DOI:10.1111/j.1750-2659.2012.00360.x
www.influenzajournal.com

Original Article

The impact of bacterial and viral co-infection in severe influenza

Christopher C. Blyth,[a,b,c] Steve A. R. Webb,[d,e] Jen Kok,[a,f] Dominic E. Dwyer,[a,f] Sebastiaan J. van Hal,[g,h] Hong Foo,[g,i] Andrew N. Ginn,[a,f] Alison M. Kesson,[j,k] Ian Seppelt,[f,l] Jonathan R. Iredell,[a,f] on behalf of the ANZIC Influenza Investigators and COSI Microbiological Investigators*[1]

[a]Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Sydney, NSW, Australia. [b]School of Paediatrics and Child Health, University of Western Australia, Princess Margaret Hospital, Perth, WA, Australia. [c]Department of Paediatric and Adolescent Medicine, Princess Margaret Hospital, Perth, WA, Australia. [d]Intensive Care Unit, Royal Perth Hospital, Perth, WA, Australia. [e]School of Medicine and Pharmacology and School of Population Health, University of Western Australia, Perth, WA, Australia. [f]Sydney Medical School, University of Sydney, Sydney, NSW, Australia. [g]Department of Microbiology and Infectious Diseases, Sydney South West Pathology Service – Liverpool, South Western Sydney Local Health Network, Sydney, NSW, Australia. [h]Antibiotic Resistance and Mobile Elements Group, Microbiology and Infectious Diseases Unit, School of Medicine, University of Western Sydney, Sydney NSW, Australia. [i]Infection Management and Control Service (IMACS), Wollongong Hospital, Wollongong, NSW, Australia. [j]Infectious Diseases and Microbiology Service, Children's Hospital Westmead, Sydney, NSW, Australia. [k]Discipline of Paediatrics and Child Health, University of Sydney, Sydney, NSW, Australia. [l]Department of Intensive Care Medicine, Nepean Hospital, Sydney, NSW, Australia.
Correspondence: Christopher C. Blyth, Faculty of Medicine, Dentistry and Health Sciences, School of Paediatrics and Child Health, The University of Western Australia, M561, Princess Margaret Hospital, Level 4, Administration Building, Roberts Road, Subiaco, WA 6008, Australia.
E-mail: christopher.blyth@uwa.edu.au
*ANZIC Influenza and COSI Microbiological Investigators are in Appendix.

Accepted 23 February 2012. Published Online 6 April 2012.

Background Many questions remain concerning the burden, risk factors and impact of bacterial and viral co-infection in patients with pandemic influenza admitted to the intensive care unit (ICU).

Objectives To examine the burden, risk factors and impact of bacterial and viral co-infection in Australian patients with severe influenza.

Patients/Methods A cohort study conducted in 14 ICUs was performed. Patients with proven influenza A during the 2009 influenza season were eligible for inclusion. Demographics, risk factors, clinical data, microbiological data, complications and outcomes were collected. Polymerase chain reaction for additional bacterial and viral respiratory pathogens was performed on stored respiratory samples.

Results Co-infection was identified in 23·3–26·9% of patients with severe influenza A infection: viral co-infection, 3·2–3·4% and bacterial co-infection, 20·5–24·7%. *Staphylococcus aureus* was the most frequent bacterial co-infection followed by *Streptococcus pneumoniae* and *Haemophilus influenzae*. Patients with co-infection were younger [mean difference in age = 8·46 years (95% CI: 0·18–16·74 years)], less likely to have significant co-morbidities (32·0% versus 66·2%, $P = 0·004$) and less frequently obese [mean difference in body mass index = 6·86 (95% CI: 1·77–11·96)] compared to those without co-infection.

Conclusions Bacterial or viral co-infection complicated one in four patients admitted to ICU with severe influenza A infection. Despite the co-infected patients being younger and with fewer co-morbidities, no significant difference in outcomes was observed. It is likely that co-infection contributed to a need for ICU admission in those without other risk factors for severe influenza disease. Empiric antibiotics with staphylococcal activity should be strongly considered in all patients with severe influenza A infection.

Keywords Co-infection, influenza, intensive care, pneumonia, *Staphylococcus aureus*, *Streptococcus pneumoniae*.

Please cite this paper as: Blyth *et al.* (2013) The impact of bacterial and viral co-infection in severe influenza. Influenza and Other Respiratory Viruses 7(2) 168–176.

Introduction

Influenza is a significant cause of morbidity and mortality worldwide.[1] Emergence of the A/H1N109 influenza virus in 2009 resulted in the first pandemic since 1968. The significant role of bacterial co-infection in past pandemics, and in seasonal influenza, has been documented.[2–7] During the 1918–1919 influenza pandemic, the organisms most frequently recovered from the sputum, lung and blood of infected patients were *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*.[2,5] In most cases, lung samples taken from patients

dying during the 1918–1919 pandemic demonstrate bacteriologic and histopathologic evidence of severe acute bacterial pneumonia.[5] During the Asian and Hong Kong influenza pandemics of 1957 and 1968, *S. pneumoniae*, *H. influenzae* and *S. aureus* were most frequently isolated from patients with bacterial pneumonia.[3,4,6,7] In 1957, *S. aureus* and *S. pneumoniae* were isolated from 59% and 15% of lung cultures, respectively.[3] Most deaths from *S. aureus* were observed in adolescents and young adults. In 1968, much of the excess mortality was attributed to the increased incidence of bacterial pneumonia: a threefold increase in the incidence of staphylococcal pneumonias and strong correlation between staphylococcal pneumonia and prior influenza infection was observed.[7]

During the 2009 influenza pandemic, initial clinical descriptions reported a severe respiratory illness with rapid progression to acute respiratory distress syndrome (ARDS).[8] Co-detection of clinically relevant bacteria with influenza A/H1N109 was infrequent, yet 31% of patients admitted to ICU had a clinical diagnosis of sepsis and 95% received antibiotics.[9] Bacterial co-infection was documented using histopathological, immunohistochemical and molecular evidence in 22 of 77 (28·6%) subjects with fatal pandemic influenza A/H1N1 2009 infection. *Streptococcus pneumoniae, S. pyogenes* and *S. aureus* were the predominant bacterial pathogens detected.[10]

Despite numerous studies examining co-infection in pandemic influenza A/H1N109, many questions remain.[10–15] Many published studies have limited number of critically ill patients enrolled,[11,14,15] have not enrolled all age groups,[12,13,15] have not examined the role of co-infecting respiratory viruses[10,12,13,15] and have not explored important risk factors such as obesity in those with and without co-infection.[11,12,15] Our objectives were (i) to determine the burden of bacterial and viral co-infections in adults and children admitted to ICUs in Australia during the first wave of the A/H1N109 pandemic (1 June–31 August 2009) using traditional and molecular techniques and (ii) to explore risk factors for and outcomes of those in whom bacteria and other viruses were detected concurrently with A/H1N109.

Patients and methods

A nested cohort study conducted in 14 Australian ICUs was performed. All ICUs contributed data to a multi-centre inception-cohort study involving 187 ICUs in Australia and New Zealand.[16] Patient demographics, risk factors, clinical data, complications and outcomes were collected prospectively.[16] Subjects admitted to 14 ICUs in New South Wales and Western Australia with influenza A confirmed by polymerase chain reaction (PCR) were identified. Results of all microbiological investigations performed during their ICU admission were collated. Laboratory results from prior to and following their influenza diagnosis (±3 days) were reviewed and collated. ICU admission criteria, diagnostic sampling, antibiotic/antiviral therapy and laboratory processing of respiratory specimens were not standardised across all ICUs or laboratories.

Respiratory tract samples stored at −20°C were retrospectively identified from associated laboratories. Nucleic acid was extracted from clinical samples using NucliSENS easyMAG (bioMérieux, Durham, NC, USA). Multiplexed tandem polymerase chain reaction (MT-PCR; AusDiagnostics Pty Ltd, Sydney, NSW, Australia) was performed on all samples to examine for other respiratory viruses including influenza B (nucleoprotein gene), respiratory syncytial virus (L gene), human parainfluenzaviruses 1–3 (hemagglutinin-neuraminidase, nucleoprotein, nucleocapsid gene, respectively), adenovirus (hexon gene), metapneumovirus (nucleoprotein gene) and picornaviruses (5′ UTR of picornavirus genome) according to the manufacturer's instructions (http://www.ausdiagnostics.com). MT-PCR for *Bordetella pertussis* (insertion sequence 481) was performed on all samples.

MT-PCR was performed on all lower respiratory tract samples for common co-infecting or colonising bacteria including *S. aureus* (*nuc*, *mecA*, *SCC-mec* genes), *S. pneumoniae* (*lytA*, *cpsAB* genes), *Pseudomonas* spp., *Enterobacteriaceae* spp., *enterococci* and other *streptococci/staphylococci* (*16S rRNA*). Serial dilution of *S. aureus* (ATCC 25923), methicillin resistant *S. aureus* (MRSA; MRSAMWS1), *S. pneumoniae* (ATCC 49615), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Escherichia faecalis* (ATCC 29212) were prepared, and standard MT-PCR curves constructed.[17]

MT-PCR was reported positive if the above bacteria were detected as the dominant signal and at a level equal to or greater than that in prepared control samples containing 10^4 cfu/ml. Controls (positive and negative) were included in each MT-PCR run. All samples positive by MT-PCR were confirmed by previously published PCR assays.[18–21]

Subjects were deemed to be assessable for viral co-infection if stored respiratory samples were available for viral MT-PCR. Subjects were deemed assessable for bacterial co-infection if (i) two or more of the following investigations were performed, (a) culture of lower respiratory tract specimen (bronchoalveolar lavage (BAL)/washing, endotracheal aspirate and sputum), (b) blood culture and (c) urinary antigen detection for *S. pneumoniae* and *Legionella pneumophila* or (ii) a lower respiratory tract specimen was available for bacterial MT-PCR. Community-acquired infection was defined by the onset of symptoms within 48 hours of hospital admission.

Data were analysed in two groups. The first group included all subjects assessable for both bacterial and viral

co-infection (Group 1). To ensure validity of the results, a second group was analysed including all subjects in group one and subjects assessable for either bacterial or viral co-infections only (Group 2). To identify the risk factors for and the impact of viral and bacterial co-infection, subjects with and without co-infection were compared.

Data were analysed using SPSS version 19.0.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were compared using the student's *t*-test. Categorical variables were compared using chi-square or Fisher's exact tests. A *P*-value of <0·05 was considered significant. In addition, we used multivariate logistic-regression models to further investigate associations with co-infection. Each centre obtained approval from their institutional ethics committee for the original cohort study.[16] Additional approval was obtained to access stored samples.

Results

Clinical data were available for 198/202 patients admitted to ICU with PCR-proven influenza A infection. This included 186 with community-acquired influenza A infection (93·9%) and 174 (87·9%) with A/H1N109 (Table 1). The median age was 38 years (range: 0–84 years). The study population was comparable to the total Australian and New Zealand ICU population diagnosed with influenza during 2009[16] except that seasonal influenza A infection was more common (10·3% versus 4·3%; $P < 0·01$) and extracorporeal membrane oxygenation was less frequently required (4·2% versus 11·6%, $P = 0·016$) in the study population.

Stored upper and/or lower respiratory specimens were available for viral MT-PCR in 131/198 (66·2%) of the study population (see Table S1A). Investigations for bacterial co-infection included blood cultures (167/198: 84·3%), culture of lower respiratory tract specimen (115/198; 58·1%), urinary antigen detection (80/198; 40·4%) and bacterial culture of pleural fluid, pericardial fluid or lung tissue (3/198; 1·5%). Lower respiratory tract specimens included BAL or washing (32/115; 27·8%), endotracheal aspirates (28/115; 24·3%) or sputum samples (55/115; 47·8%). Lower respiratory tract specimens from 34 subjects

Table 1. Demographics, risk factors, clinical features and influenza A subtype of patients including those assessable for co-infection

	Assessable for both bacterial and viral co-infection (Group 1, *n* = 93)	Assessable for any co-infection (Group 2, *n* = 176)*	All patients (*n* = 198)
Demographics			
Median age (IQ range)	48 (34–57 years)	41·5 (27–55 years)	38·0 (24–55 years)
Age < 18 years (%)	5 (5·4)	28 (15·9)	41 (20·7)
Female sex (%)	47 (50·5)	98 (55·7)	110 (55·6)
Risk factors (%)			
Any co-morbidity**	53/93 (57·0)	99/176 (53·2)	111/198 (56·1)
Pregnancy	9/92 (9·8)	14/170 (8·2)	17/192 (8·9)
Chronic lung disease	34/92 (37·0)	67/175 (38·3)	72/197 (36·5)
Chronic heart disease	12/92 (13·0)	22/174 (12·6)	25/196 (12·8)
Diabetes	12/91 (13·2)	28/173 (16·2)	31/195 (15·9)
BMI ≥ 35	24/87 (27·6)	38/153 (24·8)	39/169 (23·1)
Influenza syndrome (as decided by clinician, %)			
Viral pneumonitis	41/90 (45·5)	75/169 (44·4)	82/191 (42·9)
Secondary bacterial pneumonia	14/90 (15·6)	19/169 (11·2)	20/191 (10·5)
Exacerbation of airways disease	13/90 (14·4)	28/169 (16·6)	31/191 (16·2)
Other respiratory syndrome	9/90 (10·0)	20/169 (11·8)	24/191 (12·6)
Intercurrent illness with influenza A infection	13/90 (14·4)	27/169 (16·0)	34/191 (17·8)
Influenza subtype (%)			
Pandemic A/H1N109	79 (84·9)	152 (86·4)	174 (87·9)
Seasonal A/H3N2	10 (10·8)	19 (10·8)	19 (9·6)
Seasonal A/H1N1	1 (1·1)	1 (0·6)	1 (0·5)
Untyped	3 (3·2)	4 (2·3)	4 (2·0)

BMI, body mass index (calculated as weight in kilograms divided by height in metres squared).
*Includes 93 subjects assessable for both bacterial and viral co-infection, 38 subjects assessable for viral infection only and 45 subjects assessable for bacterial infection only.
**Any co-morbidity: Pregnancy, Chronic lung disease, Chronic heart disease, Diabetes or Obesity (BMI > 35).

were available for MT-PCR including 21 bronchoscopic specimens, nine endotracheal aspirates and four sputum samples. In total, 138/198 (69·7%) of the study population were deemed assessable for bacterial infection (see Table S1B).

Ninety-three subjects were assessable for both viral and bacterial co-infections (Group 1), and 176 subjects assessable for any co-infection (Group 2, Table 1). Viral co-infection was infrequently observed in both groups: 3/93 (3·2%) and 6/176 (3·4%), respectively (Table 2). Three of the six viral co-infections occurred in children: an infant with influenza A/H1N109 and RSV co-infection, and two children, aged between 3 and 5, with influenza A and picornavirus co-infections (one each of influenza A/H1N109 and A/H3). Only one subject had concurrent bacterial and viral co-infection with influenza A: a 50-year-old female with concurrent influenza A/H1N109, adenovirus and *S. aureus* infection.

Using both routine microbiological data and bacterial MT-PCR, the rate of bacterial co-infection ranged between 23/93 (24·7%) and 36/176 (20·5%), respectively (Table 2). Of patients with bacterial co-infection, 100% (Group 1) and 97·2% (Group 2) had community-acquired influenza infection. When only lower respiratory specimens were examined, bacterial co-infection was identified in 4/34 (11·8%) by MT-PCR compared with 2/26 by culture (7·7%).

Staphylococcus aureus and *S. pneumoniae* were the most frequent pathogens (Table 2). Samples with *S. aureus* co-infection ($n = 17$) were detected using lower respiratory tract culture ($n = 14$; 82·4%), blood cultures ($n = 3$; 17·6%), MT-PCR ($n = 2$; 11·8%) and culture of empyema fluid ($n = 1$; 5·9%). Samples with *S. pneumoniae* co-infection ($n = 10$) were detected using urinary antigen detection ($n = 7$; 70%), lower respiratory tract culture ($n = 1$; 10%), blood cultures ($n = 1$; 10%) and MT-PCR ($n = 2$; 20%). Samples with *Haemophilus* co-infection ($n = 6$) were detected by using lower respiratory tract culture ($n = 3$; 50%) and blood cultures ($n = 3$; 50%), Other bacterial co-infections ($n = 6$) were detected using lower respiratory tract culture ($n = 2$; 33%), blood culture ($n = 1$; 17%) and MT-PCR ($n = 2$; 33%).

When those with and without co-infection were compared, no significant differences were observed in clinical course, influenza type and outcomes. (Group 1: Table 3; Group 2: Table S2). The duration of symptoms prior to hospital and/or ICU admission was comparable. Requirement for endotracheal intubation, positive pressure ventilation, vasopressors, extracorporeal membrane oxygenation (ECMO) and renal replacement therapy was not significantly different between patients with and without co-infection. The proportion of patients with influenza A/H1N109 infection was not significantly different between those with and without co-infection (20/25, 80·0% versus 59/68, 86·7%). Despite a trend towards greater ICU mortality in those with co-infection (16·0% versus 5·9%), no significant differences in ICU mortality, in-hospital mortality, length of ICU stay and length of hospital stay were observed.

Significant differences in the frequency of risk factors known to be associated with ICU admission were revealed when subjects with and without co-infection were compared (Group 1: Table 3; Group 2: Table S2). When subjects in Group 1 were compared, subjects with bacterial or viral co-infection were younger [mean difference in age = 8·46 years (95% CI = 0·18–16·74 years), $P = 0·045$]. Subjects with co-infection were less likely to have significant medical co-morbidities compared to those without co-infection (32·0% versus 66·2%, $P = 0·004$, Table 3). When individual co-morbidities were examined, those with co-infection were less likely to be obese [mean difference in body mass index (BMI; weight in kilograms divided by height in metres squared) = 6·86 (95% CI = 1·77–11·96), $P < 0·01$]. These significant

Table 2. Co-infection with viruses and bacteria

	Assessable for bacterial and viral co-infection (Group 1, *n* = 93)	Assessable for any co-infection (Group 2, *n* = 176)
Viruses		
Respiratory syncytical virus	0	1
Parainfluenza virus 3	1	1
Adenovirus	1*	1*
Picornavirus	1	3
Total subjects with viral co-infection (%)	3 (3·2)	6 (3·4)
Bacteria		
Staphylococcus aureus	13*	17*
MSSA/MRSA	9/4	11/6
Streptococcus pneumoniae	7**	10**
Haemophilus influenzae	3	6
Beta haemolytic streptococci	0	2
Pseudomonas aeruginosa	1**	2**
Bordetella pertussis	2**	2**
Total subjects with bacterial co-infection (%)	23 (24·7)	36 (20·5)
Total subjects with any co-infection (%)	25 (26·9)	41 (23·3)

MRSA, methicillin resistant *S. aureus.*
*One patient had co-infection with Influenza A virus, Human adenovirus and *S. aureus.*
**Two patients had more than one bacterial co-infection identified: (i) *S. pneumoniae*, *P. aeruginosa* and *B. pertussis* and (ii) *S. pneumoniae* and *B. pertussis.*

Table 3. Risk factors for and impact of bacterial and viral co-infection in Group 1 (subjects assessable for bacterial and viral co-infection, $n = 93$)

	Any co-infection ($n = 25$)	No co-infection ($n = 68$)	Significance (P-value)
Demographics			
Median age in years (range)	45 (0–75 years)	49 (21–79 years)	0·045
Age < 18 years	5/25	0/68	0·001
Female (%)	12 (48·0)	36 (52·9)	n.s.
Risk factors (%)			
Any co-morbidity*	8/25 (32·0)	45/68 (66·2)	0·004**
Pregnancy	1/25 (4·0)	8/67 (11·9)	n.s.
Chronic lung disease	5/25 (20·0)	29/67 (43·3)	0·05
Chronic heart disease	1/25 (4·0)	11/67 (16·4)	n.s.
Diabetes	1/23 (4·3)	11/68 (16·2)	n.s.
Mean BMI	26·15	33·01	<0·01**
Length and severity of illness			
Median days from symptom onset to hospitalisation (interquartile range)	4 (1–6 days)	4 (2–6 days)	n.s.
Median days from symptom onset to ICU admission (interquartile range)	5 (2–6·5 days)	5 (3–8 days)	n.s.
Ventilation on day of diagnosis (%)	17/24 (70·8)	47/68 (69·1)	n.s.
Vasopressors on day of diagnosis (%)	9/20 (45·0)	25/47 (53·2)	n.s.
Renal replacement therapy on day of diagnosis (%)	3/20 (15·0)	6/48 (12·5)	n.s.
Outcome			
ICU mortality (%)	4/25 (16·0)	4/68 (5·9)	n.s.
In-hospital mortality (%)	4/25 (16·0)	10/68 (14·7)	n.s.
Mean length of ICU stay (days)	15·6	13·6	n.s.
Mean length of hospital stay (days)	32·5	28·1	n.s.

BMI, body mass index (calculated as weight in kilograms divided by height in metres squared).
*Any co-morbidity: pregnancy, chronic lung disease, chronic heart disease, diabetes or obesity (BMI > 35).
**The significance of these variables was maintained if only adults 18 years and older were examined ($P \leq 0·03$): Any co-morbidity: 7/20 versus 45/68, $P = 0·02$; Mean BMI: 27·17 versus 33·01, $P = 0·03$.

relationships remained when children were excluded from the analysis. A trend towards fewer co-morbidities in subjects requiring ICU admission with co-infection was observed when compared to those without co-infection: pregnancy, 4% versus 11·9%; chronic lung disease, 20·0% versus 43·3%; chronic heart disease, 4·0% versus 16·4%; diabetes, 4·3% versus 16·2% (all not significant). Obesity was the only individual risk factor of significance on multivariate analysis. Similar differences were observed in subjects assessable for any infection (Group 2, $n = 176$, Table S2).

Despite the strong microbiological evidence for bacterial or viral co-infection, treating physicians most frequently identified the predominant clinical influenza syndrome as viral pneumonitis or ARDS in co-infected patients (nine of 25; 36·0%). In the remaining subjects, the treating physician identified secondary bacterial infection (7/25; 28·0%), exacerbation of airflow limitation (2/25; 8·0%) and other respiratory syndrome (6/25; 24·0%) as the predominant clinical syndrome.

Discussion

Bacterial and viral co-infection was identified in approximately one in four adults and children admitted to intensive care units in Australia with confirmed influenza A infection in 2009. This rate is comparable to that observed in other ICU studies. Martin-Loeches *et al.*[13] identified bacterial co-infection in 113/645 adults (17·5%) admitted with confirmed pandemic influenza A/H1N109. Estenssoro *et al.*[12] identified coexisting bacterial pneumonia in 80/325 adults (25%) requiring ventilation with suspected, probable or confirmed pandemic influenza A/H1N109 infection. A similar rate of bacterial co-infection was observed in pandemic influenza A/H1N109 fatalities (18·3–29·0%),[10,22] significantly more than that observed when all hospitalised children and adults are examined (bacterial co-infection <5%).[9,23,24]

In our group, viral co-infection was infrequent and had little impact on morbidity and mortality. This is, to our knowledge, the largest study to examine both the burden

and impact of respiratory virus co-infection in subjects with severe influenza A infection requiring ICU admission during the first wave of the 2009 pandemic. In contrast, viral co-infection was observed in seven of 39 (17·9%) patients with severe influenza A/H1N109 infection (defined as infection resulting in hospitalisation or death) in Argentina by Palacios *et al.*[14] RSV was identified in 6/39 of patients with severe disease (15·4%) compared with 5/160 (3·1%, $P < 0·01$) with mild disease. It is probable that the greater proportion of children and adolescents in the study by Palacios *et al.* was reflected in a greater rate of respiratory virus co-infection (mean age: Palacios *et al.* – 24·7 years and this study – 38·0 years). Variation in the proportion of co-infecting respiratory viruses may also result from seasonal variations in respiratory virus prevalence.

What is the impact of bacterial co-infection? Data from this and previously published studies are conflicting. Estenssoro *et al.* demonstrated that *S. pneumoniae* co-infection was an independent predictor of hospital mortality yet this study and that by Martin-Loeches *et al.* failed to identify any significant difference between co-infection and ICU mortality.[12,13] Martin-Loesches *et al.*[13] found that co-infected patients were older and had higher APACHE scores, yet no differences in co-morbidities were noted. Our data demonstrate that those with co-infection were younger and had fewer co-morbidities normally associated with an increased need for ICU admission. This association was particularly strong for obesity. The significance of this finding was preserved if children were included or excluded from the analysis. Martin-Loesches *et al.* demonstrated that subjects with bacterial co-infection were more likely to require invasive ventilation and vasopressors. This was not demonstrated in this study. Despite the failure to identify differences in the severity of illness in subjects with and without co-infection, it is still possible that co-infection was a contributory factor to severe disease. As enrolment in this study required severe influenza requiring ICU admission, it is possible that co-infection was the precipitant that led to ICU admission in younger people without co-morbidities. To explore this hypothesis further, a clinically well-characterised control group not requiring ICU admission would be required.

A striking difference is the impact of *S. aureus* co-infection in Australian ICU patients with severe influenza A infection. *Staphylococcus aureus* was the major pathogen identified in more than 40% of co-infected patients and 9·6–14·0% of all patients. All subjects with staphylococcal co-infection in our study had community-acquired infection. Similar rates of co-morbidities were observed in patients with *S. aureus* co-infection compared with patients co-infected with other organisms. Methicillin resistant *S. aureus* was identified in only 35% of cases. These findings are in contrast to other studies where *S. pneumoniae* was the major cause of bacterial co-infection.[10,13,22] Despite extensive investigations including pneumococcal urinary antigen testing in addition to respiratory cultures, blood cultures and nucleic acid detection, pneumococcal co-infection was detected in ≤28% of co-infected patients and 5·7–7·5% of all patients. It is, however, possible that this is underestimated because of the frequent use of empiric antibiotics including ceftriaxone (which has excellent anti-streptococcal activity but is associated with increased incidence of *S. aureus*[25]) in patients admitted to ICU with community-acquired pneumonia (CAP), viral pneumonitis or ARDS and failure to perform culture-independent diagnostic tests (e.g. pneumococcal urinary antigen) on all patients. *Staphylococcus aureus* complicating influenza infection has been well described in previous pandemics.[3,6,26] These findings have a significant impact on both management and prevention of complications in severe influenza infection.

Treatment recommendations for adults and children with severe influenza infection include parenteral antibiotics.[27,28] The Infectious Diseases Society of America recommends a third-generation cephalosporin and a macrolide/fluoroquinolone in critically unwell adults with influenza A/H1N109 influenza.[28,29] Provision of cover against MRSA is recommended in those admitted to ICU, with necrotising/cavitatory infiltrates, empyema or microscopy demonstrating Gram-positive cocci in clusters in a respiratory specimen.[28] The British Thoracic Society recommends a beta-lactamase stable penicillin or second-/third-generation cephalosporin together with a macrolide in adults with severe influenza-related pneumonia. Empiric cover against MRSA is recommended in subjects hospitalised within the last few months or those not responding to empirical therapy.[27] These data support the use of empiric antibiotics with activity against locally circulating clones of *S. aureus* in all patients admitted to ICU with severe influenza infection.

Both Martin-Loeches *et al.* and Louie *et al.* stress the importance of preventative strategies using conjugate and polysaccharide pneumococcal vaccination.[10,13] Only three patients diagnosed with invasive pneumococcal disease would have been recommended to receive pneumococcal vaccination using current Australian guidelines.[30] Our data suggest that the impact of immunising young children, the elderly and those with underlying medical conditions would have little impact on morbidity and mortality in Australians with severe influenza infection.

The present study has several potential limitations. Despite a uniform approach to diagnosis of CAP, a pathogen is identified in <50% of cases.[31] The observational nature of this study means that the diagnostic approach varied between different patients and ICUs. These limitations

existing in other comparable studies.[12,13] Adequate lower respiratory specimens and samples for culture-independent laboratory tests (e.g. pneumococcal urinary antigen) were not routinely submitted for microbiological analysis. In addition, it is likely that many subjects were administered antibiotics prior to specimen collection. It is therefore possible that the true burden of bacterial and viral co-infection is underestimated. Despite the use of quantitative and/or semiquantitative laboratory methods, co-infecting and colonising bacteria remain difficult to differentiate. We have attempted to overcome this by further analysing lower respiratory tract specimens using quantitative nucleic acid amplification techniques, a test not routinely used for the diagnosis of CAP. This approach was hampered by limited lower respiratory sampling in the 14 ICUs enrolling subjects. It is possible, however, that the detection of bacteria colonising the lower respiratory tract at high concentrations may falsely elevate the observed rate of bacterial pneumonia. It is possible that delays in processing samples led to nucleic acid degradation. Furthermore, viral co-infection may have been underestimated as emerging viral pathogens, such as human bocavirus and coronaviruses, were not tested for. Upper respiratory tract samples were most frequently examined for viral pathogens. Additional pathogens may have been detected if upper and lower respiratory samples were available for analysis on all patients.[32] Radiology data and timing of antibiotic administration data were not collected in the study. It is probable that the burden of co-infection would be higher in subjects with radiologically confirmed pneumonia and those examined prior to receipt of antibiotics.

The burden of bacterial and viral co-infection in 198 adults and children admitted to ICU during the first wave of the influenza A/H1N109 pandemic was estimated to be 23·3–26·9%. Co-infection had little impact on severity of illness or outcome in those admitted to intensive care but may have contributed to the need for ICU admission in those without other risk factors. Empiric parenteral antibiotics with anti-staphylococcal activity should be strongly considered in those with severe influenza infection. To estimate the true burden and impact of bacterial and viral co-infection, a prospective study with uniform sampling using both culture and molecular assays is required. Further development of new diagnostic methods is urgently required, particularly to assist with diagnosing bacterial pneumonia.

Acknowledgements

The authors would like to thank that staff at the ANZIC Research Centre in particularly Belinda Howe and Siouxzy Morrison. Nicholas Conway (Princess Margaret Hospital) and Brian O'Toole (Westmead Hospital) assisted with statistical analysis.

Transparency declaration

Funding for the study was provided from a untied grant through the investigator-initiated grants program of Merck US – grant #IISP3732.

Author contributions

CCB, SARW, DED, IS and JRI were involved in the design of the study. SARW, IS and JRI were involved in the nested cohort study (Webb *et al.*, NEJM 2009). CCB, JK, SJvH, HF, AMK were involved in the processing and storage of laboratory specimens. CCB, JK, SJvH and HF extracted additional microbiological data. CCB, JK and ANG were involved in additional microbiological processing. Statistical analysis and the original draft were completed by CCB. All authors were involved in reviewing the final manuscript.

References

1 Thompson WW, Shay DK, Weintraub E *et al.* Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003; 289:179–186.
2 Brundage JF, Shanks GD. Deaths from bacterial pneumonia during 1918–19 influenza pandemic. Emerg Infect Dis 2008; 14: 1193–1199.
3 Hers JF, Masurel N, Mulder J. Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. Lancet 1958; 2:1141–1143.
4 Lindsay MI Jr, Herrmann EC Jr, Morrow GW Jr, Brown AL Jr. Hong Kong influenza: clinical, microbiologic, and pathologic features in 127 cases. JAMA 1970; 214:1825–1832.
5 Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis 2008; 198:962–970.
6 Petersdorf RG, Fusco JJ, Harter DH, Albrink WS. Pulmonary infections complicating Asian influenza. AMA Arch Intern Med 1959; 103:262–272.
7 Schwarzmann SW, Adler JL, Sullivan RJ Jr, Marine WM. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968–1969. Arch Intern Med 1971; 127:1037–1041.
8 Human infection with new influenza A (H1N1) virus: clinical observations from Mexico and other affected countries, May 2009. Wkly Epidemiol Rec 2009; 84:185–189.
9 Jain S, Kamimoto L, Bramley AM *et al.* Hospitalized patients with 2009 H1N1 influenza in the United States, April–June 2009. N Engl J Med 2009; 361:1935–1944.
10 Louie J, Jean C, Chen T-H *et al.* Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) – United States, May–August 2009. MMWR Morb Mortal Wkly Rep 2009; 58:1071–1074.
11 Dhanoa A, Fang NC, Hassan SS, Kaniappan P, Rajasekaram G. Epidemiology and clinical characteristics of hospitalized patients with pandemic influenza A (H1N1) 2009 infections: the effects of bacterial coinfection. Virol J 2011; 8:501.
12 Estenssoro E, Rios FG, Apezteguia C *et al.* Pandemic 2009 influenza A in Argentina: a study of 337 patients on mechanical ventilation. Am J Respir Crit Care Med 2010; 182:41–48.

13 Martin-Loeches I, Sanchez-Corral A, Diaz E *et al.* Community-acquired respiratory co-infection (CARC) in critically ill patients infected with pandemic 2009 influenza A (H1N1) virus infection. Chest 2011; 139:555–562.

14 Palacios G, Hornig M, Cisterna D *et al. Streptococcus pneumoniae* coinfection is correlated with the severity of H1N1 pandemic influenza. PLoS ONE 2009; 4:e8540.

15 Masia M, Padilla S, Antequera P, Ramos JM, Ruiz M, Gutierrez F. Predictors of pneumococcal co-infection for patients with pandemic (H1N1) 2009. Emerg Infect Dis 2011; 17:1475–1478.

16 Webb SA, Pettila V, Seppelt I *et al.* Critical care services and 2009 H1N1 influenza in Australia and New Zealand. N Engl J Med 2009; 361:1925–1934.

17 Szewczuk E, Thapa K, Anninos T *et al.* Rapid semi-automated quantitative multiplex tandem PCR (MT-PCR) assays for the differential diagnosis of influenza-like illness. BMC Infect Dis 2010; 10:113.

18 Glare EM, Paton JC, Premier RR, Lawrence AJ, Nisbet IT. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J Clin Microbiol 1990; 28:1982–1987.

19 Kong F, Wang W, Tao J *et al.* A molecular-capsular-type prediction system for 90 *Streptococcus pneumoniae* serotypes using partial cpsA-cpsB sequencing and wzy- or wzx-specific PCR. J Med Microbiol 2005; 54:351–356.

20 McAvin JC, Reilly PA, Roudabush RM *et al.* Sensitive and specific method for rapid identification of *Streptococcus pneumoniae* using real-time fluorescence PCR. J Clin Microbiol 2001; 39:3446–3451.

21 Ubukata K, Nakagami S, Nitta A *et al.* Rapid detection of the mecA gene in methicillin-resistant staphylococci by enzymatic detection of polymerase chain reaction products. J Clin Microbiol 1992; 30:1728–1733.

22 Martin-Loeches I, Rodriguez A, Bonastre J *et al.* Severe pandemic (H1N1)v influenza A infection: report on the first deaths in Spain. Respirology 2011; 16:78–85.

23 Louie JK, Acosta M, Winter K *et al.* Factors associated with death or hospitalization due to pandemic 2009 influenza A (H1N1) infection in California. JAMA 2009; 302:1896–1902.

24 Nguyen-Van-Tam JS, Openshaw PJ, Hashim A *et al.* Risk factors for hospitalisation and poor outcome with pandemic A/H1N1 influenza: United Kingdom first wave (May–September 2009). Thorax 2010; 65:645–651.

25 Paterson DL. ''Collateral damage'' from cephalosporin or quinolone antibiotic therapy. Clin Infect Dis 2004; 38(Suppl 4):S341–S345.

26 Robertson L, Caley JP, Moore J. Importance of *Staphylococcus aureus* in pneumonia in the 1957 epidemic of influenza A. Lancet 1958; 2:233–236.

27 Pandemic flu: clinical management of patients with an influenza-like illness during an influenza pandemic. Provisional guidelines from the British Infection Society, British Thoracic Society, and Health Protection Agency in collaboration with the Department of Health. Thorax 2007; 62(Suppl 1):1–46.

28 Bradley JS, Englund JA, File TM *et al.* Influenza H1N1: Frontline Questions and Expert Opinion Answers; in America IDSo (ed.). Arlington, VA: Infectious Diseases Society of America, 2009. Accessed 7th February 2011 (http://www.idsociety.org/Content.aspx?id=15743).

29 Mandell LA, Wunderink RG, Anzueto A *et al.* Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007; 44(Suppl 2):S27–S72.

30 Australian Technical Advisory Group on Immunisation (ATAGI). The Australian Immunisation Handbook, 9th edn. Canberra, Australia National Health and Medical Research Council, Government Department of Health and Aging, 2007.

31 Charles PG, Whitby M, Fuller AJ *et al.* The etiology of community-acquired pneumonia in Australia: why penicillin plus doxycycline or a macrolide is the most appropriate therapy. Clin Infect Dis 2008; 46:1513–1521.

32 Blyth CC, Iredell JR, Dwyer DE. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 361:2493.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Differences between subjects assessable and not assessable for (A) viral co-infection and (B) bacterial co-infection.

Table S2. Risk factors for and impact of bacterial and viral co-infection in Group 2 (subjects assessable for any co-infection, $n = 176$).

Appendix

The Australian and New Zealand Intensive Care (ANZIC) Influenza Investigators are a collaboration of the ANZIC Society Clinical Trials Group, the ANZIC Research Centre, the Australasian Society for Infectious Diseases Clinical Trials Group, the Paediatric Study Group of the ANZIC Society, and the ANZIC Society Centre for Outcome and Resource Evaluation. Clinical data were obtained from the registry maintained by the ANZIC Influenza Investigators endorsed by the ANZICS Clinical Trials Group.

ANZIC Influenza Investigators: G. Bennett, L. Ong (*Bankstown Hospital, Sydney*); K. Nand, G. Reece, T. Sara (*Blacktown Hospital, Sydney*); G. Bishop (*Campbelltown Hospital, Sydney*); M. Festa, F. Li (*Children's Hospital Westmead, Sydney*); D. Blythe, A. Palermo (*Fremantle Hospital, Fremantle*); M. Parr, S. Micallef (*Liverpool Hospital, Liverpool*); L. Hoyling, I. Seppelt, L. Weisbrodt (*Nepean Hospital, Penrith*), Y. Shehabi, M. Campbell, V. Stockdale (*Prince of Wales Hospital, Randwick*); S. Erickson (*Princess Margaret Hospital for Children, Perth*); J. Chamberlain, A. Gould, G. McEntaggart, S. Webb (*Royal Perth Hospital, Perth*); D. Inskip, D. Lamb, J. Myburgh, R. Sidoli (*St. George Hospital, Sydney*); A. Numa, G. Williams, J. Young (*Sydney Children's Hospital, Sydney*); R. Boyd, V. Nayyar, C. Skelly, E. Stachowski (*Westmead Hospital, Sydney*); M. Sterba, B. Johnson (*Wollongong Hospital*).

COSI Microbiological Investigators: A Kesson (*Children's Hospital Westmead, Sydney*); SJ van Hal (*Liverpool Hospital, Liverpool*); J Branley (*Nepean Hospital, Penrith*); WD Rawlinson, A Outhred (*Prince of Wales Hospital, Randwick*); J Flexman (*Royal Perth Hospital, Perth*); CC Blyth, DE Dwyer, H Foo, JR Iredell, J Kok, K McPhie, J Patterson (*Westmead Hospital, Sydney*); H Foo (*Wollongong*).

These data were previously presented at Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston 2010: Blyth CC, Webb SA, Kok J, Iredell JR, ANZIC Influenza Investigators and COSI Microbiology Investigators. Bacterial and Viral Co-infections in Severe Influenza Infection.