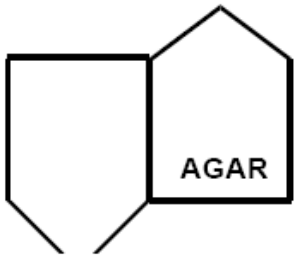

The Australian Group on Antimicrobial Resistance

Gram-negative Study

Survey 13 – 2012

Community Survey

SUMMARY

	Collect 100 consecutive isolates from **outpatient urine** samples from July 1, 2012
	Collect 70 *E. coli*, 20 *Klebsiella* species and 10 *Enterobacter* species
	Exclude isolates from nursing home patients or from catheters
	Ensure that the culture is pure, and freeze promptly
	Store cultures until determined otherwise at AGAR meetings
	Vitek 2 **AST-N246** susceptibility card to be used for susceptibility testing
	Extract Vitek data and copy into the AGAR GNB12 Excel file
	Proof read completed spreadsheet
	Forward data file to Jan Bell (jan.bell@health.sa.gov.au)
	When requested, send selected isolates to Jan Bell

Introduction

AGAR commenced biennial surveillance of *Escherichia coli* and *Klebsiella* species in 1992. In 2000, *Enterobacter* species was included. Since 2008, AGAR surveys have been conducted annually with alternating 'community' and 'hospital' collections. The 2012 survey will be a 'community' survey.

Objectives

The objectives of this study are:

1. Determine the proportion of resistance to commonly used antibiotics in *E. coli*, *Klebsiella* species and *Enterobacter* species in community-acquired urinary tract 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, and carbapenemases.

Important Dates

- Collection period: 1st July 2012 to 30th November 2012
- Complete data forwarded to Jan Bell (Jan.Bell@health.sa.gov.au) by 31st December 2012.
- Refer isolates for molecular characterisation to Jan Bell by 31st December 2012.
- Invoices to Geoff Coombs by 28th February 2013 ($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.

Specimen Source

Each participating laboratory is to collect 100 consecutive, significant clinical isolates from **outpatient urine samples**. Isolates selected should be considered to be significant if antibiotic susceptibilities were reported. No repeat isolates to be included from any patient unless a different antibiogram is observed.

Do not include:

- Isolates from hospital inpatients
- Isolates from catheter urines
- Isolates from nursing home residents (check address label)
- Duplicate isolates (isolate from the same patient with the same antibiogram)

Identification of Isolates

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

   E. coli

   Mass spectrometry

   Vitek®, Phoenix™, or MicroScan automated microbiology systems

   Chromogenic agar plus spot indole (DMACA)

API20E or ATB®
Agar replication
Minimum tests: BGA or citrate, indole, & lactose fermentation

Klebsiella spp., and *Enterobacter* spp. - all isolates must be speciated
Mass spectrometry
Vitek® (plus indole), Phoenix™ or MicroScan automated microbiology systems
API20E or ATB®
Chromogenic agar plus spot indole (DMACA)
Agar replication

Antimicrobial Susceptibility Testing

All isolates to be tested using the supplied Vitek 2 AST-N246 card[1] [Lot 646220420].

Additional Susceptibility Tests

Ertapenem MIC [TO BE CONFIRMED]

All isolates will be tested against ertapenem using Etest strips on MHA as indicated by the manufacturer. The Etest strips will be supplied by Merck Sharp & Dohme. *E. coli* ATCC 25922 to be used as the control strain: acceptable range 0.004 to 0.015 mg/L.

ESBL Confirmatory Tests

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

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

Enterobacter spp. may 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.

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 acid or AmpC[2-4] disk tests.

Carbapenemases

Isolates with a meropenem MIC >0.25mg/L will be screened for carbapenemases by a central laboratory. Laboratories wishing to screen their own isolates may use the modified Hodge test (Table 2A Supplemental Table 2, CLSI 2012, M100-S22), or MBL DDSTs. Please note that not all carbapenemase producing Enterobacteriaceae are MHT positive, and MHT-positive results may be encountered in isolates with carbapenem resistant mechanisms other than carbapenemase production.

Storage of Isolates

Participating laboratories are to store isolates immediately after they are first isolated. Store at -70°C or below until determined otherwise by the AGAR committee.

Referral of Isolates

All possible ESBL, (*E. coli* or *Klebsiella* spp. with ceftazidime or ceftriaxone MIC > 0.5 mg/L; *Enterobacter* spp. with cefepime MIC > 1 mg.L), AmpC (*E. coli* or *Klebsiella* spp. with cefoxitin resistance) or isolates with elevated meropenem MIC > 0.25mg/L will be forwarded to a central laboratory for further testing and analysis using molecular techniques at the conclusion of the study. Isolates with possible rRNA methylases or plasmid-mediated quinolone resistance genes will be also be investigated using molecular methods. **Jan Bell will contact each laboratory with a list of isolates requiring referral**. When contacted, forward the isolates to:

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

Data entry and analysis

1. All raw MIC results are to be transferred to the Excel Data Entry file AGAR GNB12.xls. The Advanced Expert System (AES) interprets MICs generated by the Vitek 2 according to either CLSI[5] or EUCAST[6] guidelines. Please note that raw data, not interpreted data is required.
2. 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 opened in Excel and then copied into the AGAR Data Entry Excel File. If you are experiencing difficulties, contact Jan Bell (Vitek2 Compact) or Narelle George (Vitek2) (narellem_george@health.qld.gov.au). BioMerieux are currently working on a data extract process for Observa software users. Further details will be forwarded when available.
3. All other information must be completed as indicated by the column headings (see below)
4. Include all QC results performed during the survey period
5. Forward data to Jan Bell (Jan.Bell@health.sa.gov.au) as soon as testing is complete

Other 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 collection
e) Patient sex
f) Date of birth (preferred) OR age if DOB unavailable
g) Patient Type (GP, Emergency Dept etc – select from drop down list)
h) Organism code and method of identification

For each potential ESBL or MBL record the appropriate information in columns N through R.

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

References

1. Vitek2 AST-N246 Package Insert
2. 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
3. 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
4. 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
5. Clinical and Laboratory Standards Institute. (2012). Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement: CLSI document M100-S22 June 2012 Update. CLSI, Villanova, PA, USA
6. EUCAST website http://www.eucast.org

JMB

June 2012

Appendix 1. Vitek2

Card: AST-N246

Inoculum: as per manufacturer's guidelines[1]

Incubation: as per manufacturer's guidelines

Quality Control: *E. coli* ATCC 25922
E. coli ATCC 35218

Antibiotics:

Antibiotic	Code	MIC range	Useful for detection of:
Amikacin	AN	2 – 64	rRNA methylases
Amoxycillin-clavulanate	AMC	2/1 – 32/16	
Ampicillin	AM	2 – 32	
Cefazolin	CZ	4 – 64	
Cefepime	FEP	1 – 64	ESBL Enterobacter
Cefoxitin	FOX	4 – 64	Plasmid-borne AmpC
Ceftazidime	CAZ	1 – 64	ESBL
Ceftriaxone	CRO	1 – 64	ESBL
Ciprofloxacin	CIP	0.25 – 4	
Gentamicin	GM	1 – 16	
Meropenem	MEM	0.25 – 16	carbapenemase
Nitrofurantoin	FT	16 – 512	
Norfloxacin	NOR	0.5 – 16	
Piperacillin-tazobactam	TZP	4/4 – 128/4	
Ticarcillin-clavulanate	TCC	8/2 – 128/2	
Tobramycin	TM	1 – 16	
Trimethoprim	TMP	0.5 – 16	
Trimethoprim-sulfamethoxazole	SXT	20 – 320	