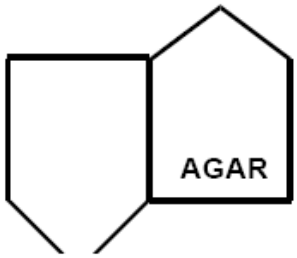

The Australian Group on Antimicrobial Resistance

Gram-negative Study

Survey 12 - 2011

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. In 2008 the decision was made to conduct surveys annually, alternating between community and hospital surveys.

The 2011 survey will be the second AGAR 'hospital' GNB survey.

Objectives

The objectives of this study are:

1. Determine resistance to commonly used antibiotics in *E. coli*, *Klebsiella* species and *Enterobacter* species in hospital-acquired infections.
2. Examine the extent of resistance and multi-resistance in these species
3. Detect emerging resistance to the carbapenems
4. Determine the mechanism of resistance with particular emphasis on extended-spectrum β-lactamases (TEM, SHV, CTX-M-types), plasmid-borne AmpC, carbapenemases (MBL, KPC, OXA-48), rRNA methylases, and plasmid-mediated quinolone resistance.

Important dates

Collection period: 1st July 2011 to 30th November 2011
Complete data forwarded to Julie Pearson by 31st December 2011.
Refer isolates for molecular characterisation to Jan Bell 31st December 2011.
Invoices to Geoff Coombs by 28th February 2012 ($5 per isolate plus GST).

Status

Compulsory study

Collection of Isolates

Each participating laboratory is to collect **up to** 70 *E. coli*, 20 *Klebsiella* species and 10 *Enterobacter* species from **100 different inpatients (hospital stay >48 hours at the time of collection)**. Hospital based laboratories to collect from one institution only. Private laboratories to collect from any institution they service (name of hospital to be entered into work sheet).

Do not include:

- Isolates from hospital out-patients or general practitioners
- Duplicate isolates (isolate from the same patient with the same antibiogram)

There are no specimen restrictions; collect consecutive 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)
Agar replication
Minimum tests: 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)
Agar replication

Antimicrobial Susceptibility Testing

Vitek 2

All isolates will be tested using the Vitek 2 AST-N149 card:

Antibiotic	Code	Concentrations	Useful for detection of:
Amikacin	AN	8, 16, 64	rRNA methylases
Amoxycillin-clavulanate	AMC	4, 16, 32	
Ampicillin	AM	4, 8, 32	
Cefazolin	CZ	4, 16, 64	
Cefepime	FEP	2, 8, 16, 32	ESBL Enterobacter
Cefoxitin	FOX	8, 16, 32	Plasmid-borne AmpC
Ceftazidime	CAZ	1, 2, 8, 32	ESBL
Ceftriaxone	CRO	1, 2, 8, 32	ESBL
Ciprofloxacin	CIP	0.5, 2, 4	
Gentamicin	GM	4, 16, 32	
Meropenem	MEM	0.5, 4, 16	carbapenemase

Nalidixic acid	NA	8, 16, 32
Nitrofurantoin	FT	16, 32, 64
Norfloxacin	NOR	1, 8, 32
Piperacillin-tazobactam	TZP	4, 16, 128
Ticarcillin-calvulanate	TCC	8, 32, 64
Tigecycline	TGC	0.75, 2, 4
Tobramycin	TM	8, 16, 64
Trimethoprim	TMP	0.5, 2, 8
Trimethoprim-sulfamethoxazole	SXT	0.5, 2, 16

The quality control organisms are:

E. coli ATCC 25922
E. coli ATCC 35218

The Vitek 2 Advanced Expert System (AES) interprets the MICs according to current CLSI guidelines. Please note that **raw** data, not interpreted data is required.

To minimise data entry errors, the Vitek 2 raw 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. Refer to the *Vitek Data Conversion* section of the AGAR website (www.antimicrobial-resistance.com) for instructions.

Ertapenem ETEST

All isolates will be tested against ertapenem using Etest strips on Mueller Hinton Agar according to the manufacturer's instructions. Etest strips will be supplied by Merck Sharp & Dohme. QC strain is *E. coli* ATCC 25922 (acceptable range 0.004 to 0.016 mg/L).

ESBL Confirmatory Tests (optional)

Any isolate with an ESBL phenotype (ie ceftazidime or ceftriaxone MIC >1 mg/L for *E. coli* and *Klebsiella* and cefepime MIC >1mg/L for *Enterobacter*) can be confirmed using commercial combination discs or Etest® strips.

- *E. coli* and *Klebsiella* spp. can be tested with both ceftazidime ± clavulanate (Oxoid, BioRad, BD, Rosco, Etest®) and cefotaxime ± clavulanate (Oxoid, BioRad, BD, Etest®).
- *Enterobacter* spp. can be tested with cefepime ± clavulanate (BioRad, Rosco, Etest®) or cefpirome ± clavulanate (Oxoid)
- MASTDISCS ID can be used to test for both ESBL and AmpC production.

The quality control organism is *K. pneumoniae* ATCC 700603 (ESBL producer SHV-18)

AmpC

Any *E. coli* or *Klebsiella* spp. that is cefoxitin resistant will be screened for plasmid-borne AmpC β-lactamase by a central laboratory using molecular techniques. Laboratories wishing to screen their own isolates may use either the boronic acid or AmpC[3-5] disk tests.

Carbapenemases

Isolates with ertapenem MIC > 0.5 mg/L or meropenem MIC >0.25mg/L will be screened for carbapenemases by a central laboratory using molecular techniques. Laboratories wishing to screen their own isolates may use the modified Hodge test[2] or MBL disc synergy tests.

Information required

Please enter in the spreadsheet the following information:

a) Your reference number or storage number

b) Lab ID: Laboratory number (optional)

c) Patients URN or initials (optional) – only required for tracking duplicate or repeat isolations. This information is not transferred to the GNB database

d) Date of admission

e) Date of collection

f) DPA. This field will calculate the number of days between DOA and DOC (≥48h for inclusion in a hospital survey so please check collection times for values of 2; discard isolate if figure is 0 or 1).

g) Patient sex

h) Date of birth (preferred) OR age if DOB unavailable

i) Hosp: Hospital the patient attended (hospital laboratories to collect from one institution only, private laboratories can collect from multiple institutions)

j) Organism code and method of identification

k) Specimen – state specimen type and site eg leg wound swab, pleural fluid

For each potential ESBL or MBL record the appropriate information in columns P through T.

Note: If 'Other' category is selected in any field please specify in the comments column.

Storage of Isolates

All isolates must be stored at -70°C or below until determined otherwise by the AGAR committee.

Referral of Isolates

After data conversion, Jan Bell will request isolates to be sent to:

Jan Bell
SA Pathology (Women's & Children's Hospital)
Microbiology and Infectious Diseases,
72 King William Road,
North Adelaide, SA, 5006.

Please do not send isolates until requested.

Data analysis

1. All raw MIC results are to be transferred to the Excel Data Entry file AGAR GNB11.xls.
2. Include QC results performed during the survey period
3. Check the file for transcription errors
4. Forwarded onto Julie Pearson (julie.pearson@health.wa.gov.au) by 31st December 2011

Checklist

	Collect 100 consecutive isolates from inpatients (>48h) – from July1, 2011
	Read Protocol
	Freeze cultures promptly - some resistance may be lost
	Store cultures until determined otherwise at AGAR meetings
	Vitek 2 AST-N149 susceptibility card to be used for primary susceptibility testing
	Perform ertapenem MIC testing using Etests
	Include QC results in worksheet
	Proof read worksheet.
	Forward appropriate isolates to Jan Bell at SA Pathology (Women's and Children's Hospital) when requested

References

1. Vitek2 AST-N149 Package Insert (REF22 221)
2. Clinical and Laboratory Standards Institute. (2011). Performance Standards for Antimicrobial Susceptibility Testing; Twenty first Informational Supplement: CLSI document M100-S21. CLSI, Villanova, PA, USA
3. Yagi, T., J. Wachino, H. Kurokawa, S. Suzuki, K. Yamane, Y. Doi, N. Shibata, H. Kato, K. Shibayana, and Y. Arakawa. 2005. Practical Methods using Boronic acid compounds for Identification of Class C β-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli*. *J. Clin. Microbiol*. 43:2551-2558
4. Coudron, P.E. 2005. Inhibitor-based Methods for Detection of Plasmid-Medicated AmpC-β-lactamases in *Klebsiella* spp., *Escherichia coli*, and *Proteus mirabilis. J. Clin. Microbiol*. 43:4163-4167
5. Jacoby, G.A., K.E. Walsh, and V.J. Walker. 2006. Identification of Extended-Spectrum, AmpC, and Carbapenem-Hydrolyzing β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* by Disk tests*. J. Clin. Microbiol*. 1971-1976

JMB

24th May 2006

Modified June 2011