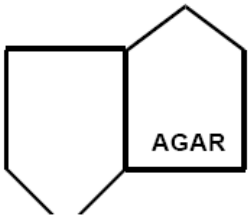

The Australian Group on Antimicrobial Resistance

2007 Antibiotic Susceptibility Survey for

Hospital-Onset *Staphylococcus aureus*

Introduction

The Australian Group for Antimicrobial Resistance (AGAR) was established in 1986, initially to monitor resistance in *Staphylococcus aureus* throughout Australia. This surveillance role has become more important with the multiple waves of methicillin-resistant *S. aureus* (MRSA) that have occurred in Australia since the 1960s. Results of previous *S aureus* surveys have been published. AGAR performs alternate hospital- and community-onset surveys.

The 2007 survey is a hospital survey.

Objectives

The objectives of this study are:

1. Determine the proportion of resistance to commonly used antibiotics in *S aureus* in Australian hospitals.
2. Characterise the epidemic and community MRSA clones isolated in Australian hospitals using a range of molecular techniques.
3. Determine the proportion of MRSA in Australian hospitals that carry the Panton-Valentine leukocidin determinants.

Study Period

1st July 2007 to 31st December 2007

Status

Compulsory study

Organisms to be Tested

100 consecutive isolates of *S. aureus* (MRSA included) from **100 different inpatients (hospital stay >48 hours at the time of collection)** will be tested by each laboratory. Collect isolates from one institution only.

Specimen Source

Each *S. aureus* (whether MRSA or not) should be an individual patient isolate judged to have come from a potentially infected site and an organism for which the laboratory would normally perform antibiotic susceptibility testing.

The following should **not** be included:

- Coagulase-negative staphylococci.
- Repeat isolates from the same patient.
- Organisms isolated from specimens received for the purpose of gathering surveillance data (eg. infection control).
- Isolates from peripherally separate health care facilities (with the exception of the Women's and Children's Hospital, Melbourne, which is considered as one institution)
- Isolates from outpatients
- Isolates from inpatients where hospital stay is <48 hours at time of sample collection.

Identification of Isolates

The minimum tests for identification of staphylococci include at least two of the following:

(1) Slide coagulase test (A commercial staphylococcal slide test may be substituted for the standard slide test)

(2) Tube coagulase test

(3) Demonstration of deoxyribonuclease production (Use DNAse agar).

For identification of an organism as *S. aureus,* two tests should be positive.

Additional tests such as fermentation of mannitol on mannitol-salt agar may be performed for confirmation if preferred.

Isolate Information Required

For all *S. aureus* enter information in columns B to K:

a) Your reference number or storage number

b) Date of admission

c) Date of collection

d) Lab ID: Laboratory number (optional)

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

f) Site of Infection – state specimen type and site eg leg wound swab, brain pus

g) Invasive or Non-invasive – eg. synovial fluid is invasive, superficial knee wound swab is non-invasive

h) Hosp: Hospital the patient attended (collect from one institution only)

i) Patient sex

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

For each MRSA record the appropriate information in column L:

- MRSA: new or old (known patient)?

Antimicrobial Susceptibility Testing

All isolates will be tested using the Vitek 2 AST-P545 card

Antibiotic	Well Concentration	MIC Range
Benzylpenicillin	0.125, 0.25, 1, 2, 8, 64	0.03 – 0.5
Oxacillin	0.5, 1, 2	0.25 – 4
Cefazolin	4, 8, 16	4 - 64
Vancomycin	2, 4, 6	1 - 32
Rifampicin	0.25, 0.5, 2	0.5 - 32
Fusidic acid	0.5, 1, 4	0.5 - 32
Gentamicin	8, 16, 64	0.5 - 16
Erythromycin	0.25, 05, 2	0.25 - 8
Clindamycin	0.5, 1, 2	0.25 - 8
Tetracycline	0.5, 1, 2	1 - 16
Trimethoprim/Sulphamethoxazole	8/152, 16/304, 32/608	10 - 320
Ciprofloxacin	1, 2, 4	0.5 - 8
Quinupristin/dalfopristin	0.25, 0.5, 2	0.25 - 16
Teicoplanin	1, 4, 8, 16	0.5 – 32
Linezolid	0.5, 1, 2	0.5 – 8
Imipenem	2, 4, 8	1 - 16
Nitrofurantoin	16, 32, 64	16 – 152

The Advanced Expert System (AES) interprets MICs generated by the Vitek 2 according to 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. Procedure involved is outlined in Appendix 1.

Additional Susceptibility Tests

Disc Diffusion

Performed by CLSI **or** CDS disc diffusion

Antimicrobials to be Tested

Mupirocin 5µg

Cefoxitin 30µg (CLSI) or 10 µg (CDS)

CLSI Disc Diffusion

Reference: Clinical and Laboratory Standards Institute (2007). Performance Standards for Antimicrobial Disk Susceptibility Tests—Ninth Edition: Approved Standard M2-A9. CLSI, Villanova, PA, USA

Method

- Mueller-Hinton Agar plates (20-25ml per 9 cm round plate agar 4 ± 0.5 mm depth)
- Remove excess moisture from agar by placing in an incubator (35°C) or laminar flow hood at room temperature until excess surface moisture is lost by evaporation (10 to 30 minutes). Agar surface should be moist but no droplets should be apparent on agar surface or on petri dish cover
- Organism preparation: suspended organism from an 18 to 24 hr blood agar culture into broth or saline to turbidity equivalent to the McFarland 0.5 turbidity standard.
- Inoculate onto Mueller-Hinton agar plate, within 15 minutes of inoculum preparation, by dipping a swab into the organism suspension and spreading in three directions or on a rotary inoculator (swab from McFarland 0.5). Ensure the agar surface is dry before adding the discs.
- Add the discs within 15 minutes of inoculation
- Within 15 minutes of discs being dispensed, incubate at 35ºC for 18-24 hours (mupirocin), full 24 hours for cefoxitin. Note: testing at temperatures above 35°C may not detect methicillin resistant staphylococci
- Measure and record the zone diameters

Interpretive Criteria

- 5µg mupirocin disc (susceptible breakpoint ≥19mm)
  (Reference: Comité de l'Antibiogramme de la Société Française de Microbiologie, Communiqué 2006. www.sfm.asso.fr/

- 30µg cefoxitin disc (susceptible breakpoint ≥22mm)

CDS DISC DIFFUSION

Reference: Antibiotic Susceptibility Testing by the CDS Method. A Manual for Medical and Veterinary Laboratories 2006. 4th Edition.

Method

- Sensitest Agar plates (20 ml per 9cm round plate agar)
- Dry the plates in a 35 to 37°C incubator for 15 to 20 mins prior to inoculation.
- Organism preparation: suspended organism from an 18 to 24 hr blood agar culture into saline to turbidity equivalent to the McFarland 0.5 opacity standard.
- Dilute the 0.5 McFarland suspension 1:5 by adding 0.5 ml to 2 ml of saline.
- Inoculate by flooding the plate with the 2.5 ml diluted suspension; then drain the fluid completely. Ensure the agar surface is dry before adding the discs.
- Add the discs
- Incubate at 35ºC for 24 hours
- Measure and record annular radius

Interpretive Criteria

- 5µg mupirocin disc (breakpoint ≥6mm)
- 10µg cefoxitin disc (breakpoint ≥6mm)

TIGECYCLINE ETEST

All isolates will be tested against tigecycline by Etest. Use MHA at a depth of +/- 0.5mm. For CLSI users, the Etest strip can be placed on the same plate as that used for disk diffusion in such a way as minimize the chance of overlap of the E-test ellipse with the zones around the other two disks, e.g. strip on one half of the plate and the two disks a sufficient distance apart on the other half. Etest strips will be supplied by Wyeth. QC is ATCC 29213: acceptable range 0.032 to 0.25mg/L (there will be a trailing endpoint – read at 80%).

BETA LACTAMASE TEST

All isolates that are penicillin susceptible should be tested for the production of ß-lactamase

Cefoxitin will be used as an inducer for ß-lactamase. The growth around the cefoxitin disc will be used for this test

The ß -lactamase test is to be performed by using the nitrocefin method. Use Cefinase Discs (BBL Cat No. 31650). Follow the manufacturer's instructions.

Storage of Isolates

Participating laboratories will collect isolates and store isolates immediately after they are first isolated. They are to be tested, using methods as described.

The participating laboratories should retain all cultures in glycerol broth or a suitable medium and stored at -70°C until determined otherwise at AGAR meetings.

Referral of Isolates

All possible MRSA (oxacillin and/or cefoxitin resistant *S. aureus*) and mupirocin resistant *S. aureus* will be forwarded to Julie Pearson for further testing and analysis using molecular techniques at the conclusion of the study.

Department of Microbiology and Infectious Diseases
PathWest Laboratory Medicine WA
Royal Perth Hospital
6th Floor A Block
Wellington Street
Perth. WA 6000

Please: ***Label cultures with your laboratory code e.g. "6RPH 2, 6RPH 32". The number of your isolate is in Column A of the SAP06 worksheet ie label cultures with the same identification as entered into the data file***

Data analysis

Julie Pearson will provide the updated data entry programme (SAP07.xls).

Please: ***Read the instructions on the software***

1. All **raw** MIC results are to be transferred to the Excel Data Entry file AGAR SAP07.xls as indicated in Appendix 1. All other information must be completed as indicated
2. Include all QC results performed during the survey period
3. Check the file for transcription errors
4. Check for unusual resistances eg vancomycin, linezolid, quinupristin/dalfopristin. Re-test and confirm with another method eg disc diffusion, Etest
5. Forwarded onto Julie Pearson (julie.pearson@health.wa.gov.au) as soon as testing is complete

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Appendix 1. Extraction of data from the Vitek AST-P545 card

The Vitek2 AST-P545 card antibiotic codes areas follows:

Benzylpenicillin	peng
Oxacillin	ox
Cefazolin	cz
Vancomycin	va
Rifampicin	rif
Fusidic acid	fa
Gentamicin	gm
Erythromycin	e
Clindamycin	cc
Tetracycline	tet
Trimethoprim/sulphamethoxazole	sxt
Ciprofloxacin	cip
Quinupristin/dalfopristin	qda
Teicoplanin	tpn
Linezolid	lnz
Imipenem	imi
Nitrofurantoin	ftn

The extraction of raw data and conversion process depends on which machine the card was processed on; Vitek 2 or Vitek 2 compact.

Suggested workflow to allow recovery of the raw data will be provided in a separate document. However; where possible, please use the same reference number as entered into the Excel data file (eg 3SVH1). This will help in the logbook report and conversion process.

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 or using a visual basic script written by Peter Lawson (Vitek 2 compact). On completion the conversion, data will be sent back in the SAP07 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).

For queries regarding the extraction process:

Vitek 2 compact: Jan Bell
Women's and Children's Hospital, Adelaide
jbell@wcharl.net

Vitek 2: Narelle George
QHPS Central, Brisbane
Narellem_george@health.qld.gov.au

Appendix 2. Check List

	Collect 100 consecutive isolates from one institution only – from July1, 2007
	Read Protocol
	Freeze cultures promptly - some resistance may be lost
	Store cultures till determined otherwise at AGAR meetings
	Vitek 2 AST-P545 Susceptibility card to be used for primary susceptibility testing
	Disc diffusion susceptibility testing (CLSI or CDS) for mupirocin and cefoxitin
	Test all isolates against tigecycline using the Etest strips provided by Wyeth
	Test all penicillin-susceptible strains for β-lactamase
	Proof read worksheet. ***Confirm rare resistances ie vancomycin, linezolid and quinupristin/dalfopristin.***
	Virus scan computer discs before using them in your computer or sending data
	Forward requested isolates to Royal Perth Hospital