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Efficacy of face masks and respirators in preventing upper respiratory tract bacterial colonization and co-infection in hospital healthcare workers

C. Raina MacIntyre [a,b], Quanyi Wang [c], Bayzidur Rahman [a], Holly Seale [a], Iman Ridda [a,b], Zhanhai Gao [a], Peng Yang [c], Weixian Shi [c], Xinghuo Pang [c], Yi Zhang [c], Aye Moa [a,*], Dominic E. Dwyer [d]

[a] School of Public Health and Community Medicine, UNSW Medicine, University of New South Wales, Australia
[b] National Centre for Immunization Research and Surveillance, The Children's Hospital at Westmead, Sydney, Australia
[c] The Beijing Center for Disease Prevention and Control, Beijing, China
[d] Centre for Infectious Diseases and Microbiology Laboratory Services, Institute for Clinical Pathology and Medical Research, Westmead Hospital and University of Sydney, Australia

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ABSTRACT

Objective. We compared the efficacy of medical masks (MM) and N95 respirators (N95) in preventing bacterial colonization/infection in healthcare workers (HCWs).

Methods. A cluster randomized clinical trial (RCT) of 1441 hospital HCWs randomized to medical masks or N95 respirators, and compared to 481 control HCWs, was performed in Beijing, China, during the winter season of 2008–2009. Participants were followed for development of clinical respiratory illness (CRI). Symptomatic subjects were tested for *Streptococcus pneumoniae*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* or *Haemophilus influenza* type B by multiplex polymerase chain reaction (PCR).

Results. The rate of bacterial colonization was 2.8% in the N95 group ($p = 0.02$), 5.3% among medical mask users ($p < 0.01$) and 7.5% among the controls ($p = 0.16$). N95 respirators were significantly protective (adjusted RR 0.34, 95% CI: 0.21–0.56) against bacterial colonization. Co-infections of two bacteria or a virus and bacteria occurred in up to 3.7% of HCWs, and were significantly lower in the N95 arm.

Conclusions. N95 respirators were significantly protective against bacterial colonization, co-colonization and viral-bacterial co-infection. We showed that dual respiratory virus or bacterial-viral co-infections can be reduced by the use of N95 respirators. This study has occupational health and safety implications for health workers.

Introduction

Healthcare workers (HCWs) are at a significantly increased occupational risk for a range of infections. These include infections that cause substantial illness and occasional deaths in HCWs (Decker and Schaffner, 1996; Eriksen et al., 2005; Klevens et al., 2007), or are associated with healthcare associated infections (the majority of which are caused by bacteria). Various infectious agents can be transmitted from patients to HCWs and vice versa (Weber et al., 2010). As droplet transmission is a major mode of transmission of some pathogens, standard infection control measures like hand washing alone may not be enough to prevent HCW transmission or outbreaks. HCWs can transmit infections such as tuberculosis, varicella, and influenza by the airborne route (Weber et al., 2010); it is less well appreciated that airborne and other routes of transmission of certain bacterial pathogens may occur.

There is a low awareness of bacterial infections as an occupational health risk for HCWs. In addition, antibiotic resistant bacteria are a very significant problem facing hospitals, and HCWs play a role in their transmission. Bacterial respiratory tract infections are generally not considered a major occupational problem for HCWs. A growing body of evidence suggests that the risk of bacterial respiratory infections is increased by co-infection with viruses and vice-versa, and this has been studied mostly around the relationship between influenza and pneumococcus (Klugman et al., 2009; Madhi and Klugman, 2004; MMWR, 2009; Zhou et al., 2012). Bacterial load in the nasopharynx is also thought to be related to risk of invasive disease or bacterial–viral co-infection (Klugman et al., 2009). A meta-analysis showed frequent bacterial co-infections during influenza outbreaks (Wang et al., 2011). *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus* spp. and other *Streptococcus* spp. are the commoner causes of bacterial secondary infection following an influenza-like illness (ILI) (Wang et al., 2011).

Case studies documenting the role of HCWs in transmission of *S. pneumoniae* are absent, possibly because this is usually not an outbreak-associated disease, and because the pathogenesis of invasive

* Corresponding author at: School of Public Health and Community Medicine, UNSW Medicine, University of New South Wales, Gate 11, Botany Street, Randwick, NSW 2052, Australia.
E-mail address: a.moa@unsw.edu.au (A. Moa).

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disease is complex (including the relationship with prior colonization). Further, HCWs with invasive pneumococcal disease may go unreported in the occupational context (Sherertz et al., 2001). On the other hand, *Bordetella pertussis* outbreaks among HCWs have been widely reported (Addiss et al., 1991; Gehanno et al., 1999; Pascual et al., 2006), with such outbreaks attributed to airborne transmission through droplets (Nouvellon et al., 1999). In another study, evidence of acute infection with *Chlamydia pneumoniae* was detected in 2% of HCWs (Hyman et al., 1995). Outbreaks of *Mycoplasma pneumoniae* among HCWs have been observed in Finland, where 44% (n = 97) of HCWs tested positive for the pathogen without detectable *M. pneumoniae*-specific antibody, suggesting acute infection (Kleemola and Jokinen, 1992). *Legionella* has also been described as an occupational risk factor for HCWs (Borella et al., 2008; Rudbeck et al., 2009). In contrast to these outbreaks, there are few prospective studies of bacterial respiratory infections or colonization and the clinical implications for HCWs.

There has been recent interest in the role of medical masks and respirators in preventing respiratory infections in HCWs and the general community (MacIntyre et al., 2009, 2011, 2013). Medical masks (MMs) are unfitted devices worn by an infected person, HCW, or member of the public to reduce transfer of potentially infectious body fluids between individuals. They were originally designed for surgeons in order to attenuate wound contamination, but have not been demonstrated to have their intended efficacy (Mitchell and Hunt, 1991; Orr, 1981; Tunevall, 1991). Of note, MMs have not been shown to clearly provide respiratory protection in the community or HCW setting (Aiello et al., 2012; Cowling et al., 2009; MacIntyre et al., 2009, 2011). This may be attributed to lower filtration efficiency and poorer fit than respirators which, in contrast, are specifically designed to provide respiratory protection (Balazy et al., 2006; Lawrence et al., 2006; Weber et al., 1993). We have previously shown that a N95 respirator provides significantly better protection against clinical respiratory infection than medical masks in HCWs (MacIntyre et al., 2011, 2013). Although our previous work tested clinical efficacy in preventing infection, the relative importance of different routes of transmission (airborne, aerosol, and direct hand-to-mouth contact) in the clinical efficacy of respiratory protection is unknown. That is, a mask may provide protection against more than one mode of transmission. The only bacterial infection for which respirators are considered and recommended for HCWs is tuberculosis (Chen et al., 1994; Nicas, 1995). In this study, our aim was to determine the efficacy of respiratory protection in preventing bacterial colonization and co-infections or co-colonization in HCWs.

Methods

A prospective, cluster randomized trial of N95 respirators (fit tested and non-fit tested) and medical masks compared to each other and to controls who did not routinely wear masks was conducted in frontline HCWs during the winter of 2008–2009 (December to January) in Beijing, China. The methodology and consort diagram used in the study and the primary clinical and viral infection outcomes have been previously described (MacIntyre et al., 2011). We also measured bacterial colonization/infection and co-infections in symptomatic trial subjects, which has not been previously reported. This study describes the efficacy of the interventions (N95 respirators and medical masks) in preventing bacterial colonization and co-infection in HCWs.

Recruitment commenced on December 1, 2008 and final follow-up completed on January 15, 2009. 1441 HCWs in 15 hospitals were randomized to one of three intervention arms: (1) Medical masks (3M™ medical mask, catalog number 1820); (2) N95 fit tested mask (3M™ flat-fold N95 respirator, catalog number 9132); (3) N95 non-fit tested mask (3M™ flat-fold N95 respirator, catalog number 9132) (MacIntyre et al., 2011). A secure computerized randomization program was used to randomize the hospitals to each intervention. A convenience control group of 481 HCW who did not routinely wear masks were recruited and prospectively followed up in the same way as the trial participants for the development of symptoms. The study protocol was approved by the Institutional Review Board (IRB), Human Research Ethics Committee of the Beijing Ministry for Health. Staff who agreed to participate provided informed consent.

The primary study endpoint was the presence of laboratory-confirmed bacterial colonization of the respiratory tract in subjects who were symptomatic. We tested for *S. pneumoniae*, *Legionella* spp., *B. pertussis*, *Chlamydia*, *M. pneumoniae* or *H. influenzae* type B by multiplex PCR. These organisms have been reported in the HCW setting (Kurt et al., 1972; Rudbeck et al., 2009; Wang et al., 2011). We also looked at co-colonization with more than one bacteria, and co-infection with a laboratory-confirmed viral infection and bacterial colonization. Laboratory-confirmed viral respiratory infection was defined as detection of adenoviruses, human metapneumovirus, coronaviruses 229E/NL63 and OC43/HKU1, parainfluenza viruses 1, 2 and 3, influenza viruses A and B, respiratory syncytial viruses A and B, or rhinovirus A/B by nucleic acid testing (NAT) (MacIntyre et al., 2011).

Eligibility

Nurses or doctors who worked full time in the emergency or respiratory wards at the participating hospitals were eligible. HCWs were excluded if they: (1) were unable or refused to consent; (2) had beards, long mustaches or long facial hair stubble; (3) had a current respiratory illness, rhinitis and/or allergy; and (4) worked part-time or did not work in the selected wards/departments (MacIntyre et al., 2011).

Intervention

Subjects were randomized to masks or respirators, and wore the mask or respirator on every shift (8–12 h) for four consecutive weeks and were shown how to wear it and fit it correctly. Participants were supplied daily with three masks for the medical mask group or two N95 respirators. They were asked to store the mask in a paper bag every time they removed it (for toilet breaks, tea /lunch breaks and at the end of every shift) and place the bagged mask or respirator in their locker. All participants were instructed on the importance of hand hygiene prior to/ after the removal of medical masks and respirators, as described (MacIntyre et al., 2011). Participants in the fitted N95 arm underwent a fit testing procedure using a 3M™ FT-30 Bitrex Fit Test Kit according to the manufacturers' instructions (3M™, St Paul, MN, USA) (MacIntyre et al., 2011).

Follow-up

All participants were followed up for four weeks for development of respiratory symptoms, and for an additional week after mask wearing had ceased (to account for incubation of infections acquired in week 4). Validated diary cards were provided for the four-week period to record daily the (1) number of hours worked; (2) mask/respirator usage; and (3) recognized CRI (MacIntyre et al., 2011).

Participants were contacted daily by the study team either by phone or face-to-face contact to actively identify incident cases of viral respiratory infection. CRI was defined as at least two respiratory symptoms (cough, sneezing, runny nose, shortness of breath, sore throat) or one respiratory symptom and one systemic symptom (including fever, headache, and lethargy). If any respiratory symptom was present, subjects were tested, following collection of a nose and throat swab, for bacterial and viral pathogens.

Sample collection and laboratory testing

Subjects with respiratory symptoms had two pharyngeal swabs collected by a trained nurse or doctor. Double rayon-tipped, plastic-shafted swabs were used to scratch both tonsil areas and the posterior pharyngeal wall. These were transported immediately after collection to the laboratory, or at 4 °C if transport was delayed within 48 h. Pharyngeal swabs were tested at the Laboratories of the Beijing Centers for Disease Control and Prevention. A multiplex PCR (Seegen Inc., Seoul, Korea) was used to detect *S. pneumoniae*, *M. pneumoniae*, *B. pertussis*, *Legionella* spp., *Chlamydia* and *H. influenza* type B. After preheating at 95 °C for 15 min, 40 amplification cycles were carried out under the following conditions in a thermal cycler (GeneAmp PCR system 9700, Foster City, CA, USA): 94 °C for 30 s, 60 °C for 1.5 min, and 72 °C for 1.5 min. Amplification was completed at the final extension step at 72 °C for 10 min. The multiplex PCR products were visualized by electrophoresis on an ethidium bromide-stained 2% agarose gel. Laboratory-confirmed viral respiratory infection, defined as detection of adenoviruses, human metapneumovirus, coronaviruses 229E/NL63 and OC43/HKU1, parainfluenza viruses 1, 2 and 3, influenza viruses A and B, respiratory syncytial viruses A and B, or rhinovirus A/B by nucleic acid testing (NAT)

using a commercial multiplex polymerase chain reaction (PCR) (Seegen, Inc., Seoul, Korea) as previously described (MacIntyre et al., 2011).

Analysis

The endpoint of interest, bacterial colonization and co-infection with two bacteria or virus and bacteria were analyzed by intention-to-treat analysis. The two N95 arms (fit-tested and non-fit-tested) were combined for analysis, given that there was no significant difference between them and because rates of fit test failure were extremely low in the fit tested arm (5/461 fit test failures — in other words, the majority of HCWs who underwent fit-testing were wearing the mask correctly prior to fit testing, and fit testing did not add a significant benefit, allowing us to combine data from the fit tested and non-fit tested arms) (MacIntyre et al., 2011). We calculated the relative risk and efficacy of the N95 arms using medical mask group as the reference category, and also the efficacy of N95 and medical mask group using control as the reference category.

We fitted a multivariable log binomial model, using generalized estimating equation (GEE) to account for clustering by hospital, to estimate relative risk (RR) after adjusting for potential confounders. In the initial model, we included all the variables along with the main exposure variable (randomization arm) that were significant ($p < 0.25$) in the univariable analysis. A backward elimination method was used to remove the variables that did not have any confounding effect, that is, could not make meaningful change ($\pm 10\%$) in the RR of the N95 arms (Kleinbaum et al., 2007, 2010; Vittinghoff et al., 2012). In the multivariable analysis we estimated RR for N95 and medical mask arms compared to the control arm.

Results

A total of 1441 nurses and doctors in 15 hospitals were recruited into the intervention arms, and 481 nurses and doctors in 9 hospitals were recruited into the control group (Fig. 1). The distribution of socio-demographic variables was generally similar between arms, as previously reported (MacIntyre et al., 2011).

Fig. 2 illustrates the rates of bacterial detection in symptomatic HCWs by trial arm, and shows increasing rates with decreasing level of respiratory protection. Table 1 shows bacterial and viral infections, as well as co-infections or co-colonization with multiple pathogens, including co-infection with bacteria and virus. The rates of bacterial detection were lower for N95 respirators compared to MM (2.8% and 5.3% respectively), and was highest (7.5%) among the controls. By intention to treat analysis, N95 respirators were significantly more protective than MM against the laboratory-confirmed presence of bacteria, with an efficacy of 46% against medical masks and 62% against control. MMs had no significant efficacy against any outcome compared to control (Table 1).

Rates of all types of co-infection were significantly lower in the N95 group. N95 (but not MM) demonstrated efficacy against multiple bacterial pathogen colonization as well as co-infection with a virus and bacteria, and against dual virus infection (Table 1). There were no dual virus infections in controls (0/481), 2/949 in the N95 group and 5/492 in MM group. The MM arm had a higher rate of dual virus infection than controls, but the difference between MM and control did not reach statistical significance. The most common bacteria identified was *S. pneumoniae*; 2.5% for N95; 4.7% for MM, and 6.2% for control arm, followed by *H. influenzae* type B; 2%, 3.7%, and 5% respectively (data not shown). These differences were statistically significant across all three arms. *B. pertussis* was also detected in three HCWs.

In a multivariable cluster adjusted log binomial model, when compared to the control group, the N95 group was significantly protective against bacterial colonization (Table 2). We demonstrated 59% efficacy of N95 respirators against any co-infection (Table 3), and 67% against bacterial and viral co-infection (Table 4) in adjusted multivariate analyses. The only other significant variable for bacterial infection and

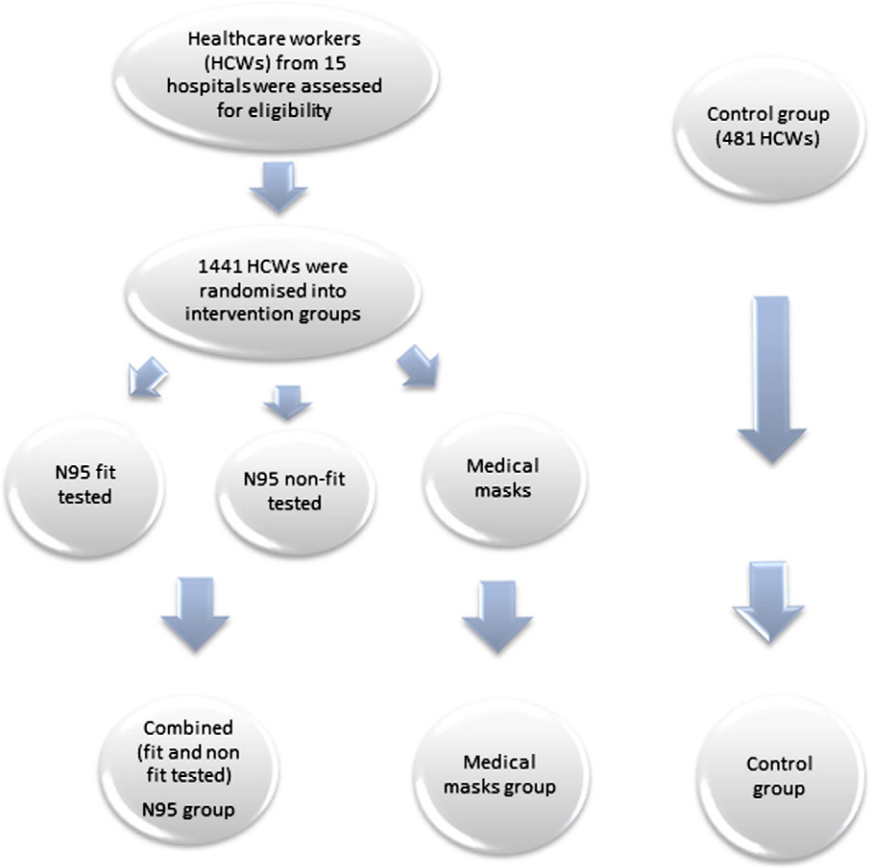
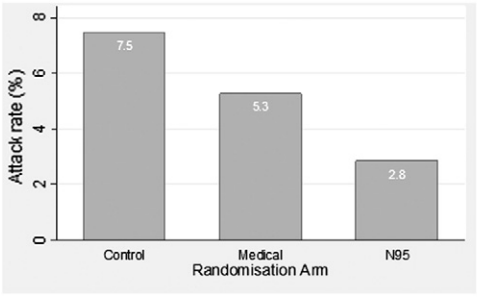


Fig. 1. A consort diagram for the study selection.



[1] P = 0.02, from cluster adjusted chi-squared test for differences in rates between arms

Fig. 2. Bacterial colonization by trial arm.[1].

Table 2
Multivariable cluster adjusted log binomial model of bacterial infection compared with control group.

Variables in the model	Relative risk (95% CI)
N95	0.34 (0.21–0.56)[a,†]
Medical mask	0.67 (0.38–1.18)
Hospital level	1.48 (0.91–2.42)
High-risk procedure	1.34 (0.84–2.13)
Influenza vaccine	1.03 (0.58–1.83)
Hand washing	0.82 (0.47–1.43)
Respiratory ward vs other	2.15 (1.39–3.31)[†]

[a] Efficacy 66%.
[†] Significant p values ($p < 0.01$).

bacterial and viral co-infection was the respiratory ward, which significantly increased the risk of colonization or co-infection compared to other wards (Tables 2 and 4).

In addition, univariable analyses of infection and co-infection rates by other factors, such as, smoking (current vs non-smoker), staff type (doctor vs nurses) and ward type (respiratory vs other) were conducted in the analysis. For bacterial infection, HCWs working in a respiratory ward were significantly at higher risk of infection than HCWs in other wards (7.3% vs 3.5%, $p < 0.001$). For bacterial co-infection, nurses had a significantly higher risk than doctors (3.2% vs 1.4%, $p = 0.02$) and the rate was also significantly higher in respiratory wards (4.4% vs 1.8%, $p = 0.001$). Respiratory wards had a higher rate of bacteria–virus co-infection than other wards (2.5% vs 1%, $p = 0.02$).

Discussion

We have previously shown that N95 respirators protect against clinical respiratory illness (MacIntyre et al., 2011, 2013). N95 respirators, but not medical masks, were significantly protective against bacterial colonization, co-colonization, viral-bacterial co-infection and dual virus infection in HCWs. We also showed a statistically significant decrease in rates of bacterial respiratory colonization with increasing levels of respiratory protection. The lowest rates were in the N95 group, followed by the medical mask group, and the highest rates were in HCWs who did not wear a mask. Although the clinical significance of this finding is unknown in terms of the implications for HCWs, we have shown that such colonization can be prevented by the use of N95 respirators. These findings are consistent with other work we have published, which shows a reduction in bacterial colonization following use of N95 respirators (MacIntyre et al., 2013).

While the role of nosocomial viral respiratory infections is accepted, bacterial infections are less well understood. Our findings suggest that bacterial respiratory tract colonization or infection in HCWs should be studied further. Bacterial colonization may be a precursor to viral and bacterial co-infections and invasive bacterial infections in individuals with influenza or other respiratory viral infections. It is possible that the onset of upper respiratory tract bacterial colonization may itself cause mild respiratory tract symptoms, given that only symptomatic HCWs were swabbed in our study. This requires further investigation, in particular comparison with an asymptomatic HCW group. We believe that these results may have occupational health implications for HCWs, given the body of evidence that supports a complex, synergistic and poorly understood pathogenic relationship between bacterial and viral respiratory infection (Klugman et al., 2009; Madhi and Klugman, 2004; MMWR, 2009; Zhou et al., 2012). The finding that bacterial colonization and co-infections were a greater risk on respiratory wards than

Table 1
Intention to treat analysis of bacterial, viral and bacterial–viral co-infections. Bold indicates "significant p value".

	N95 (n = 949)		Medical (n = 492)		Control (n = 481)	
All infections		Efficacy of N95 vs medical masks % (95% CI)[a]		Efficacy of N95 vs control % (95% CI)[b]		Efficacy of medical mask vs control % (95% CI)[b]
Bacteria	2.8% (27/949)	**46.2 (8.8–68.2) p = 0.02**	5.3% (26/492)	**62.0 (38.0–77.0) p = 0.001**	7.5% (36/481)	29.0 (0.0–57.0) p = 0.16
Virus	1.4% (13/949)	48.2 (0.0–75.8) p = 0.085	2.6% (13/492)	**56.1 (8.4–78.9) p = 0.024**	3.1% (15/481)	15.3 (0.0–59.2) p = 0.657
Bacteria or virus	3.3% (31/949)	**49.8 (18.7–69.0) p = 0.004**	6.3% (32/492)	**59.7 (36.3–74.5) p < 0.001**	8.1% (39/481)	19.8 (0.0–48.9) p = 0.336
Co-infections						
≥2 bacteria	1.7% (16/949)	48.2 (0.0–74.4) p = 0.064	3.1% (15/492)	**57.8 (16.9–78.5) p = 0.010**	3.7% (18/481)	18.5 (0.0–58.5) p = 0.550
Virus and bacteria	1.0% (9/949)	33.3 (0.0–75.0) p = 0.415	1.4% (7/492)	**62.0 (10.4–83.9) p = 0.022**	2.5% (12/481)	43.0 (0.0–77.4) p = 0.227
Co-infection						
≥2 viruses	0.1% (2/949)	**72.3 (0.0–96.0) p = 0.05**[d]	1.0% (5/492)	Incalculable[c] p = 0.553[d]	0.0% (0/481)	Incalculable[c] p = 0.062[d]

[a] Efficacy and p-values were calculated using medical group as the referent category.
[b] Efficacy and p-values were calculated using control group as the referent category.
[c] Efficacy could not be calculated because zero events in the control group.
[d] Fisher's exact test was used to calculate the p-value because of small expected cell frequencies.

Table 3
Multivariable cluster adjusted log binomial model of any co-infections compared with control group.

Variables in the model	Relative risk (95% CI)
N95	0.41 (0.23–0.75)[a,†]
Medical mask	0.87 (0.44–1.73)
Hospital level	1.41 (0.77–2.56)
High-risk procedure	1.45 (0.84–2.50)
Influenza vaccine	0.90 (0.46–1.78)
Hand washing	1.07 (0.51–1.23)

[a] Efficacy 59%.
[†] Significant p values ($p < 0.01$).

other clinical settings also supports the fact that occupational transmission is occurring in HCWs. Interestingly, smoking was not a risk factor for colonization or co-infection. We also found that nurses had significantly higher rate of bacterial co-infection than doctors. This may be due to higher patient contact or differences in use of infection control measures and personal protection (Chan, 2010; Chan et al., 2002).

The clinical significance of bacterial colonization in HCWs is uncertain, and this is an under-studied and unrecognized risk in HCWs. The significant protection against this afforded by N95 respirators mirrors the same trend seen in our previous study for clinical outcomes (MacIntyre et al., 2011, 2013). Outbreaks of bacterial respiratory infection do occur in HCWs (Kleemola and Jokinen, 1992; Ong et al., 2006; Pascual et al., 2006). Therefore, the observed reduction in bacterial colonization may translate to clinical protection against infection. *S. pneumoniae* was the most common bacteria identified in the upper respiratory tract. Invasive pneumococcal disease is thought to occur shortly after acquisition of colonization (Boulnois, 1992; Gray et al., 1980), and the infection can be transmitted by a colonized, asymptomatic individual. The rate of pneumococcal colonization demonstrated in our study was 6% (30/481 in controls), which is within the range described in adults (who have lower rates of colonization than children) (Austrian, 1986; Kadioglu et al., 2008; Obaro et al., 1996; Ridda et al., 2011). In an earlier study of frail elderly adults, only 1/315 subjects carried *S. pneumonia* (Ridda et al., 2011), although rates of adult carriage in the pre-vaccine era of up to 28% have been described (Hammitt et al., 2006). Bacterial load in the nasopharynx, not measured in this study, may be important in predicting the risk of invasive disease or viral co-infection and warrants further study (Klugman et al., 2009). We demonstrated that N95 respirators prevent carriage with *S. pneumoniae*. Although *S. pneumoniae* is not typically associated with outbreaks, nosocomial transmission and invasive disease in hospital patients from a carrier HCW have been reported (Guillet et al., 2012). In addition, transmission of bacterial pathogens from patients to HCWs during high-risk procedures has been described (Baba et al., 2009).

Table 4
Multivariable cluster adjusted log binomial model of bacterial and viral co-infection compared with control group.

Variables in the model	Relative risk (95% CI)
N95	0.33 (0.14–0.78)[a,†]
Medical mask	0.59 (0.20–1.73)
Hospital level	1.93 (0.80–4.62)
High-risk procedure	1.22 (0.52–2.86)
Influenza vaccine	1.60 (0.64–4.01)
Hand washing	1.24 (0.37–4.11)
Respiratory ward vs other	2.85 (1.30–6.26)[†]

[a] Efficacy 67%.
[†] Significant p values ($p = 0.01$).

The issue of co-infection is not well studied in HCWs, therefore our findings are quite novel. We have shown that all combinations of co-infection or co-colonization, with bacteria, viruses and both bacteria and virus, occur in symptomatic HCWs. These co-infections also display the same trend of decreasing frequency with increasing respiratory protection. Whatever their clinical significance, co-infection can be reduced by respiratory protection, and this may have implications for both patient safety, control of outbreaks and occupational health and safety of HCWs in hospitals. Co-infections, particularly bacterial–viral co-infection and dual viral infections can be more clearly implicated in causing disease in HCWs than colonization with a single bacterial species. This aspect of our findings, as well as the increased risk for staff in respiratory wards, therefore, has more direct clinical implications.

We demonstrated 59% efficacy against control of N95 respirators against any co-infection, and 67% against bacterial/viral co-infection. Medical masks were not protective and may in fact increase the risk of viral co-infections (5/492 compared to 0/481 in controls and 2/949 in N95). This finding, while not reaching statistical significance, may be due to chance, but is concerning and should certainly be investigated further. It is possible that the physical conditions of a medical mask may increase moisture or other parameters to increase risk of co-infection.

The limitations of this study include the fact that we did not test asymptomatic subjects, and therefore cannot examine the relationship of bacterial colonization to symptoms. Quantitative data on bacterial load would also have strengthened the study. Finally, the mechanisms of protection of a mask against respiratory tract colonization may be multi-modal. A mask may protect against respiratory transmission of pathogens, but may also act as a barrier to reduce hand to nose or hand to face contact, and may reduce infection in this way. Barrier precautions have been shown to reduce the rate of nasopharyngeal bacterial colonization (Safdar et al., 2006), so it would be expected that the barrier provided by a mask may have the same effect. A limitation of this study is that we cannot differentiate the relative contributions of prevention of airborne, droplet or direct contact transmission, but the study provided clinical efficacy estimates regardless of the different potential mechanisms of protection. If masks act by preventing multiple modes of transmission, they could have utility in preventing multidrug-resistant bacteria colonization of the nasopharynx of HCWs. Organisms such as methicillin-resistant *S. aureus* (MRSA) are a serious hospital infection control problem for HCWs (Morgan et al., 2012). Rates of clinical infections in HCWs with MRSA of 5.1% have been described, as has transmission of MRSA from HCWs to patients (Elie-Turenne et al., 2010; Sherertz et al., 2001; Verwer et al., 2012; Wang et al., 2011). A future research question could be the role of masks in preventing MRSA colonization in HCWs.

In summary, we have described novel data on bacterial infection and co-infections in HCWs, something which has not widely been documented or accepted previously, and shown that N95 respirators consistently provide protection against bacterial colonization and co-infections of the respiratory tract of hospital HCWs. The risk of such colonization is higher in ward types where more respiratory infections are expected (such as respiratory wards). The documented nosocomial outbreaks of bacterial infections such as pertussis and even *S. pneumoniae* in HCWs (Guillet et al., 2012; Pascual et al., 2006), as well as the efficacy against co-infections suggest there may be occupational safety benefits to HCWs in high-risk settings using a respirator, and that more studies are needed to better understand potential bacterial nosocomial respiratory pathogens.

Conflict of interest statement

The masks/respirators used in this study were provided by mask manufacturer 3M. The investigators have also partnered with 3M on an Australian Research Council Linkage Grant on masks. Prof MacIntyre also receives funding from influenza vaccine manufacturers GSK and CSL Biotherapies for investigator-driven research. Dr Holly Seale holds an NHMRC Australian based Public Health Training Fellowship (1012631) and has received funding for investigator-driven research/invitations to present from GSK, CSL and Sanofi-Pasteur.

Dr Iman Ridda holds an NHMRC Early career (630739) and has received funding for Investigator initiated research from GSK and for consultation from Merck. The remaining authors declare that they have no competing interests.

Contribution of each author

Professor C Raina MacIntyre: As a lead investigator Prof. MacIntyre was responsible for conception and design of the trial, overseeing the whole study, analyzing data, writing the report.

Professor Quanyi Wang: Study implementation, contribution to design, analysis and drafting of paper.

Dr. Bayzidur Rahman: Statistical analysis and drafting of paper.

Dr. Holly Seale: Study design, form/database development, monitoring, review and drafting of paper.

Dr. Iman Ridda: Literature review and drafting of manuscript.

Dr. Zhanhai Gao: Statistical analysis and drafting of paper.

Dr. Peng Yang: Study design, acquisition of data and drafting of paper.

Dr. Weixian Shi: Study design, Laboratory testing, review of the paper.

Dr. Xinghuo Pang: Study implementation, acquisition of data and review of the paper.

Dr. Yi Zhang: Database management and analysis.

Ms Aye Moa: Literature review and drafting of manuscript.

Professor Dominic E Dwyer: Study design, clinical and laboratory technical assistance and drafting of paper.

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Ethics statement

The study protocol was approved by the Institutional Review Board (IRB), Human Research Ethics Committee of the Beijing Ministry for Health, and National Ethics Application Form (NEAF), National Health and Medical Research Council (NHMRC), Australia.

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