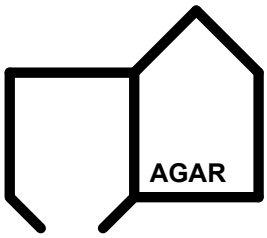


The Australian Group on Antimicrobial Resistance
http://antimicrobial-resistance.com

Haemophilus influenzae Survey 2006

Antimicrobial Susceptibility Report

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

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

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)

Members of AGAR who participated in this study:

Victoria	
Alfred Hospital	Denis Spelman, Clare Franklin
Gribbles Pathology	John Andrew, Di Olden
Monash Medical Centre	Tony Korman, Despina Kotsanas
Royal Women's and Children's Hospital	Suzanne Garland, Gena Gonis
St Vincent's Hospital	Mary Jo Waters, Linda Joyce
New South Wales	
John Hunter Hospital	John Ferguson, Jo Anderson
Nepean Hospital	James Branley, Donna Barbaro
Royal North Shore Hospital	George Kotsiou, Clarence Fernandes
Sydney South West Pathology Service, Liverpool	Iain Gosbell, Helen Ziochos
Australian Capital Territory	
The Canberra Hospital	Peter Collignon, Susan Bradbury
South Australia	
Gribbles Pathology	PC Lee, Barbara Koldej
Institute of Medical and Veterinary Science	Ivan Bastian, Rachael Pratt
Women's and Children's Hospital	John Turnidge, Jan Bell
Western Australia	
PathWest, QE2 Medical Centre	Clay Golledge, Barbara Henderson
PathWest, Royal Perth Hospital	Keryn Christiansen, Geoffrey Coombs
Queensland	
Pathology Queensland, Prince Charles Hospital	Chris Coulter, Sonali Gribble
Pathology Queensland, Princess Alexandra Hospital	Joan Faoagali, Gwen Lye
Pathology Queensland, Royal Brisbane Hospital	Graeme Nimmo, Narelle George
Sullivan Nicolaides Pathology	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 4
2 ..... Introduction 5
2.1 ..... Objectives of the Programme 5
2.2 ..... Importance of *H. influenzae* 5
2.3 ..... Antimicrobials tested 5
2.3.1 ß-lactams 5
2.3.2 Tetracycline 6
2.3.3 Trimethoprim-sulphamethoxazole: 6
3 ..... Methods 6
3.1 ..... Study Period 6
3.2 ..... Collection of Isolates 6
3.3 ..... Identification of Isolates 6
3.4 ..... Information Collected 7
3.5 ..... Antimicrobial Susceptibility Testing 7
3.6 ..... Data analysis 7
4 ..... Demographics 7
4.1 ..... Regional Source of Isolates 7
4.2 ..... Age 8
4.3 ..... Source of isolates 8
5 ..... Discussion 14
6 ..... References 17

1 Executive Summary

The Australian Group on Antimicrobial Resistance (AGAR) performs regular multicentre period-prevalence studies to monitor changes in antimicrobial resistance. In 2006, 19 laboratories participated in national surveillance of *Haemophilus influenzae* resistance.

One thousand eight hundred and twenty five strains of *H. influenzae* were collected prospectively and tested by disk diffusion and Etest. Results were compared to one previous AGAR sponsored study performed between 1988 and 1990. Only thirty seven (2.0%) isolates were from invasive sites with the majority being respiratory (77.8%) in origin. The number of invasive isolates was much less than in the previous AGAR study as this previous study predated the widespread introduction of capsular *H. influenzae* type b vaccine to the childhood immunisation schedule. β-lactamase production and ampicillin resistance (BLP) was present in 21.9% of all isolates. Western Australia (WA) had the lowest rate of BLP (16.0%) compared to all other states. Isolates collected from sinuses had the highest overall rates of BLP (37.5%). Nationally, 8.5% of strains were β-lactamase negative ampicillin resistant (BLNAR). BLNAR were more commonly isolated from eyes (14.0%). Molecular confirmation was not performed on BLNAR strains.

Amoxicillin/clavulanate, chloramphenicol and cefaclor resistance remained low (1.9%, 2.5% and 7.5% respectively) and did not increase significantly from the first AGAR survey in 1998-1990. Tetracycline and trimethoprim-sulphamethoxazole resistance was 11.8% and 20.1% respectively; a significant increase from the 4.1% and 4.6% reported in the first AGAR survey.

2 Introduction

2.1 Objectives of the Programme

The objective of the 2006 *H. influenzae* surveillance program was to determine the prevalence of antimicrobial resistance in clinical isolates of *H. influenzae* throughout Australia, with particular emphasis on:

1. estimate the incidence of β-lactamase production (BLP)
2. estimate the incidence of β-lactamase-negative ampicillin-resistant (BLNAR) strains
3. determine tetracycline susceptibility as it is recommended as standard therapy for adults in the "Antibiotic Guidelines"[1] for conditions where *H. influenzae* is common
4. report other antimicrobial agents where these were included as part of normal laboratory testing procedures for that laboratory. Antibiotics tested included amoxicillin/clavulanate, cefaclor, chloramphenicol and chloramphenicol trimethoprim/sulphamethoxazole
5. assess changes in resistance patterns in Australia over time in comparison to the one previous *H. influenzae* AGAR study performed[2]

2.2 Importance of *H. influenzae*

With the introduction of the *H. influenzae* capsular type b conjugate vaccines into the Australian Childhood Immunisation Schedule in 1993 the occurrence of life threatening meningitis, epiglottitis, cellulitis and other invasive syndromes due to *H. influenzae* type b infection have reduced dramatically from 502 cases in 1992 to an average of 15 cases per year currently being notified[3]. Nonetheless non capsulated *H. influenzae* remain one of the major bacterial pathogens of respiratory tract infections in children and adults and have an aetiological role in conjunctivitis, sinusitis, otitis media, community acquired pneumonia, and exacerbations of chronic bronchitis and bronchiectasis. Such infections account for the majority of outpatient visits in primary care practices. Most acute respiratory infections (ARIs) have a viral aetiology and thus antibiotics are not indicated however bacterial infections do occur and antibiotic prescriptions to cover both upper and lower respiratory tract disease is responsible for a significant amount of antibiotic prescribing nationally. The high prevalence of antimicrobial resistance in major respiratory pathogens has driven selection of broad spectrum antimicrobial agents for empirical treatment of ARI worldwide. Although β-lactamase (BLP) production is the main mechanism of ampicillin resistance the occurrence of β-lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* raises concern regarding the clinical utility of other β-lactams, including some cephalosporins[4,5].

2.3 Antimicrobials tested

2.3.1 ß-lactams

Resistance to β-lactam antibiotics for most *Haemophilus influenzae* strains is due to the production of plasmid-mediated TEM or ROB β-lactamases. However, another ampicillin resistance mechanism involves production of altered penicillin-binding proteins (PBPs). The molecular basis of β-lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* is mutations in the *fts*I gene producing alterations in Penicillin Binding Protein 3 (PBP3). The most common mutations result in alterations in amino acids positions 517 and 526 although many different and multiple mutations have been characterized. There are

difficulties in detecting and defining BLNAR *H. influenzae* strains phenotypically. When a genetic definition is used (e.g N526K or R517H amino acid substitution in PBP3) "BLNAR" strains may have ampicillin MICs as low as 0.25 – 0.5 mg/L. This creates considerable overlap with MIC's of baseline susceptible strains without a resistance mechanism, and makes them difficult to separate[6]. Using strict CLSI criteria, BLNAR strains have ampicillin MICs ≥ 4mg/L, although some advocate including non susceptible (NS) isolates with MICs ≥ 2mg/L. Other bodies such as BSAC (amoxicillin) or CDS use other antibiotics and breakpoints. This is especially so with disc diffusion because of the poor correlation between MIC and zone size, especially for BLNAR strains. The definition used obviously influences the reported prevalence of these strains in surveys. Although β-lactamase negative ampicillin-resistant (BLNAR) *H. influenzae* isolates are uncommon in most western countries, their detection is important. These isolates show broader resistance to β-lactams, including reduced susceptibility to first and second-generation cephalosporins and uncommon treatment failures have been reported with these antibiotics. BLNAR strains have become increasingly prevalent in certain countries[7].

2.3.2 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 potential discoloration of young teeth, tetracyclines are usually restricted to adults and children over the age of 8 years.

2.3.3 Trimethoprim-sulphamethoxazole:

Trimethoprim-sulphamethoxazole (co-trimoxazole) is a folate synthesis (dihydrofolate reductase) inhibitor. This has been used in the past for treatment of *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 *H. influenzae* resistance.

3 Methods

3.1 Study Period

Consecutive clinical isolates were collected from 1st January 2006 to 31st December 2006.

3.2 Collection of Isolates

Each participating laboratory collected up to 100 consecutive, significant clinical isolates of *H. influenzae*. Isolates selected were considered to be significant if antibiotic susceptibilities or β-lactamase results were reported. No repeat isolates were included from any patient unless a different antibiogram was observed from routine susceptibility results.

3.3 Identification of Isolates

Isolates were confirmed as *H.influenzae* if they were gram-negative cocco-bacilli with typical morphology and demonstrated requirement for either X- and V factors or ALA negative, and V factor –dependence.

3.4 Information Collected

For each isolate, a reference number, date of collection, date of birth, specimen source including blood, CSF, joint, other sterile body fluid, sputum/tracheal aspirate (but not nasopharyngeal aspirates), middle ear fluid/discharge (ear), sinus pus (antral washout), eye or other were recorded. The serotype (if known) of isolates from normally sterile sites (blood, CSF, joint aspirates) was also recorded.

3.5 Antimicrobial Susceptibility Testing

All isolates were tested for β-lactamase activity using either the chromogenic cephalosporin (e.g. nitrocefin disk), acidometric or iodometric method.

Using Haemophilus Test Medium (HTM) and the appropriate standardized method (CLSI or CDS), zone diameters or annular radius results were recorded for the following antimicrobial agents.

Antimicrobial Agent	**CLSI Disc Diffusion**	**CDS**
Ampicillin	10 µg	5 µg
Cefaclor	30 µg	30 µg
Tetracycline	30 µg	30 µg
Trimethoprim-sulphamethoxazole[b]	1.25/23.75 µg	1.25/23.75 µg
Chloramphenicol [b]	30 µg	10 µg
Amoxicillin-clavulanate [b]	20/10 (30) µg	10/5 (15) µg

[a] For detection of BLNAR
[b] Optional

No more than four discs per plate were tested. As growth rates for *H. influenzae* can vary significantly and may affect zone sizes irrespective of susceptibility, the age of cultures used for testing was critical. Colonies from a 20-24h chocolate agar plate were selected for preparing the suspension because higher inoculum concentrations can lead to false resistant results with some β- lactam antimicrobial agents. Hazy growth within the inhibition zones may be seen with incorrect inoculum density.

Ampicillin MICs (Etests) using HTM were performed for any isolate that was β-lactamase negative and was ampicillin intermediate or resistant, or cefaclor intermediate or resistant.

3.6 Data analysis

Zone diameters (to the nearest whole mm) were recorded on an excel spread sheet. QC results performed during the survey period were also recorded.

4 Demographics

4.1 Regional Source of Isolates

Both public (16) and private laboratories (3) participated in the study. Participants included New South Wales and the ACT (5), Queensland (4), Victoria (5), South Australia (3) and Western Australia (2). There were 1,825 isolates from 19 institutions (Table 1). Isolates from NSW and ACT were combined.

Table 1: Isolates by Region:

Region	Number of Institutions	Total	%
New South Wales (NSW) Australian Capital Territory (ACT)	5	455	24.9
Queensland (Qld)	4	400	21.9
South Australia (SA)	3	272	14.9
Victoria (Vic)	5	498	27.3
Western Australia (WA)	2	200	11.0
Total	19	1,825	100

4.2 Age

12.3% of the patients included in this survey were younger than 2 years. Almost one third of patients were 65 years or older (Table 2). The hospitalization status was not available but the literature suggests that nearly *all H.influenzae* infections appear to have a community onset.

Table 2: Age Range

Age Range (years)	n	%
<2	225	12.3
2-4	94	5.2
5-14	93	5.1
15-29	166	9.1
30-64	647	35.5
≥65	595	32.6
Unknown	5	0.3
Total	**1,825**	**100**

4.3 Source of isolates

More than three quarters of isolates (77.8%) originated from the respiratory tract and included sputum, bronchial washings or tracheal aspirates (Table 3). Conjunctival swabs from eyes were the next most common source comprising 10.6% of isolates. 3.9% and 1.8% came from ear swabs or sinuses respectively. Only 37 isolates (2.0%) were from sterile sites. 22 of these were from blood cultures, 2 isolates were from joint fluid, 1 isolate was from CSF, 1 from the peritoneum and 1 came from vitreous fluid culture. The remaining sterile sites (10) were not specified. Typing data was included for only 6 sterile site isolates. Five isolates were non-typeable and a single blood culture isolate from a 2

year old was capsular type b. Included in “other” sites were urines, genital and skin swabs as well as a placental swab. In the less than 2 year age group the eye was the most common source of isolates whereas in all other age categories a respiratory source was the most common site. This peaked in the 65 years and older category where a respiratory site was the source of 95.5% of isolates. Almost all of the ear isolates came from infants and children 14 years and younger (see Table 3).

Table 3: Source of Isolates (% of column) by age range

Age	**<2**	**2-4**	**5-14**	**15-29**	**30-64**	**≥65**	**Unknown**	**Total**
Specimen Source								
Respiratory	26 (11.6%)	33 (35.1%)	60 (64.5%)	142 (85.5%)	588 (90.9%)	568 (95.5%)	3 (60.0%)	1420 (77.8%)
Eye	133 (59.1%)	24 (25.5%)	10 (10.8%)	7 (4.2%)	11 (1.7%)	6 (1.0%)	2 (40.0%)	193 (10.6%)
Ear	35 (15.6%)	21 (22.3%)	10 (10.8%)	0 (0.0%)	3 (0.5%)	2 (0.3%)	0 (0.0%)	71 (3.9%)
Blood, CSF, joint, sterile site	7 (3.1%)	4 (4.3%)	2 (2.2%)	1 (0.6%)	13 (2.0%)	10 (1.7%)	0 (0.0%)	37 (2.0%)
Sinus pus	8 (3.6%)	6 (6.4%)	3 (3.2%)	4 (2.4%)	7 (1.1%)	4 (0.7%)	0 (0.0%)	32 (1.8%)
Other	16 (7.1%)	6 (6.4%)	8 (8.6%)	12 (7.2%)	25 (3.9%)	5 (0.8%)	0 (0.0%)	72 (3.9%)
Total	**225**	**94**	**93**	**166**	**647**	**595**	**5**	**1,825**

Table 4: Proportion of isolates non-susceptible by disc diffusion (CLSI and CDS)

Drug	NSW/ACT	Qld	SA	Vic	WA	Aus
Ampicillin*	122/455	105/400	91/272	134/498	38/200	490/1825
	(26.8%)	**(26.3%)**	**(33.5%)**	**(26.9%)**	**(19.0%)**	**(26.8%)**
Amoxicillin/Clavulanic	11/455	7/399	10/272	1/398	4/200	33/1724
acid	**(2.4%)**	**(1.8%)**	**(3.7%)**	**(0.2%)**	**(2.0%)**	**(1.9%)**
Cefaclor	32/455	47/400	17/272	33/498	8/200	137/1825
	(7.0%)	**(11.8%)**	**(6.3%)**	**(6.6%)**	**(4.0%)**	**(7.5%)**
Tetracycline	66/355	40/400	36/272	53/498	9/200	204/1725
	(18.6%)	**(10.0%)**	**(13.2%)**	**(10.6%)**	**(4.5%)**	**(11.8%)**
Trimethoprim-Sulphamethoxazole	82/356	78/400	28/172	92/398	26/199	306/1525
	(23.0%)	**(19.5%)**	**(16.3%)**	**(23.1%)**	**(13.1%)**	**(20.1%)**
Chloramphenicol	17/356	7/400	1/179	7/398	4/100	36/1433
	(4.8%)	**(1.8%)**	**(0.6%)**	**(1.8%)**	**(4.0%)**	**(2.5%)**

* Includes 11 isolates that were ampicillin susceptible by disc diffusion but β-lactamase positive

4.3 Antimicrobial susceptibility results

Results for the six antibiotics tested are tabulated in Table 4. Seventeen laboratories used CLSI[8,9] methodology and two laboratories used the CDS[10] method.

4.3.1 Ampicillin

Overall, non-susceptibility for ampicillin was 26.8% ranging from 19.0% in Western Australia to 33.5% in South Australia (p=0.0005). For 11 isolates ampicillin disc zone sizes were in the susceptible range however a β-lactamase was detected and the results overridden.

4.3.2 Amoxicillin/Clavulanic acid

Overall, only thirty three isolates (1.9%) were amoxicillin/clavulanate resistant ranging from 0.2% in Victoria to 3.7% in South Australia (p=0.0008).

4.3.3 Cefaclor

Cefaclor resistance rates were 7.5% overall, ranging from 4.0% in Western Australia to 11.8% in Queensland (p=0.0015).

4.3.4 Tetracycline

Of the 1,725 isolates tested, 11.8% were non susceptible overall ranging from 4.5% in Western Australia to 18.6% in NSW/ACT (p<0.0001).

4.3.5 Trimethoprim-Sulphamethoxazole

Non susceptibility rates for trimethoprim-sulphamethoxazole were 20.1% overall, ranging from 13.1% in Western Australia to 23.1% in Victoria (p=0.0033).

4.3.6 Chloramphenicol

Non susceptibility rates for chloramphenicol were 2.5% overall, ranging from 0.6% in South Australia to 4.8% in NSW/ACT (p=0.0094).

Table 5: Ampicillin status* of isolates by region

	β-lactamase negative, ampicillin resistant (BLNAR)	β-lactamase negative, ampicillin susceptible (BLNAS)	β-lactamase positive (BLP)	Total
NSW/ACT	37	318	100	455
	(8.1%)	**(69.9%)**	**(22.0%)**	
Qld	48	261	91	400
	(12.0%)	**(65.3%)**	**(22.8%)**	
SA	27	183	59	269
	(10.0%)	**(68.0%)**	**(21.9%)**	
Vic	37	344	117	498
	(7.7%)	**(69.1%)**	**(23.5%)**	
WA	6	162	32	200
	(3.0%)	**(81.0%)**	**(16.0%)**	
Aus	155	1,268	399	1,822
	(8.5%)	**(69.6%)**	**(21.9%)**	

* CLSI: Isolates that are β-lactamase negative with a zone diameter of <27mm to ampicillin 10µg and <21mm to cefaclor 30µg are considered to be BLNAR[11]. CDS: Isolates that are β-lactamase negative with a zone diameter of <6mm to ampicillin 5µg and <6mm to cefaclor 30µg are considered to be BLNAR.

Table 6: Ampicillin status of isolates by source

	β-lactamase negative, ampicillin resistant (BLNAR)	β-lactamase negative, ampicillin susceptible (BLNAS)	β-lactamase positive (BLP)	Total
Respiratory	114	997	306	1,417
	(8.0%)	**(70.4%)**	**(21.6%)**	
Eye	27	115	51	193
	(14.0%)	**(59.6%)**	**(26.4%)**	
Ear	1	58	12	71
	(1.4%)	**(81.7%)**	**(16.9%)**	
Blood, CSF, joint, sterile site	2	29	6	37
	(5.4%)	**(78.4%)**	**(16.2%)**	
Sinus pus	1	19	12	32
	(3.1%)	**(59.4%)**	**(37.5%)**	
Other	10	50	12	72
	(13.9%)	**(69.4%)**	**(16.7%)**	
Aus	155	1,268	399	1,822
	(8.5%)	**(69.6%)**	**(21.9%)**	

Figure 1: Ampicillin 10µg zone diameter (mm)

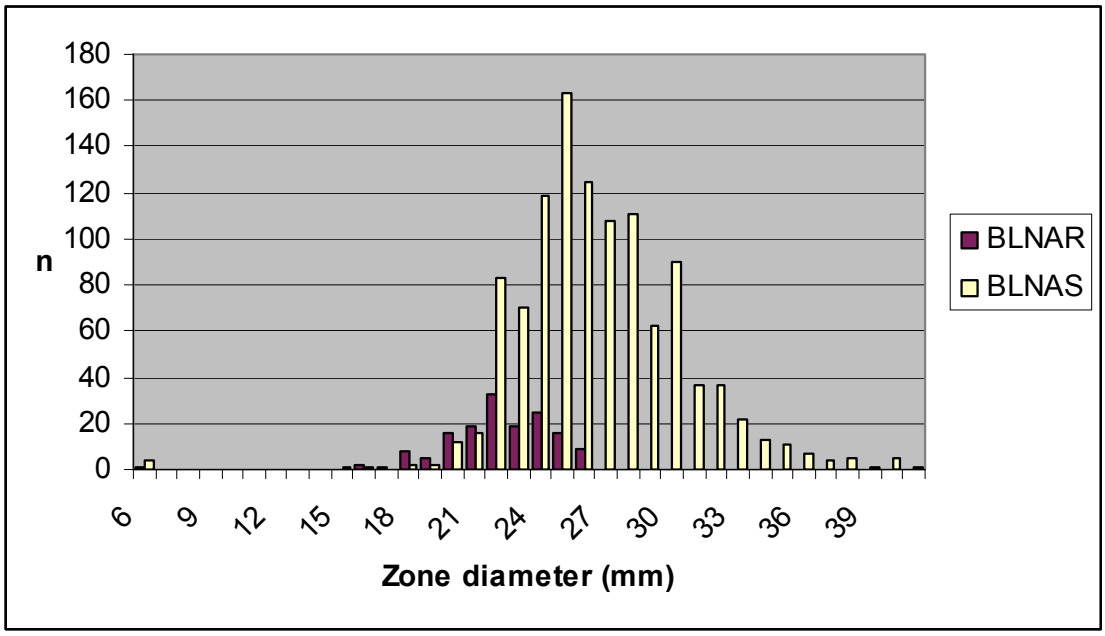


Figure 2: Cefaclor 30µg zone diameter (mm)

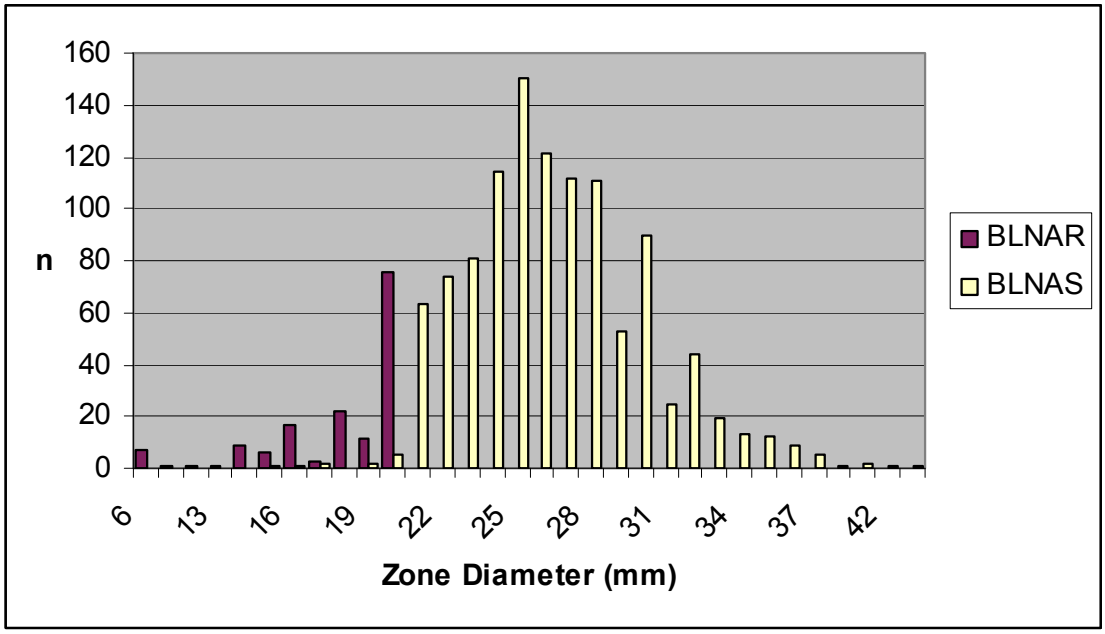
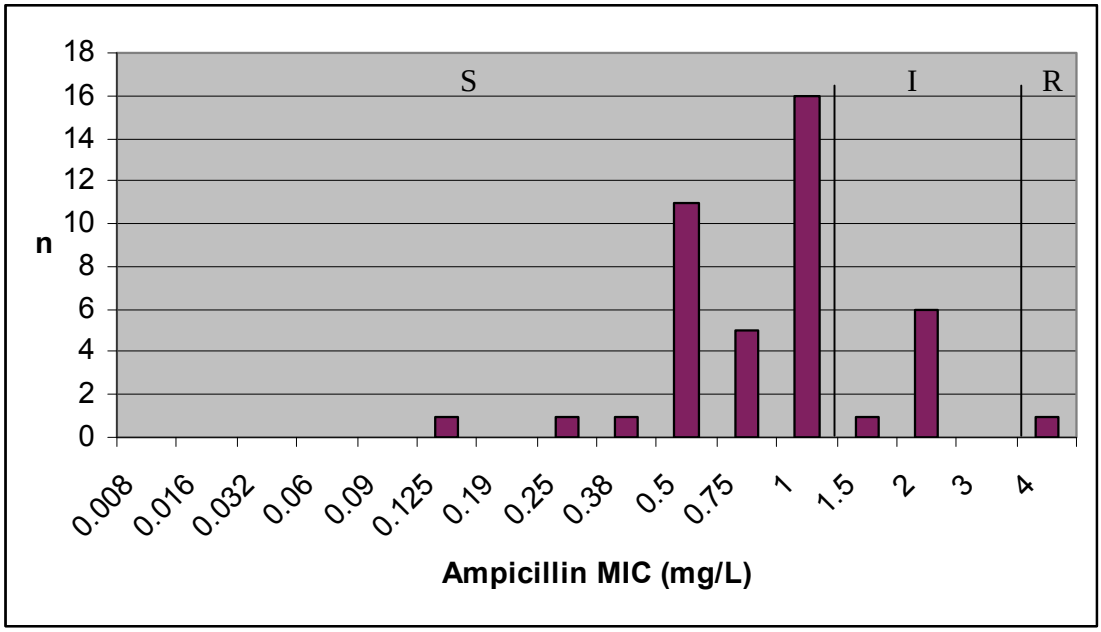


Figure 3: Ampicillin MIC (Isolates β-lactamase negative and non-susceptible to ampicillin and/or cefaclor by disc diffusion)



5 Discussion

Overall there has been little change in resistance rates for amoxicillin/clavulanic acid, cefaclor and chloramphenicol in *H. influenzae* in Australia between the two AGAR studies, the first in 1988-1990 and this one in 2006. β-lactamase production and resistance rates for ampicillin, tetracycline and trimethoprim-sulphamethoxazole have increased significantly between the two surveys.

In *H. influenzae*, the resistance to ampicillin and other β-lactam antibiotics is generally limited to either the production of a β-lactamase or, in the case of β-lactamase negative ampicillin-resistant (BLNAR) strains, the presence of altered PBPs with lowered affinity for β-lactams. Occasionally strains possess both mechanisms and are referred to as β-lactamase positive amoxicillin-clavulanate resistant (BLPACR) strains. Neither lowered cell permeability nor efflux systems are thought to represent significant mechanisms of resistance to β-lactams.

Both the more common TEM-1 and the less common ROB-1 β-lactamases are plasmid-mediated class A serine enzymes with similar substrate profiles that confer resistance to ampicillin and are effectively inhibited by β-lactamase inhibitors such as clavulanate. Susceptibility to all β- lactams in *H. influenzae* is generally predicted by susceptibility to ampicillin as defined by the CLSI MIC breakpoints, which are as follows: ≤1 mg/L susceptible; 2 mg/L, intermediate; and ≥4 mg/L resistant. In clinical isolates, β-lactamase mediated ampicillin resistance is usually easily detected, as strains positive for either enzyme exhibit ampicillin MICs well above the resistant breakpoint, with MIC_{90}s of ≥32 mg/L, and both enzymes are positive for nitrocefin hydrolysis. In this survey 11 β-lactamase positive isolates (0.6%) tested susceptible using CLSI or CDS disk diffusion breakpoints. The ampicillin MIC by Etest for one of these isolates fell in the sensitive range at 0.25 mg/L.

In this survey, the current rate of β-lactamase production (BLP) *H. influenzae* throughout Australia in 2006 was 21.9% ranging from 16.0% in Western Australia to 23.5% in Victoria. The only previous AGAR survey was performed in 1988-1990[2]. In this survey, β-lactamase production was 15.7%; ranging from 4.5% in Adelaide to 28.6% in Canberra. More recently, SENTRY surveillance from 1998 to 2004 suggested β-lactamase production in Australia to be 20-25%. In that survey, four (1.3%) strains contained ROB-1 type β-lactamase gene[12]. The type of β-lactamase production was not further delineated in this study. There is however a distinct association between the presence of ROB-1 and higher cefaclor MICs in *H. influenzae.* In this survey 36/399 (9.0%) β-lactamase positive strains displayed cefaclor MICs in the resistant range (≤ 19mm CLSI or ≤4 mm CDS) raising the possibility that these might be a ROB-1 β-lactamase. In 2004 in a survey of 4,320 clinical isolates from around Queensland[13], β-lactamase production was found in 22.8%. In summary there has been a significant (p=0.0001) increase in β-lactamase production in the 16 years between the two AGAR studies although the character of the testing laboratories has changed to include more laboratories that service the community.

International surveillance data in a study of almost 3,000 strains from 1999 to 2000 showed a prevalence of 16.6% β-lactamase positive strains, ranging from as low as 2% Italy to as high as 65% in South Korea[14]. In some countries, the prevalence of β-lactamase positive strains has begun to decline, with Canada down from 24% in 1997-1998 to 19% in 2001-2002[15], Spain down from 23% in 1997-1998 to 14% in 2002-2003[16], the United States down from 36% in 1994 to 29% in 2002[17], and Japan down from 10% in 1999 to 5% in 2003[18].

Some studies use the ampicillin-resistant breakpoint and the absence of β-lactamase to define BLNAR strains; others include ampicillin-intermediate strains as BLNAR strains. Additionally BLNAR strains have been characterised by genotyping and documenting mutations in the *ftsI* gene resulting in at least 24 different amino acids in the transpeptidase domain of PBP3. In this situation the MIC of some organisms that bear these mutations have MICs as low as 0.25mg/L. In addition, there is no international consensus on ampicillin breakpoints: for example, BSAC and

the Australian Calibrated Dichotomous Sensitivity method have a resistance breakpoint of ≥1.0 mg/L. The lack of a consensus definition and the broad range of ampicillin MICs associated with BLNAR strains makes comparison of the prevalence of BLNAR across surveys very difficult. BLNAR strains show reduced susceptibility not only to ampicillin but also to other β-lactam antibiotics, particularly cephalosporins.

Cefaclor resistance has been suggested is a better indicator of a BLNAR strain than ampicillin resistance[19]. The CLSI recommends that BLNAR strains also be considered resistant to amoxicillin-clavulanic acid, cefaclor, and cefuroxime, despite apparent in vitro susceptibility of some strains to these antibiotics. In this survey the definition of BLNAR was for CLSI users: isolates that are BLN with a zone diameter of <27mm to ampicillin 10µg and <21mm to cefaclor 30µg, and for CDS users: isolates that are BLN with a zone diameter of <6mm to ampicillin 5µg and <6mm to cefaclor 30µg. Even though susceptibility to cefaclor remains at reasonable levels use of cefaclor is not encouraged because of significant adverse reaction profile.[20]

The occurrence of BLNAR as defined above in this study was 8.5% ranging from 3.0% in WA to 12.0% in Queensland. BLNAR were significantly less common for ear and sinus isolates. In the original AGAR study only three β-lactamase negative strains were ampicillin intermediate (0.4%)[2] and in the SENTRY surveillance from 1998 to 2004 low-BLNAR strains were detected at <5%; and BLNAR strains emerged in 2003[12].

When the binding affinities for the different *H. influenzae* PBPs (in a susceptible strain with unmodified PBPs) and various β-lactam antibiotics were examined, cefotaxime and other cephalosporins had the highest affinity for PBP3A and PBP3B, whereas ampicillin had a higher affinity for PBP1A and PBP4[21] . In fact, it is the high affinity of cephalosporins for PBP3 that is central to their good activity against this organism, so under the selective pressure of cephalosporin use, mutations in *ftsI* are selected[22]. It has been proposed that the much higher rates of BLNAR strains found in Japan than in Europe and the United States is a consequence of different prescribing habits. BLNAR strains with multiple substitutions are associated with decreased susceptibility to cephalosporins with an approximate 60-fold increase in cefotaxime MICs (to 4.0 mg/L) compared with wild type susceptible strains.[23]

The clinical relevance of BLNAR strains can be debated. There are very few reports of clinical failures when patients infected with BLNAR strains were treated with ampicillin or a cephalosporin. However, clinical data cannot be generated until there is a standardized reference method and a universal definition of MIC criteria for the BLNAR category.

There is also no clear definition of BLPACR strains, but most authors use the resistant CLSI amoxicillin-clavulanate breakpoint of ≥8 mgL (amoxicillin component) to define these strains. Fifteen (15/1724, 0.9%) isolates were both BLP and resistant on disc testing to amoxicillin-clavulanate. Another 18 isolates although BLN, were resistant on disc testing to amoxicillin-clavulanate.

Tetracyclines exert an antimicrobial effect by binding to the 30S subunit of bacterial ribosomes and preventing tRNA from binding to the A or P sites. Tetracycline resistance in *H.influenzae* is associated with an efflux mechanism encoded by the *tet*(B) gene which is usually located on conjugative plasmids. A bimodal MIC distribution with a clearly defined susceptible population below the breakpoint and a clearly defined resistant population, typically with defined resistance mechanisms, is the case with tetracycline- resistant population associated with the *tet*(B) gene. In this survey tetracycline resistance was 11.8% nationally. In the 1990 AGAR survey this rate was 4.1%: a significant difference ($p<0.0001$). In the large Queensland survey in 2004 tetracycline resistance was around 20%. Drug usage of the tetracycline group has halved over the period 1992 to 2006 from 7.2 DDD/1000 population/ year to 3.2 in 2006.

Resistance to trimethoprim/sulfamethoxazole among strains of *H. influenzae* is common and is caused by an increase in the production of DHFR with altered affinity for trimethoprim. The national rate of resistance in this survey is 20.1%. In 1990, trimethoprim/sulfamethoxazole resistance was only 4.8% and in the large Queensland survey in 2004 it was 14.8%.

Chloramphenicol resistance in *H. influenzae* is usually associated with plasmid-mediated production of chloramphenicol acetyltransferase (CAT) encoded by the *cat* gene, with occasional strains having a permeability barrier. Resistance rates are low however this drug is not likely to have a therapeutic role in the future given its association with inducing aplastic anaemia.

Acknowledgements: Data on antibiotic utilization supplied by Drug Utilisation Subcommittee, Pharmaceutical Benefits Branch, Health Access and Financing Division, Commonwealth Department of Health and Ageing. Copyright "Commonwealth of Australia".

6 References

1. Therapeutic Guidelines Antibiotics Version 13 2006

2. Collignon P, Bell JM, MacInnes SJ, Gilbert GL, Toohey M, and the Australian Group for Antimicrobial Resistance (AGAR). A national collaborative study of antimicrobial resistance in *Haemophilus influenzae* in Australian hospitals. *J Antimicrob Chemother* 1992;30:153-163.

3. http://www9.health.gov.au/cda/source/Rpt_3_sel.cfm (accessed 6th April 2010)

4. Mendelman P M, Chaffin DO, Krilov LR, Kalaitzoglou G, Serfass DA, Onay O, Wiley EA, Overturf GD, and Rubin LG. Cefuroxime treatment failure of nontypable *Haemophilus influenzae* meningitis associated with altered penicillin-binding proteins. *J. Infect. Dis*. 1990;162:1118–1123.

5. Rubin LG, Yolken R, Medeiros A, and Moxon ER. Ampicillin treatment failure of apparently beta-lactamase-negative *Haemophilus influenzae* type B meningitis due to novel beta-lactamase. Lancet 1981:ii;1008–1010.

6. Tristram S, Jacobs MR, Appelbaum PC *Antimicrobial resistance in Haemophilus influenzae*. *Clin Microbiol Rev*. 2007;20(2):368-89.

7. Hasegawa K, Chiba N, Kobayashi R, Murayama SY, Iwata S, Sunakawa K, Ubukata K. Rapidly increasing prevalence of beta lactamase non-producing Ampicillin Resistant *Haemophilus influenza* Type B in patients with Meningitis. *Antimicrob Agents Chemother* 2004;48:1509-14.

8. Clinical and Laboratory Standards Institute (2009). Performance Standards for Antimicrobial Disk Susceptibility Tests—Tenth Edition: Approved Standard M2-A9. CLSI, Villanova, PA, USA

9. Clinical and Laboratory Standards Institute. (2011). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-first Informational Supplement: CLSI document M100-S21. CLSI, Villanova, PA, USA

10. Bell, S. M., B. J. Gatus, J. N. Pham & D. L. Rafferty. Antibiotic Susceptibility Testing by the CDS Method. A Manual for Medical and Veterinary Laboratories 2004. 3rd Edition

11. Ubukata K, Chiba N, Hasegawa K, Shibasaki Y, Sunakawa K, Nonoyama M, Iwata S, Konno M. Differentiation of β-lactamase-negative ampicillin-resistant *Haemophilus influenzae* from other *H. influenzae* strains by disc method. *J. Inf. Chemother.* 2002;8:50-58.

12. Bell J. M., G. N. Borlace, and J. D. Turnidge. Antimicrobial resistance among *Haemophilus influenzae* from Australia – an emerging problem. Poster presented at the 5th Annual Scientific Meeting of the Australian Society for Antimicrobials, February 2004, Sydney, Australia

13. Bell R, Allison S, Robson J. Ampicillin 2 µg, Ampicillin 10 µg and Cefaclor 30 µg discs for the detection of beta-lactamase negative ampicillin resistant (BLNAR) strains of *Haemophilus influenzae*. Poster presented at Antimicrobials, 2006, Sydney, Australia

14. Hoban D, Felmingham D. The PROTEKT surveillance study: antimicrobial susceptibility of *Haemophilus influenzae* and *Morexella catarrhalis* from community-acquired respiratory tract infections. *J Antimicrob Chemother.* 2002;50:S1 49-59.

15. Zhanel G, Palatnick L, Nichol K, Low D, The CROSS Study Group, Hoban D. Antimicrobial resistance in *Haemophilus influenzae* and *Morexella catarrhalis* respiratory tract isolates: results of the Canadian respiratory organism susceptibility study, 1997 to 2002. *Antimicrob Agents Chemother.* 2003;47:1875-1881.

16. Fluit A, Florijn, Verhoef J, Milatovic D. Susceptibility of European β-lactamase-positive and –negative *Haemophilus influenzae* isolates from the periods 1997/1998 and 2002/2003. *J Antimicrob Chemother.* 2005;56:133-138.

17. Heilmann K, Beekman S, Dickson E, Doern G. Antimicrobial resistance among clinical isolates of *Haemophilus influenzae* in the United States. *Annals of Emer Med.* 2004;44:S13.

18. Goto H, Shimadu K, Ikemoto H, Oguri T. Antimicrobial susceptibility of pathogens isolated from more than 10 000 patients with infectious respiratory disease: a 25-year longitudinal study. *J. Inf. Chemother.* 2009;15:347-360

19. Karlowsky J, Verma G, Zhanel G, Hoban D. Presence of ROB-1 beta-lactamase correlates with cefaclor resistance among recent isolates of *Haemophilus influenzae. J Antimicrob Chemother.* 2000;45:871-5

20. Parra F, Igea J, Martin J, Alonso M, Lezaun A, Sainz T. Serum sickness-like syndrome associated with cefaclor therapy. *Allergy* 1992;47(4):439-440

21. Ubukata K, Shibasaki Y, Yamamoto K, Chiba N, Hasegawa K, Takeuchi Y, Sunakawa K, Inoue M, Konno M. Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*. *Antimicrob. Agents Chemother*. 2001;45:1693–1699.

22. Hasegawa, K, Kobayashi R, Takada E, Ono A, Chiba N, Morozumi M, Iwata S, Sunakawa K, Ubukata K. High prevalence of type b {beta}-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* in meningitis: the situation in Japan where Hib vaccine has not been introduced. *J. Antimicrob. Chemother*. 2006;57:1077–1082

23. Garcia-Cobos S, Campos J, Lazaro E, Roman F, Cercenado E, Garcia-Rey C, Perez-Vazquez M, Oteo J and Abajo F. Ampicillin-resistant non-B-lactamase=producing *Haemophilus influenzae* in Spain: recent emergence of clonal isolates with increased resistance to cefotaxime and cefixime. *Antimicrob. Agents Chemother*. 2007;57:2564–2573.