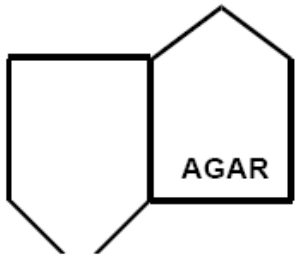


The Australian Group on Antimicrobial Resistance

Gram-negative Study

Survey 8 - 2006

Introduction

AGAR commenced surveillance of *Escherichia coli* and *Klebsiella* species in 1992. Surveys have been conducted biennially since then. In 2000, *Enterobacter* species was included. Custom made broth microdilution panels MicroScan® prepared by Dade Behring have been used, with three panel formats (1992, 1994; 1996-1998; and 2000-2004). For the 2006 study, the Vitek 2, an automated susceptibility testing system enabling rapid (4-7 h) determinations of MICs by the analysis of growth kinetics of bacteria with antibiotics in test cards, will be utilized.

Objectives

The objectives of this study are:

1. Determine the proportion of resistance to commonly used antibiotics in *E. coli*, *Klebsiella* species, *Enterobacter* species and *Acinetobacter* species in Australian institutions.
2. Examine the extent of resistance and multi-resistance in these species
3. Detect emerging resistance to newer agents such as carbapenems and tigecycline

Particular emphasis on detection and of extended-spectrum β-lactamases (TEM, SHV, CTX-M), plasmid-borne AmpC, and carbapenemases will be made.

Study Period

1st July 2006 to 31st December 2006

Status

Compulsory study

Collection of Isolates

Each participating laboratory is to collect **up to** 25 *E. coli*, 25 *Klebsiella* species, 25 *Enterobacter* species and 25 *Acinetobacter* (non *A. lwoffii*) species from different patients.

Specimen Source

Only one specimen type per patient (both inpatients and outpatients allowed). No repeat isolates to be included from any patient unless a significantly different antibiogram is observed from routine susceptibility test results.

- For *E. coli* and *Klebsiella* spp. the following restrictions apply:

Source	maximum number
Blood	no limit
Sterile body sites*	no limit
Cathether urine	<=5
Non-cathether urine	<=7
Tracheal aspirate	<=5
Wounds/pus	<=5
Expectorated sputum	<=2
Other sites	<=1

* pleural fluid, CSF, joint fluid, peritoneal dialysate

- For *Enterobacter* spp. and *Acinetobacter* spp. collect all consecutive, non-repeat, clinically significant isolates

Identification of Isolates

1. Identification. One of the following methods must be used.

E. coli

API20E
Vitek® or Phoenix™ Automated Microbiology System
MicroScan
ATB®
Chromogenic agar plus spot indole (DMACA) (urinary tract isolates)
Agar replication
Minimum tests for urine isolates: BGA or citrate, indole, & lactose fermentation

Klebsiella spp., and *Enterobacter* spp. - all isolates must be speciated

API20E
MicroScan
Vitek® (plus indole) or Phoenix™ Automated Microbiology System
ATB®
Chromogenic agar plus spot indole (DMACA) (urinary tract isolates)
Agar replication

Acinetobacter spp. (saccharolytic) – non *A. lwoffi* (non-saccharolytic)

API20NE
MicroScan
Vitek® or Phoenix™ Automated Microbiology System
Agar replication
Browning effect on Columbia Horse Blood D-glucose Agar,[1] refer to Appendix 1.

Information Required

The following information is required for each isolate

a) Your reference number
b) Date of collection
c) Specimen source
    i) Blood
    ii) Sterile body site (pleural fluid, CSF, joint fluid, peritoneal dialysate)
    iii) Catheter urine
    iv) Non-catheter urine
    v) Tracheal aspirate. Note: Do not include nasopharyngeal aspirates
    vi) Wound/pus
    vii) Expectorated sputum
    viii) Other – specify

Antimicrobial Susceptibility Testing

1. All isolates will be tested using the Vitek 2 AST-N044 card.[2]
2. The Advanced Expert System (AES) interprets MICs generated by the Vitek 2 according to CLSI guidelines[3]
3. To minimise data entry errors, the Vitek 2 MIC data is extracted via the logbook report to a txt file format that can be readily converted into the current AGAR Data Entry Excel File. Procedure involved is outlined in Appendix 3.

Additional susceptibility tests

All isolates will be tested against:

1. Tigecycline Etest [Etest strips will be supplied to each laboratory by Wyeth Australia]
2. colistin 10 µg (as sulphate) disc set up according to CLSI guidelines4
    a. Incubate Enterobacteriaceae for 16-18 h and *Acinetobacter* spp for 20-24 h at 35°C
    b. Quality control: ATCC 25922
        Acceptable quality control range 13 – 16 mm 5
        (note: not same range as quoted as M100-S16)
    c. any zone diameter <13 mm will be referred to the central testing laboratory
3. Test both tigecycline and colistin on one MHA plate as indicated in Appendix 4

ESBL Confirmatory Tests

Any isolate that the Vitek AES flags with ESBL phenotype should be confirmed using commercial combination discs or Etest® strips.

E. coli and *Klebsiella* spp. must be tested with both ceftazidime ± clavulanate (Oxoid, BioRad, BD, Rosco, Etest®) and cefotaxime ± clavulanate (Oxoid, BioRad, BD, Etest®).

Enterobacter spp. must be tested with cefepime ± clavulanate (BioRad, Rosco, Etest®) or cefpirome ± clavulanate (Oxoid)

AmpC

Any *E. coli* or *Klebsiella* spp. that is cefoxitin resistant will be screened for plasmid-borne AmpC β-lactamase by a central laboratory and confirmed by molecular techniques. Laboratories wishing to screen their own isolates may use either the boronic acid6,7,8 or AmpC9 disk tests.

Storage of Isolates

All isolates must be stored at -70^{o}C or below until determined otherwise by the AGAR committee.

Referral of Isolates

All possible ESBL, AmpC or isolates with elevated meropenem MIC > 0.5mg/L will be forwarded to a central laboratory for further testing and analysis using molecular techniques at the conclusion of the study. The isolates to be referred will be identified by the AGAR Scientific Officer, who will contact each laboratory.

Data analysis

1. All raw MIC results are to be transferred to the Excel Data Entry file AGAR GNB06.xls as indicated in Appendix 3. All other information must be completed as indicated
2. Zone diameters (to the nearest whole mm) must be recorded
3. Include all QC results performed during the survey period
4. Check the file for transcription errors
5. Forwarded onto Julie Pearson (julie.pearson@health.wa.gov.au) as soon as testing is complete

Appendix 1. Browning Effect

Purpose

To identify glucose-oxidizing acinetobacters on Blood Agar in the presence of D-glucose by unique browning effect.

Principle

Acinetobacters do not possess unique biochemical characteristics to allow for unambiguous phenotypic identification. The genus is characterized by properties it does not possess, i.e., being oxidase negative, non-motile, and non-fermentative.
Glucose-oxidizing acinetobacters are able to cause a unique brown discoloration of blood agar into which glucose is incorporated.

Specimen

Any isolated pure single colony on primary or subculture plates

Medium

Columbia Agar Base with 5% defibrinated sheep, or horse blood, in the presence of 0.22M (4% wt/vol) D-glucose (CBG)

Procedure

1. Streak a colony across the Columbia Base + 5% sheep blood + 4% D-glucose Agar
2. Inoculate both positive and negative control strains whenever test is performed
3. Incubate at 35°C for 24 h

Interpretation

Positive reaction: light-brown discoloration of the surrounding agar
Negative reaction: no discoloration

Columbia Sheep Blood Agar + 4% glucose

Columbia Horse Blood Agar + 4% glucose

Quality Control

Acinetobacter baumannii = positive

Pseudomonas aeruginosa = negative

Acinetobacter lwoffii = negative

Appendix 2. Vitek2

Card: AST-N044

Inoculum: as per manufacturer's guidelines[2]

Incubation: as per manufacturer's guidelines

Quality Control: *E. coli* ATCC 25922
E. coli ATCC 35218
K. pneumoniae ATCC 400603 (ESBL producer SHV-18)

Antibiotics:

Antibiotic	Concentrations	Useful for detection of:
Amikacin	8, 16, 64	
Amoxycillin-clavulanate	4, 16, 32	
Ampicillin	4, 8, 32	
Cefazolin	4, 16, 64	
Cefepime	2, 8, 16, 32	ESBL Enterobacter
Cefoxitin	8, 16, 32	Plasmid-borne AmpC
Ceftazidime	1, 2, 8, 32	ESBL
Ceftriaxone	1, 2, 8, 32	ESBL
Cephalothin	2, 8, 32	
Ciprofloxacin	0.5, 2, 4	
Gentamicin	4, 16, 32	
Meropenem	0.5, 4, 16	carbapenemase
Nalidixic acid	8, 16, 32	
Nitrofurantoin	16, 32, 64	
Norfloxacin	1, 8, 32	
Piperacillin-tazobactam	4, 16, 128	
Ticarcillin-calvulanate	8, 32, 64	
Tobramycin	8, 16, 64	
Trimethoprim-sulfamethoxazole	0.5, 2, 16	
Trimethoprim	0.5, 2, 8	

Appendix 3. Extraction of data from the Vitek2 AST-N044 card

If possible, use the same reference number as entered into the Excel data file (eg 3SVH1). This will help in the logbook report.

When you require data to be converted:

1. run the Vitek 2 'logbook report'. Depending on how you have entered the request, and when it was performed, the report can be generated for a particular collection date, or by the examID field (eg contains 3SVH).
2. The text file that is generated should be saved with the AGAR laboratory prefix (eg 3SVH) included in the name
3. Send the file electronically to Jan Bell (jbell@wcharl.net)

The conversion is done very quickly through Access. On completion the data will be sent back in Julie's Excel file to both the laboratory that sent the text file (the Vitek 2 operators) and to the laboratory to which the data originated from for completion of the data entry. The Vitek 2 data fills the respective antibiotic fields (MIC values), and the Excel file does the interpretation. You will also see extra fields in the file which contain the Vitek 2 Expert Interpreted data as well (for your own information).

The Vitek2 AST-N044 card antibiotic codes are:

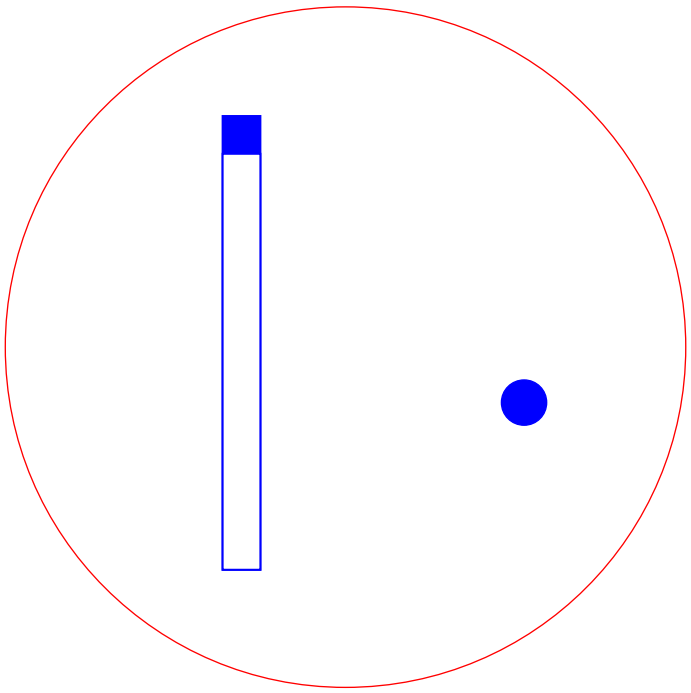
an	amikacin	gm	gentamicin
amc	amoxycillin-clavulanate	mem	meropenem
am	ampicillin	na	nalidixic acid
cz	cefazolin	ft	nitrofurantoin
fep	cefepime	nor	norfloxacin
fox	cefoxitin	tzp	piperacillin-tazobactam
caz	ceftazidime	tcc	ticarcillin-calvulanate
cro	ceftriaxone	tm	tobramycin
cf	cephalothin	tmp	trimethoprim
cip	ciprofloxacin	sxt	trimethoprim-sulfamethoxazole

For queries regarding the conversion process:

Jan Bell
Women's and Children's Hospital, Adelaide
jbell@wcharl.net

Appendix 4. Template for Additional Susceptibility Test

Medium: Mueller-Hinton Agar (4 mm depth)
Inoculation: CLSI Methodology3
Etest strip: tigecycline
Disc: colistin 10 µg
Incubation: 16-18 h Enterobacteriaceae
20-24 h *Acinetobacter* spp
Quality Control: ATCC 25922 *Escherichia coli*
Tigecycline: 0.032 – 0.25mg/L
Colistin: 13-16 mm



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24th May 2006