Prevalence of *Escherichia coli* Carriage in the Oropharynx of Ambulatory Children and Adults with and without Upper Respiratory Symptoms

To the Editor:

Escherichia coli is a common gut commensal and an important human pathogen that rarely causes pneumonia in the community. However, *E. coli* increasingly causes hospital-acquired pneumonia, particularly among intensive care unit (ICU) patients (1, 2). *E. coli* pneumonia, similar to other hospital-acquired and ventilator-associated pneumonias, is responsible for longer ICU and hospital stays, high morbidity and mortality, and increased antibiotic consumption and hospital costs (1, 2). This is particularly true since the recent global emergence and spread of *E. coli* strains producing extended-spectrum β-lactamases, which constitute a major public health concern (3).

E. coli identified in protected bronchoalveolar lavage specimens from ICU patients with ventilator-acquired pneumonia is often genetically identical to the strains found in their dental plaque and oropharyngeal cavity (4, 5). Yet little is known about oropharyngeal colonization by *E. coli,* with most studies dating back 30 years ago (6–9). Because no recent work has addressed *E. coli* carriage in the community, we aimed to fill this gap in the literature by estimating the *E. coli* carriage rates among otherwise healthy adults and children with and without upper respiratory symptoms. We detected *E. coli* using quantitative PCR (qPCR), which is more sensitive than culture.

Methods

Using a collection of oropharyngeal samples from participants in two community cohort studies ongoing in Southeast Michigan during the 2012–2013 and 2013–2014 influenza season, the Household Influenza Vaccination Effectiveness Study (HIVE) (10) and the eX-FLU study (11), we estimated the prevalence of *E. coli* oropharyngeal carriage among 589 subjects. HIVE participants (range, 2–70 yr old) were recruited among households with at least two children (for details, see Reference 10). eX-FLU participants were college students (aged 18–21 yr; mean, 19.2 yr) living in dormitories and enrolled using a social network referral model. Students suffering from upper respiratory symptoms defined as cough and fever/feverishness, body aches, or chills (cough plus one of fever/feverishness, chills, or body aches) were requested to provide samples at onset. Their healthy social contacts (no illness in the previous 14 d) were also sampled.

The Household Influenza Vaccine Effectiveness Study is supported by the Centers for Disease Control and Prevention (U01 IP000474) and the National Institute of Allergy and Infectious Diseases (R01 AI097150). The eX-FLU study is supported by the Centers for Disease Control and Prevention (U01 CK00018501). This work was made possible thanks to an Advancing Science through Pfizer-Investigator Research Award 2013 (B.F.).

Author Contributions: Conception and design: V.d.L. and B.F.; sample collections: R.E.M. and A.E.A.; experiments: V.d.L.; analysis and interpretation: V.d.L. and B.F.; and drafting the manuscript: V.d.L., R.E.M., A.E.A., and B.F.

We used a highly sensitive qPCR assay for *E. coli* detection, as bacterial load was anticipated to be lower than among hospitalized patients. We limited our analysis to oropharyngeal swabs collected from 152 adults and 186 children from the HIVE study at the time of acute respiratory illness (an illness <7 d duration with cough, fever or feverishness, nasal congestion, chills, headache, body aches, or sore throat) and to 251 college students from the eX-FLU study, 130 of whom (52%) had upper respiratory symptoms and 121 of whom were the healthy contacts.

Dual throat swabs were collected from participants and conserved in universal transport media and in sterilized skim milk at −80°C. After bacterial DNA extraction from the universal media sample, qPCR was performed using primers specific to the 16S ribosomal genes in *E. coli* previously designed by our laboratory (12). All assays were conducted in triplicate. A no-template control and *E. coli* MG1655 positive control were included in every run. Negative samples were those with cycle threshold (C_T) values higher than 40. *E. coli* MG1655 was used to determine the accurate limit of detection of qPCR by comparing cultures on MacConkey agar with qPCR results for sequential dilutions starting with a Macfarlane 5 dilution. In parallel, we cultured on MacConkey agar a subset of skim milk specimens both from subjects with qPCR results of more than 10^4 genomic copies/mL and from *E. coli*-negative subjects.

Results

Altogether, 21/338 (6.2%) HIVE and 20/251 (7.9%) eX-FLU participants carried *E. coli*, using a detection limit of 10^3 genomic copies/mL ($P = 0.4$) (Table 1). Mean bacterial load for positive samples was 4.3 Log genomic copies/mL (SD, 0.32), with a maximum of 5.7 Log genomic copies/mL. Using sequential dilutions of the MG1655 strain, we estimated the limit of detection by culture to be approximately 10^5 genomic copies/mL of skim milk, which is two Logs higher than the qPCR assay. None of the skim milk samples grew *E. coli* on MacConkey agar. No difference was found either in carriage rates or relative abundance of *E. coli* among positive samples between adults and children, by age group in the HIVE cohort, or between subjects with and without acute upper respiratory symptoms in the eX-FLU study.

Discussion

In two different large cohorts of ambulatory children and adults with and without acute respiratory symptoms, we found that carriage of *E. coli* in the throat was a rare event, despite use of a highly sensitive detection method. *E. coli* carriage did not differ by age, age group, or the existence of upper respiratory symptoms. Relative bacterial loads were low and unaffected either by age or upper respiratory symptoms.

Our findings are similar to those of previous studies of subjects from the community (6, 8, 9). A study conducted among 250 individuals in Texas published in 1969 found an increased prevalence in oropharyngeal carriage of gram-negative bacteria by severity of illness, from 2% in healthy subjects to 57% in

Table 1. Oropharyngeal carriage of *Escherichia coli* in subjects from the community in southeast Michigan participating in the Household Influenza Vaccine Effectiveness Study (participants in a household study with acute respiratory illness during 2013–2014 influenza season) and the eX-FLU study (college students with upper respiratory symptoms and their healthy contacts during the 2012–2013 influenza season)

Study by Selected Characteristics	Total Number of Participants (*n*)	Prevalence of *Escherichia coli* [*n* (%)]
Household Influenza Vaccination Effectiveness Study (n=338)		
Age, yr		
2–3	20	2 (10)
4–6	74	5 (6.8)
7–15	92	7 (7.6)
21–30	8	0
31–40	81	4 (4.9)
41–50	56	3 (5.4)
51–60	6	0
61–70	1	0
All ages	338	21 (6.2)
eX-FLU (n=251)*		
Upper respiratory symptoms†	130	10 (7.7)
Healthy contact	121	10 (8.3)
All	251	20 (7.9)

Escherichia coli was detected using quantitative polymerase chain reaction.
*Participants in the eX-FLU study were aged 18–21 years, with a mean age of 19.2 years.
†Defined as cough + at least fever/feverishness, body aches, or chills.

"moribund" patients (9). A 1994 study following 114 healthy infants from birth to 2 years detected, using culture, a prevalence of *E. coli* throat carriage ranging from 0% to 13% (13). The prevalence of gram-negative rods in a 1975 study among 100 healthy individuals aged 3–70 years (mean age, 43 years) who did not take antibiotics in the previous year was 17% (14).

Although our study population was weighted heavily toward children and young adults, our sample did include 63 adults aged 41–70 years. In this largest cohort of older adults assessed to date, the prevalence of oropharyngeal carriage of *E. coli* was 4.8% (3/63). This older population is more likely to have chronic illnesses and be hospitalized in an adult ICU. In addition, others have found higher rates of *E. coli* carriage in hospitalized children (including some in ICU) than in the community (6). The large age span covered by our population of subjects confirms previous findings that *E. coli* colonization is rare in ambulatory subjects.

Carriage rates of *E. coli* in the oropharynx of patients in the ICU are far greater (even as detected using culture) than those observed in the community (9, 15, 16). More severely ill ICU patients, and those with diabetes or alcoholism (7, 9), are at greater risk for colonization with gram-negative bacteria, especially if their treatment includes mechanical ventilation (15). Further, a study published in 1994 found that patients colonized with gram-negative bacteria (including *E. coli*) were more likely to develop gram-negative bacterial pneumonia during their ICU stay (15). In 2012, 132 French ICU patients were screened for *E. coli* using culture: 37 (28.0%) were colonized in their oropharynx, 13 also had lung colonization, and 12 patients had both lung colonization and clinical *E. coli* pneumonia (16). Others have shown that colonization by *E. coli* occurs in the first 4 days after hospitalization (15). Therefore, a major shift in *E. coli* carriage seems to occur upon admission to an ICU. This probably explains why ICU patients are at higher risk of developing *E. coli* pneumonia.

We hypothesize that *E. coli* oropharyngeal colonization is a multifactorial process promoted by prolonged supine position, inhalation from the stomach, modified gastric pH resulting from the common use of proton inhibitors, and multiple interactions with healthcare workers and/or potentially from some disturbed local immunity. These factors may explain why such low rates of *E. coli* are found in the oropharynx of ambulatory subjects compared with those found in ICU patients, in turn explaining the increased risk for *E. coli* pneumonia in ICU subjects, as colonization appears to precede inhalation in the lungs (15, 16). One report suggests that *E. coli* colonizing the oropharynx and subsequently responsible for ventilator-associated pneumonia originates from the subjects' own gut (16). Further studies are needed to determine the dynamics of and risk factors for *E. coli* colonization of the oropharynx and how frequently oropharyngeal and intestinal *E. coli* are identical to determine effective prevention and control strategies among ICU patients.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Acknowledgment: The authors thank the Household Influenza Vaccine Effectiveness study and eX-FLU participants and staff for allowing us to use the oropharyngeal samples. V.d.L. would like to thank the Philippe Foundation and the Assistance-Publique Hôpitaux de Paris for financial support.

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