

The Australian Group on Antimicrobial Resistance
<http://antimicrobial-resistance.com>

***Streptococcus pneumoniae* Survey**

2005 Antimicrobial Susceptibility Report

A/Prof Thomas Gottlieb
Senior Specialist in Infectious Diseases and Microbiology
Department of Microbiology and Infectious Diseases
Concord Hospital
Concord, NSW

Prof Peter Collignon
Director Infectious Diseases Unit and Microbiology Department
The Canberra Hospital
Woden, ACT
Professor, Canberra Clinical School
Australian National University
Acton, ACT

Dr Jenny Robson
Microbiologist and Infectious Diseases Physician
Microbiology Department
Sullivan Nicolaides Pathology
Taringa, QLD

Ms Julie Pearson
Scientific Officer
Department of Microbiology and Infectious Diseases
PathWest Laboratory Medicine WA
Royal Perth Hospital
Perth, WA

Ms Jan Bell
Scientist
Microbiology and Infectious Diseases
Children, Youth & Women's Health Service
Women's & Children's Hospital
North Adelaide, SA

On behalf of the Australian Group for Antimicrobial Resistance (AGAR)

Address correspondence to: Ms Julie Pearson c/o AGAR

August 2006

Antimicrobial Susceptibility Report of *Streptococcus pneumoniae* Isolates from the Australian Group on Antimicrobial Resistance (AGAR)

2005 Surveillance Report

Members of AGAR who participated in this study:

Victoria

Alfred Hospital
Austin Hospital
St Vincent's Hospital

Denis Spelman, Clare Franklin
Barrie Mayall, Peter Ward
Mary Jo Waters, Linda Joyce

New South Wales

Concord Hospital
Royal North Shore Hospital
Royal Prince Alfred Hospital
South Western Area Pathology Service
John Hunter Hospital
Nepean Hospital

Thomas Gottlieb, Glenn Funnell
George Kotsiou, Clarence Fernandes
Richard Benn, Barbara Yan
Iain Gosbell, Helen Ziochos
Sue Tiley, Jo Anderson
James Branley, Samantha Ryder

Australian Capital Territory

The Canberra Hospital

Peter Collignon, Susan Bradley

South Australia

SouthPath
Gribbles Pathology (SA)
Institute of Medical and Veterinary Science
Women's and Children's Hospital

David Gordon, Hendrik Pruul
PC Lee, Barbara Koldej
Ivan Bastian, Rachael Pratt
John Turnidge, Jan Bell

Western Australia

PathWest, QE2 Medical Centre
PathWest, Royal Perth Hospital
St John of God Pathology

Barbara Henderson
Keryn Christiansen, Geoffrey Coombs
Susan Benson, Janine Fenton

Queensland

Queensland Health Pathology Service, Princess
Alexandra Hospital
Queensland Health Pathology Service, Royal
Brisbane Hospital
Sullivan Nicolaidis Pathology

Graeme Nimmo, Jacqueline Schooneveldt
Joan Faoagali, Narelle George
Jenny Robson, Renee Bell

The AGAR group has been funded by the Commonwealth of Australia, Department of Health and Ageing since 2001

Table of Contents

1	Executive Summary	5
2	Introduction.....	6
2.2	Objective of the Programme.....	6
2.3	Importance of <i>Streptococcus pneumoniae</i>	6
2.4	Antimicrobials Tested and Resistance	6
2.4.1	β -lactams.....	6
2.4.2	Other Antimicrobial Classes	7
3	Methods.....	8
3.2	Species Identification	8
3.3	Susceptibility Testing Methodology	8
3.3.1	Disc Tests.....	9
3.3.2	MIC (Etest)	9
3.4	Quality Control.....	9
3.5	Statistical Analysis.....	9
4	Demographics	9
4.2	Regional Source of Isolates	9
4.3	Age.....	10
5	Specimen Source	10
6	Susceptibility Testing Results: 2005 Study.....	11
6.2	Penicillin	11
6.3	Cefotaxime/Ceftriaxone.....	14
6.4	Erythromycin and Clindamycin.....	16
6.5	Clindamycin Genotype	19
6.6	Tetracycline	19
6.7	Trimethoprim-sulphamethoxazole	21
6.8	Fluoroquinolones.....	22
6.8.1	Levofloxacin	23
6.8.2	Moxifloxacin.....	23
6.9	Resistance Rates – Invasiveness and Hospitalisation Status.....	24
7	Multi-resistance	25
8	Limitations of the Study	25
9	Discussion	25
10	References	28
11	Acknowledgements	30
Appendix 1	Trends in Resistance 1994-2005	31
Appendix 2	Antibiotic Profiles by Frequency	32

1 Executive Summary

The Australian Group on Antimicrobial Resistance (AGAR) performs regular multicentre point-prevalence studies to monitor changes in antimicrobial resistance. In 2005, 20 laboratories participated in national surveillance of *Streptococcus pneumoniae* resistance. One thousand seven hundred and seventy six strains of *S. pneumoniae* were collected prospectively and tested by disc diffusion, Etest and/or agar dilution. Results were compared to four previous AGAR sponsored studies performed in 1989, 1994, 1999 and 2002. Three hundred and forty one (19.2%) were from blood cultures, which formed the majority of invasive isolates. Non-susceptibility to penicillin was present in 28.0%, with percentages of intermediate susceptibility (MIC: 0.125mg/L – 1 mg/L) and resistance (MIC \geq 2 mg/L) being 16.3% and 11.7% respectively. Twenty-nine isolates (1.6%) had a penicillin MIC \geq 4 mg/L. Non-susceptibility for invasive isolates was 16.5% compared with non invasive isolates where non-susceptibility was 30.6%. For β -lactam antibiotics in particular, Queensland (33.8%) and NSW (31.7%) tended to have higher percentages of non-susceptibility than other states (21.4% combined). In non-invasive isolates resistance to macrolides (24.6%) and tetracyclines (21.3%) was also higher, compared with invasive isolates (rates of 15.1% and 6.3% respectively). Almost two-thirds of macrolide resistant strains are also clindamycin resistant (MLS resistance). Fluoroquinolone resistance remains rare with 0.2% of isolates demonstrating resistance to levofloxacin and 0.1% to moxifloxacin. Multi-resistance, defined as resistance to 3 or more antibiotic classes, was identified in 6% of invasive and 20.1% of non-invasive strains. Resistance rates for penicillin and macrolides were higher among the young (< 5 years old), and the elderly (>65 years old), whereas tetracycline resistance was highest in the elderly.

When compared to previous AGAR studies, the greatest increases in penicillin non-susceptibility were observed between 1994 and 1999, rising from 6.7% to 21.7%. A further rise in resistance between 2002 and 2005 has occurred primarily in the subset of isolates with high level penicillin resistance (6.8% to 11.7%). Erythromycin resistance has continued a step-wise increase from 8.7% in 1994, to 22.7% in 2005. No significant rise in tetracycline resistance was demonstrated between 1999 and 2005, whilst a decrease was observed for trimethoprim-sulphamethoxazole. Resistance rates for most antibiotics used in the therapy of *S. pneumoniae* have continued to rise since the early 1990's but at a slower rate since 1999. Overall, the trends in susceptibility match trends seen in other developed countries although the rates seen in Australia in 2005 remain substantially lower than those seen in many areas in Europe and particularly the rates seen in many developing countries in Asia.

2 Introduction

2.2 Objective of the Programme

The objective of the 2005 surveillance program was to determine the prevalence of antimicrobial resistance in clinical isolates of *S. pneumoniae* throughout Australia, with particular emphasis on:

1. Obtaining accurate information on the minimum inhibitory concentrations to penicillin and third generation cephalosporins in order to ascertain whether beta-lactams are likely to remain effective therapy for *S. pneumoniae* infection in Australia.
2. To assess susceptibility to non β -lactam antibiotics and investigate any developing resistance to new fluoroquinolones.
3. Assess changes in resistance patterns over time in comparison to previous *S. pneumoniae* AGAR surveys.

AGAR commenced surveillance of *S. pneumoniae* in 1989.¹ Three similar surveys have been performed in 1994, 1999 and 2002^{2,3} although the number of participating laboratories and testing methodologies have changed somewhat over this timeframe.

2.3 Importance of *Streptococcus pneumoniae*

Worldwide *S. pneumoniae* is the commonest bacterium causing pneumonia and meningitis. Invasive pneumococcal infection, (primarily bacteraemia and meningitis), occurs most commonly in young children <5 years of age and older adults >65 years of age. Bronchitis and sinusitis in adults and otitis media in children are frequently caused by *S. pneumoniae*, and these conditions are responsible for a significant proportion of the antibiotic prescriptions in the community. Otitis media is a particular problem as few antibiotics reach therapeutic levels in the middle ear particularly when *S. pneumoniae* and other bacterial pathogens have raised MICs. Community antibiotic use, in non-invasive infections is seen as an important driver for selection pressure of emerging resistance. Emergence of multi-drug resistance varies among pneumococcal serotypes and correlates with clonal spread. Subsequent to completion of this survey the 7-valent pneumococcal conjugate vaccine (7vPCV), first introduced in 2001 for children at high risk of invasive pneumococcal disease, was funded for all children as part of the Australian Vaccination Schedule commencing 2005. These surveys, along with serotype studies, will serve as important baselines to monitor changes that may occur in response to introduction of this vaccine.

2.4 Antimicrobials Tested and Resistance

2.4.1 β -lactams

This group of agents are the mainstay of treatment for *S. pneumoniae*, being the drugs of choice both for invasive infections (eg. meningitis), and non-invasive infections (eg. bronchitis, otitis media, sinusitis).⁴

Penicillin: Penicillin testing is used to predict resistance to penicillin, ampicillin and amoxicillin. Susceptibility reporting has conventionally been divided into susceptible, intermediate resistant and resistant (in this report intermediate and resistant are classed as non-susceptible). Penicillin-intermediate (MIC 0.125mg/L - 1mg/L) strains have epidemiological significance as an indicator of evolving β -lactam resistance. However the usual intravenous doses of penicillin and oral amoxicillin given in higher doses of 20-30mg/kg/tds (1 gram

tds in adults) result in peak blood levels at least 4 times greater than the MIC for greater than 40% of the dosing interval. For therapeutic use, therefore, penicillin and amoxicillin continue to be the preferred agents for infection caused by susceptible and intermediate resistant *S. pneumoniae* other than in the therapy of meningitis where penicillin use is associated with a higher failure rate when isolates have raised MICs to penicillin.

Ceftriaxone/cefotaxime: The third-generation cephalosporin antibiotics are regarded as drugs of choice for pneumococcal meningitis, as attainable spinal fluid levels are approximately tenfold higher than for intravenous penicillin. When strains with raised penicillin MICs are or might be encountered, these β -lactams are often used as therapy for lower respiratory tract infections. However their benefit over penicillin has not been demonstrated for strains of *S. pneumoniae* with penicillin MIC <4 mg/L.⁵

For both β -lactam classes, antibiotic resistance develops due to mutations to penicillin binding proteins, altering the binding affinity of β -lactam antibiotics. Although resistance to penicillins and cephalosporins may occur differentially, it is usual for MIC changes to increase in parallel, with third generation cephalosporin MIC results frequently only 1-2 dilutions below that of penicillin.

Laboratory definitions for penicillin and third generation cephalosporin resistance⁷:

	Resistant	Intermediate	Susceptible
Penicillin	≥ 2 mg/L	0.125 – 1mg/L	≤ 0.064 mg/L
Cefotaxime/ceftriaxone (Non-meningitis)	≥ 4 mg/L	2mg/L	≤ 1 mg/L
Cefotaxime/ceftriaxone (Meningitis)	≥ 2 mg/L	1mg/L	≤ 0.5 mg/L

2.4.2 Other Antimicrobial Classes

Erythromycin and Clindamycin: Erythromycin is a macrolide antibiotic. In vitro, erythromycin is used to test for susceptibility to other macrolides (roxithromycin, clarithromycin) and azalides (azithromycin). In common with lincosamides and streptogramins, (MLS antibiotics) it shares an overlapping binding site on the 23S rRNA portion of the 50S ribosomal subunit. Changes in the target site, eg methylation due to acquisition of erythromycin ribosome methylase (*erm*) genes, result in high-level resistance to all MLS antibiotics (MLS_B phenotype). Lincosamide (clindamycin) resistance may be constitutive or inducible. Inducible resistance is detected by the placement of an erythromycin disc adjacent to a clindamycin disc.

Genes that encode pumps that mediate macrolide efflux (*mef*) do not confer resistance to clindamycin (M phenotype). Resistance due to *ermB* is associated with higher erythromycin MICs (>64 mg/L) than when due to efflux (1-16 mg/L), and with a greater likelihood of failure of macrolide therapy. In bacteraemic patients however any resistance >1mg/L has been associated with failure of macrolide therapy⁶. Erythromycin resistance has co-evolved with penicillin and tetracycline resistance, and cross-resistance to two or more antibiotic classes occurs frequently.

Tetracycline: Tetracycline is used to test for susceptibility to doxycycline and minocycline, the most commonly used tetracycline agents. Until recently only

oral tetracyclines have been available for therapy, and use has been primarily in upper respiratory tract infections. Because of discoloration of young teeth, tetracyclines are usually restricted to adults and children over the age of 8 years.

Trimethoprim-sulphamethoxazole: Trimethoprim-sulphamethoxazole (co-trimoxazole) is a folate synthesis (dihydrofolate reductase) inhibitor. This has been used in the past for treatment of *S. pneumoniae* and *H. influenzae* infections, however its use as a respiratory antimicrobial has declined over the past two decades, in part due to its adverse reaction profile and increased *S. pneumoniae* resistance.

Fluoroquinolones: Older generation fluoroquinolones such as ciprofloxacin demonstrate borderline MICs and have not traditionally been recommended for treatment of *S. pneumoniae* infection. Resistance is usually the result of modification in the genes *gyrA* and *parC*, coding respectively for the target enzymes gyrase and topoisomerase IV. Mutations in *gyrB* and *parE* may also contribute to resistance. In addition an efflux mechanism has been described. The presence of acquired mutations lowers antibiotic affinity. The MICs increase sequentially as numbers of these quinolone resistance determining region (QRDR) mutations accumulate.

Different agents have varying enzyme affinity. The greater difficulty in selecting mutants to newer generation fluoroquinolones, such as moxifloxacin and gatifloxacin, suggests these agents act substantially through both target enzymes and two or three mutations may be required to develop clinical resistance. Clinical use of ciprofloxacin and levofloxacin (the latter not available in Australia) antibiotics with less activity against *S. pneumoniae* due to lower enzyme affinity, may encourage selection of first step *gyrA* gene mutants, sufficient to result in high-level resistance. Sequential mutations lead to stepwise increases in resistance and cross-resistance to all fluoroquinolones. Unless the MICs of newer quinolones approach breakpoint levels, routine testing of these agents may not detect rising resistance. Because of potential cartilage damage, fluoroquinolone use is usually restricted to adults.

3 Methods

Twenty institutions from the 5 mainland states and the Australian Capital Territory (ACT) participated in the *S. pneumoniae* AGAR survey. Starting 1st January 2005, each laboratory collected up to 100 consecutive significant clinical isolates. Only one isolate per patient was tested. If *S. pneumoniae* was isolated from more than one site, then the isolate from the most significant clinical site was tested. For each isolate the following information was obtained: date of collection, age, sex, specimen site, and inpatient or outpatient status.

3.2 Species Identification

Alpha-haemolytic, optochin sensitive, and/or bile-soluble, Gram-positive cocci were identified as *S. pneumoniae*. Any strain with an optochin zone of inhibition of between 6 and 14mm in CO₂ was tested for bile solubility.

3.3 Susceptibility Testing Methodology

Participating laboratories performed antimicrobial susceptibility tests according to each laboratory's routine standardised methodology^{7,8,9,10,11} (CDS, CLSI or BSAC disc diffusion, Vitek2, agar dilution or MIC testing).

3.3.1 Disc Tests

Antimicrobials tested included: oxacillin, erythromycin, clindamycin, tetracycline, levofloxacin, (gatifloxacin, if the CDS disc testing method was used) and trimethoprim-sulphamethoxazole. Clindamycin and erythromycin discs were placed side by side to look for clindamycin inducibility (MLS_B phenotype).

3.3.2 MIC (Etest)

Penicillin and moxifloxacin MICs were determined for all isolates using Etest[®] strips. Four hundred and seventy one (95%) of the 497 isolates that were penicillin intermediate or resistant (MIC >0.064 mg/L) were also tested with either a ceftriaxone or cefotaxime Etest[®] strip.

3.4 Quality Control

S. pneumoniae ATCC 49619 was the quality control strain for this survey.

3.5 Statistical Analysis

P values were calculated using Fischer's exact test (GraphPad[®] Prism Software).

4 Demographics

Both public (17) and private laboratories (3) participated in the study. Participants included New South Wales (6), ACT (1), Queensland (3), Victoria (3), South Australia (4) and Western Australia (3). There were 1776 isolates from 20 institutions (Table 1). Isolates from NSW and ACT were combined.

The ages of patients reflect the incidence of *S. pneumoniae* infection, with 25.1% of patients below age 5, and 27.4% in the elderly, \geq age 65 (Table 2). 741 (41.7%) of the patients were classified as hospital inpatients at time of collection, 937 (52.8%) were outpatients. Hospitalisation status was not available on 98 (5.5%)

4.2 Regional Source of Isolates

Table 1. Isolates by Region

Region	Number of Institutions	Total	%
New South Wales (NSW)	7	583	32.8
Australian Capital Territory (ACT)			
Queensland (Qld)	3	284	16.0
South Australia (SA)	4	392	22.1
Victoria (Vic)	3	221	12.4
Western Australia (WA)	3	296	16.7
Total	20	1776	100

4.3 Age

Table 2. Age of Patients

Age Range (years)	n	%
<2	299	16.8
2-4	146	8.3
5-14	104	5.9
15-29	115	6.5
30-64	623	35.1
≥65	487	27.4
Unknown	2	0.2
Total	1776	100

5 Specimen Source

The majority of isolates (54.9%) were from the respiratory tract (Table 3). Invasive isolates (19.9%) includes 341 isolates (19.2%) from blood cultures. Other common sites of isolation were from eye and ear specimens.

Table 3. Source of Isolates

Specimen Source	n	%
Respiratory	975	54.9
Blood	341	19.2
Ear	199	11.2
Eye	168	9.5
Wound	24	1.4
Urine	12	0.7
CSF	6	0.3
Other	51	2.9
Total	1776	
Invasive	354	19.9
Non-Invasive	1413	79.6
Not Specified	9	0.5

6 Susceptibility Testing Results: 2005 Study

6.2 Penicillin

In this report, the combined penicillin intermediate and resistant categories are referred to as non-susceptible (NS). For the purposes of determining antibiograms and multi-resistance, NS has been treated as resistant. Overall 28% of isolates were non-susceptible to penicillin (Table 4). Higher rates were detected in Queensland (33.8%) and New South Wales/ACT (31.7%) than in the other states combined (21.4%).

Overall, there was a significantly higher rate of non-susceptibility detected in non-invasive isolates compared with invasive isolates ($P < 0.001$). Queensland and New South Wales/ACT also had the highest rates of non-susceptibility demonstrated in non-invasive strains (>36%). Although Victoria had the lowest rates of non-susceptibility in non-invasive strains, it had the highest rate in invasive strains (21.9% and 20.8% respectively). Nationally there were trends to higher rates of resistance among the young (< 5 years old), and the elderly (> 65 years old) (Table 5).

Penicillin 'high-level' resistance (MIC ≥ 2 mg/L) was present in 11.7% of isolates (Figure 1), with higher rates demonstrated in Queensland and New South Wales/ACT (range: 14.2 -17.3%) than in the other states (range: 7.1 – 9.7%) (Figure 2). Nationally twenty-nine isolates (1.6%) had a penicillin MIC ≥ 4 mg/L, a group that may define better the numbers that may fail penicillin treatment for non-meningeal infection. Intermediate resistance (0.125mg/L – 1 mg/L) did not vary greatly between states, with a range of 14.5%-17.5% nationally.

Figure 3 documents trends in penicillin non-susceptibility between 1989 and 2005. Overall, the most substantial rise in penicillin non-susceptibility was observed between 1994 and 1999, with a rise from 6.7% to 21.7%. Since 1999, this trend has continued, but less abruptly, with 28% non-susceptibility detected in the current study. Between the last two surveys, whilst intermediate resistance has stayed relatively unchanged, the rise seen has been predominantly in the proportion of strains demonstrating resistance (≥ 2 mg/L), rising from 6.8% to 11.7%, with a similar trend for both invasive (3.4% to 5.4%) and non-invasive (7.5% to 13.3%) isolates.

Because of changes in participating laboratories, these studies may not be fully comparable and hence P values have not been reported. However an analysis of results for 8 core laboratories that have participated in all AGAR *S. pneumoniae* studies since 1994 have confirmed a similar trend to above. In the studies since 1994 a common methodology (Etest) was used to measure penicillin MICs in all labs.

Table 4. Penicillin non-susceptibility: Number non-susceptible/total tested (%).

	Qld	NSW/ACT	Vic	SA	WA	Aus
All	96/284 (33.8%)	185/583 (31.7%)	49/221 (22.2%)	101/392 (25.8%)	66/296 (22.3%)	497/1776 (28.0%)
Invasive	7/40 (17.5%)	30/160 (18.8%)	5/24 (20.8%)	9/73 (12.3%)	7/54 (13.0%)	58/351 (16.5%)
Non-invasive	89/244 (36.5%)	150/415 (36.1%)	43/196 (21.9%)	92/319 (28.8%)	59/242 (24.4%)	433/1416 (30.6%)

Table 5: Penicillin non-susceptibility by age range: Number non-susceptible/total tested (%).

Age Range			Significance of Results		
<5yo	5-64	>65	Between <5 and 5-64	Between 5-64 and >65	Between <5 and >65
128/445 (28.8%)	207/842 (24.6%)	161/487 (33.1%)	NS	P<0.005	NS

Figure 1: Penicillin MIC Distribution

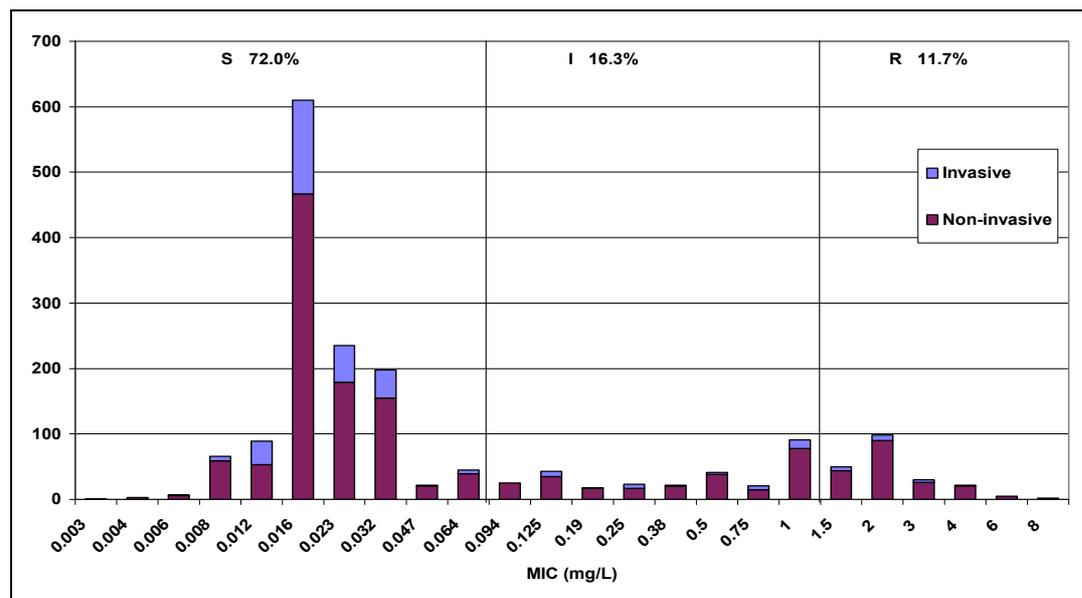


Figure 2: Penicillin Non-susceptibility by Region

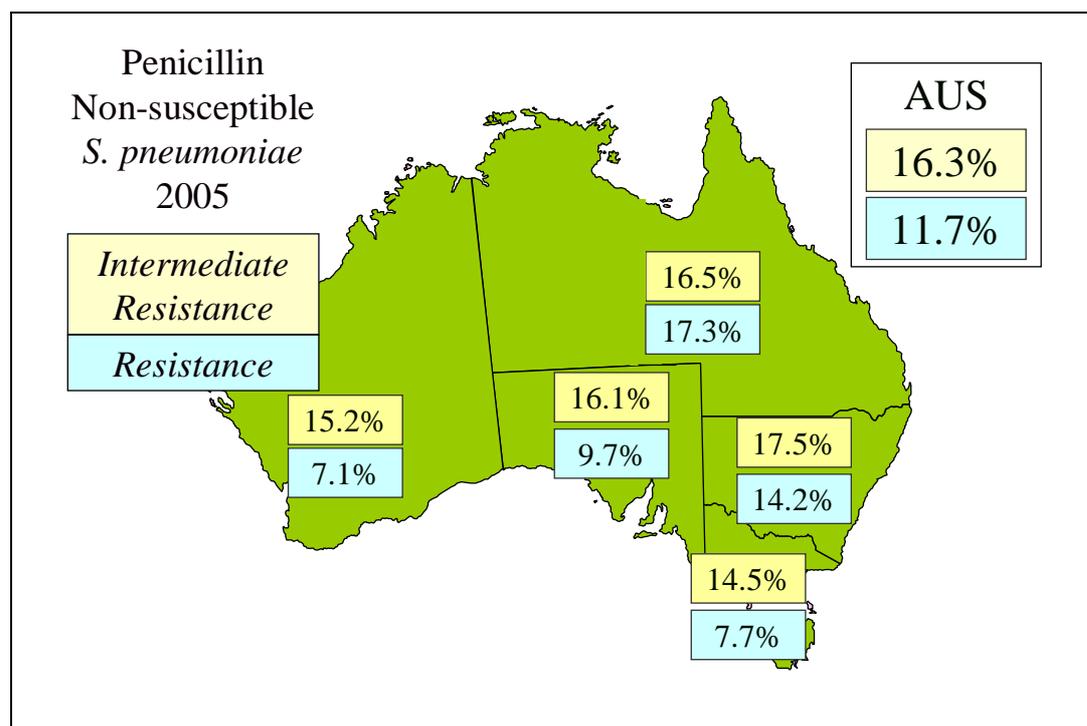
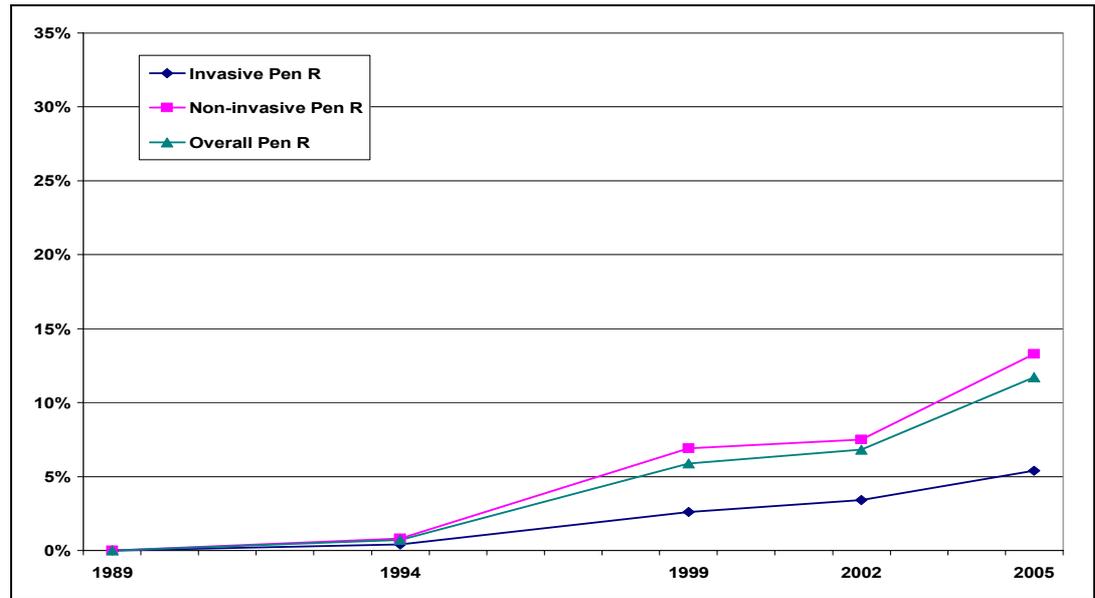
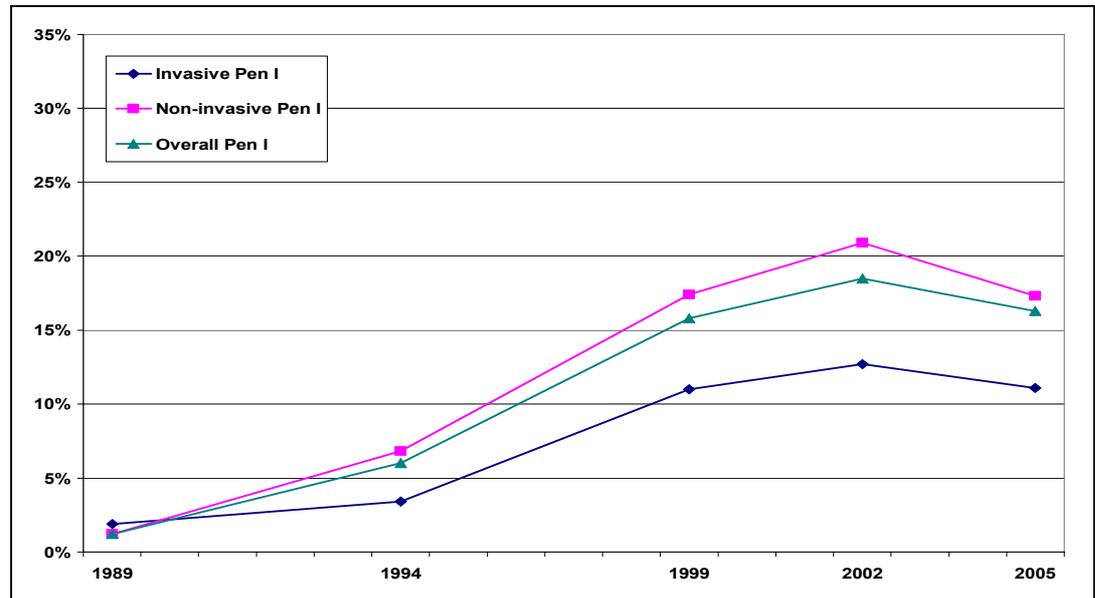


Figure 3a: AGAR surveys 1989 to 2005: Trend in penicillin resistant strains (MIC ≥ 2 mg/L)



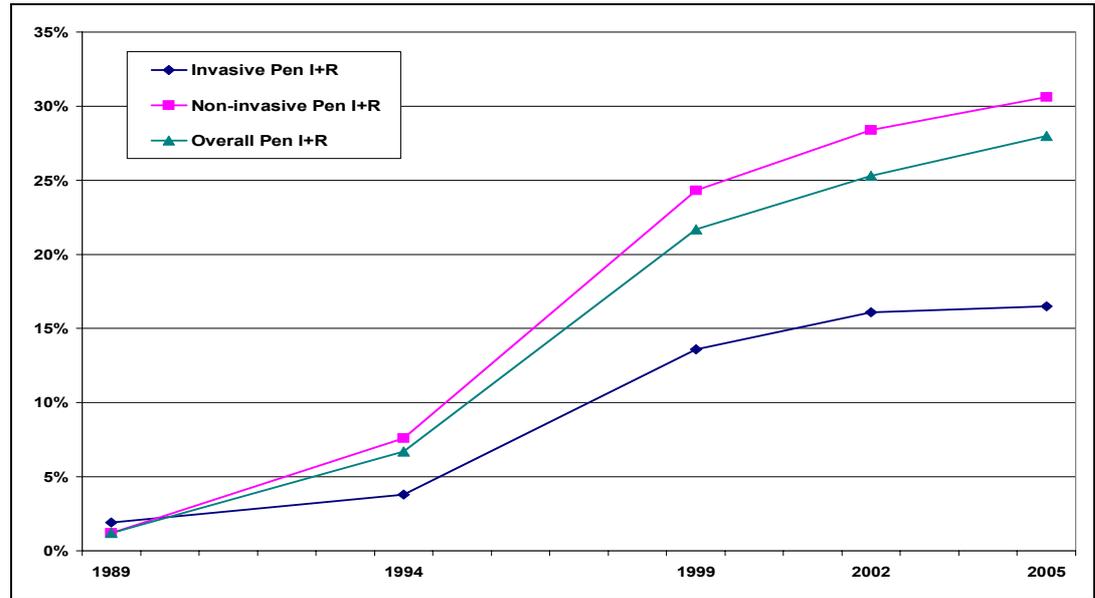
1989: Invasive n=105, non-invasive n=1717, overall n=1822. 1994: Invasive n=532, non-invasive n=1835, overall n=2385. 1999: Invasive n=381, non-invasive n=1167, overall n=1548. 2002: Invasive n=292, non-invasive n=717, overall n=1009. 2005: Invasive n=351, non-invasive n=1416, overall n=1776

Figure 3b: AGAR surveys 1989 to 2005: Trend in penicillin intermediate strains (MIC 0.06-1mg/L)



1989: Invasive n=105, non-invasive n=1717, overall n=1822. 1994: Invasive n=532, non-invasive n=1835, overall n=2385. 1999: Invasive n=381, non-invasive n=1167, overall n=1548. 2002: Invasive n=292, non-invasive n=717, overall n=1009. 2005: Invasive n=351, non-invasive n=1416, overall n=1776

Figure 3c: AGAR surveys 1989 to 2005: Trend in penicillin non-susceptible strains

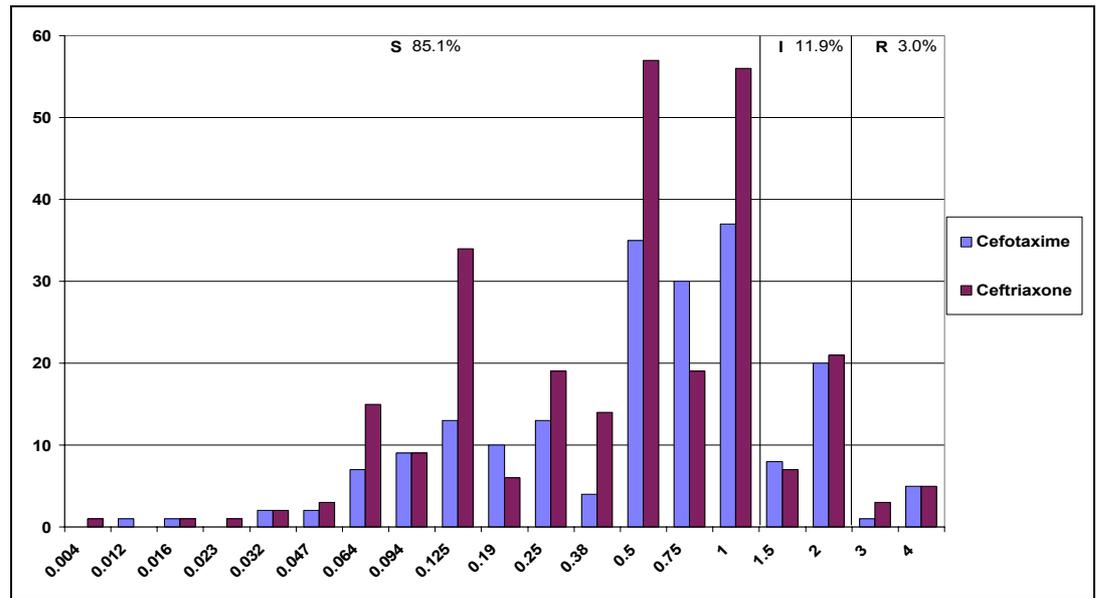


1989: Invasive n=105, non-invasive n=1717, overall n=1822. 1994: Invasive n=532, non-invasive n=1835, overall n=2385. 1999: Invasive n=381, non-invasive n=1167, overall n=1548. 2002: Invasive n=292, non-invasive n=717, overall n=1009. 2005: Invasive n=351, non-invasive n=1416, overall n=1776

6.3 Cefotaxime/Ceftriaxone

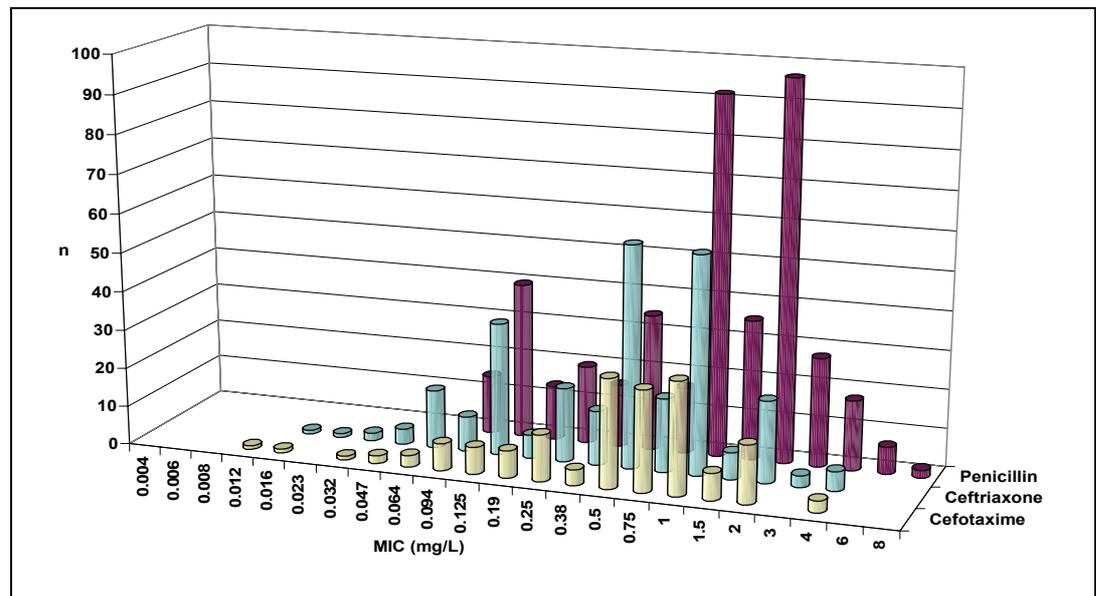
Cefotaxime or ceftriaxone MIC was determined on 471 (95%) of penicillin non-susceptible isolates (MIC >0.064mg/L). Non-susceptibility to the third generation cephalosporins was present in 14.9% of those strains tested (Figure 4). As the cephalosporin intermediate and resistant breakpoints are set at a higher level, compared with penicillin, a reading of overall proportion does not accurately reflect relationship between β -lactam MICs. Figure 5 confirms that for a majority of isolates, cephalosporin MICs present a similar distribution, about two dilutions below that of penicillin. Of 14 cefotaxime/ceftriaxone resistant isolates (MICs >2 mg/L), 13 were also resistant to penicillin (2 – 8 mg/L), and one had intermediate resistance (0.38 mg/L) (Tables 5 and 6). Of these, 13 were also resistant to macrolides, and 11 to tetracycline, but none were resistant to the fluoroquinolones. All six CSF samples were susceptible to ceftriaxone or cefotaxime using the lower (<0.5 mg/L) meningitis breakpoint.

Figure 4. Cefotaxime or Ceftriaxone MIC Distribution for 471 penicillin non susceptible isolates (MIC >0.064 mg/L)



Cefotaxime/ceftriaxone non-meningitis breakpoints: Susceptible ≤ 1 mg/L; Intermediate resistance MIC 2mg/L, Resistant MIC ≥ 4 mg/L.

Figure 5. Penicillin, cefotaxime and ceftriaxone MIC distribution (where penicillin MIC >0.064mg/L)



Penicillin: susceptible MIC ≤ 0.064 mg/L, intermediate resistance MIC 0.12-1mg/L, resistance MIC ≥ 2 mg/L.

Table 6 : Ceftriaxone MICs

Penicillin	Ceftriaxone			Total
	S (≤ 1 mg/L)	I (2 mg/L)	R (≥ 4 mg/L)	
I (0.12 – 1 mg/L)	154 (98.1%)	2 (1.3)	1 (0.6%)	157
R (≥ 2 mg/L)	83 (71.6%)	26 (22.4%)	7 (6.0%)	116
	237	28	8	273

Table 7: Cefotaxime MICs

Penicillin	Cefotaxime			Total
	S (≤ 1 mg/L)	I (2 mg/L)	R (≥ 4 mg/L)	
I (0.12 – 1 mg/L)	108 (97.3%)	3 (2.7%)	0	111
R (≥ 2 mg/L)	56 (64.4%)	25 (28.7%)	6 (6.9%)	87
	164	28	6	198

6.4 Erythromycin and Clindamycin

Erythromycin (macrolide) resistance was detected in 22.7% of isolates overall (Table 8). As for the β -lactam antibiotics, resistance was significantly higher ($P < 0.001$) in non-invasive (24.6%) compared with invasive strains (15.1%) with the exception of Queensland, which had similar rates for both (28.3% and 27.5% respectively). The highest rates of resistance in non-invasive strains were seen in NSW/ACT, with rates of 32%. The lowest rates of resistance for both invasive and non-invasive isolates were in Victoria (4.2% and 15.8% respectively). Resistance was highest among the elderly, and least in the 5-64 age group, paralleling likely macrolide antibiotic use in the community (Table 9).

Erythromycin resistance increased from 1994-1999 for both invasive and non-invasive isolates. Comparison with the previous 2 studies, revealed a rise in resistance for non-invasive isolates (from 19.5% to 24.6%), between 1999 and 2005, but this was not observed for invasive isolates (Figure 7).

Clindamycin resistance was relatively uncommon among invasive strains (5.4% overall), with little variance across Australia (Table 10). A presumptive assessment of resistance genotype was made based on clindamycin resistance and the clindamycin disc induction test among erythromycin resistant isolates (Table 11). Of 364 erythromycin resistant isolates, 222 (61%) had a MLS_B constitutive resistant phenotype and 6 (1.6%) had inducible resistance. These results suggest the presence of an *ermB* mechanism of resistance, associated with higher erythromycin MICs than when due to efflux, in 62.6% of Australian macrolide resistant isolates. All clindamycin resistant isolates were also resistant to the macrolides.

Table 8: Erythromycin resistance: Number Resistant/Total Tested (%).

	Qld	NSW/ACT	Vic	SA	WA	Aus
All	80/284 (28.2%)	162/583 (27.8%)	35/221 (14.5%)	82/392 (20.9%)	48/296 (16.2%)	404/1776 (22.7%)
Invasive	11/40 (27.5%)	26/160 (16.2%)	1/24 (4.2%)	9/73 (12.3%)	6/54 (11.1%)	53/351 (15.1%)
Non-invasive	69/244 (28.3%)	133/415 (32.0%)	31/196 (15.8%)	73/319 (22.9%)	42/242 (17.4%)	348/1416 (24.6%)

Figure 6: Erythromycin resistance by region

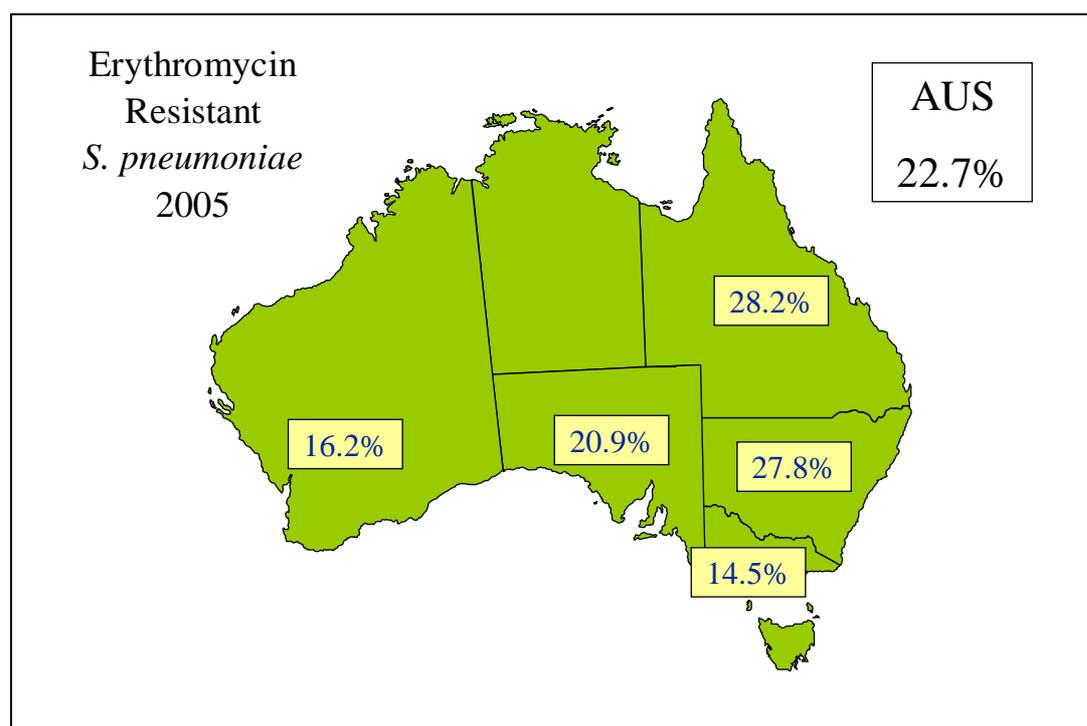
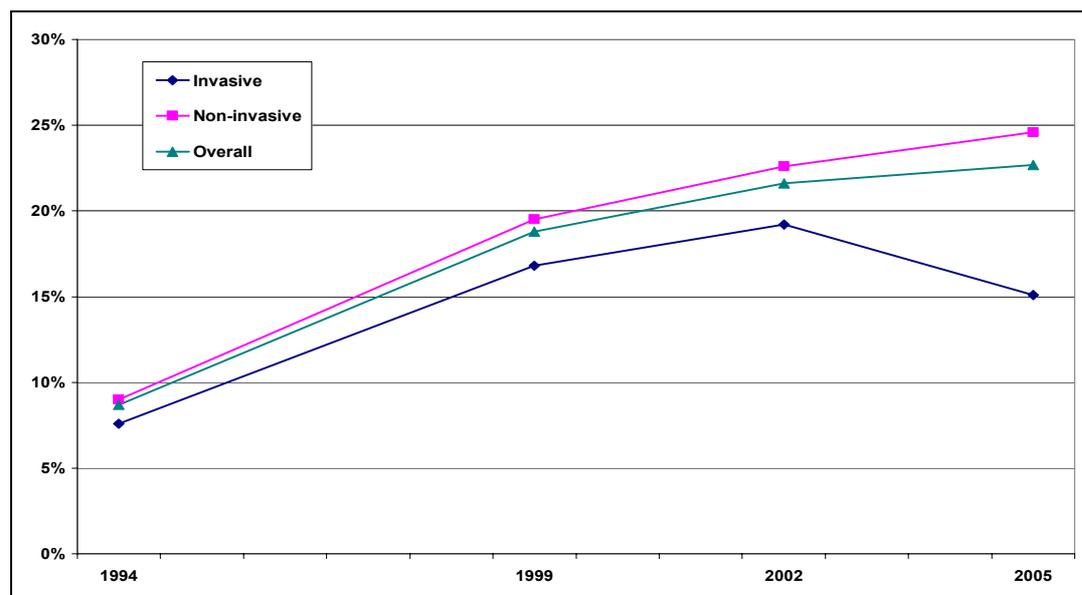


Table 9: Erythromycin resistance by age range: Number non-susceptible/total tested (%).

Age Range			Significance of Results		
<5yo	5-64	>65	Between <5 and 5-64	Between 5-64 and >65	Between <5 and >65
103/445 (23.1%)	162/842 (19.2%)	139/487 (28.5%)	NS	P<0.001	NS

Figure 7: AGAR surveys 1994 – 2005: Trends in erythromycin resistance

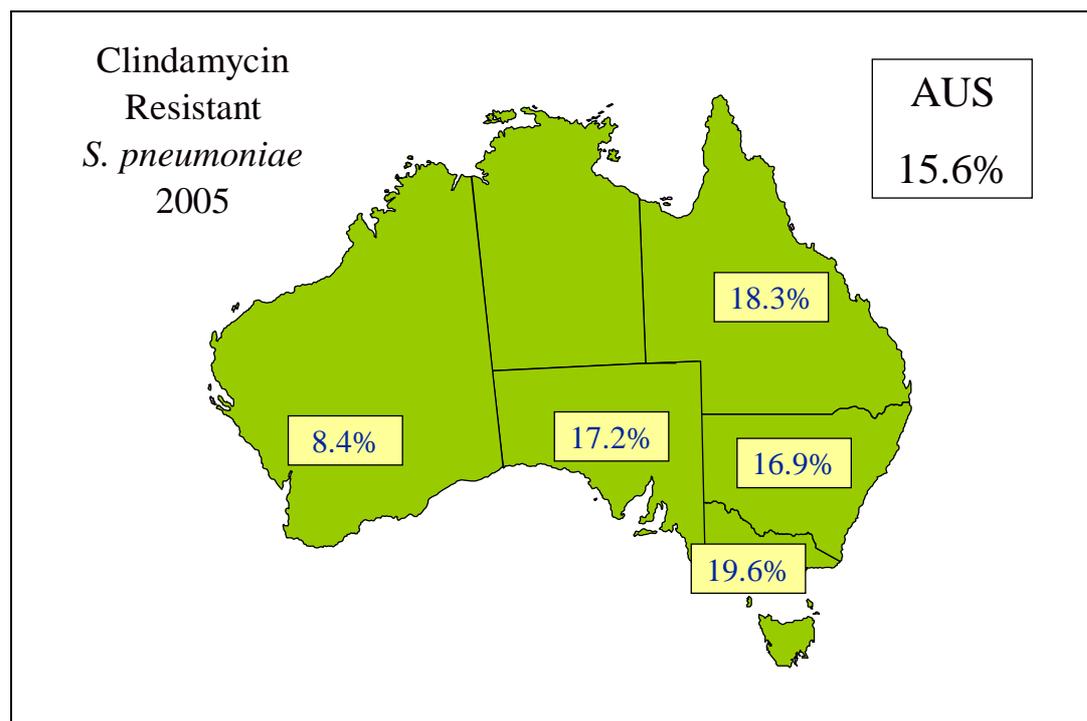


1994: Invasive n=532, non-invasive n=1835, overall n=2385. 1999: Invasive n=381, non-invasive n=1167, overall n=1548. 2002: Invasive n=292, non-invasive n=717, overall n=1009. 2005: Invasive n=351, non-invasive n=1416, overall n=1776

Table 10: Clindamycin resistance: Number Resistant/Total Tested (%).

	Qld	NSW/ACT	Vic	SA	WA	Aus
All	52/284 (18.3%)	88/522 (16.9%)	10/51 (19.6%)	53/309 (17.2%)	25/296 (8.4%)	228/1462 (15.6%)
Invasive	5/40 (12.5%)	7/143 (4.9%)	1/9 (11.1%)	2/67 (3.0%)	2/54 (3.7%)	17/313 (5.4%)
Non-invasive	47/244 (19.3%)	80/376 (21.3%)	9/42 (21.4%)	51/242 (21.1%)	23/242 (9.5%)	210/1146 (18.3%)

Figure 8: Clindamycin resistance by Region



6.5 Clindamycin Genotype

Table 11: Presumptive genotype of erythromycin non-susceptible strains

	Erythromycin R	%	Presumptive genotype
Clindamycin S	136	37.4%	<i>mef</i>
Clindamycin inducible resistance	6	1.6%	<i>erm</i>
Clindamycin R	222	61.0%	<i>erm</i>

6.6 Tetracycline

There was a significant difference in tetracycline resistance among non-invasive (21.3%) and invasive (6.3%) strains ($P < 0.001$). Compared with β -lactam and macrolide resistance, tetracycline resistance in invasive isolates was consistently $< 10\%$ across all Australian states. Queensland and New South Wales/ACT had the highest rates of resistance demonstrated in non-invasive strains (24.6% and 25.5% respectively). Unlike rates for penicillins and macrolides, resistance was not higher in the young (< 5 years), as would be predicted by lack of tetracycline use in this age group. Rates were again highest in the elderly. Tetracycline resistance increased from 1994-1999, all of the change being seen in non-invasive isolates. No significant rise in tetracycline resistance was demonstrated between 1999 and 2005 (16.9% and 18.4% respectively).

Table 12: Tetracycline resistance: Number Resistant/Total Tested (%).

	Qld	NSW/ACT	Vic	SA	WA	Aus
All	63/284 (22.2%)	121/583 (20.8%)	38/221 (17.2%)	60/391 (15.3%)	44/296 (14.9%)	326/1775 (18.4%)
Invasive	3/40 (7.5%)	12/160 (7.5%)	2/24 (8.3%)	2/73 (2.7%)	3/54 (5.6%)	22/351 (6.3%)
Non-invasive	60/244 (24.6%)	106/415 (25.5%)	36/196 (18.4%)	58/318 (18.2%)	41/242 (16.9%)	301/1415 (21.3%)

Figure 9: Tetracycline resistance by region

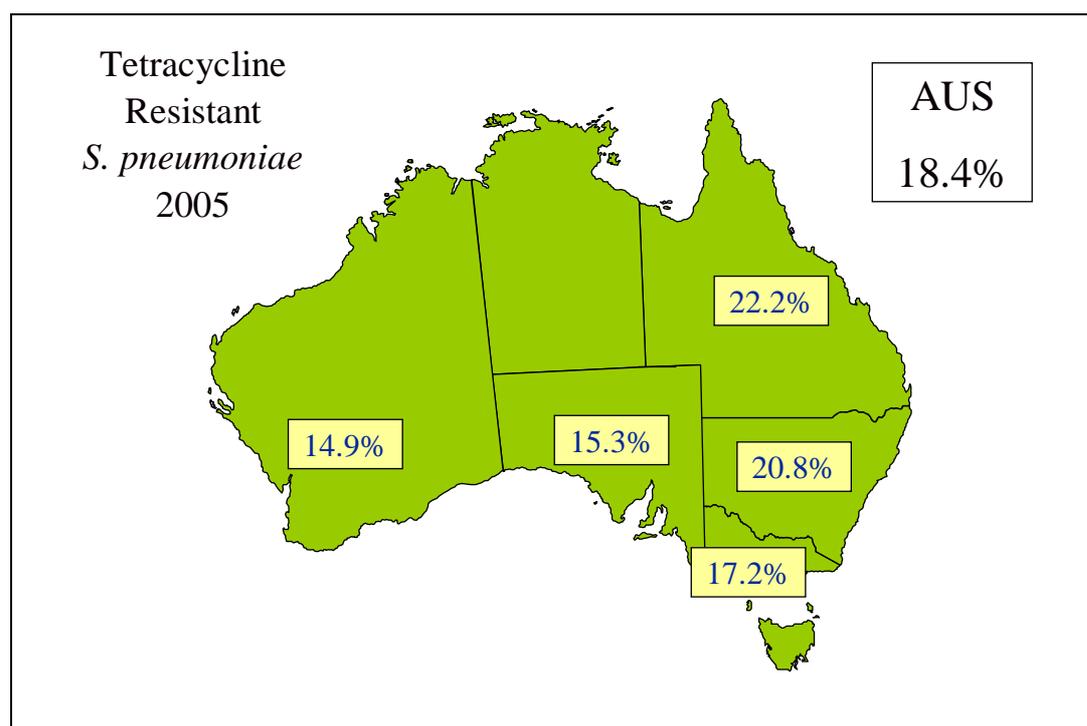
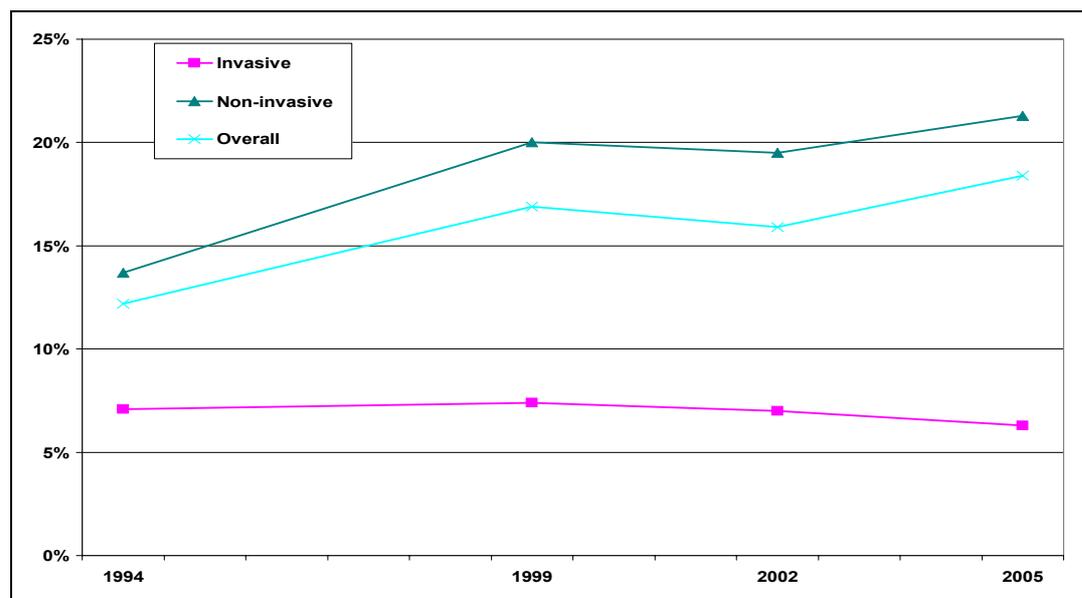


Table 13: Tetracycline resistance by age range: Number non-susceptible/total tested (%).

Age Range			Significance of Results		
<5yo	5-64	>65	Between <5 and 5-64	Between 5-64 and >65	Between <5 and >65
75/444 (16.9%)	132/842 (15.7%)	119/487 (24.4%)	NS	P<0.005	P<0.005

Figure 10: AGAR surveys 1994 – 2005: Trends in tetracycline resistance



1994: Invasive n=532, non-invasive n=1835, overall n=2385. 1999: Invasive n=381, non-invasive n=1167, overall n=1548. 2002: Invasive n=292, non-invasive n=717, overall n=1009. 2005: Invasive n=351, non-invasive n=1416, overall n=1776

6.7 Trimethoprim-sulphamethoxazole

Unlike other antibiotic classes, trimethoprim-sulphamethoxazole (TMP-SMX) resistance appears to have peaked in the previous decade, and in this study has decreased Australia-wide, compared with previous studies. Trimethoprim-sulphamethoxazole resistance decreased from 1999-2005 for both invasive and non-invasive isolates (Figure 12). Nonetheless, rates of resistance of 25.6% for invasive and 32.2% for non-invasive strains limit the use of TMP-SMX in infections caused by *S. pneumoniae* (Table 14). The trends to higher resistance in non-invasive strains in the eastern states, particularly NSW and Queensland, is maintained for TMP-SMX. The resistance rates among invasive strains across Australia were generally lower and did not vary as greatly between states.

Table 14: Trimethoprim-sulphamethoxazole resistance: Number Resistant/Total Tested (%).

	Qld	NSW/ACT	Vic	SA	WA	Aus
All	101/284 (35.6%)	198/583 (34.0%)	65/221 (29.4%)	122/391 (31.2%)	64/296 (21.6%)	550/1775 (31.0%)
Invasive	8/40 (20.0%)	43/160 (26.9%)	7/24 (29.2%)	22/73 (30.1%)	10/54 (18.5%)	90/351 (25.6%)
Non-invasive	93/244 (38.1%)	151/415 (36.4%)	58/196 (29.6%)	100/318 (31.4%)	54/242 (22.3%)	456/1415 (32.2%)

Figure 11: Trimethoprim-sulphamethoxazole resistance by region

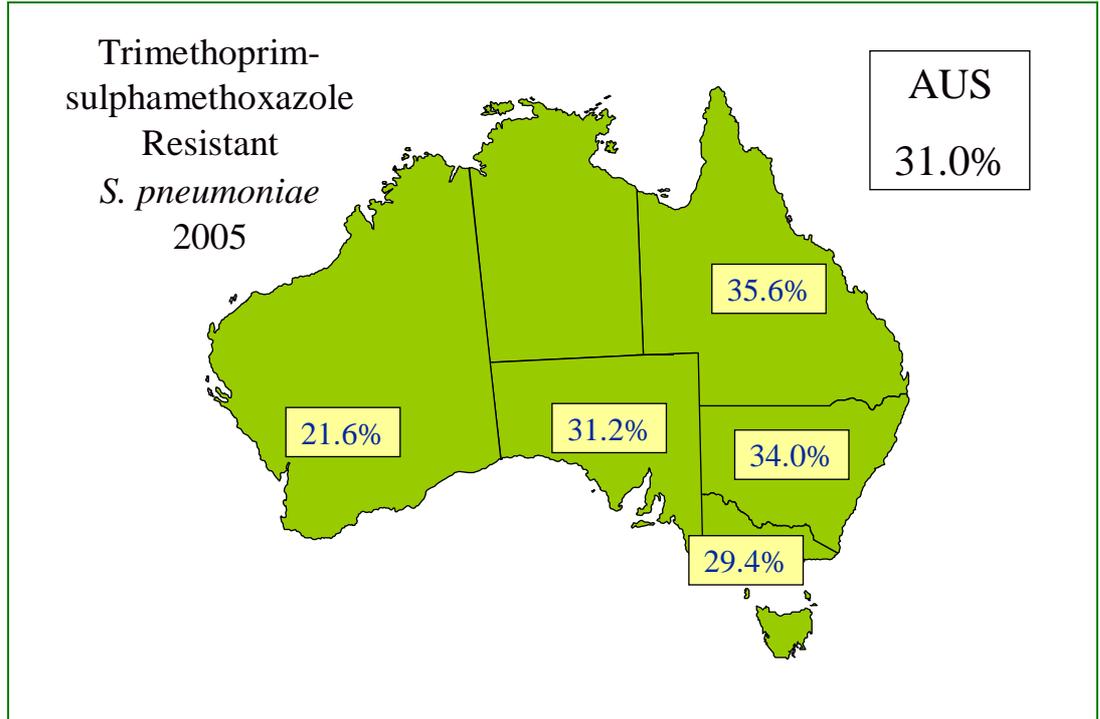
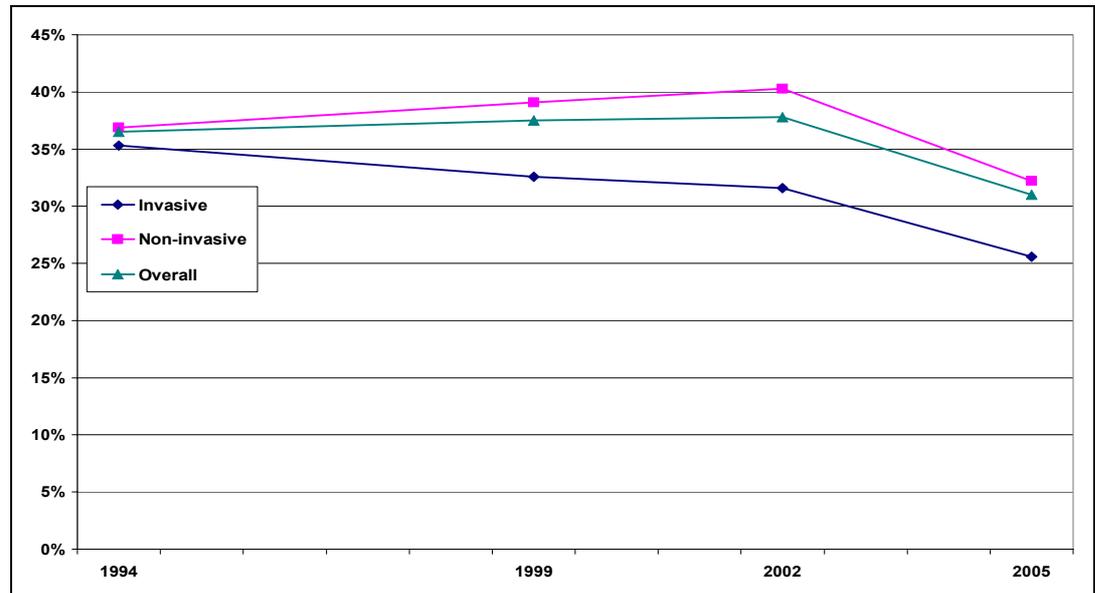


Figure 12: AGAR surveys 1994 – 2005: Trends in trimethoprim-sulphamethoxazole resistance



1994: Invasive n=532, non-invasive n=1835, overall n=2385. 1999: Invasive n=381, non-invasive n=1167, overall n=1548. 2002: Invasive n=292, non-invasive n=717, overall n=1009. 2005: Invasive n=351, non-invasive n=1416, overall n=1776

6.8 Fluoroquinolones

In 2005 fluoroquinolone resistance remains uncommon in Australia. By disc testing, levofloxacin resistance was detected in only four of 1775 (0.2%) isolates tested (Table 15). Intermediate resistance to levofloxacin was detected in another four isolates. All eight were non-invasive isolates and were detected in NSW (4), South Australia (3) and Queensland (1). Moxifloxacin resistance was present in two isolates with MICs of 3 mg/L and 4 mg/L. We are unable to confirm whether

moxifloxacin resistance in these patients was acquired after pre-treatment with fluoroquinolones or if they may have recently been overseas.

6.8.1 Levofloxacin

Table 15: Levofloxacin resistance: Number Resistant/Total Tested (%).

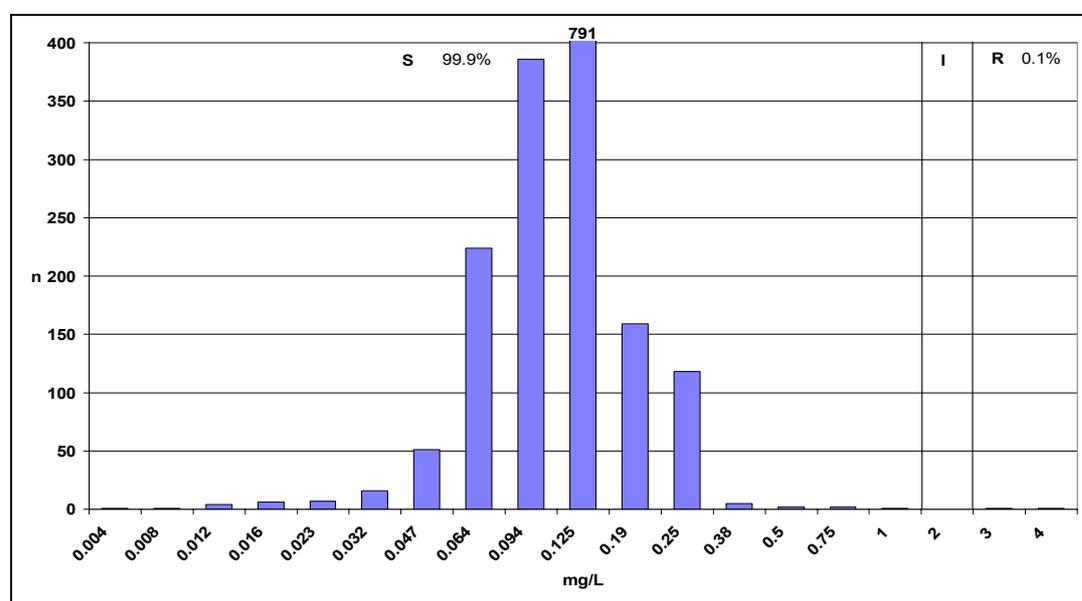
	Qld	NSW/ACT	Vic	SA	WA	Aus
All	1/284 (0.4%)	2/583 (0.3%)	0/221 (0%)	1/391 (0.3%)	0/296 (0%)	4/1775 (0.2%)
Invasive	0/40 (0%)	0/160 (0%)	0/24 (0%)	0/73 (0%)	0/54 (0%)	0/351 (0%)
Non-invasive	1/244 (0.4%)	2/415 (0.5%)	0/196 (0%)	1/318 (0.3%)	0/242 (0%)	4/1415 (0.3%)

6.8.2 Moxifloxacin

Table 16: Moxifloxacin resistance: Number Resistant/Total Tested (%).

	Qld	NSW/ACT	Vic	SA	WA	Aus
All	1/284 (0.4%)	0/583 (0%)	0/221 (0%)	1/392 (0.3%)	0/296 (0%)	2/1776 (0.1%)
Invasive	0/40 (0%)	0/160 (0%)	0/24 (0%)	0/73 (0%)	0/54 (0%)	0/351 (0%)
Non-invasive	1/244 (0.4%)	0/415 (0%)	0/196 (0%)	1/318 (0.3%)	0/242 (0%)	2/1416 (0.1%)

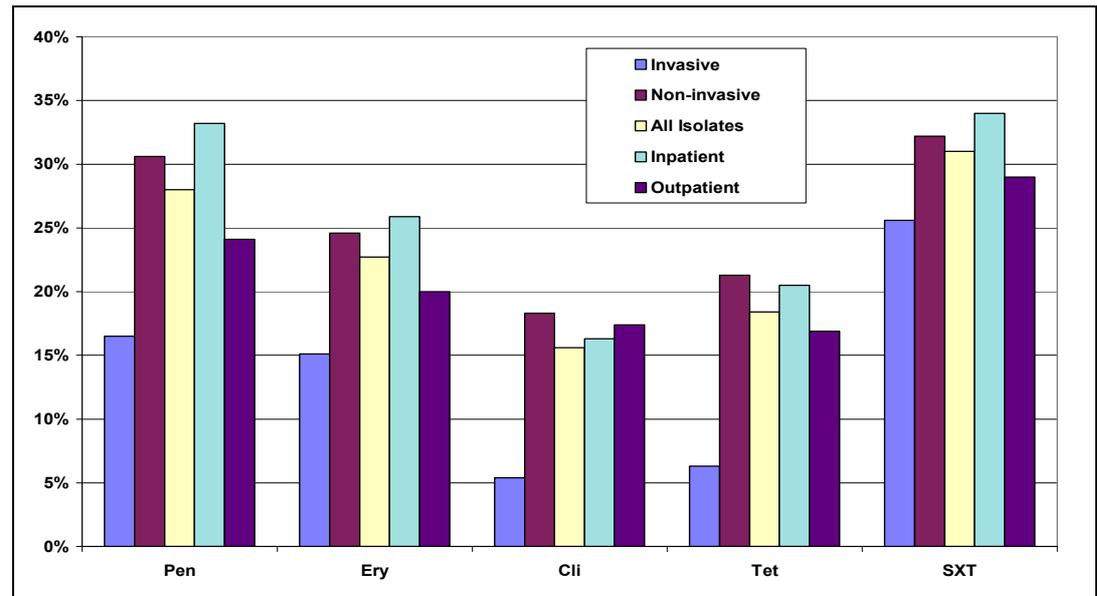
Figure 12: Moxifloxacin MIC Distribution



6.9 Resistance Rates – Invasiveness and Hospitalisation Status

Figure 13 compares the difference in resistance rates for invasive and non-invasive isolates for all antibiotics, other than the fluoroquinolones. For all antibiotics there are statistically significant higher levels of resistance among non-invasive isolates. For penicillin, erythromycin and trimethoprim-sulphamethoxazole there are statistically significant higher levels of resistance in inpatients than outpatients.

Figure 13: Proportion of resistant isolates Australia-wide by Source and Hospitalisation



Pen= penicillin (non-susceptible), Ery = erythromycin; Cli = clindamycin Tet = tetracycline; SXT = trimethoprim-sulphamethoxazole

7 Multi-resistance

The most problematic strains of *S. pneumoniae* are those with multiple acquired resistances. Although there is no agreed benchmark for the definition of multi-resistance in *S. pneumoniae*, we have chosen acquired resistance to > 2 drug classes to define multi-resistance in this survey.

Table 17: Multi-resistance in *S. pneumoniae*

Region	No	Non-multiresistant				Multi-resistant		
		0	1	2	%	3	4	%
Qld	284	144	48	31	78.5%	13	48	22.5%
NSW/ACT	583	307	90	62	78.7%	42	82	21.3%
Vic	221	140	31	16	84.6%	15	19	15.4%
SA	392	223	75	40	86.2%	5	49	13.8%
WA	296	191	46	24	88.2%	12	23	11.8%
Australia	1776	1005	290	173	82.7%	87	221	17.3%
Invasive	351	216	77	37	94.0%	12	9	6.0%
Non-invasive	1416	786	211	135	79.9%	75	209	20.1%

Antibiotics included: penicillin (intermediate or resistant); erythromycin, tetracycline, trimethoprim-sulphamethoxazole, levofloxacin. Antibiotics excluded: clindamycin, cefotaxime, ceftriaxone.

8 Limitations of the Study

There have been changes in participating laboratories in the AGAR *S. pneumoniae* surveys over time from 1989 through to 2005 with the more recent inclusion of a number of private pathology laboratories.

9 Discussion

Internationally changes in susceptibility in *S. pneumoniae* have evolved rapidly in the past two decades for both β -lactam and other antibiotic classes, with potential to compromise treatment efficacy. Susceptibility rates have varied widely according to reporting countries, clinical site of infection (eg. invasive versus non-invasive infection), and according to pneumococcal serotype. Timely national surveillance studies that examine trends in antimicrobial resistance are essential for the formulation of appropriate, and up to date evidence-based therapeutic guidelines and in monitoring their ongoing relevance. This has implications for prescribing in situations where *S. pneumoniae* remains the major pathogen; primarily community acquired lower respiratory tract infections and upper respiratory tract infections, including sinusitis and otitis media, as well as the empiric treatment of community acquired meningitis. The Australian Group for Antimicrobial Resistance (AGAR), a network of Australian laboratories, has conducted studies in 1989, 1994, 1999, 2002 and 2005 to monitor changes in *S. pneumoniae* susceptibility.

In this article we report on the results of the 2005 survey conducted by 20 laboratories nationally. We have also briefly summarised overall trends in susceptibility compared to previous AGAR studies. Validation of trend data was made potentially difficult by changes in laboratory representation through successive studies. We therefore conducted an analysis of data for 8 laboratories that participated in all the studies since 1994. The results for these laboratories mirrored the trend results obtained when all the contributing laboratories were combined. We have therefore presented the data for all participating laboratories in the trend analysis.

In AGAR sponsored studies, penicillin non-susceptibility has continued to increase albeit at a slower rate than first observed in the early 1990s, reaching a national level in this survey of 28%. This increase has occurred in both the penicillin intermediate and resistant categories. In 1989 only 1% of strains tested were penicillin non-susceptible and only intermediate resistance to penicillin was detected. No high level resistant strains were found.¹ In 1994, 6.7% of isolates were non-susceptible, the majority with intermediate resistance. Although by 2005 the penicillin intermediate group has increased to 16.3%, this level of resistance has remained steady over the past 6 years. In this period however the rate of *S. pneumoniae* resistance has doubled from 5.9% in 1999 to 11.7% in 2005.^{2,3}

As has been noted previously, in 2005 higher rates of resistance were seen in strains causing non-invasive infection compared to invasive infection. This may be due to a number of contributing factors, including the differing serotypes that are involved in invasive and bloodstream infections and the selective pressure of frequent antibiotic use in upper respiratory tract infections. As observed worldwide, the latter appears to be the major factor involved in development of antibiotic resistance in *S. pneumoniae*. Cephalosporin, macrolide and more recently fluoroquinolone usage data have been linked with corresponding rises in penicillin, erythromycin, quinolone and multidrug resistance.

The changes in resistance rates will impact on empiric β -lactam therapy but mainly for meningitis. In non-invasive pneumococcal infection in Australia, the currently recommended antibiotic of choice for oral therapy is amoxicillin.⁴ When higher doses are employed, amoxicillin achieves adequate tissue levels and is active against penicillin-intermediate strains. Between 1999 and 2005 there was an almost twofold increase in the number of non-invasive isolates with a MIC ≥ 2 mg/L from 6.9% to 13.3%. The only states not to show increases in this category were NSW/ACT and SA, though NSW/ACT already had established high levels of resistance. These ongoing changes require continuing observation but amoxicillin prescribed at the upper dosing range, still continues to be appropriate therapy for the vast majority of non-invasive infections occurring in Australia.

Despite lower rates compared with non-invasive strains, increasing numbers of invasive strains also demonstrate high-level resistance to penicillin. The rates in invasive strains rose from 2.6% to 5.4% between 1999 and 2005. In non-meningeal infection, the relationship of penicillin resistance to outcomes continues to be a source of controversy. Recent studies document a higher mortality associated with penicillin non-susceptibility¹² though the reasons are not clear as other studies have failed to associate worsening outcome with raised penicillin MICs. Where pneumococci are β -lactam susceptible or in the intermediate range, therapy with these agents is appropriate. Invasive strains with an MIC ≥ 2 mg/L remain relatively uncommon (5.4%). Reviews of the literature on the clinical implications of penicillin resistance^{13,14} suggest that failures of therapy are not predicted until MIC levels are ≥ 4 mg/L. As strains with MICs ≥ 4 mg/L are rare (1.6%) in Australia, data from the most recent AGAR study support current recommendations for high dose parenteral penicillin in non-meningeal invasive *S. pneumoniae* infection.

The 16.5% penicillin non-susceptibility rate in invasive strains (58/351) mitigates against the use of penicillin in empiric therapy of meningitis. Similar rates (13.5%) were described in a 2004 review of Australian invasive pneumococcal isolates.¹⁵ Third generation cephalosporins in combination with vancomycin are recommended as empiric therapy for meningitis.⁴ Changes in therapy can be instituted once the MIC is available (usually about 48 hours after patient presentation). New meningitis breakpoints were adopted by the CLSI recently. When isolates have cefotaxime or ceftriaxone MIC's ≥ 2 mg/L, even cephalosporins may be ineffective. However, of note in the 2005 study, all CSF isolates were susceptible to the 3rd generation cephalosporins using the CLSI meningial breakpoints.

In 2005 erythromycin resistance was documented in 22.7% of isolates, a rise of 5% since 1999. Increases were primarily in non-invasive isolates. Of 364 erythromycin resistant

isolates, 228 (62.6%) had a MLS_B phenotype suggestive of an *ermB* resistance mechanism, and associated with high level erythromycin resistance (Table 11). This is consistent with previous Australian data^{16,17} and similar to European data for macrolide resistance, in which *ermB* resistance predominates. It differs to data from the USA^{18,19} where up to 72% of macrolide resistance in the US was on the basis of macrolide efflux (*mef*) and in which some argue that macrolides may still be effective in some non-invasive infections. However it has now been clearly documented that there is an association between macrolide resistance and therapeutic failures in bacteraemic *S. pneumoniae* infection even when resistance is mediated by a macrolide efflux pump (*mef*) and MICs are lower (often between 1 to 16 µg/ml).^{6,20} The AGAR study data suggests that if macrolides are used as sole empiric therapy for respiratory tract infections when pneumococci may be implicated, many of these isolates (24.6% of non-invasive strains) are unlikely to respond to this class of antibiotics. The current Australian Antibiotic Guidelines⁴ do not recommend macrolides as empiric therapy associated with otitis media, sinusitis, acute exacerbations of chronic bronchitis or as empiric therapy for community acquired pneumonia except where additional cover for atypical organisms is required.

Tetracycline resistance in invasive isolates was below 10% across all Australian states. This may reflect the restricted use of oral tetracyclines for non-invasive infections in adults, and its restricted use for all infections in children.

A fall in resistance was demonstrated for trimethoprim-sulphamethoxazole which has fallen from 37.8% in 2002 to 31.0% in 2005. Whilst it is considered an unsuitable drug for use in respiratory tract infection, the fall may reflect the reduction in antibiotic pressure due to a decline in prescribing of this drug in the community. It is notable that the tetracycline and folate synthesis inhibitor drugs were the two classes of antibiotics to show significant reduction in community prescribing over the past 14 years, 53% and 54% reduction respectively (unpublished data).²¹

The new “respiratory” fluoroquinolones, such as moxifloxacin are valuable drugs for the therapy of *S. pneumoniae* infections, particularly for infection by multi-drug resistant strains. However in countries where fluoroquinolones (particularly levofloxacin) have been used widely for respiratory tract infections, rising resistance levels are seen. Levels of 14% have been measured for levofloxacin in Hong Kong.²² In some regions of France resistance levels of 3% to levofloxacin have been observed²³. In Europe and Canada a clear relationship is documented between the levels of fluoroquinolone use and rising resistance rates.^{24,25,26} As for macrolides, quinolone resistance has been linked to failure of pneumonia therapy.²⁷ Because of the recent introduction of the new fluoroquinolones into clinical use, the AGAR 2005 *S. pneumoniae* study undertook an evaluation of resistance rates using levofloxacin discs and moxifloxacin Etest MIC testing to detect early development of resistance. The results demonstrated extremely low levels of resistance. Of 1776 isolates tested, only 2 moxifloxacin resistant non-invasive isolates were detected (0.1%). Both isolates were multiresistant. The low fluoroquinolone resistance levels are likely to be related to the relatively low volumes of these drugs that have so far been used in Australia, because of restrictions placed on their use (eg the need for an Authority prescription in the community and restricted formularies in most public hospitals). Australian guidelines have not endorsed fluoroquinolone use as first line therapy in community acquired pneumonia.

Overall the level of penicillin resistance in *S. pneumoniae* in Australia appears to have stabilised over the last 6 years, or is only slowly rising (Figure 3). The reason this has occurred is unclear as in many other countries penicillin resistance rates as high as 80 to 90% are seen. In Vietnam in 2000/01 92% penicillin non-susceptibility was reported, 71% of which was high level resistance.²⁸

Total antibiotic prescriptions in the Australian outpatient community have shown a steady and progressive fall from 1994 through to 2003 i.e 25.6 to 19.8 DDD/1000 population/day. In the last two years this trend has reversed but still remains 15% below 1994 levels i.e 21.7 DDD/1000 population/day.²¹ This overall reduction in antibiotic prescribing in the

Australian community is likely to be a contributing factor to the relatively lower resistance levels seen in Australia compared with other regions such as Eastern Europe and East Asia.

Although rising levels of antibiotic resistance are often regarded as inevitable, a number of studies have shown a decline in resistance trends after implementation of community prescribing restrictions. A study in Canberra involving young children showed that when not exposed to antibiotics for a period of 6 months or more, carriage rates of penicillin-resistant pneumococci fell to the rate seen in the general community.²⁹

Immunisation has been effective not only in decreasing disease in children but also in their parents and grandparents.^{30,31,32} In reducing disease burden it also has the potential to reduce antibiotic resistance rates by decreasing community driven antibiotic selection pressure.

Regular AGAR surveillance studies of *S. pneumoniae* resistance have allowed a much clearer picture of changing susceptibility in this important community pathogen. After a rapid emergence and rise in resistance between 1989 and 1999, recent studies have documented a gradual rise or decrease for non- β -lactam antimicrobials. Rates of "high-level" penicillin resistance and multi-resistance continue to rise. Overall, based on AGAR studies, Australia has lower rates of resistance in *S. pneumoniae* than many other countries. Introduction and maintenance of effective vaccination programmes as well as continuing promotion of prudent use of antibiotic prescribing in the community are crucial to maintaining effective control on resistance. If Australia can adopt these measures, there is an opportunity to curb this rise and to effect a reduction in resistance rates and preserve current antibiotics into the future. Ongoing surveillance studies such as the ones performed by the AGAR group are an important tool in measuring continuing trends in antibiotic resistance and as an indirect measure of the successes and failures of our health policies.

10 References

1. Collignon P, Bell J. *Streptococcus pneumoniae*: how common is penicillin resistance in Australia? *J Aust NZ Med* 1992;22:473-6.
2. Collignon P, Bell J, on behalf of the Australian Group for Antimicrobial Resistance (AGAR). Drug-resistant *Streptococcus pneumoniae*: the beginning of the end for many antibiotics? *Med J Aust* 1996;164:64-7.
3. Nimmo G, Bell J, Collignon P, on behalf of the Australian Group for Antimicrobial Resistance (AGAR). Fifteen years of surveillance by the Australian Group for Antimicrobial Resistance (AGAR). *Commun Dis Intell* 2003;27:547-54.
4. Therapeutic guidelines: antibiotic. Thirteenth edition. Melbourne: Therapeutic Guidelines Ltd; 2006.
5. Pallares R, Liñares J, Vadillo M, Cabellos C, Manresa F, Viladrich P, Martin R, Gudiol, F. Resistance to penicillin and cephalosporin and mortality from severe pneumococcal pneumonia in Barcelona, Spain. *N Engl J Med* 1995;333:474-80.
6. Daneman N, McGeer A, Green K, Low D. Macrolide resistance in bacteremic pneumococcal disease: implications for patient management. *Clin Infect Dis* 2006;43:432-8.
7. Clinical and Laboratory Standards Institute (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard – seventh edition. M7-A7. CLSI, Villanova, PA, USA.
8. National Committee on Clinical Laboratory Standards (2003). Performance standards for antimicrobial disk susceptibility tests: approved standard – eighth edition. M2-A8. NCCLS, Wayne, PA, USA.

9. Clinical and Laboratory Standards Institute (2006). Performance standards for antimicrobial susceptibility testing; fifteenth Informational Supplement. M100-S16. CLSI, Villanova, PA, USA.
10. Bell S, Gatus B, Pham J, Rafferty D. Antibiotic susceptibility testing by the CDS method: A manual for medical and veterinary laboratories. Third edition 2004. www.med.unsw.edu.au/pathology-cds
11. BSAC disc diffusion method for antimicrobial testing. Version 3.1 2004. www.bsac.org.uk
12. Tleyjah I, Thaygeh H, Hejal R, Montori V, Baddour L. The impact of penicillin resistance on short-term mortality in hospitalized adults with pneumococcal pneumonia: a systematic review and meta-analysis. *Clin Infect Dis* 2006;42:788-97.
13. Peterson L. Penicillins for treatment of pneumococcal pneumonia: Does in vitro resistance really matter? *Clin Infect Dis*. 2006;42:224-33.
14. Chiou C. Does penicillin remain the drug of choice for pneumococcal pneumonia in view of emerging in vitro resistance? *Clin Infect Dis*. 2006;42:234-37.
15. Roche P, Krause V, Bartlett M, Coleman D, Cook H, Davis C, Fielding J, Holland R, Giele C, Gilmour R, Kampen R, Brown M, Gilbert L, Hogg G, Murphy D, for the Pneumococcal Working Party. Invasive pneumococcal disease in Australia. *Comm Dis Intell* 2006;30(1):80-92.
16. Watson M, Roche P, Bayley K, Bell J, Collignon P, Gilbert G, Hogg G, Keil A, Krause V, Murphy D, Smith H, Brown M, Stylianopoulos, Turnidge J. Laboratory Surveillance of Invasive Pneumococcal Disease in Australia, 2003 – predicting the future impact of the universal childhood conjugate vaccine program. *Commun Dis Intell* 2004;28:455-64.
17. Gosbell I, Neville S. Antimicrobial resistance in *Streptococcus pneumoniae*: a decade of results from south western Sydney. *Comm Dis Intell*. 2000;24:340-3.
18. Jones R, Pfaller M. Macrolide and fluoroquinolone (levofloxacin) resistances among *Streptococcus pneumoniae* strains: significant trends from the SENTRY antimicrobial surveillance program (North America, 1997-1999). *J Clin Microbiol* 2000;38:4298-9.
19. Doern G, Richter S, Miller A, Miller N, Rice C, Heilmann K, Beekman S. Antimicrobial resistance among *Streptococcus pneumoniae* in the United States: have we begun to turn the corner on resistance to certain antimicrobial classes? *Clin Infect Dis* 2005;41:139-148.
20. Lonks J, Garau J, Medeiros A. Implications of antimicrobial resistance in the empirical treatment of community-acquired respiratory tract infections: the case for macrolides. *J Antimicrob Chemother* 2002;50 S2:87-92.
21. Drug Utilisation Subcommittee, Pharmaceutical Benefits Branch, Health Access and Financing Division, Commonwealth Department of Health and Ageing.
22. Canton R, Morosini M, Enright M, Morrissey I. Worldwide incidence, molecular epidemiology and mutations implicated in fluoroquinolone-resistant *Streptococcus pneumoniae*: data from the global PROTEKT surveillance programme. *J Antimicrob Chemother* 2003;52:944-52.
23. Reinert R, Reinert S, van der Linden M, Cil M, Al-Lahham A, Appelbaum P. Antimicrobial susceptibility of *Streptococcus pneumoniae* in eight European countries from 2001 to 2003. *Antimicrob Agents Chemother* 2005;49:2903-13.
24. Goldstein E, Garabedian-Ruffalo S. Widespread use of fluoroquinolones versus emerging resistance in pneumococci. *Clin Infect Dis*. 2002;35:1505-11.
25. Chen D, McGeer A, de Azavedo J, Low D. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. *N Engl J Med*. 1999;341:233-9.

26. Pallares R, Fenoll A, Liñares J, The Spanish Pneumococcal Infection Study Network. The epidemiology of antibiotic resistance in *Streptococcus pneumoniae* and the clinical relevance of resistance to cephalosporins, macrolides and quinolones. *Int J Antimicrob Agents* 2003;22:S15-24.
27. Davidson R, Cavalcanti R, Brunton J, Bast D, Azavedo J, Kibsey P, Fleming C, Low D. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. *N Engl J Med* 2002;346:747-50.
28. Song J, Jung S, Ko K, Kim N, Son J, Chang H, Ki H, Oh W, Suy J *et al.* High prevalence of antimicrobial resistance among clinical *Streptococcus pneumoniae* isolates in Asia (an ANSORP study). *Antimicrob Agents Chemother* 2004;48:2101-7.
29. Nasrin D, Collignon P, Roberts L, Wilson E, Pilotto L, Douglas R. Effects of beta lactam antibiotic use in children on pneumococcal resistance to penicillin: prospective cohort study. *Brit Med J* 2002;324:28-30.
30. Musher D. Pneumococcal vaccine – direct and indirect (“Herd”) effects. *N Engl J Med* 2006;354:1522-4.
31. Dagan R, Givon-Lavi N, Zamir O, Fraser, D. Effect of a nonavalent conjugate vaccine on carriage of antibiotic-resistant *Streptococcus pneumoniae* in day-care centers. *Paed Infec Dis* 2003;22:532-9.
32. Kyaw M, Lynfield R, Schaffner W, Craig A, Hadler J, Reingold A, Thomas A, Harrison L *et al.* Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006;354:1455-63.

11 Acknowledgements

AGAR thanks Bayer Health for supplying the moxifloxacin Etests.

Appendix 1 Trends in Resistance 1989-2005

Invasive	1989	1994	1999	2002	2005
PEN	n=105	n=532	n=381	n=292	n=351
I	1.9%	3.4%	11.0%	12.7%	11.1%
R	0%	0.4%	2.6%	3.4%	5.4%
Ery	NT	n=528 7.6%	n=380 16.8%	n=313 19.2%	n=351 15.1%
Tet	NT	n=422 7.1%	n=379 7.4%	n=313 7.0%	n=351 6.3%
SXT	NT	n=462 35.3%	n=380 32.6%	n=313 31.6%	n=351 25.6%

Non-Invasive	1989	1994	1999	2002	2005
PEN	n=1702	n=1835	n=1167	n=717	n=1416
I	0.8%	6.8%	17.4%	20.9%	17.3%
R	0%	0.8%	6.9%	7.5%	13.3%
Ery	NT	n=1835 9.0%	n=1172 19.5%	n=774 22.6%	n=1416 24.6%
Tet	NT	n=1562 13.7%	n=1167 20.0%	n=774 19.5%	n=1415 21.3%
SXT	NT	n=1608 36.9%	n=1171 39.1%	n=774 40.3%	n=1415 32.2%

All Isolates	1989	1994	1999	2002	2005
PEN	n=1822	n=2385	n=1548	n=1009	n=1776
I	1.1%	6.0%	15.8%	18.5%	16.3%
R	0%	0.7%	5.9%	6.8%	11.7%
Ery	NT	n=2381 8.7%	n=1552 18.8%	n=1087 21.6%	n=1776 22.7%
Tet	NT	n=2000 12.2%	n=1546 16.9%	n=1087 15.9%	n=1775 18.4%
SXT	NT	n=2088 36.5%	n=1551 37.5%	n=1087 37.8%	n=1775 31.0%

NT: Not tested

Appendix 2 Antibiotic Profiles by Frequency

Profile	n	%
- - - - -	1005	56.6%
PenEryTetSXT -	220	11.3%
- - - SXT -	142	8.0%
Pen - - SXT -	113	6.4%
- Ery - - -	71	4.0%
Pen - - - -	64	3.6%
PenEry - SXT -	33	1.9%
- EryTet - -	29	1.6%
PenEryTet - -	28	1.6%
Pen - TetSXT -	22	1.2%
- - Tet - -	12	<1%
PenEry - - -	9	<1%
- Ery - SXT -	9	<1%
Pen - Tet - -	7	<1%
- - TetSXT -	5	<1%
- EryTetSXT -	3	<1%
- - - - Lev	1	<1%
- - - SXTLev	1	<1%
PenEry - SXTLev	1	<1%
- Ery - SXTLev	1	<1%
Total	1776	

Antibiotics included: Pen, Ery, Tet, SXT, Lev

Antibiotics excluded: Ctr, Ctx, Cli, Mox