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

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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 23–32% of patients with severe influenza A infection: viral co-infection, 3–3% and bacterial co-infection, 20–24%. *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 A. 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*.

Introduction

Influenza is a significant cause of morbidity and mortality worldwide. 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. 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*. In most cases, lung samples taken from patients...
dying during the 1918–1919 pandemic demonstrate bacteriologic and histopathologic evidence of severe acute bacterial pneumonia. 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. In 1957, *S. aureus* and *S. pneumoniae* were isolated from 59% and 15% of lung cultures, respectively. 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.

During the 2009 influenza pandemic, initial clinical descriptions reported a severe respiratory illness with rapid progression to acute respiratory distress syndrome (ARDS). 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. Bacterial co-infection was documented using histopathological, immunohistochemical and molecular evidence in 22 of 77 (28%) subjects with fatal pandemic influenza A/H1N1 2009 infection. *Streptococcus pneumoniae*, *S. pyogenes* and *S. aureus* were the predominant bacterial pathogens detected.

Despite numerous studies examining co-infection in pandemic influenza A/H1N109, many questions remain. Many published studies have limited number of critically ill patients enrolled. have not enrolled all age groups, have not examined the role of co-infecting respiratory viruses and have not explored important risk factors such as obesity in those with and without co-infection. 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. Patient demographics, risk factors, clinical data, complications and outcomes were collected prospectively. 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* (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.

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 10⁶ 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. 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 confirmed by previously published PCR assays.
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 200916 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 (IQR 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, inhospital 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 (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 (0)                                                 | 1 (1)                                             |
| Parainfluenza virus 3 | 1 (1)                                                   | 1 (1)                                             |
| Adenovirus           | 1* (1*)                                                  | 1* (1*)                                           |
| Picornavirus         | 1 (1)                                                   | 3 (3)                                             |
| Total subjects with viral co-infection (%) | 3 (3·2)                                             | 6 (3·4)                                           |
| **Bacteria**         |                                                               |                                                  |
| *Staphylococcus aureus* | 13* (13)                                             | 17* (17)                                          |
| MSSA/MRSA            | 9/4 (9·4)                                                | 11/6 (11·6)                                       |
| Streptococcus pneumoniae | 7** (7·7)                                             | 10** (10·3)                                       |
| *Haemophilus influenzae* | 3 (3)                                                 | 6 (6)                                             |
| *Beta haemolytic streptococci* | 0 (0)                                                 | 2 (2)                                             |
| *Pseudomonas aeruginosa* | 1** (1·2)                                             | 2** (2·2)                                         |
| Bordetella pertussis | 2** (2·2)                                                | 2** (2·2)                                         |
| Total subjects with bacterial co-infection (%) | 23 (23·4)                                           | 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.
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 pneumonia 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. identified bacterial co-infection in 113/645 adults (17.5%) admitted with confirmed pandemic influenza A/H1N109. Estenssoro et al. 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%).

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 of bacterial and viral co-infection.
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-Löeches et al. failed to identify any significant difference between co-infection and ICU mortality.12,13 Martin-Löeches et al. found that coinfected 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-Löeches 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) 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-Löeches 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. 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. 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.

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Transparency declaration

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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.

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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.

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