotherapy*. 43(4):245-53, 1997
8. Bevins CL. Martin-Porter E. Ganz T. Defensins and innate host defence of the gastrointestinal tract. *Gut*. 45(6):911-5, 1999
9. Moellering RC. Strategies to discover novel agents for bacterial infections. Proceedings of the 3rd International Symposium on Antimicrobial Agents and Resistance, 12-13 April 2001, pp 383-86.
10. Silvestri L. Mannucci F. van Saene HK. Selective decontamination of the digestive tract: a life saver. *Journal of Hospital Infection*. 45(3):185-90, 2000
11. Glauser MP. Pathophysiologic basis of sepsis: considerations for future strategies of intervention. *Critical Care Medicine*. 28(9 Suppl):S4-8, 2000
12. Rahal JJ. Urban C. Horn D. Freeman K. Segal-Maurer S. Maurer J. Mariano N. Marks S. Burns JM. Dominick D. Lim M. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella* *JAMA*. 280(14):1233-7, 1998