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Trivalent inactivated influenza vaccine effective against influenza A(H3N2) variant viruses in children during the 2014/15 season, Japan

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The 2014/15 influenza season in Japan was characterised by predominant influenza A(H3N2) activity; 99% of influenza A viruses detected were A(H3N2). Subclade 3C.2a viruses were the major epidemic A(H3N2) viruses, and were genetically distinct from A/New York/39/2012(H3N2) of 2014/15 vaccine strain in Japan, which was classified as clade 3C.1. We assessed vaccine effectiveness (VE) of inactivated influenza vaccine (IIV) in children aged 6 months to 15 years by test-negative case-control design based on influenza rapid diagnostic test. Between November 2014 and March 2015, a total of 3,752 children were enrolled: 1,633 tested positive for influenza A and 42 for influenza B, and 2,077 tested negative. Adjusted VE was 38% (95% confidence intervals (CI): 28 to 46) against influenza virus infection overall, 37% (95% CI: 27 to 45) against influenza A, and 47% (95% CI: -2 to 73) against influenza B. However, IIV was not statistically significantly effective against influenza A in infants aged 6 to 11 months or adolescents aged 13 to 15 years. VE in preventing hospitalisation for influenza A infection was 55% (95% CI: 42 to 64). Trivalent IIV

that included A/New York/39/2012(H3N2) was effective against drifted influenza A(H3N2) virus, although vaccine mismatch resulted in low VE.

Introduction

Influenza vaccination is the most effective method of preventing influenza virus infection and its potentially severe complications. Based on the results of randomised controlled trials [1,2] and observational studies [3,4] the vaccine effectiveness (VE) of inactivated influenza vaccine (IIV) in healthy children has been reported to be 40% to 70%.

During the 2014/15 season, a variant strain of influenza A(H3N2) virus that was classified as phylogenetic clade 3C.2a and was genetically distinct from the 2014/15 A/Texas/50/2012(H3N2)-like clade 3C.1 vaccine reference strain appeared in the northern hemisphere. Consistent with the substantial vaccine mismatch, no or low VE against A(H3N2) was reported as interim estimates in Canada, the United Kingdom (UK), and the United States (US) [5-7].

There have been many reports of VE in studies conducted by a test-negative case-control (TNCC) design. Most of the subjects of the studies were adults and the elderly, and VE in children was not fully elucidated, especially the VE of IIV in children. In 2014, it was clearly recommended in the US that live attenuated influenza vaccine (LAIV) be used in healthy children from 2 to 8 years of age [8]. However, the effectiveness of LAIV against influenza A(H1N1)pdm09 in the 2013/14 season was found to be poor [9,10]. Moreover, although one large randomised trial reported superior relative efficacy of LAIV over IIV against antigenically drifted influenza A(H3N2) viruses [11], neither LAIV nor IIV provided significant protection against the drifted influenza A(H3N2) viruses in children in the 2014/15 season, and LAIV did not provide greater protection than IIV against these viruses [8]. Accordingly, LAIV is no longer recommended over IIV in children aged 2–8 years in the US [12].

In the past, Japan's strategy for controlling influenza was to vaccinate schoolchildren, based on the theory that this could reduce influenza epidemics in the community, and a special programme to vaccinate schoolchildren against influenza was begun in 1962. However, the programme was discontinued in 1994 because of lack of evidence that it had limited the spread of influenza in the community [13]. At present in Japan, influenza vaccination is officially recommended for elderly and high-risk patients with underlying conditions. However, ca 50% of children receive an influenza vaccination every year on their parents' initiative, paid for out of pocket [14].

Only trivalent IIV was approved for use in children in Japan until the 2014/15 season, and we have previously reported on the VE of IIV in children in Japan based on the results of influenza rapid diagnostic tests (IRDT) during the 2013/14 season [14], when influenza A(H1N1)pdm09 and B viruses were the main epidemic strains. VE was high against influenza A (63%, 95% CI: 56 to 69), and especially high (77%, 95% CI: 59 to 87) against influenza A(H1N1)pdm09, but was only 26% against influenza B (95% CI: 14 to 36).

A large influenza epidemic caused by A(H3N2) occurred in the 2014/15 season, and that provided an excellent opportunity to test VE against A(H3N2) virus infection in children. Influenza A(H3N2) outbreaks were reported throughout Japan since week 44 of 2014. The epidemic peaked between week 51 of 2014 and the week 1 of 2015. The start and peak of the influenza epidemic in the 2014/15 season occurred 3 weeks earlier than in the average year [15]. The vaccine strain used in Japan for influenza A(H3N2) was A/New York/39/2012(H3N2), which is different from A/Texas/50/2012; however, it belongs to the same clade, 3C.1.

We investigated the VE of trivalent IIV in children during the large epidemic caused by the drifted influenza

A(H3N2) virus by conducting a study by using the TNCC design and based on IRDT results.

Methods

Epidemiology

According to FluNet [16], 5,070 influenza A(H3N2) viruses were detected in Japan from week 45 of 2014 to week 14 of 2015, but only 50 A(H1N1) pdm09 viruses and 598 influenza B viruses were detected during the same period. In the 2014/15 season, over 99% of the influenza A viruses detected were A(H3N2) viruses (5,070/5,120).

Phylogenetic analysis

Influenza A(H3N2) viruses were isolated by using MDCK or MDCK-AX4 cells at the Yokohama City Institute of Public Health, Yokohama, Kanagawa, Japan [17]. The nucleotide sequences of the haemagglutinin (HA) genes were subjected to phylogenetic analysis, and phylogenetic trees were constructed using MEGA 6 software (The Biodesign Institute, Arizona, USA) and the neighbour-joining method [18]. The viruses were isolated in the 2014/15 influenza seasons. The nucleotide sequences determined are available from the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database. Accession numbers for the HA genes are EPI679784-EPI679834, respectively (Table 1).

Study enrolment and location

Children aged 6 months to 15 years with a fever of 38°C or over and cough and/or rhinorrhoea and who had received an IRDT in an outpatient clinic of one of 20 hospitals between 10 November 2014 and 31 March 2015 were enrolled in this study. In Japan, the cost of IRDT is covered by public health insurance, and almost all children with a high fever of 38°C or over receive an IRDT during an influenza epidemic. Our hospitals were located in six (Gunma, Tochigi, Saitama, Tokyo, Kanagawa, and Shizuoka prefectures) of the 47 prefectures in Japan, mainly in the Greater Tokyo Metropolitan area.

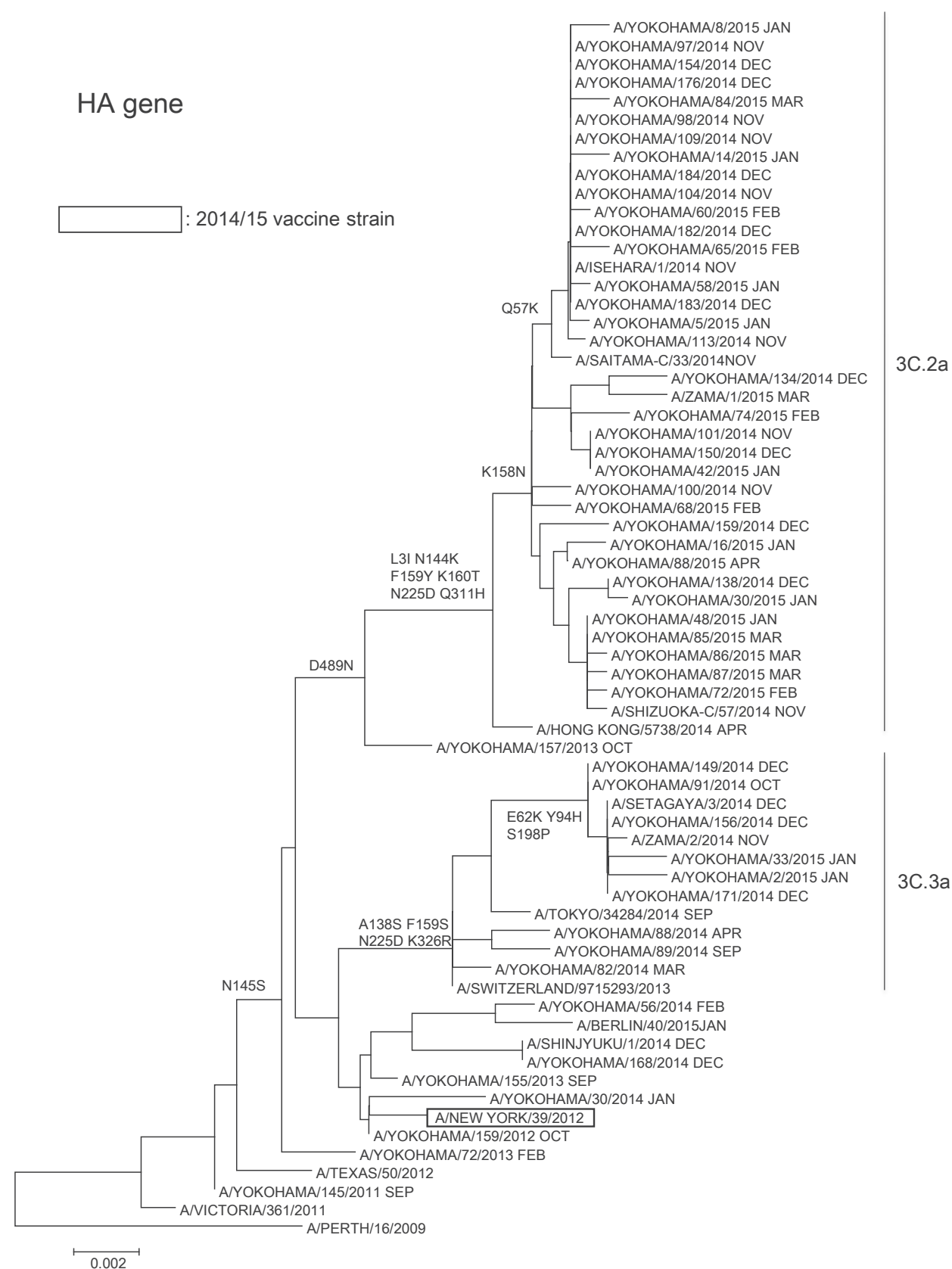
Patients who met the symptom criteria were eligible if they had not received antiviral medication before enrolment. Patients who had been vaccinated against influenza less than 14 days before illness onset were excluded from this study. A TNCC design was used to estimate VE based on IRDT results as previously described [14].

Diagnosis of influenza

Nasopharyngeal swabs were obtained from all of the enrollees. Several different IRDT kits, including the Espline Influenza A and B-N kit (Fujirebio Inc., Tokyo, Japan), ImmunoAce FLU kit with LineJudge pdm kit (Tauns Laboratories, INC, Shizuoka, Japan), Quick Chaser Flu A, B kit (Mizuho Medy Co., Ltd., Saga, Japan), and QuickNavi-Flu kit (DENKA SEIKEN Co., Ltd., Tokyo, Japan), all of which are capable of differentiating between influenza A and influenza B, were used

FIGURE 1

Phylogenetic analysis with sequences of the HA1 subunit of the haemagglutinin gene from reference viruses and influenza A(H3N2) sequences derived from children aged 6 months to 15 years, Yokohama, Japan, November 2014 to March 2015

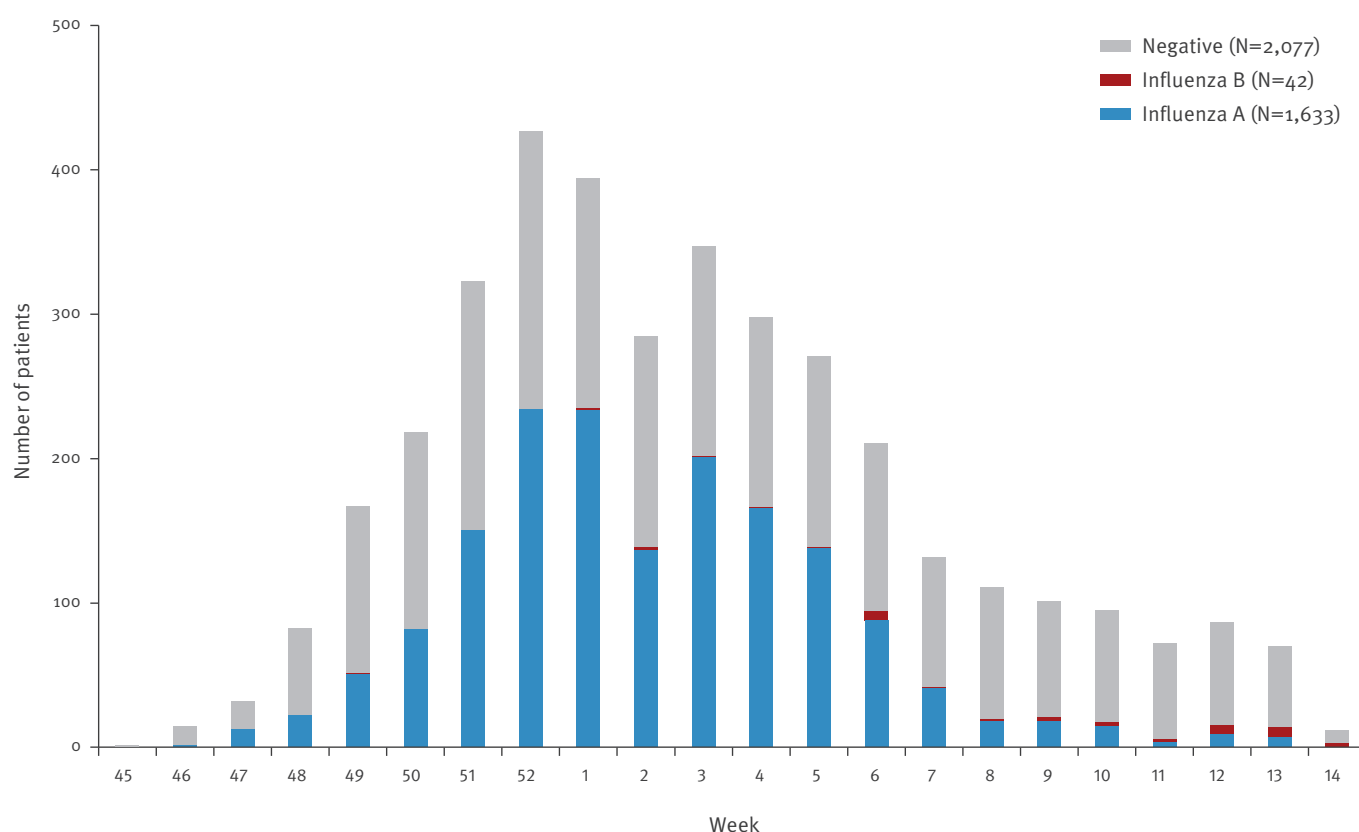


The amino acids are described with the H1 numbering. The amino acid substitutions relative to the A/PERTH/16/2009 virus are shown to the left of the nodes.

Reference viruses downloaded from the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database (EPI679784-EPI679835).

FIGURE 2

Influenza patients aged 6 months to 15 years diagnosed with influenza rapid diagnostic tests by week and type of virus in influenza vaccine effectiveness evaluation, Japan, November 2014 to March 2015



in the hospitals. Two of the 20 participating hospitals used the LineJudge pdm kit, which enables differentiation between influenza A, influenza B, and influenza A(H1N1)pdm09. According to their respective manuals, all of the IRDT kits used in this study have similar sensitivities (88–100%) and specificities (94–100%) [19].

Case and control patient identification

The IRDT-positive patients were enrolled as case patients and the IRDT-negative patients as control patients. Their medical charts were reviewed, and information regarding symptoms, influenza vaccination, number of vaccine doses (one or two), influenza complications and hospitalisations, sex, age, comorbidities, and treatment with neuraminidase inhibitors (NAIs) was collected and recorded. Children were excluded if definite information on influenza vaccination was found to be unavailable.

When a child was brought to one of our clinics, the parents or guardians were asked about the child's influenza vaccination status; the status was then usually confirmed by consulting the Maternal and Child Health Handbook provided by local governments, in which all vaccinations are recorded by the doctors in charge.

Vaccine

A trivalent inactivated subunit-antigen vaccine was used to vaccinate children in Japan during the 2014/15 season. The vaccine strains used to produce the vaccine for use in the 2014/15 season were: A/California/7/2009(X-179A) for protection against A(H1N1)pdm09, A/New York/39/2012(X-233A) for protection against A(H3N2), and B/Massachusetts/02/2012(BX-51B) for protection against type B, Yamagata lineage.

In Japan, two 0.25 ml doses of vaccine 2 to 4 weeks apart are recommended for children aged 6 months to 2 years, and two 0.5 ml doses of vaccine 2 to 4 weeks apart are recommended for children aged 3–12 years. Only one 0.5 ml dose of vaccine is recommended for children aged 13 years and over.

Test-negative case-control design

We estimated VE by TNCC design. VE was defined as $1 - \text{OR}$ (odds ratio), and was calculated as described below.

Statistical analysis

Statistical analysis was performed by using SPSS 22.0 software (IBM, US) and Ekuseru-Toukei 2015 for Windows software programme (Social Survey Research Information Co., Ltd., Tokyo, Japan).

TABLE 1A

Details of the influenza A(H3N2) haemagglutinin sequences obtained from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu database used in the phylogenetic analysis for this study.

Segment ID	Isolate name	Collection date	Country	Originating laboratory	Submitting laboratory	Authors
EPI679784	A/YOKOHAMA/30/2014	27/1/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679785	A/YOKOHAMA/56/2014	29/2/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679786	A/YOKOHAMA/82/2014	9/3/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679787	A/YOKOHAMA/88/2014	13/4/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679788	A/YOKOHAMA/168/2014	12/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679789	A/YOKOHAMA/100/2014	5/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679790	A/YOKOHAMA/101/2014	15/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679791	A/YOKOHAMA/104/2014	18/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679792	A/YOKOHAMA/109/2014	25/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679793	A/YOKOHAMA/113/2014	25/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679794	A/YOKOHAMA/134/2014	1/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679773 / EPI679795	A/YOKOHAMA/138/2014	2/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679774 / EPI679796	A/YOKOHAMA/14/2015	13/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679797	A/YOKOHAMA/150/2014	1/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679798 / EPI679775	A/YOKOHAMA/154/2014	5/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679799 / EPI679776	A/YOKOHAMA/159/2014	4/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679800 / EPI679777	A/YOKOHAMA/16/2015	13/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679801	A/YOKOHAMA/176/2014	15/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679802	A/YOKOHAMA/182/2014	23/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679803	A/YOKOHAMA/183/2014	20/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679804	A/YOKOHAMA/184/2014	25/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679805	A/YOKOHAMA/30/2015	16/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679806	A/YOKOHAMA/42/2015	23/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679807	A/YOKOHAMA/48/2015	29/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679808	A/YOKOHAMA/5/2015	6/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679809 / EPI679778	A/YOKOHAMA/58/2015	26/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679810 / EPI679779	A/YOKOHAMA/60/2015	4/2/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679811	A/YOKOHAMA/65/2015	6/2/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679812 / EPI679780	A/YOKOHAMA/68/2015	6/2/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679813	A/YOKOHAMA/72/2015	16/2/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T

TABLE 1B

Details of the influenza A(H3N2) haemagglutinin sequences obtained from the Global Initiative on Sharing All Influenza Data (GISAID)'s EpiFlu database used in the phylogenetic analysis for this study.

Segment ID	Isolate name	Collection date	Country	Originating laboratory	Submitting laboratory	Authors
EPI679814	A/YOKOHAMA/74/2015	13/2/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679815	A/YOKOHAMA/8/2015	10/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679816	A/YOKOHAMA/84/2015	14/3/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679781 / EPI679817	A/YOKOHAMA/85/2015	19/3/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679818	A/YOKOHAMA/86/2015	20/3/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679819	A/YOKOHAMA/87/2015	27/3/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679820	A/YOKOHAMA/88/2015	18/4/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679821	A/YOKOHAMA/97/2014	13/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679822	A/YOKOHAMA/98/2014	17/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679823	A/YOKOHAMA/149/2014	6/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679824	A/YOKOHAMA/156/2014	5/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679825	A/YOKOHAMA/171/2014	12/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679826	A/YOKOHAMA/2/2015	5/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679827	A/YOKOHAMA/33/2015	16/1/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679828	A/YOKOHAMA/89/2014	27/9/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679829	A/YOKOHAMA/91/2014	20/10/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679830 / EPI679782	A/SHINJYUKU/1/2014	30/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679831 / EPI679783	A/SETAGAYA/3/2014	20/12/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679832	A/ZAMA/1/2015	20/3/2015	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679833	A/ZAMA/2/2014	20/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizuno T
EPI679834	A/ISEHARA/1/2014	17/11/2014	Japan	Yokohama City Institute of Public Health	Yokohama City Institute of Public Health	Kawakami C, Usuku S, Sasao T, Mizun

VE was adjusted for age group (6–11 months, 1–2 years, 3–5 years, 6–12 years, and 13–15 years), comorbidity (yes or no), area of the Kanto region of Japan, i.e. north area: Gunma Prefecture and Tochigi Prefecture; middle area: Saitama Prefecture and Tokyo Prefecture; and south area: Kanagawa Prefecture and Shizuoka Prefecture, and month of illness onset.

The influenza season was divided into an early phase (November, December and January) and a late phase (February and March), and the VE for each phase was compared. We also estimated VE according to the number of doses of vaccine administered. The Breslow-Day test was used to assess the homogeneity of the odds ratios in several 2 x 2 contingency tables. P

value of < 0.05 was considered to indicate statistical significance.

VE against hospitalisation

We calculated the VE against hospitalisation using the TNCC design. The cases included patients with positive IRDT results who were admitted to hospital. These cases were divided into an in-patient group that had received the influenza vaccine and a in-patient group that had not received a vaccine. The control group included all patients who were not admitted to hospital, whether they received an influenza vaccine or not. Admitted patients with negative IRDT results were excluded from the analysis.

Ethics

This study was approved by the Keio University Ethics Committee in 2013 (Approval Number 20130216) and by the Institutional Review Board (IRB) at each hospital. Eligible patients and their guardians (usually parents) were verbally informed of the objective and methods of the study in the outpatient departments. The requirement for obtaining written consent was waived by the IRBs because performing an IRDT is standard practice in Japan.

Results

Influenza A(H3N2) virus characterisation

The HA sequences of the majority of the 128 influenza A(H3N2) viruses in the 2014/15 season that were sequenced (113/128; 88.3%) were further characterised within this clade as belonging to subclade 3C.2a of clade 3C.2, with fewer (15/128; 11.7%) belonging to clade 3C.3 (Figure 1). These subclade 3C.2a viruses are considered genetically distinct from both the A/New York/39/2012 (H3N2) clade 3C.1 vaccine strain used in Japan and the A/Texas/50/2012 WHO vaccine reference strain.

Characteristics of the enrollees

A total of 3,896 children were enrolled in this study, of whom 144 were subsequently excluded from the analysis for the following reasons: 117 were <6 months old or >15 years old, or their age was unknown; two had a fever <38°C; 24 had an unclear influenza vaccination history and the date of one patient's clinic visit had not been recorded.

Of the remaining 3,752 patients who were eligible for inclusion in the analysis in this study, 1,633 had influenza A (1 had influenza A(H1N1)pdm09 infection, and the remaining 1,632 had influenza A, subtype unknown); and 42 patients had influenza B. Of the 3,752 patients included, 2,077 were IRDT-negative. Figure 2 shows the total numbers of cases of influenza diagnosed by week at the 20 hospitals as a whole. The first case of influenza A was diagnosed in week 45 of 2014. The number of influenza A cases diagnosed per week increased towards the end of 2014, and peaked in week 52, after which time the number of cases diagnosed per week gradually decreased. A small number of influenza B cases were seen after week 6 of 2015.

Of the children with positive IRDT, 95.1% (1,545/1,625) had been brought to the hospital or clinic and diagnosed within 48 hours of illness onset, and 96.5% (1,231/1,276) of the children with a positive IRDT were treated with NAIs (Table 2).

Vaccine effectiveness against influenza

The adjusted VE of the influenza vaccine was 38% (95% CI: 28 to 46) against influenza virus infection overall (Table 3), 37% (95% CI: 27 to 45) against influenza A infection, and 47% (95% CI: -2 to 73) against influenza B infection (Table 3).

VE by age group was analysed only in regard to influenza A. Statistically significant adjusted VE was not demonstrated in the infant group aged 6 months to 11 months, in which it was -5% (95% CI: -139 to 54), but statistically significant adjusted VE was seen in the 1- to 12-year-old group. Moderate adjusted VE against influenza A was demonstrated in the 1- to 2-year-old group (40%, 95% CI: 18 to 56) and in the 3- to 5-year-old group (55%, 95% CI: 41 to 65). Adjusted VE against influenza A in the 6- to 12-year-old group was lower (25%, 95% CI: 6 to 41), and it was not statistically significant in the 13- to 15-year-old group (41%, 95% CI: -0.1 to 65). Crude VE against influenza A was 29% (95% CI: 11 to 43) in the 6- to 12-year-old group and was significantly lower than the 55% (95% CI: 42 to 65) in the 3- to 5-year-old group ($p=0.0089$, Breslow-Day test).

VE against influenza B was not analysed by age group because of the small number of cases.

Protection against hospitalisation

Patients admitted to the hospitals with influenza A were divided into an unvaccinated group ($n=231$) and a vaccinated group ($n=104$) (Table 4). The control group consisted of patients who were not admitted to the hospital, including 1,447 unvaccinated patients and 1,439 vaccinated patients. Influenza vaccination was effective in preventing hospitalisation for influenza A virus infection (55%, 95% CI: 42 to 64) (Table 4), but VE was not statistically significant in preventing hospitalisation for influenza B virus infection because of the small number of cases.

Admitted patients with negative IRDT results ($n=143$) were excluded from this analysis.

Vaccine effectiveness by month of illness onset

Crude VE against influenza A infection decreased markedly in the late phase of the influenza epidemic, from 46% (95% CI: 37 to 54) in the 3-month period November, December, and January to 13% (95% CI: -18 to 36) in the 2-month period February and March (Table 5).

Weekly changes in vaccine effectiveness

Crude VE against influenza A first became statistically significant in week 49, when it reached 69% (95% CI: 46 to 82) (Table 6). VE then gradually decreased from 60% (95% CI: 47 to 70) in week 51 of 2014 to 42% (95% CI: 34 to 50) in week 8 of 2015 and stabilised.

VE against influenza B, on the other hand, was rather unstable because of the small number of patients (data not shown).

Number of doses of vaccine

Two doses of influenza vaccine did not provide better protection against influenza A in children of 6 months to 12 years of age than a single dose, even though two doses of trivalent IIV were recommended for that age

TABLE 2

Characteristics of the children aged 6 months to 15 years enrolled in influenza vaccine effectiveness study, Japan, November 2014 to March 2015 (n = 3,752)

Characteristic	Any influenza (%)		Influenza A (%)	Influenza B (%)	Influenza negative (%)	Difference between 'any influenza' and 'influenza negative'
Sex	Female	799 (48)	777 (48)	22 (52)	965 (46)	p = 0.4575 ^a
	Male	876 (52)	856 (52)	20 (48)	1,111 (54)	
	Total	1,675	1,633	42	2,076	
Age	6–11 mo	47 (3)	44 (3)	3 (7)	136 (7)	p < 0.001 ^b
	1–2 y	229 (14)	224 (14)	5 (12)	738 (36)	
	3–5 y	410 (24)	402 (25)	8 (19)	574 (28)	
	6–12 y	793 (47)	772 (47)	21 (50)	519 (25)	
	13–15 y	196 (12)	191 (12)	5 (12)	110 (5)	
	Total	1,675	1,633	42	2,077	
Comorbidity	No	1,343 (82)	1,307 (82)	36 (86)	1,585 (79)	p = 0.0251 ^a
	Yes	293 (18)	287 (18)	6 (14)	418 (21)	
	Total	1,636	1,594	42	2,003	
Area of Kanto region ^c	North	125 (7)	121 (7)	4 (10)	170 (8)	p = 0.4007 ^d
	Middle	781 (47)	766 (47)	15 (36)	996 (48)	
	South	769 (46)	746 (46)	23 (55)	911 (44)	
	Total	1,675	1,633	42	2,077	
Month of illness onset	Nov 2014	38 (2)	38 (2)	0 (0)	93 (4)	p < 0.001 ^e
	Dec 2014	646 (39)	644 (39)	2 (5)	699 (34)	
	Jan 2015	742 (44)	737 (45)	5 (12)	614 (30)	
	Feb 2015	188 (11)	175 (11)	13 (31)	385 (19)	
	Mar 2015	61 (4)	39 (2)	22 (52)	286 (14)	
	Total	1,645	1,633	42	2,077	
Clinic visit (hours after symptom onset)	< 12 h	551 (34)	541 (34)	10 (24)	602 (31)	p = 0.0348 ^f
	12–48 h	994 (61)	968 (61)	26 (63)	1,114 (57)	
	> 48 h	80 (5)	75 (5)	5 (12)	251 (13)	
	Total	1,625	1,584	41	1,967	
	> 12 h	1,074	1,043	31	1,365	
Received vaccine in 2014/15 season	No	978 (58)	952 (58)	26 (62)	930 (45)	p < 0.001 ^g
	Yes	697 (42)	681 (42)	16 (38)	1,147 (55)	
	Total	1,675	1,633	42	2,077	
Vaccine doses received in 2014/15 season	None	978 (59)	952 (58)	26 (63)	930 (45)	p < 0.001 ^g
	One	224 (13)	220 (14)	4 (10)	336 (16)	
	Two	464 (28)	457 (28)	11 (27)	807 (39)	
	Total	1,670	1,629	41	2,073	
Treatment with neuraminidase inhibitors	No	45 (4)	44 (4)	1 (3)	1,409 (98)	p < 0.001 ^h
	Yes	1,231 (96)	1,201 (96)	30 (97)	29 (2)	
	Total	1,276	1,245	31	1,438	

^a Chi-squared test.

^b Chi-squared test, Cramer's V = 0.3188.

^c Area of Kanto region. North: Gunma Prefecture and Tochigi Prefecture; Middle: Saitama Prefecture and Tokyo Prefecture; South: Kanagawa Prefecture and Shizuoka Prefecture.

^d Chi-squared test, Cramer's V = 0.0221.

^e Chi-squared test, Cramer's V = 0.2367.

^f Chi-squared test, comparing the number of patients who came to the clinic < 12 hours after the onset with the number who came later.

^g Chi-squared test, Cramer's V = 0.1379.

^h Chi-squared test, Cramer's V = 0.9453.

TABLE 3

Effectiveness of trivalent inactivated influenza vaccine, influenza vaccine effectiveness study, Japan, November 2014 to March 2015 (n = 3,752)

Category VE% (95% CI)		Any influenza ^a		Influenza A ^a		Influenza B ^{a,b}	
		Vaccinated/cases (Vaccinated/controls)	VE% (95% CI)	Vaccinated/cases (Vaccinated/controls)	VE% (95% CI)	Vaccinated/cases (Vaccinated/controls)	
All ages 6 months to 15 years	Crude	42 (34 to 49)	697/1,675 (1,147/2,077)	42 (34 to 49)	681/1,633 (1,147/2,077)	50 (6 to 73)	16/42 (1,147/2,077)
	Adjusted _{c, d}	38 (28 to 46)		37 (27 to 45)		47 (-2 to 73)	
	Adjusted _{c,d,e}	39 (30 to 47)		39 (29 to 47)		51 (4 to 75)	
	Adjusted _{c,d,f}	39 (27 to 49)		38 (26 to 48)		65 (21 to 85)	
Age 6–11 months	Crude	-8 (-137 to 51)	11/47 (30/136)	-18 (-161 to 47)	11/44 (30/136)	NA	
	Adjusted _c	3 (-119 to 57)		-5 (-139 to 54)			
Age 1–2 years	Crude	42 (21 to 57)	106/229 (440/738)	40 (19 to 56)	105/224 (440/738)		
	Adjusted _c	41 (20 to 57)		40 (18 to 56)			
Age 3–5 years	Crude	54 (41 to 65)	181/410 (364/574)	55 (42 to 65)	176/402 (364/574)		
	Adjusted _c	54 (40 to 65)		55 (41 to 65)			
Age 6–12 years	Crude	29 (11 to 43)	336/793 (264/519)	29 (11 to 43)	327/772 (264/519)		
	Adjusted _c	26 (7 to 41)		25 (6 to 41)			
Age 13–15 years	Crude	41 (5 to 64)	63/196 (49/110)	40 (3 to 63)	62/191 (49/110)		
	Adjusted _c	41 (1 to 65)		41 (0 to 65)			

NA: not analysed.

^a One hospital had no information on comorbidity.

^b Not analysed by age because few patients developed influenza.

^c Adjusted for comorbidity (yes or no), area (north area, middle area, south of the Kanto region), month of onset.

^d Adjusted for age (0–15 years).

^e Adjusted for time tested after the onset (<12, 12–48 and >48 hours).

^f Only patients tested >12 hours after onset.

TABLE 4

Effectiveness of trivalent inactivated influenza vaccine in preventing influenza hospitalisation, influenza vaccine effectiveness study, Japan, November 2014 to March 2015 (n=3,228)

Influenza type	Vaccination status	No hospitalisation	Hospitalisation for influenza	Effectiveness in preventing influenza hospitalisation	95% CI
Any Influenza	Unvaccinated	1,447	236	55	43 to 64
	Vaccinated	1,439	106		
Type A	Unvaccinated	1,447	231	55	42 to 64
	Vaccinated	1,439	104		
Type B	Unvaccinated	1,447	5	60	-108 to 92
	Vaccinated	1,439	2		

CI: confidence interval.

range. The OR of two doses (cases/controls, 451/800) vs one dose (164/294) was 1.01 (95% CI: 0.81 to 1.26) for influenza A and 1.35 (95% CI: 0.37 to 4.86) for influenza B (crude data).

Vaccine coverage

The proportion of vaccine coverage calculated for the IRDT-negative enrollees was 55% (1,147/2,077). By age group, it was: 6–11 months, 22% (30/136); 1–5 years, 61% (804/1,312); for 6–12 years, 51% (264/519); and 13–15 years, 45% (49/110).

Discussion

Estimations of the effectiveness of influenza vaccine by a TNCC design have been reported annually in recent years [20–22], and the TNCC design has become the standard design for assessing VE. In this study, we used the results of IRDTs as a basis for estimating VE using the TNCC design in children who had received trivalent IIV during the 2014/15 season, since almost all children with a fever receive an IRDT during an influenza epidemic [23], resulting in a large enrolment for this study.

TABLE 5

Effectiveness of trivalent inactivated influenza vaccine, by phase of the influenza season, influenza vaccine effectiveness study, Japan, November 2014 to March 2015 (n=3,752)

Phase of the influenza season	Any influenza		Influenza A		Influenza B	
	VE% (95%CI)	Vaccinated/cases (Vaccinated/controls)	VE% (95% CI)	Vaccinated/cases (Vaccinated/controls)	VE% (95% CI)	Vaccinated/cases (Vaccinated/controls)
Nov 2014 –Jan 2015	46 (38 to 54)	573/1,426 (781/1,406)	46 (37 to 53)	572/1,419 (781/1,406)	87 (-11 to 98)	1/7 (781/1,406)
Feb–Mar 2015	17 (-11 to 38)	124/249 (366/671)	13 (-18 to 36)	109/214 (366/671)	38 (-24 to 69)	15/35 (366/671)
Total	42 (34 to 49)	697/1,675 (1,147/2,077)	42 (34 to 49)	681/1,633 (1,147/2,077)	50 (6 to 73)	16/42 (1,147/2,077)

CI: confidence interval; VE: vaccine effectiveness.

VE against any influenza and VE against influenza A were higher early in the season than late in the season (Breslow-Day, $p < 0.05$).

The overall adjusted VE for prevention of laboratory-confirmed medically attended influenza illness in this large study of 3,752 children was 38% (95% CI: 28 to 46). Most cases (97.5%) had been infected by influenza A virus, and VE was 37% (95% CI: 27 to 45) in the influenza A group. Because over 99% of the influenza A viruses detected in Japan in the 2014/15 season were A(H3N2) viruses, the results of our study demonstrated that trivalent IIV was effective against the drifted influenza A(H3N2) in children. VE against influenza B, on the other hand, was not statistically significant because there were only 42 influenza B patients.

The majority, 88.3%, of the haemagglutinin (HA) sequences of the influenza A(H3N2) viruses isolated during the 2014/15 season and analysed at the Yokohama City Institute of Public Health belonged to subclade 3C.2a of clade 3C.2, and the National Institute of Infectious Diseases has reported that subclade 3C.2a accounted for the major epidemic A(H3N2) viruses in Japan in the 2014/15 season [15]. Consequently there have been genetic and antigenic mismatches between most epidemic A(H3N2) strains in Japan and the vaccine strains that have been used, as has been reported in Canada [5], the UK [6], and the US [7]. The low VE in the 2014/15 season, when the dominant influenza virus was A(H3N2), was postulated to be attributable to mutations in the egg-adapted A(H3N2) vaccine strain [24] as well as to a mismatch due to antigenic drift of the virus.

According to the interim estimates of 2014/15 VE in Canada [5], little or no VE was observed, because the adjusted VE against influenza A(H3N2) for all ages was -8% (95% CI: -50 to 23). Based on the end-of-season VE results for 2014/15 in the UK [25], the adjusted VE for all ages against influenza A(H3N2) was 29.3% (95% CI: 8.6 to 45.3). It was 29.4% for those 18 years of age and over, which was attributable to the effect of the IIV alone, but for those aged under 18 years, it was only 19.1%, which was attributable to the combined

effect of both the LAIV and IIV, and was not statistically significant. The end-of-season VE results for 2014/15 in the US [7] showed that the adjusted VE for all ages against influenza A(H3N2) was 13% (95% CI: 2 to 23). However, none of these recent reports [5,7,25] clearly demonstrated VE of IIV in children. The results of our study showed that trivalent IIV provided low but significant protection against influenza A(H3N2) virus infection in children in the 2014/15 season in Japan, despite marked antigenic drift in the epidemic virus. In a previous paper, we reported having found that trivalent IIV was highly effective in protecting against influenza A(H3N2) virus infection irrespective of whether there had been marked antigenic drift [3].

The widespread circulation of influenza A(H3N2) viruses in the 2014/15 season provided an opportunity to compare VE according to age group. Although significant protection against influenza A(H3N2) illness was demonstrated in the 1- to 12-year-old group, VE was not statistically significant in the 6- to 11-month-old group or 13- to 15-year-old group. Similarly low or no effectiveness was observed in both the 6- to 11-month-old group and 13- to 15-year-old group in our study of VE in the 2013/14 season [14].

The results of the present study showed that the influenza vaccine was not effective against influenza A (-5%, 95% CI: -139 to 54) in 6- to 11-month-old infants. Similarly, no significant VE was shown against influenza A in infants in the 2013/14 season (21%, 95% CI: -87 to 67) [14]. Our studies in these two consecutive seasons showed that trivalent IIV was not effective against influenza A(H1N1)pdm09 or A(H3N2) in infants. However, the number of infants enrolled was relatively small, and further studies are needed.

We unexpectedly found that VE was low in adolescents (the 13–15 years age group), in the two consecutive seasons 2013/14 and 2014/15. In the 2013/14 season, both influenza A(H3N2) and A(H1N1)pdm09 were circulating

TABLE 6

Effectiveness of trivalent inactivated influenza vaccine against influenza A in children aged 6 months to 15 years, cumulative data, by week, influenza vaccine effectiveness study, Japan, November 2014 to March 2015 (n=3,752)

Year	Week	Type A positive		Influenza-negative		Vaccine effectiveness (95% CI)
		Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	
2014	45	0	0	0	1	NA
	46	0	2	4	10	NA
	47	3	12	16	17	73 (-12 to 94)
	48	12	26	42	51	44 (-24 to 75)
	49	23	66	110	98	69 (46 to 82)
	50	50	121	182	162	63 (46 to 75)
	51	104	218	281	235	60 (47 to 70)
2015	52	199	358	381	327	52 (40 to 62)
	1	307	484	476	391	48 (37 to 57)
	2	368	560	554	459	46 (35 to 55)
	3	446	683	633	525	46 (36 to 54)
	4	515	780	710	579	46 (37 to 54)
	5	580	853	790	631	46 (37 to 53)
	6	623	898	849	688	44 (35 to 51)
	7	644	918	901	726	43 (35 to 51)
	8	656	924	949	769	42 (34 to 50)
	9	668	930	983	815	40 (32 to 48)
	10	674	939	1,031	844	41 (33 to 49)
	11	675	942	1,068	873	41 (33 to 49)
	12	676	950	1,112	900	42 (34 to 50)
	13	681	952	1,141	927	42 (34 to 49)
	14	681	952	1,147	930	42 (34 to 49)

CI: confidence interval; NA: not analysed; VE: vaccine effectiveness.

in Japan [26], and no statistically significant VE against influenza A was observed in the 13- to 15-year-old group [14]. VE against influenza B was not statistically significant either [14]. Although we cannot explain this low or absent VE in adolescents, similar results, including low VE of trivalent IIV against influenza A(H3N2) and B in adolescents, were reported during the 2012/13 season in the US [27].

A meta-analysis showed no convincing evidence that influenza vaccine reduces mortality, hospitalisations, or serious complications in children [28]. However, the results of our previous study demonstrated that influenza vaccination was highly effective in reducing hospitalisation of children infected with influenza A in the 2013/14 season. In the present study, which covered the period of the widespread epidemic caused by the drifted influenza A(H3N2), it reduced such admissions of children infected with influenza A by 55%. Although the criteria for hospitalisation vary from country to country, our studies conducted two years in row demonstrated VE in reducing hospitalisation for influenza A in children in Japan, where over 90% of the children with influenza-like illness (ILI) enrolled in the present study were brought to clinics within 48 hours after the onset of illness and 96% were treated with NAIs if their IRDT was positive. There are recent reports from other countries showing that influenza vaccination

was associated with reduced hospitalisations [29] and reduced clinical severity in children [30].

Our previous study showed that VE against influenza A and B decreased by ca 10% in the latter half of the epidemic [14]. The present study showed that VE against influenza A declined greatly over the course of the epidemic, from 46% in November, December, and January to 13% in February and March. Thus, persistence of VE depends on the type and subtype of influenza viruses and the match between vaccine strain and epidemic virus.

The weekly changes in VE shown in this study demonstrated the major advantage of a TNCC design based on IRDT results. It is easy to calculate VE every week in Japan. VE against influenza A gradually declined every week from 69% in week 49 of 2014 to 42% in week 8 of 2015.

Two doses of influenza vaccine have been reported to be necessary to provide sufficient protection in children [4,31-33], and our previous study [14] showed that two doses were needed to optimise protection against influenza A in children. However, the results of the present study show that a single dose of influenza vaccine was as effective as two doses of vaccine in protecting

against influenza A in children. The difference between the results in the two seasons can be explained by the fact that the epidemic in the 2014/15 season started and peaked much earlier than the 2013/14 epidemic [15] and even though many children received only one dose in the 2014/15 season, adequate VE was maintained. If the 2014/15 epidemic had started later, there might have been a difference in VE between two doses and one dose.

The limitations of this study need to be considered. Unlike most previous TNCC studies based on RT-PCR data, our study was based on the results of IRDTs. Although using IRDTs in TNCC studies has been reported to possibly result in underestimations of VE [34,35], Suzuki et al. found no difference between VE estimated on the basis of IRDT results and VE estimated on the basis of PCR data [36], and the VE results in our previous study were consistent with the results based on RT-PCR findings reported in another study [14]. VE estimates have been found to be much less influenced when the sensitivity of the diagnostic method used is over 80%, although low specificity has been found to cause greater bias in VE estimates [35]. The sensitivity of the IRDT kit used in this study (Espline Influenza A and B-N kit) is 85.1% to 92.4% for influenza A and 71.6% to 91.2% for influenza B, and its specificity is 97.6% to 100% [37]. Moreover, over 90% of the children with ILI were brought to our clinics within 48 hours of illness onset. By contrast, in most of the TNCC studies based on the RT-PCR tests, the patients were enrolled within 7 days after illness onset, suggesting that influenza virus could not have been detected even by the RT-PCR tests [38,39].

A TNCC design based on IRDT results is limited from an epidemiological standpoint, since the VE against each subtype of influenza A or especially against each lineage of influenza B cannot be determined. However, from a clinical standpoint, a TNCC design based on IRDT results has various advantages. VE can be communicated easily to the Japanese population during the very early stages of an influenza epidemic, and more importantly, VE against hospitalisation can be easily calculated.

In the near future, VE estimated by a TNCC assessment based on IRDT results will be reported weekly in many areas of Japan. The large number of patients in Japan who receive an IRDT makes it possible to estimate VE with considerable precision, and the most appropriate vaccination policy will be established based on the data obtained.

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Conflict of interest

NS has received speakers' honoraria from Astellas, Daiichi Sankyo, Denka Seiken and Takeda, none of which was in connection with the work presented here.

MS has received speakers' honoraria from Astellas, Daiichi Sankyo, Japan Vaccine and MSD; and grant support from Japan Vaccine, none of which was in connection with the work presented here.

KM has received speakers' honoraria from Japan Vaccine and MSD, none of which was in connection with the work presented here.

For other authors, none declared.

Authors' contributions

NS was involved in the original methodological design of the study. TT led the study group. MS undertook the statistical analysis. CK was responsible for phylogenetic analysis. YY, MY, HB, MI, MK, SS, TK, KM, MF, OK, NY, KT, AN, YN, AS, NT, HF, MT, MM and IO were responsible for the coordination of the study at the local level, and were involved in data collection and analyses. NS and MS wrote the paper.

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Herd effect from influenza vaccination in non-healthcare settings: a systematic review of randomised controlled trials and observational studies

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Influenza vaccination programmes are assumed to have a herd effect and protect contacts of vaccinated persons from influenza virus infection. We searched MEDLINE, EMBASE, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Global Health and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to March 2014 for studies assessing the protective effect of influenza vaccination vs no vaccination on influenza virus infections in contacts. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) using a random-effects model. Of 43,082 screened articles, nine randomised controlled trials (RCTs) and four observational studies were eligible. Among the RCTs, no statistically significant herd effect on the occurrence of influenza in contacts could be found (OR: 0.62; 95% CI: 0.34–1.12). The one RCT conducted in a community setting, however, showed a significant effect (OR: 0.39; 95% CI: 0.26–0.57), as did the observational studies (OR: 0.57; 95% CI: 0.43–0.77). We found only a few studies that quantified the herd effect of vaccination, all studies except one were conducted in children, and the overall evidence was graded as low. The evidence is too limited to conclude in what setting(s) a herd effect may or may not be achieved.

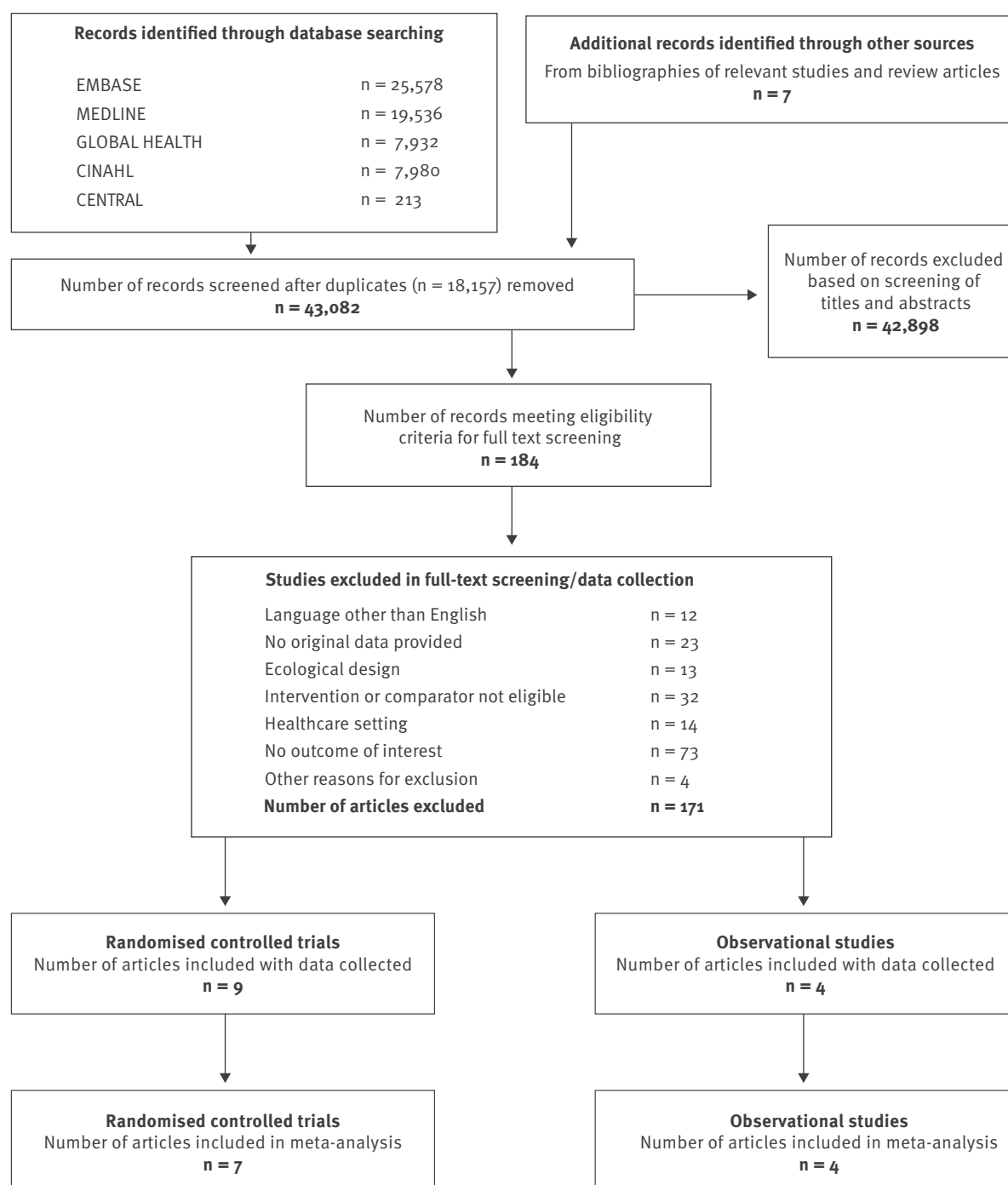
Introduction

Influenza is a major cause of morbidity and mortality worldwide [1–3]. Many countries recommend vaccination against influenza to prevent influenza infections, in particular for groups at high risk for complications [4–7]. Some high risk groups, such as young children and elderly persons (commonly defined as those above 65 years of age), experience decreased influenza vaccine effectiveness compared with healthy adults [8,9], complicating influenza prevention strategies. Moreover, because such groups represent a minority of the population at large, the population-wide impact of vaccination of risk groups may be limited [7,10].

Influenza vaccine modelling and ecological studies identifying benefits of herd effect have informed seasonal and pandemic influenza vaccine policies [10,11], herd effect being usually defined as the indirect protection of individuals susceptible to infection when a sufficient proportion of the population is immune to the pathogen. Vaccinating persons most likely to respond to the influenza vaccine and relying on herd effect to reduce the chance of exposure to influenza may protect unvaccinated or high-risk individuals. Herd effect may therefore mitigate the consequences of impaired vaccine response in some high-risk groups [12–14].

FIGURE 1

Flowchart of included and excluded randomised control trials and observational studies identified in a systematic review of herd effect from influenza vaccination in non-healthcare settings



^a Two randomised control trials did not report all numerator and denominator data and therefore could not be included in the meta-analysis.

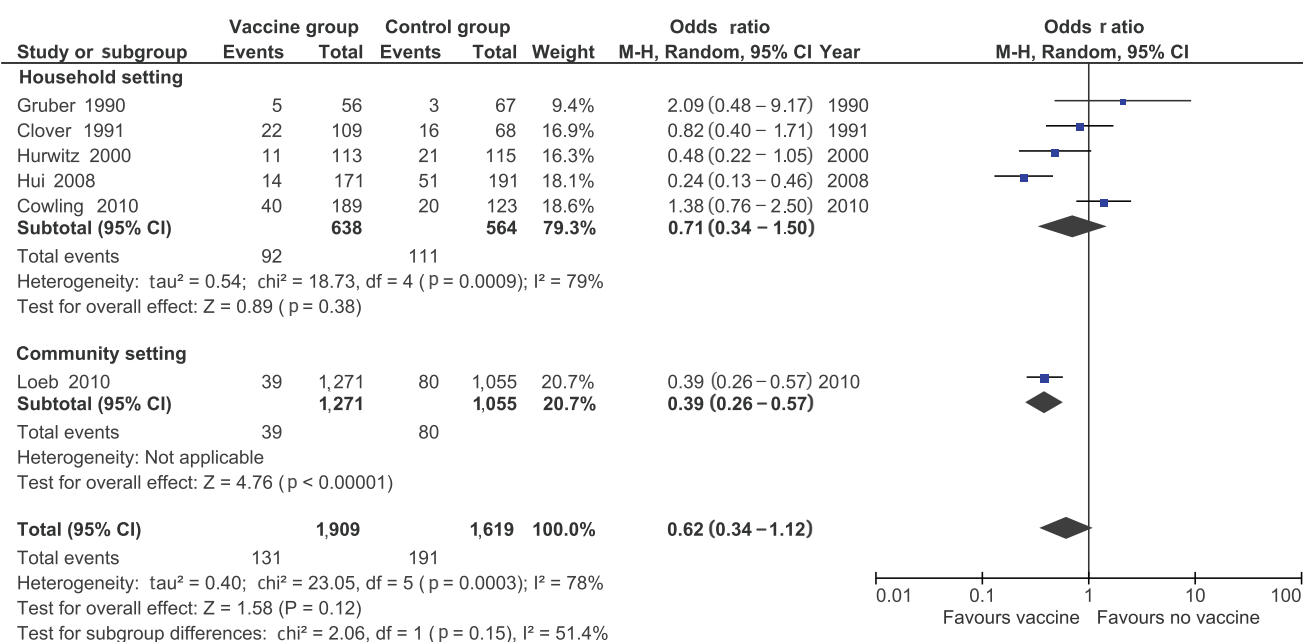
The purpose of this systematic review was to summarise the evidence on herd effect from influenza vaccination outside healthcare settings. These data may help to inform public health on influenza vaccine research and policy development.

Methods

All decisions regarding eligibility criteria, search strategy, study selection, assessment of risk for bias, explanation for heterogeneity, data collection and analysis were established before data collection. The protocol was registered with the international prospective register of systematic reviews (PROSPERO) [15]

FIGURE 2

Meta-analysis of seven included randomised controlled trials reporting on influenza infections in contacts of influenza vaccinated vs unvaccinated individuals in non-healthcare settings



CI: confidence interval; df: degrees of freedom; M-H: Mantel-Haenszel.

(CRD42014009401) and was reported in accordance with the PRISMA statement [16].

Eligibility criteria and outcomes assessed

Studies assessing the protective effect of influenza vaccination vs no influenza vaccination (either no vaccination, placebo or alternative vaccine) on contacts of any age group in a non-healthcare setting were eligible. The definition of contacts was broad and included anyone in the same community, school or household. Study designs included randomised controlled trials (RCTs) and observational studies with a non-influenza vaccine comparator group. For the latter study type, quasi-experimental (before–after) studies, cohort studies, case–control studies and cross-sectional studies were eligible. Ecological studies and modelling studies were excluded. We also excluded studies conducted within healthcare institutions, such as nursing homes and hospitals, and studies in languages other than English.

The primary outcome was influenza in non-vaccinated contacts exposed to persons vaccinated against influenza vs those not vaccinated. Influenza included both laboratory-confirmed influenza (defined by one or more of the following: nucleic acid amplification testing, viral culture, antigen detection, pre-/post-season or acute/convalescent serology) or non-laboratory-defined evidence. Non-laboratory-defined evidence required the presence of influenza-like illness (ILI, as per the study definition) within a period of time when laboratory-confirmed influenza was circulating in the

study area. Secondary outcomes included hospitalisation, pneumonia and death.

Search strategy, study selection and data extraction

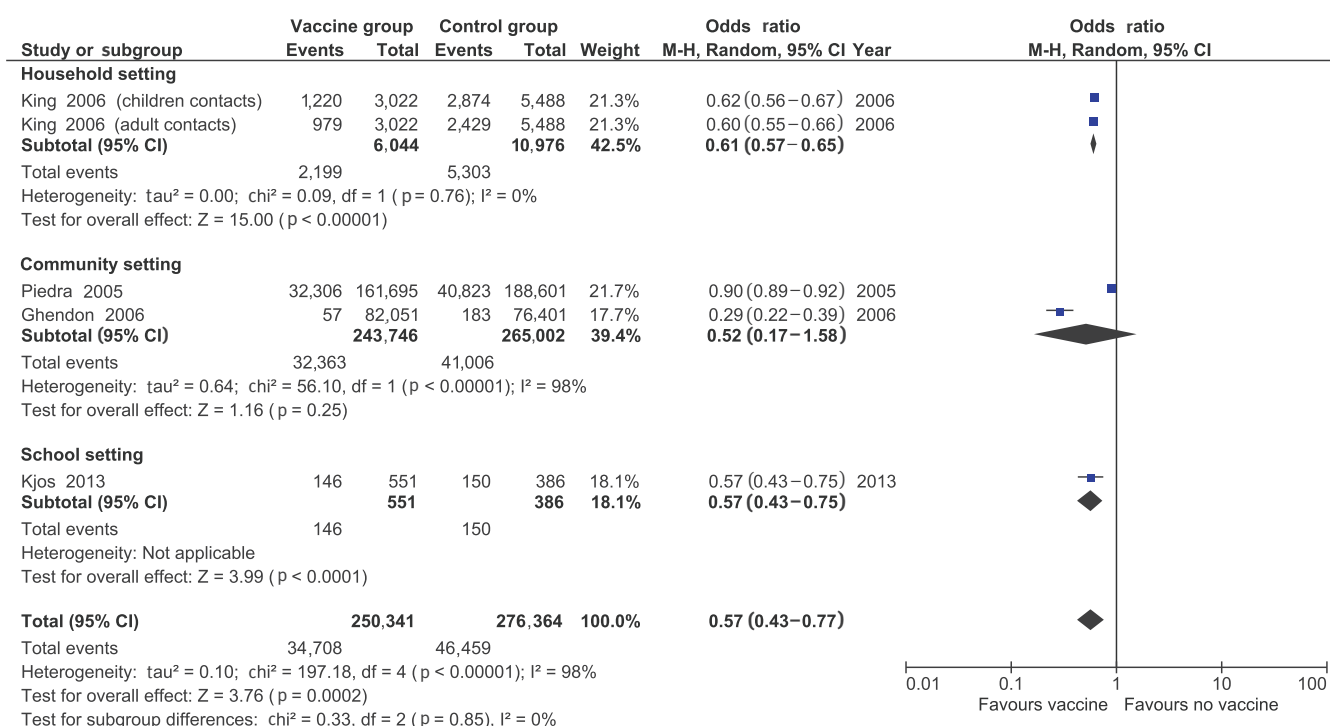
We searched MEDLINE (since 1950), EMBASE (since 1980), the Cumulative Index to Nursing and Allied Health Literature (CINAHL) (since 1982), Global Health (since 1973) and the Cochrane Central Register of Controlled Trials (CENTRAL) up to 7 March 2014. We also searched reference lists of identified articles and those of review articles for eligible studies.

Multiple teams of two reviewers independently screened titles and abstracts and, for studies identified by at least one reviewer to be of potential interest, full-text articles were screened. Data from eligible studies were extracted independently by two reviewers using a database. Any disagreement between the reviewers was resolved by consensus or arbitration by a third reviewer. We attempted to contact the first and corresponding author of the original article whenever potentially important information was missing.

Assessment of the risk of bias and of the overall quality of evidence was also conducted by two reviewers independently. We used the Cochrane Review Collaboration's tool [17] to assess the risk of bias for RCTs, and the Newcastle-Ottawa scale (NOS) [18] to assess the quality of observational studies. The overall quality of evidence was assessed using the grading of recommendations assessment, development and

FIGURE 3

Meta-analysis of four included observational studies reporting on influenza infections in contacts of influenza vaccinated vs unvaccinated patients in non-healthcare settings



CI: confidence interval; df: degrees of freedom; M-H: Mantel-Haenszel.

evaluation (GRADE) criteria [19]. Given the small number of studies, no formal assessment of the risk of publication bias could be conducted [20].

Data analysis

We performed meta-analyses of RCTs and observational studies separately. We calculated odds ratios (ORs) and corresponding 95% confidence intervals (CIs) as summary estimates using random-effects modelling (using RevMan 5.3 [21]).

We planned a priori to conduct two subgroup analyses. First, we examined herd effect by study setting, comparing the effect in household studies, school-based studies (where the impact on non-vaccinated school-children was measured) and community studies. For community studies, those comparing geographically defined areas with different vaccination strategies were considered. We hypothesised that the closer the contact was to vaccinated persons, the stronger the effect would be. Second, we assessed whether the herd effect of the vaccination in young children (up to 5 years of age) was different from that in older children and teenagers (5–18 years), and in adults.

Heterogeneity was evaluated using χ^2 and I^2 statistics [22]. We considered a χ^2 of <0.10 or an I^2 statistic

of >50% to reflect significant heterogeneity. If significant heterogeneity was found, we planned to perform additional subgroup analyses. Our a priori hypotheses to explain heterogeneity beyond the planned subgroup analyses were: laboratory-confirmed vs non-laboratory-confirmed influenza cases, and cases confirmed by nucleic acid amplification testing and viral culture vs cases confirmed by other laboratory methods. We also analysed the predominant circulating type/sub-type (influenza A(H3N2) or A(H1N1), and influenza B).

Results

After removing 18,157 duplicates, we screened a total of 43,082 titles and abstracts, reviewed 184 full-text articles and included nine RCTs and four observational studies in our systematic review (Figure 1). Of the 13 RCTs and observational studies, seven were conducted in North America, and two each in Italy and Russia, and one in Malaysia and Hong Kong Special Administrative Region, respectively (Table 1).

Findings from randomised controlled trials

Of the nine RCTs included, seven were conducted in a household setting, one in a school and one in a community setting (Table 1). The intervention group consisted of children in all but one study. The total sample

TABLE 1

Study characteristics of studies included in a systematic review of herd effect arising from influenza vaccination in non-healthcare settings

First author [source]	Study location	Study period	Predominant influenza virus type or subtype	Intervention group	Setting	Number of vaccinees	Number of contacts	Laboratory confirmation of influenza
Randomised control trials								
Gruber [29]	United States	1985/86	B	Children aged 3–18 years	Household	133	123	Yes
Clover [33]	United States	1986/87	A(H1N1)	Children aged 3–19 years	Household	194	177	Yes
Rudenko ^b [23]	Russia	1989–91	A(H3N2)	Children aged 7–14 years	School	11,071	Not available	No
Hurwitz [13]	United States	1996/97	Influenza B	Children aged 2–5 years	Household	127	228	No
Esposito [34]	Italy	2000/01	H1N1	Children aged 0.5–9 years	Household	127	349	No
Principi ^b [24]	Italy	2001/02	Influenza B	Children aged 0.5–5 years	Household	303	1,098	No
Hui [31]	Malaysia	2005	Not reported	Adults aged 18–64 years	Household	346	362	No
Cowling [30]	Hong Kong SAR	2008/09	A(H3N2)	Children aged 6–15 years	Household	119	312	Yes
Loeb [12]	Canada	2009	A(H3N2)	Children aged 1.5–15 years	Community	947	2,326	Yes
Observational studies (all cohort studies)								
Piedra [26]	United States	1998–2001	A(H3N2)	Children aged 1.5–18 years	Community	ca 40,000	350,296	No
Ghendon [25]	Russia	2001–03	A(H3N2)	Children aged 3–17 years	Community	87,221	158,451	No
King [14]	United States	2004/05	A(H3N2)	Children aged 5–14 years	Household	2,717	3,022 ^c	No
Kjos [27]	United States	2010/11	A(H3N2)	Children, age unavailable	Elementary school (5–10 year-olds)	1,012	937	No

SAR: Special Administrative Region.

^a The definition of contacts was broad and included anyone in the same community, school or household.

^b The randomised control trial did not report all numerator and denominator data and therefore could not be included in the meta-analysis.

^c In this study, the number of contacts was not reported. The number shown is the number of households (3,022) included in the analysis in intervention schools; there were 5,488 households in control schools).

size of contacts was 4,975, with one study –the largest– not reporting the total number of contacts [23].

A total of six RCTs provided data for the primary analysis comparing influenza-like illness in contacts of vaccinated vs unvaccinated persons (Figure 2). Overall, no statistically significant herd effect was found (OR: 0.62; 95% CI: 0.34–1.12), with significant statistical heterogeneity ($I^2=78\%$). Only one study, by Loeb et al., assessed contacts for influenza virus infection at community level: vaccination of children reduced the influenza infection rate for the community (OR: 0.39; 95% CI: 0.26–0.57) [12]. In contrast, there was no statistically significant effect in the subgroup of RCTs assessing household contacts (OR: 0.71; 95% CI: 0.34–1.50). No other differences between subgroups were found ($p = 0.15$ for subgroup differences). There was an 86% reduction in the odds of 5–17 year-old contacts of vaccinated individuals becoming infected as compared with contacts of unvaccinated individuals (OR: 0.14; 95% CI: 0.03–0.70), while no statistically significant differences were found when contacts were less

than five years-old or adults. This difference across age groups was not statistically significant ($p=0.26$).

Given the significant amount of statistical heterogeneity in the primary analyses, we conducted additional subgroup analyses. Subgrouping by whether or not influenza was laboratory confirmed did not significantly reduce statistical heterogeneity (p for subgroup differences was 0.06; $I^2=70.8\%$), with a significant effect on influenza infections in contacts in RCTs with no laboratory confirmation (OR: 0.33; 95% CI: 0.17–0.64; $I^2=43\%$; $n=2$) and no effect in RCTs using laboratory confirmation (OR: 0.87; 95% CI: 0.40–1.89; $I^2=81\%$; $n=4$). Subgrouping by type of laboratory confirmation or by influenza virus type/subtype could not further explain the statistical heterogeneity.

Two RCTs provided data on hospitalisation of contacts, with no statistically significant difference seen (OR 0.83; 95% CI: 0.17–4.1). Only the RCT by Loeb et al. [12] reported on mortality and pneumonia in contacts, with no effect of the vaccine on either of these outcomes in

TABLE 2

Risk of bias in nine included randomised controlled trials reporting on influenza infections in contacts of influenza vaccinated vs unvaccinated individuals in non-healthcare settings

First author [source]	Risk of bias						
	Sequence generation	Allocation concealment	Blinding of patients	Blinding of healthcare provider	Blinding of outcome adjudicators	Incomplete data addressed	Selective reporting
Gruber [29]	NK	NK	Low	Low	Low	Low	Low
Clover [33]	NK	NK	Low	NK	Low	Low	Low
Rudenko [23]	NK	NK	Low	NK	Low	Low	Low
Hurwitz [13]	NK	NK	Low	NK	NK	NK	Low
Esposito [34]	Low	NK	Low	Low	Low	Low	Low
Principi [24]	NK	NK	High	High	NK	Low	Low
Hui [31]	NK	NK	High	High	Low	Low	Low
Cowling [30]	Low	NK	Low	Low	Low	Low	Low
Loeb [12]	Low	Low	Low	Low	Low	Low	Low
Percentage low risk of bias ^a	33	11	22	33	78	89	100

NK: not known, as either unclear or not reported.

^a The percentage low risk of bias for each domain was calculated by dividing the number of randomised controlled trials (RCTs) at low risk of bias by the total number of RCTs (n=9).

community contacts. Because of the limited number of studies reporting these outcomes, no subgroup analyses could be performed.

Two other RCTs demonstrated a herd effect of influenza vaccination, but the data provided in the publications did not report the numerators and denominators needed for our meta-analysis, and we were unable to obtain further data or information from the authors. Principi et al. concluded that influenza vaccination significantly reduced the direct and indirect influenza-related costs in healthy children and their unvaccinated family members [24]. Rudenko et al. found that the use of a live attenuated influenza vaccine was associated with a lower rate of influenza-like illness in school staff and non-vaccinated children when comparing schools that had vs schools that did not have an institutional influenza vaccination programme [23].

Findings from observational studies

A total of four observational studies were identified (Table 1). The intervention groups consisted of children in all the studies. Two studies were conducted in a community setting, and one each in the household and school setting. The total sample size of contacts was more than 500,000. The level of analysis was the household, and not the individual person, in one of the studies [14].

Meta-analysis showed a significant reduction of influenza illness in contacts of vaccinated patients (OR 0.57; 95% CI: 0.43–0.77) (Figure 3). Heterogeneity was very high ($I^2=98\%$); however, the direction of the effect was identical in all studies, only the amount of the effect size varied across studies. No age-specific data were available. When comparing the three study

settings, no significant subgroup effect was found ($p = 0.85$ for subgroup differences). Given that all studies were lacking laboratory confirmation, and all were conducted during influenza A(H3N2)-predominant influenza seasons, no further subgroup analyses could be performed.

Only Ghendon et al. [25] reported on pneumonia, and found a significant reduction in contacts of influenza vaccinated patients (OR: 0.38; 95% CI: 0.30–0.50). Hospital admission was only reported in one study [14]; showing higher hospital admission rates in contacts of vaccinated persons (OR: 1.92; 95% CI: 1.17–3.14). There were no studies reporting on mortality endpoints.

Risk of bias and grading of evidence

The most common potential risks of bias in the included RCTs were lack of appropriate generation of the randomisation sequence, lack of allocation concealment and lack of blinding of patients and healthcare providers (Table 2). The RCTs scored a mean of 4.3 (range: 2–7) when assessed against seven domains.

The observational studies were awarded a mean of 6.25 points of a maximum of nine on the Newcastle-Ottawa scale, i.e. they were in a middle range of risk of bias (7 for Piedra et al. [26] and Ghendon et al. [25], 6 for Kjos [27] and 5 for King et al. [14]).

Applying GRADE criteria, we decreased the level of evidence for the primary outcome because of serious limitations in the quality of the studies (i.e. risk of bias in RCTs and observational design in non-RCTs) and inconsistency with significant statistical heterogeneity. Therefore, the overall level of evidence supporting a herd effect of influenza vaccines in preventing

influenza virus infection in contacts in non-healthcare settings was considered to be low.

Discussion

We found an overall low level of evidence supporting an indirect or herd effect of influenza vaccination in preventing influenza virus infection in vaccinated persons' contacts. In all but one study we identified, children were vaccinated. While observational studies showed a significant effect, the summary estimates from RCTs did not show a statistically significant effect. Few data were available on herd effect of influenza vaccination preventing hospital admission, pneumonia and death.

Point estimates of four of the six RCTs that reported on the prevention of influenza virus infection in contacts of vaccinated persons pointed towards a potential benefit of vaccination, but no significant effect was found overall. In an RCT by Loeb et al. involving Hutterite communities [12], vaccination of children in an enclosed community significantly reduced influenza infections in contacts. The uptake of influenza vaccination in that RCT, which had a low risk of bias in all domains assessed, was ca 83%. The RCT confirmed the findings from an observational study by Monto et al. that found a similar effect at the population level by vaccinating schoolchildren in one community in Michigan, United States [28]. However, no strong evidence was found in a household setting [29,30]. A possible explanation is that vaccinating only one child per household, as done in the study by Cowling et al., may have been insufficient to have a measurable effect [30]. In the study by Gruber et al., in contrast, all children three years of age and older received the vaccine, but again there was no effect on household contacts. However, the study was limited by the low attack rate and was therefore likely underpowered [29]. Furthermore, the authors argued that the non-vaccinated contacts were likely to be immune to the predominant influenza B strain that circulated in previous years. It is therefore unclear what key factors are needed to achieve a herd effect in the household, particularly given the importance of the broader community as a potential source of infection of the non-vaccinated. Notably, the only study that investigated herd effect of influenza vaccination of adults did find a statistically significant effect [31]. However, this study had significant methodological limitations, including lack of blinding. It should be acknowledged that two studies that both reported a significant herd effect of influenza vaccination could not be included in the meta-analysis because of the lack of detail reported in the published article, and no additional information could be obtained from the authors [23,24].

In contrast to our findings from RCTs, we found evidence of herd effect following influenza vaccination in observational studies, which was corroborated by a recent observational study by Pannaraj et al., who found that unvaccinated children may be protected in schools with vaccination rates approaching 50% [32].

Our extensive screening of over 40,000 studies found very few studies that were designed to measure herd effects of influenza vaccination. One reason for this may be the cost of community influenza surveillance as well as the cost of clinical trials. While modelling studies demonstrate that herd immunity can be achieved by vaccinating young children [10], we are surprised by how few studies with laboratory-confirmed influenza as an outcome support the modelling literature. Moreover, there are very limited data available to estimate herd effect of influenza vaccination programmes. As indirect benefits would increase the cost-effectiveness of these programmes, such data would be highly valuable for vaccine advisory bodies and decision makers evaluating whether to initiate or expand influenza vaccine programmes.

Our review highlights the need for more rigorous studies using laboratory-confirmed influenza virus infections as an outcome. Data on a herd effect on outcomes other than influenza virus infection were sparse, due either to outcomes not being measured or to inadequate power to detect a difference. Although the effect of influenza vaccination on mortality has been demonstrated through modelling [10], high-quality studies would better support the ability of influenza vaccination to prevent hospital admissions, pneumonia or death in contacts through herd effect.

Strengths of this systematic review include a systematic, protocol-driven and comprehensive review with extensive literature search strategy including RCTs and observational studies. In addition, rigorous assessment of eligibility ensured high reliability of the results. All subgroup analyses were defined a priori. A rigorous use of the GRADE approach ensured a transparent and comprehensive approach to evaluate overall quality of the studies. An important limitation, however, was the presence of statistically significant heterogeneity that could not be explained by a priori defined subgroup analyses. We assume that differences in study designs and clinical heterogeneity in terms of study population, outcome assessment and health service resources may have resulted in differences in outcomes that could not be explained by the intervention per se. Furthermore, differences in vaccine effectiveness in case of mismatch and existing immunity if the circulating strain had been dominant for several seasons may have introduced heterogeneity across the included studies. Another major limitation was the potential risk of bias in the majority of studies, which further decreased the level of evidence. Finally, all but one study vaccinated children, thus, no generalisation to vaccination programmes in adults can be made, and the evidence is too limited to conclude in what setting(s) a significant herd effect may or may not be achieved.

In summary, herd effects are assumed with influenza vaccine programmes, but there are few studies that quantify the herd effect of vaccination. We found low-level evidence supporting a herd effect of vaccination

on influenza virus infection in contacts of vaccinated persons. Further rigorous studies are needed in order to better understand under which circumstances vaccination may prevent influenza and its complications in contacts.

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Conflict of interest

SPK received travel grants from Pfizer and Novartis. DT received grant funding from GSK Canada. SAF, PL, JS, SAA, MS, JN, JJ, JRO, DM, ML: none declared.

Authors' contributions

Conception and design (DM, JRO, ML), data acquisition (SAF, PL, DT, JS, SAA, MS, SPK, JN, JJ), interpretation of data (DM, JRO, ML), drafting the manuscript (DM, ML), revising manuscript for important intellectual content (SAF, PL, DT, JS, SAA, MS, SPK, JN, JJ, JRO). All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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Prolonged colonisation with *Escherichia coli* O25:ST131 versus other extended-spectrum beta-lactamase-producing *E. coli* in a long-term care facility with high endemic level of rectal colonisation, the Netherlands, 2013 to 2014

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The extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* clone ST131 (ESBL-ST131) has spread in healthcare settings worldwide. The reasons for its successful spread are unknown, but might include more effective transmission and/or longer persistence. We evaluated the colonisation dynamics of ESBL-producing *E. coli* (ESBL-EC), including ESBL-ST131, in a long-term care facility (LTCF) with an unusually high prevalence of rectal ESBL-EC colonisation. During a 14-month period, rectal or faecal samples were obtained from 296 residents during six repetitive prevalence surveys, using ESBL-selective culture. Transmission rates, reproduction numbers, and durations of colonisation were compared for ESBL-ST131 vs other ESBL-EC. Furthermore, the likely time required for ESBL-ST131 to disappear from the LTCF was estimated. Over time, the endemic level of ESBL-ST131 remained elevated whereas other ESBL-EC returned to low-level prevalence, despite comparable transmission rates. Survival analysis showed a half-life of 13 months for ESBL-ST131 carriage, vs two to three months for other ESBL-EC ($p < 0.001$). Per-admission reproduction numbers were 0.66 for ESBL-ST131 vs 0.56 for other ESBL-EC, predicting a mean time of three to four years for ESBL-ST131 to disappear from the LTCF under current conditions. Transmission rates were comparable for ESBL-ST131 vs other ESBL-EC. Prolonged rectal carriage explained the persistence of ESBL-ST131 in the LTCF.

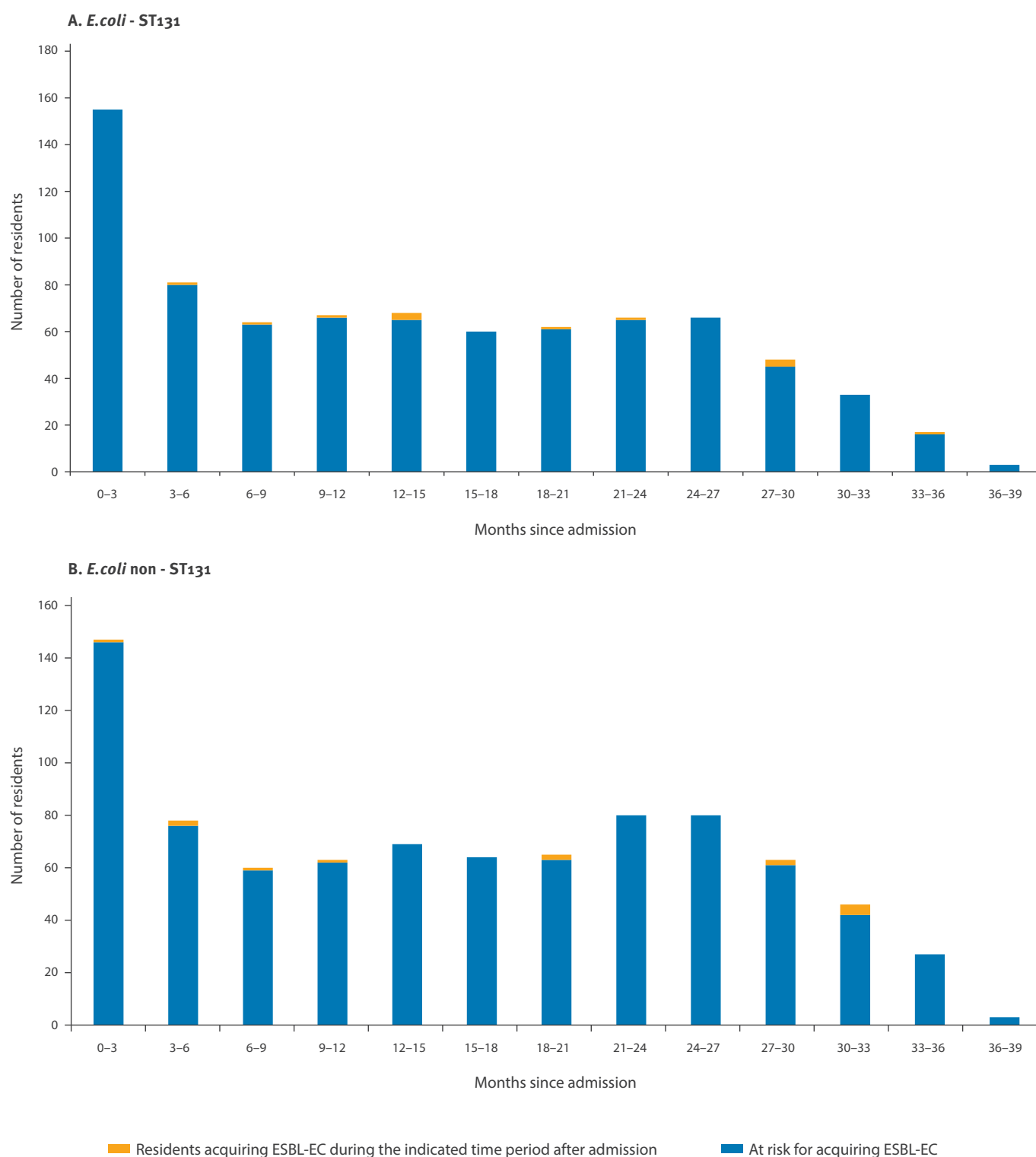
Introduction

The prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* is increasing rapidly worldwide [1,2]. Infections with these and other resistant bacteria are associated with higher morbidity, mortality, and healthcare costs [3,4]. *Enterobacteriaceae* colonising the gut are the most important reservoirs for infection and transmission of ESBL-producing *Enterobacteriaceae* [5,6].

The first reports of outbreaks with ESBL-producing *Enterobacteriaceae* came from hospitals. However, more and more outbreaks are reported in long-term care facilities (LTCFs) [7,8]. Residents of LTCFs are mainly frail, elderly people, with underlying diseases who often have medical devices and need regular medical care. Among these residents, a low functional status and higher medical and nursing dependence are associated with a greater risk of ESBL carriage [9]. For their residents, LTCFs emphasise the quality of life, including participation in social activities, over healthcare. Therefore, the amount of interaction between LTCF residents is higher than between hospitalised patients. This may be an important factor for transmission since the risk of transmission of ESBL-producing *Enterobacteriaceae* is greater among household contacts than among hospital inpatients [10]. Furthermore, our own experience shows that diagnostic sampling frequency in LTCFs is low and infection control measures are not as strict as in hospitals. We assume

FIGURE 1

Acquisition of extended-spectrum beta-lactamase-producing *Escherichia coli* colonisation at various lengths of stay, long-term care facility, the Netherlands, March 2013 to April 2014

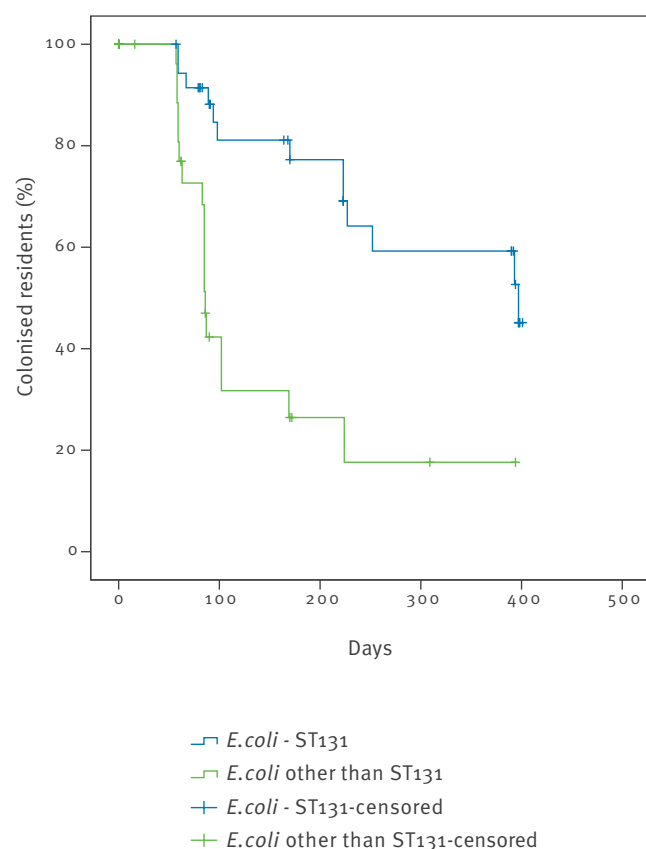


ESBL-ST₁₃₁: ESBL-producing *E. coli* isolates belonging to sequence type ST₁₃₁; Other ESBL-EC: ESBL-producing *E. coli* isolates not belonging to ST₁₃₁.

The histogram shows the number of residents during the study period with a length of stay equal to or greater than the indicated number of months. Residents already admitted at the start of our survey 'entered' the histogram at the time of their first negative culture.

FIGURE 2

Survival curve for remaining colonised with extended-spectrum beta-lactamase-producing *Escherichia coli*, long-term care facility, the Netherlands, March 2013 to April 2014



ESBL: extended-spectrum beta-lactamase.

Day 0 is the day of a resident's first rectal culture with ESBL-producing *Escherichia coli*. Follow-up ends on the day of the first negative rectal culture (drop of the line) or at the day the resident dies or leaves the facility (+ sign on the curve).

that, under these conditions, transmission of ESBL-producing organisms between residents could often be overlooked.

From June to July 2012, a routine prevalence survey involving nine LTCFs in the southern Netherlands identified one facility with an unusually high prevalence of rectal ESBL carriage of 21%, compared with 0–10% for six of the other LTCFs [11] and ca 5% for hospitalised patients [12]. The two remaining LTCFs of the 2012 prevalence survey housed a small number of residents and had a prevalence of ESBL carriage of 14 and 18%. In the high ESBL-prevalence LTCF, strain typing showed the presence of one large cluster of ESBL-producing *Escherichia coli* from sequence type O25:ST131 (i.e. ESBL-ST131), along with other smaller clusters and unique strains. In accordance with Dutch guidelines [13], this prompted outbreak containment measures, including frequent prevalence surveys.

The ESBL-ST131 clonal lineage is a major driver of the current worldwide spread of ESBLs [14–16]. It is associated with presence of multiple virulence factors [17] and with community-acquired infections. Older age and LTCF residence have been implicated as independent risk factors for ESBL-ST131 colonisation and infection [18]. ST131 was the most prevalent clone in a recent study of antimicrobial resistance in another Dutch LTCF [19].

Here we evaluated, over a period of 14 months, the epidemiology of various ESBL-producing *E. coli* (ESBL-EC), including ESBL-ST131, in a LTCF with a high endemic level of ESBL carriage. Specifically, we assessed whether ESBL-ST131 strains were more transmissible or more persistent colonisers than other ESBL-EC. Both factors are theoretical explanations for the successful worldwide spread of ESBL-ST131.

Methods

Study period and setting

We evaluated the dynamics of colonisation with ESBL-producing *Enterobacteriaceae* among residents of a LTCF in the Netherlands over 14 months, from March 2013 to April 2014. The LTCF comprised four semi-separate buildings (A, B, C, and D), each divided into one to three separate wards (A1–3, B1–2, C1–3, and D). Each ward housed ca 20 residents and contained two kitchens and communal areas. Sanitary facilities were shared by several residents each. Nursing staff was dedicated to specific wards. The building contained communal recreation and therapy areas where residents from all buildings and wards met regularly.

During the study period, improved infection control measures, improved emphasis on hand hygiene, and improved cleaning strategies were implemented in all wards, irrespective of the prevalence or clonal distribution of ESBL colonisation. No attempts were made to actively decolonise residents.

Specimen collection

Over the 14 month study period, six cross-sectional surveys were performed at intervals of two to three months by culturing faeces or rectal swabs from all residents. For residents admitted during the study, efforts were made to culture them similarly within one week after admission.

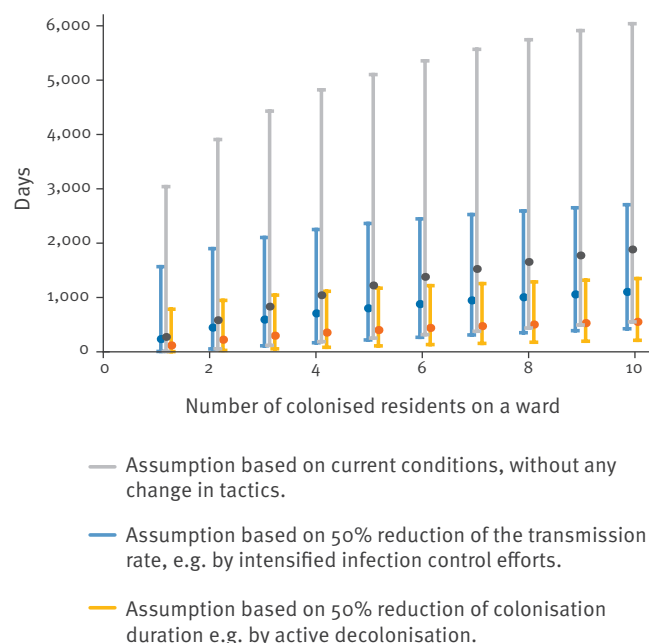
In order to assess possible routes of transmission, the following cultures were obtained concurrently with the faecal surveys in residents: environmental cultures (5 times), hand cultures (twice from all available staff, once from residents), and air sedimentation cultures (twice near ESBL-colonised residents and a selection of non-colonised residents).

Identification and detection of resistant strains

Faecal and rectal samples were collected using ESwab (Copan diagnostics, Brescia, Italy). For environmental

FIGURE 3

Estimated time for all extended-spectrum beta-lactamase-producing *Escherichia coli*-ST131 to disappear from a ward and effect of transmission rate and duration of colonisation, long-term care facility, the Netherlands, March 2013 to April 2014



ESBL: extended-spectrum beta-lactamase

Dots: mean time for all ESBL-ST131 to disappear from a ward.

Vertical lines: 95% confidence intervals

cultures, standardised surfaces of 10x10 cm were sampled thoroughly using ESwab medium in the first two surveys, and a sterile 10x10 cm pad soaked in sterile isotonic saline solution for the subsequent three surveys. Hands of staff members were cultured by having the workers dip and rub their hands into tryptic soy broth (TSB). Residents' hands were cultured using ESwab, with special attention paid to palms, fingers, nails, and jewellery. Air sedimentation cultures were performed by placing five selective agar plates (EbSA agar, Cepheid Benelux, Ledeberg, Belgium) in close proximity to residents while they were being washed and getting dressed.

The sterile pads for environmental cultures and all ESwabs were incubated for 16 to 18 hours at 37°C in 15 mL of TSB containing 8 mg/L vancomycin and 0.25 mg/L cefotaxime. Then, 10 µL of the broth was inoculated and incubated on an EbSA agar plate (Cepheid Benelux, Ledeberg, Belgium), selective for ESBL-producing *Enterobacteriaceae*. The TSB used to rinse staff members' hands was incubated directly, as were the agar plates used for air sedimentation culture.

Identification of all oxidase-negative, Gram-negative bacteria was performed by matrix-assisted laser desorption/ionization–time (MALDI-TOF, BioMérieux, Marcy l'Etoile, France). Susceptibility testing was performed by VITEK2 (BioMérieux, Marcy l'Etoile, France) using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [20], and ESBL production was confirmed by a double disk method [21].

Typing

All phenotypically confirmed ESBL-EC underwent phylogroup-defining PCR [22]. Group B2 *E. coli* underwent O25:ST131-specific PCR [23].

ESBL-EC obtained from colonisation cultures, environmental cultures, hand cultures and air sedimentation cultures underwent ESBL genotyping using a microarray (Check-MDR CT103, CheckPoints, Wageningen, the Netherlands) [24,25] and strain typing by using amplified fragment length polymorphism (AFLP) [26]. Clusters were defined based on both visual and computerised interpretation of AFLP patterns.

Of residents with repetitive positive colonisation cultures with similar ESBL-EC, only the first isolate was genotyped. Similarity was defined as identical species, identical phylogroup and O25:ST131 status, and absence of major differences in susceptibility (i.e. susceptible vs resistant) for the 25 antibiotics tested.

Statistical analysis

Acquisition was defined as detection of an ESBL-producing organism in a previously culture-negative resident. Transmission was defined as acquisition of an ESBL-EC strain identical according to AFLP profile and ESBL-variant to one already present on the ward where the individual resided before the acquisition. Routine prevalence surveys in several LTCFs [11] and a hospital (data not shown) in the same area as the LTCF studied showed little clustering of ESBL-EC and low prevalence of colonisation with O25:ST131 *E. coli*. Consequently, it is unlikely for newly admitted residents to be colonised with the same strain as present on the ward they are admitted to. Transmission was thus also assumed for residents who were admitted during the study period, stayed on a ward over 14 days before being cultured, and, who were found to be colonised with an ESBL-EC strain already present on that ward when first cultured.

We used differences in length of stay (LOS) as a marker for inter-individual differences in susceptibility to colonisation. We reasoned that if differences in susceptibility were present, residents less susceptible to colonisation should remain non-colonised for a longer LOS than other residents and consequently acquisition risk should be lower for patients with a longer LOS. For the analysis, LOS was grouped into three-month periods in which residents could be ESBL-culture-negative and at risk for acquisition, or could have acquired ESBL-EC. Differences in acquisition risk between a LOS

TABLE 1

Number of residents colonised with extended-spectrum beta-lactamase-producing *Escherichia coli* at various study points in a long-term care facility, the Netherlands, March 2013 to April 2014 (n=296)

Organism category	Residents colonised at start of the survey (number positive during entire survey ^a)	Residents with colonisation acquired during study period (number with presumed in-ward transmission)	Residents colonised when admitted during the study period	Total number colonised at any point
ESBL-ST131 ^b	24 (10)	14 (12)	3	41
Other ESBL-EC ^c	11 (1)	17 (10)	5	33
Total	35 (11)	29 ^d (22)	8	69 ^{d,e}

EC: *Escherichia coli*; ESBL: extended-spectrum beta-lactamase.

^a Only residents who remained in the facility for the entire study period could be positive during the entire survey; this in contrast to residents who were lost to follow-up.

^b ESBL-ST131: ESBL-producing *E. coli* isolates belonging to sequence type ST131.

^c Other ESBL-EC: ESBL-producing *E. coli* isolates not belonging to ST131.

^d Two residents acquired both an ESBL-ST131 and a non-ST131 ESBL-EC.

^e Three residents were positive with ESBL-ST131 at the start of the survey, and acquired a non-ST131 ESBL-EC later.

shorter vs one longer than 12 months were assessed by Chi-Square analysis.

Median duration of colonisation was calculated from the first positive culture using Kaplan-Meier survival analysis, with status 'loss of colonisation' as the primary outcome. Differences between ESBL-ST131 and other ESBL-EC were tested with Log-Rank analysis. Residents acquiring colonisation in the final prevalence survey were excluded.

Transmission rates and corresponding reproduction numbers were calculated for ESBL-ST131 and other ESBL-EC separately, taking into account the ward-level infection pressure and assuming that transmission occurred only at the ward. Residents were considered to have newly acquired or lost colonisation on the day of the culture that detected their changed colonisation status. Weighted days at risk were calculated by multiplying, for each day, the number of positive (i.e. colonised) residents per ward by the number of non-colonised residents on the same ward. Weighted days at risk were summed over all wards, separately for all combinations of AFLPplus ESBL-variant. Per-day transmission rates were calculated by dividing the number of presumed transmissions by weighted days at risk. A per-admission reproduction number was calculated by multiplying the number of residents on a ward (n=20) by the per-day transmission rates of ESBL-ST131 and other ESBL-EC and the corresponding mean durations of colonisation obtained from the Kaplan-Meier survival analysis [27].

The time for ESBL-ST131 to disappear from the LTCF was estimated by using a mathematical model that incorporated the per-day transmission rate and a constant decolonisation rate equal to the mean duration of colonisation obtained from the Kaplan-Meier survival analysis. The model randomly simulated one million scenarios. This was repeated for situations with one

to 10 colonised residents per ward. Additionally, the effects of alterations in the transmission rates and mean duration of colonisation on time for all ESBL-ST131 to disappear from the LTCF were calculated.

Ethical considerations

Data for this study were obtained as part of outbreak containment. Frequent prevalence surveys are part of the measurements recommended by the Dutch guidelines [13]. No informed consent was obtained, but residents were informed about the surveys and had the option to refuse sampling.

Results

Colonisation cultures

During the study period, the LTCF housed a total of 296 residents, 126 male and 170 female, with an average of 173 residents at the time of the prevalence surveys. During the study period, 125 residents were newly admitted and 120 residents were lost to follow-up due to transfer to other facilities, transfer to home, or death. The average age at time of the prevalence surveys was 78 years (range: 46–98 years, SD: 11 years). The participation rate was 93.7% (964/1,029) for intended culturing at the prevalence surveys and 66.9% (83/125) at admittance. Only four residents declined to participate at all culture points.

In total, 1,050 rectal or faecal samples were obtained. Of these, 188 (17.9%) yielded one or more ESBL-EC, including 131 (12.5%) with ESBL-ST131 and 57 (5.4%) with other ESBL-EC. The 131 ESBL-EC-positive rectal samples were obtained from 69 different residents (23.3% of 296). Table 1 shows the number of residents who were colonised at the start of the survey, acquired colonisation during the study, or were already colonised when admitted during the study period.

TABLE 2

Prevalence of surface contamination with extended-spectrum beta-lactamase-producing *Escherichia coli*, long-term care facility, the Netherlands, March 2013 to April 2014

Surface	Total number of cultures ^a	Number of cultures positive ^a (row %)		
		Total	ESBL-ST131	Other ESBL-EC
Toilet or bedside commode	103	11 (10.7)	3 (2.9)	8 (7.8)
Sink	54	2 (3.7)	1 (1.9)	1 (1.9)
Kitchen area	48	3 (6.3)	0	3 (6.3)
Common living area	58	1 (1.7)	0	1 (1.7)
Total	285	17 (6.0)	4 (1.4)	13 (4.6)
Ward-related ^b	285	9 (3.2)	4 (1.4)	5 (1.8)

ESBL-ST131: ESBL-producing *E. coli* isolates belonging to sequence type ST131; Other ESBL-EC: ESBL-producing *E. coli* isolates not belonging to ST131.

^a Only the results from the sterile gauze method are shown.

^b Number of isolates that by genomic profiling were similar to isolates obtained from residents' colonisation cultures during the same time-period.

All ESBL-ST131 isolates (100%; 131 isolates from 69 residents) were resistant to ciprofloxacin vs 25 of 57 (44%; $p < 0.001$) other ESBL-EC isolates obtained from 15 of 32 (47%; $p < 0.001$) residents colonised with other ESBL-EC. In contrast, only 19 of 131 (15%) ESBL-ST131 isolates, obtained from nine of 69 residents (13%), were resistant to co-trimoxazole vs 43 of 57 (75%; $p < 0.001$) other ESBL-EC isolates, obtained from 26 of 32 (81%; $p < 0.001$) colonised residents. No resistance to colistin, meropenem or imipenem was observed in any of the isolates.

The prevalence of ESBL-EC colonisation was unevenly distributed across the LTCF. At study onset, wards B-1, B-2, and C-2 had large clusters with ESBL-ST131 (29 carriers, all with isolates from the same AFLP cluster; ward prevalence 39–45%). Wards A-2, A-3, and C-3 had smaller clusters of other ESBL-EC plus sporadic carriage of non-related isolates (16 carriers; ward prevalence 11–23%). The remaining three wards had only sporadic cases of ESBL-EC colonisation (2 carriers; ward prevalence <5%). During the follow-up period, the endemic level of ESBL-ST131 remained high, and on ward A-3 new colonisation and transmission of ESBL-ST131 appeared. However, in the same period the prevalence of other ESBL-EC decreased with some sporadic cases remaining. On wards C-1 and D, the prevalence of ESBL colonisation remained at zero.

Environmental surveys

Of 485 standardised environmental cultures, 17 (3.5%) yielded ESBL-EC, including 17 (6.0%) of 285 done in the last three of five surveys using the sterile gauze method, vs none (0%) done in the first two surveys using the Eswab method ($p < 0.001$). Isolates from only nine of the 17 positive cultures matched isolates obtained from residents on the same ward during the same survey. Three identical environmental ESBL-EC isolates were obtained from ward D, but in that time period, no prevalence survey was performed on this

ward. Toilets were the sites most likely to yield any ESBL-EC (11 of 17 positive sites), and overall ESBL-ST131 was less often cultured than were other ESBL-EC (4 vs 13 of 285 cultures; Table 2).

Hand and air sedimentation cultures

Hand cultures from four (2.7%) of 148 cultured staff members yielded ESBL-producing *Enterobacteriaceae*. All four individuals worked on ward C-2, a ward with a high endemic level of ESBL-ST131. However, only one of them carried ESBL-ST131 on the hands; the other three all carried *bla*CTX-M9-producing *Enterobacter cloacae*, another strain present in a colonised resident on ward C-2.

Of 176 residents, 168 (95.5%) underwent hand culturing. At the time of hand culture, 30 (17.9%) of these residents were colonised with ESBL-EC, and three (1.8%) had unknown colonisation status. Hand cultures of only two residents yielded an ESBL-producing organism, in each instance, non-*E. coli*. For only one of these residents the cultured strain, a *bla*CTX-M9-producing *Enterobacter cloacae*, corresponded with a strain found in rectal colonisation cultures obtained from two other ward residents.

Air sedimentation cultures were obtained near 52 residents, including all 26 ESBL carriers plus 26 non-colonised residents. In the vicinity of three of these residents, air sedimentation cultures were positive for the ESBL-ST131 strain they were colonised with. Repeated air sedimentation cultures for these three residents, and for 12 other ESBL-carriers, were negative.

Length of stay as marker for inter-individual differences in acquiring extended-spectrum beta-lactamase-producing *Escherichia coli* colonisation

The risk of acquiring ESBL-EC did not vary in relation to LOS; prolonged LOS did not select for residents

less susceptible to acquiring ESBL-colonisation (Figure 1). For both ESBL-ST₁₃₁ and other ESBL-EC, acquisition risk did not differ between residents with a LOS shorter vs longer than 12 months ($p=0.13$ and $p=0.84$, respectively).

Duration of colonisation

During the study, conversion to ESBL-negative was observed for 13 of 39 ESBL-ST₁₃₁ carriers, vs 18 of 29 carriers of other ESBL-EC ($p=0.03$). Survival analysis showed that the half-life of carriage for ESBL-ST₁₃₁ was 13 months, compared with two to three months for other strains ($p<0.001$; Figure 2).

Transmission rates

During the study, we documented 12 transmissions involving ESBL-ST₁₃₁ and 10 involving other ESBL-EC. The ratio of per-day transmission rates for ESBL-ST₁₃₁ vs other ESBL-EC was 0.59 (95% CI: 0.26–1.32), indicating that other ESBL-EC spread as fast as or more readily compared with ESBL-ST₁₃₁. The corresponding reproduction numbers were 0.66 (95% CI: 0.25–1.09) for ESBL-ST₁₃₁ and 0.56 (95% CI: 0.20–1.01) for other ESBL-EC.

Estimated duration for extended-spectrum beta-lactamase-producing *Escherichia coli*-ST₁₃₁ to disappear from the long-term care facility

Figure 3 shows the estimated time for ESBL-ST₁₃₁ to disappear from a ward, based on the number of colonised residents, the mean duration of colonisation, and the estimated reproduction numbers. In the situation observed during the study, with a maximum of six colonised residents per ward, the mean expected time for all ESBL-ST₁₃₁ to disappear from the LTCF would be more than 1,000 days, or three to four years. Halving the duration of colonisation, e.g. by active decolonisation, would reduce the average expected time to ca 400 days (1 year), whereas halving the transmission rate, e.g. by improved hygiene, would reduce it only to 800 days (2 to 3 years).

Discussion

We performed a prospective cohort study of ESBL colonisation, comparing ESBL-ST₁₃₁ with other ESBL-EC, in a LTCF. In the studied LTCF a high endemic level of ESBL-ST₁₃₁ colonisation persisted in spite of measures taken, while colonisation with other ESBL-EC returned to a more normal level over time. We documented prolonged colonisation of individual residents with ESBL-ST₁₃₁, with a half-life of ca 13 months compared to two to three months for other ESBL-EC ($p<0.001$). This appeared to sustain the high endemic level of ESBL-ST₁₃₁ colonisation.

As alternative explanations for the persistence of ESBL-ST₁₃₁, we examined the environment, hands of staff members, and direct resident-to-resident contact as possible transmission routes. Strikingly, we found that ESBL-ST₁₃₁ were nearly absent from the

corresponding cultures, whereas other ESBL-EC were more often detected; thus environmental contamination with ESBL-ST₁₃₁ did not explain the sustained ESBL-ST₁₃₁ colonisation. Furthermore, transmission rates did not differ between ESBL-ST₁₃₁ and other ESBL-EC, which excluded another possible explanation for the findings. Prolonged gut colonisation appeared to be the sole explanation for the sustained high prevalence of ESBL-ST₁₃₁.

The reasons for prolonged ESBL-ST₁₃₁ colonisation are unclear but can be speculated upon. ESBL-ST₁₃₁ may have an intrinsically better ability to persist in the gut than other ESBL-EC, and this may be even more pronounced in elderly or functionally dependent individuals who constitute most of the population in LTCFs. Further research should be performed to elucidate the mechanisms underlying prolonged colonisation by ESBL-ST₁₃₁.

Since the study data were collected in the context of infection prevention-related interventions, data on patient-specific factors such as functional status or indwelling catheters could not be obtained. We used difference in acquisition risk for different LOS as a surrogate marker for differences in acquisition risk between residents. However, longer LOS did not select for residents less susceptible to acquisition of ESBL-EC, indicating that differences in susceptibility between patients with ESBL-ST₁₃₁ vs other ESBL-EC were unlikely.

The per-admission reproduction numbers for ESBL-ST₁₃₁ and other ESBL-EC were comparable at 0.66 (95% CI: 0.25–1.09) and 0.56 (95% CI: 0.20–1.01), respectively. Both were less than one, indicating that, with the infection control measures in place, the prevalence of ESBL carriage should eventually return to baseline. In the situation where residents have a long average LOS and an endemic strain (ESBL-ST₁₃₁) is present that causes persistent colonisation, and infection control measures are in place, the estimated duration for ESBL-ST₁₃₁ to disappear from the LTCF is three to four years. This indicates that prolonged periods of increased prevalence do not necessarily mean that infection control measures are ineffective.

Statistical modelling predicted that the time required for ESBL-ST₁₃₁ to disappear from the LTCF would be affected only minimally by improved infection control measures. In contrast, a shortened duration of colonisation e.g. by decolonisation would, have a larger impact. Unfortunately, reliably effective decolonisation strategies for ESBL carriage are unavailable. Probiotics [28] and donor faeces infusion [29] have been used in experimental settings, and selective bowel decontamination (SDD) regimes have been proposed [30,31]. However, some studies observed only a temporary suppression of ESBL carriage during SDD treatment, with a rapid rebound one week after the end of treatment [32].

Few reports of prolonged colonisation with ESBL-EC have been published. Alsterlund et al. reported five residents who remained colonised for 41 to 59 months after an infection caused by ESBL-EC [33]. Other authors reported colonisation durations of 1.4 months [34], more than three months [35], and of 179 days (i.e. ca 6 months) [36]. Prolonged carriage of ESBL-producing bacteria after travel has also been documented [37]. To our knowledge, only one study compared duration of colonisation for different types of ESBL-producing *Enterobacteriaceae*. Titelman et al. found that faecal carriage of ESBL-EC persisted in 26 of 61 patients one year after infection, and that prolonged carriage is associated with *E. coli* phylogroup B2 [38]. In our study, the prolonged duration of colonisation was ascribed solely to ESBL-ST131 (phylogroup B2), with a 13-month colonisation half-life, vs three to four months for other phylogroup B2 *E. coli*.

Differences in transmission rates between different types of ESBL-EC have been investigated previously. Hilty et al. suggested that *E. coli* phylogroups B2 and D are more often transmitted within households than phylogroups A and B1 [10]. However, these differences were not statistically significant ($p=0.10$), and clonal typing (e.g. to identify ST131) was not done. Adler et al. found that CTX-M-27 (CTX-M-9 group)-producing ST131 *E. coli* spread more efficiently than the CTX-M-15 ST131 *E. coli* [39]. Since our cohort included only few CTX-M-9 group-positive ST131 isolates, we could not reliably compare these two ST131 subgroups.

Our analysis has several limitations. Firstly, we assume that all residents are equally contagious over time, whereas, hypothetically, superspreading events or periods of increased infectiousness may occur. Secondly, we used a conservative definition for 'transmission' that presumed that transmission occurred only between residents on the same ward and disregarded the possibility of plasmid transmission. The resulting transmission numbers, which might have been underestimates, were used to calculate reproduction numbers, which if too low could have resulted in underestimation of the average duration for ESBL-ST131 to disappear from the LTCF. Thirdly, the possibility of new introductions of ESBL-ST131, for example through food, was not taken into account, which could also have resulted in an underestimation of the average duration of ESBL-ST131 to disappear from the LTCF. On the other hand, the method used to type the isolates (AFLP) is not as specific as, for example, whole genome sequencing (WGS). Theoretically, this might have led to an overestimation of transmissibility by assigning isolates to the same clonal complex that actually represented different clonal lineages. However, when used in prevalence surveys in other healthcare facilities in the same area and time period, AFLP revealed hardly any clonal relatedness. Therefore, the clonal relatedness in this specific LTCF is likely to represent clonal spread.

Another limitation is the setting, i.e. a specific LTCF during a period of elevated endemic levels of ESBL colonisation that triggered intensified infection control measures. Transmission rates and duration of colonisation might be different in other situations. However, we suspect that the observed differences in colonisation duration between ESBL-ST131 and other ESBL-EC can be extrapolated reasonably to other settings. Lastly, we had no data on underlying disease or use of medical devices or antimicrobials during the study period. From a previous study we know that in this LTCF use of antimicrobial and medical devices is infrequent [11]. Therefore we think that these factors cannot explain why ESBL-ST131 has caused such a high endemic level of colonisation in the LTCF.

Our study also had notable strengths. Most important is the length of follow-up (14 months), with clearly defined intervals at which standardised cultures were taken, and the high participation rate (90.6%).

In conclusion, we found that ESBL-ST131 can colonise LTCF residents for prolonged periods, with an estimated half-life of 13 months, which contrasts with the two to three month half-life of other ESBL-EC. Furthermore, calculated transmission rates did not differ between ESBL-ST131 and other ESBL-EC, and environmental contamination was actually more abundant for other ESBL-EC than for ESBL-ST131. Therefore, duration of colonisation was the main identified factor that contributed to the success of ESBL-ST131 in this LTCF under the current infection control measures. We postulate that prolonged colonisation may also be the key to success of this clone worldwide, which merits further study. Our models predict that implementing additional infection control measures aimed at limiting the spread of ESBL-ST131 would have only a minor effect on high colonisation prevalence levels, whereas effective decolonisation strategies should have a much more profound effect. Therefore, in addition to implementing infection control measures, development of effective decolonisation strategies is warranted to contain the spread of ESBL-ST131 worldwide.

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Conflict of interest

None declared.

Authors' contributions

Designed the study: IO, AM, WC, JK. Executed the survey: YH, CV, AM, WC. Prepared and analysed data: IO, MH, MB, JV.

Interpreted the results: IO, MH, MB, JV, JJ, JK. Wrote the first draft: IO.

All authors reviewed, provided comments and approved the final manuscript.

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ECDC launches public consultation on rotavirus vaccination in infancy

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On 17 October, the European Centre for Disease Prevention and Control (ECDC) launched a public consultation on the preliminary scientific advice document 'Expert opinion on rotavirus vaccination in infancy'. The consultation is open until 28 November 2016.

The aim of the consultation is to harvest input for the ECDC expert opinion which should provide European Union/European Economic Area Member States with scientific opinion and expert opinion to support the decision-making process on the possible introduction and monitoring of routine vaccination against rotavirus-induced gastroenteritis in infants.

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