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Trends in HIV surveillance data in the EU/EEA, 2005 to 2014: new HIV diagnoses still increasing in men who have sex with men

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Human immunodeficiency virus (HIV) transmission remains significant in Europe. Rates of acquired immunodeficiency syndrome (AIDS) have declined, but not in all countries. New HIV diagnoses have increased among native and foreign-born men who have sex with men. Median $CD4^+$ T-cell count at diagnosis has increased, but not in all groups, and late diagnosis remains common. HIV infection and AIDS can be eliminated in Europe with resolute prevention measures, early diagnosis and access to effective treatment.

Global goals to end acquired immunodeficiency syndrome (AIDS) by 2030 have been set by the Joint United Nations Programme on HIV/AIDS (UNAIDS) [1]. Despite growing evidence of effective measures to prevent human immunodeficiency virus (HIV) infections and eliminate AIDS, such as early treatment and new prevention interventions, HIV transmission continues at considerable levels in Europe [2-5]. To better understand transmission patterns and identify key populations where prevention efforts need strengthening, we analysed HIV and AIDS surveillance data for the years 2005 to 2014 [6]*. These data are reported annually by the 31 countries of the European Union and European Economic Area (EU/EEA) to a joint database for HIV/AIDS coordinated by the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) Regional Office for Europe.

HIV and AIDS diagnoses in the EU/EEA in 2014

In 2014, 29,992 people were newly diagnosed with HIV in the EU/EEA, a rate of 5.9 diagnoses per 100,000 population. The majority of cases (76.8%) were men (9.2 per 100,000 population vs 2.6 in women). This was largely driven by HIV transmission attributed to sex between men, which accounted for 12,677 (42%) of all HIV diagnoses. A total of 9,833 cases (33%) were attributed to heterosexual contact, 1,244 (4%) to injecting drug use, and for 5,908 (20%) of new diagnoses, the transmission mode was not reported or unknown. Migrant status was defined as native (born in the reporting country) or foreign-born (born outside the reporting country). We adjusted for reporting delay, defined as the time between HIV diagnosis and the report of this event, using reverse Cox proportional hazards models. Linear regression models were used to test for trends, whereby the significance level was set at 0.05. Analysis of migrant status showed that more than one third (37%) of cases were foreign-born. The transmission patterns and migrant status of cases varied considerably between EU/EEA countries (Figure 1).

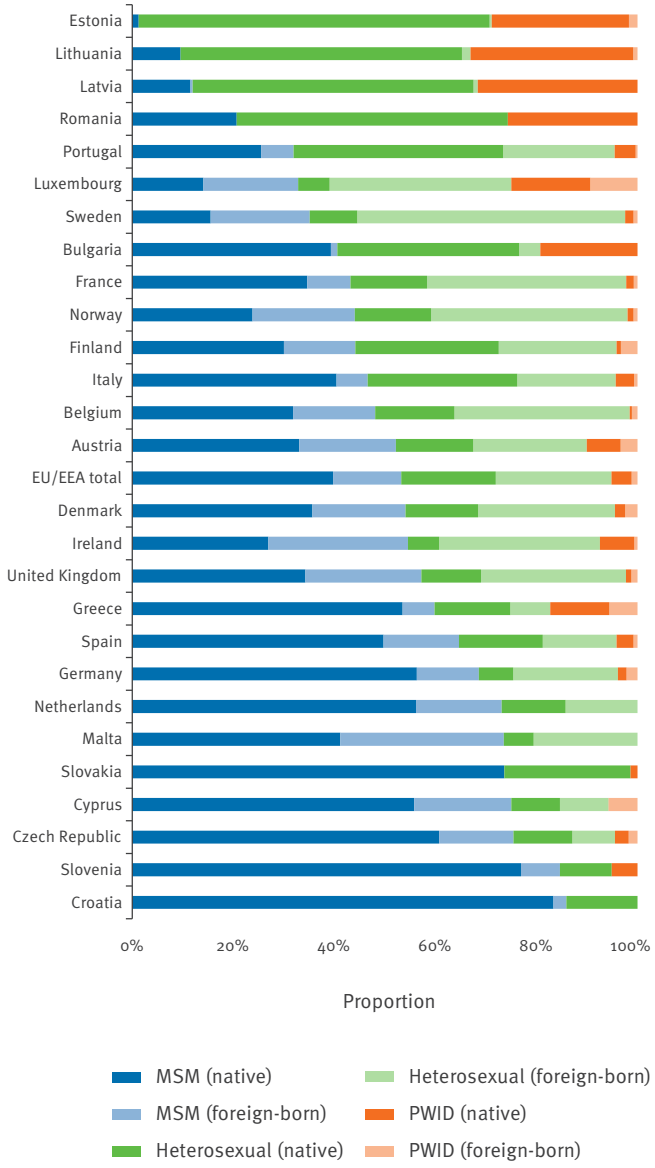
In 2014, 4,020 persons in 30 EU/EEA countries were diagnosed with AIDS, resulting in a rate of 0.8 per 100,000 population. AIDS rates varied markedly from 0.1 in Slovakia to 8.5 in Latvia. The most common route of HIV acquisition among persons diagnosed with AIDS was heterosexual contact (1,771 cases, 44%), while 1,130 cases (28.1%) were in men who have sex with men (MSM) and 588 (14.6%) in people who inject drugs (PWID). Just over one quarter (27.8%) of those diagnosed with AIDS in 2014 were foreign-born.

Trends in new HIV and AIDS diagnoses

Among the 27 countries reporting on new HIV diagnoses and transmission consistently between 2005 and 2014, the number and proportion of cases with known information on transmission and migrant status that were attributed to MSM increased significantly in native men from 5,319 (20% of cases) to 6,265 (29%) and in foreign-born men from 1,438 (6%) to 2,783 (10%) (p value for trend < 0.001 and 0.005, respectively) (Figure 2).

Figure 1

Proportion of new HIV diagnoses with known mode of transmission, by country, transmission route and migration status, EU/EEA, 2014 (n = 22,443)

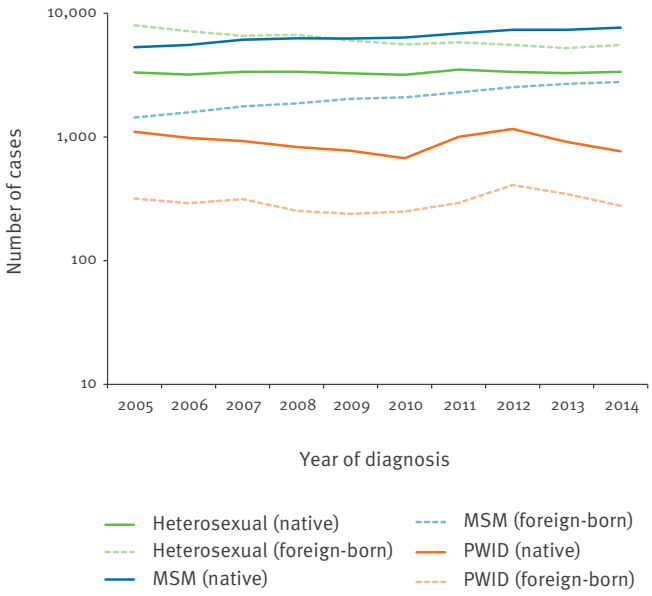


EU/EEA: European Union and European Economic Area; HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Unknown route of transmission was excluded from proportions presented here. Data from Poland and Hungary were excluded due to missing information on country of birth. Liechtenstein and Iceland reported one case each.

Figure 2

New HIV diagnoses, by year of diagnosis, transmission and migration status, adjusted for reporting delay, EU/EEA, 2005–2014 (n = 193,761)



EU/EEA: European Union and European Economic Area; HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Logarithmic scale. Data reported by Estonia, Poland and Hungary were excluded due to incomplete reporting on transmission mode. Data from Italy and Spain were excluded due to increasing surveillance coverage during the period.

The proportion of cases attributed to heterosexual contact was stable among native cases, with 3,322 (17%) in 2005 and 3,368 (16%) in 2014, but decreased among foreign-born cases from 7,991 cases (31%) to 5,548 (21%) over the same period ($p<0.001$). Native cases attributed to injecting drug use decreased over the period from 1,103 (4.2%) to 766 (2.9%) and cases in foreign-born PWID remained stable, with the exception of a slight increase in both groups in 2011 and 2012 due to localised outbreaks in Athens and Bucharest (not tested for trend).

The overall rate of AIDS cases reported in the EU/EEA has halved from 2.0 per 100,000 (9,203 cases) reported in 2005, but has increased by 50% or more since 2005 in Bulgaria, the Czech Republic, Hungary, Latvia, Lithuania and Slovenia. The number of AIDS cases has declined in all transmission groups, but most notably in native PWID ($p<0.001$) and MSM ($p<0.001$) and in native and foreign-born persons infected heterosexually ($p<0.001$ for both groups) (Figure 3).

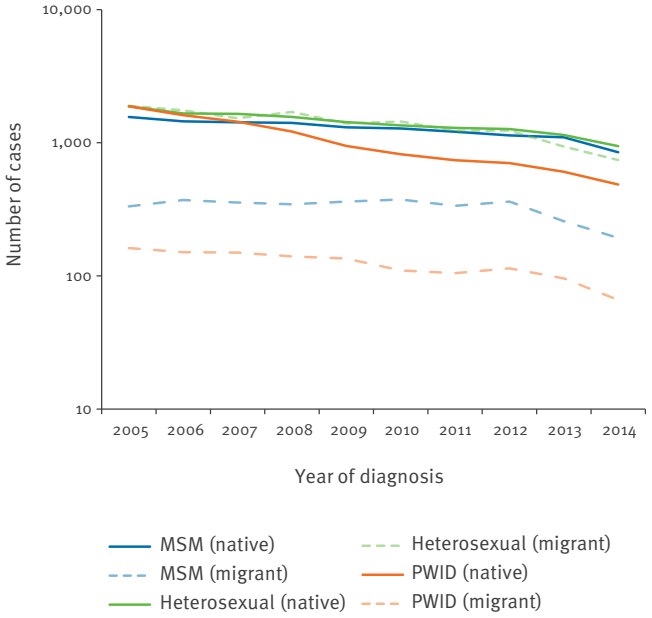
Late diagnosis and AIDS

Twenty-two EU/EEA countries reported information on $CD4^+$ T-cell count/mm^3 at HIV diagnosis for 18,467 (62%) of all cases diagnosed in 2014. Of these, 8,606 individuals (47%) had a $CD4^+$ T-cell count at diagnosis of less than 350 cells/mm^3, including 5,069 (27%) with evidence of advanced disease ($CD4^+$ T-cells<200/mm^3) (Figure 4).

When stratifying by transmission group and migrant status, the highest proportion of people presenting at a later stage of HIV infection ($CD4^+$ T-cells<350 cells/mm^3) was observed in foreign-born and native PWID (63% and 60%, respectively) and the lowest

Figure 3

New AIDS diagnoses, by transmission and migration status, EU/EEA, 2005–2014 (n = 55,760)

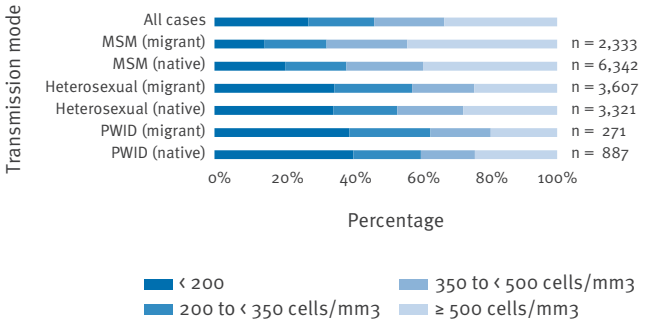


AIDS: acquired immunodeficiency syndrome; EU/EEA: European Union and European Economic Area; MSM: men who have sex with men; PWID: people who inject drugs.

Logarithmic scale. Data from Sweden were excluded due to inconsistent reporting during the period

Figure 4

Proportion of HIV diagnoses by CD4+ T-cell count/mm³ category at diagnosis, all cases and by transmission mode and migration status, EU/EEA, 2014 (n = 18,467)



EU/EEA: European Union and European Economic Area; HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Data from EU/EEA countries not reporting CD4+ T-cell count at diagnosis were excluded (Croatia, Germany, Hungary, Iceland, Lithuania, Malta, Norway, Poland and Sweden). The category all cases includes 16,414 cases for whom region of origin was known, and an additional 2,053 cases for whom region of origin was unknown or not reported.

proportion in foreign-born and native MSM (33% and 39%, respectively).

The median CD4+ T-cell count/mm³ by transmission group over time (Figure 5) increased in both native and foreign-born MSM ($p = 0.075$ and $p = 0.001$, respectively) and in foreign-born heterosexuals ($p < 0.001$), but not in native heterosexuals ($p = 0.323$). For cases who acquired HIV through drug injection, there was variation over the period, with a temporary peak in native PWID in 2011.

Discussion and conclusions

Our analysis indicates that HIV diagnoses continue to increase among native and foreign-born MSM, who represent the largest proportion of new HIV diagnoses in the EU/EEA but are diagnosed earlier than other groups. Although new diagnoses have declined in heterosexuals, influenced by a large decline in cases among foreign-born individuals, this remains the second most common transmission group in the EU/EEA. Transmission related to injecting drug use has declined, with the exception of the outbreak reported in two countries in 2011 and 2012, however late diagnosis remains a significant problem in PWID.

At national level, the diversity of HIV epidemic patterns is apparent. Differences in the population groups most at risk would require diversified and targeted approaches to best address particular national and sub-national variations. For countries with large, and in many cases growing, HIV epidemics in MSM, there is a pressing need to significantly scale up more effective multi-component prevention programmes based on the cornerstones of accessible and effective testing and treatment policies. They should further consider introducing new approaches to prevention, such as pre-exposure prophylaxis [7,8].

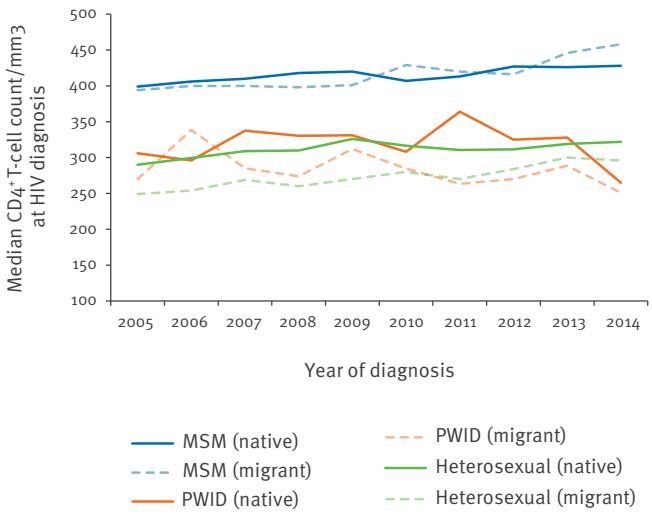
Despite the decline over the last decade, migrants still constitute a considerable proportion (37%) of new HIV diagnoses in the EU/EEA in 2014, reaching more than half in some countries. There is growing evidence that a substantial proportion of migrants, even those originating from HIV-endemic areas, acquire HIV after arrival in the EU/EEA, indicating the need for targeted interventions directed at this vulnerable population [9-12].

In all transmission categories, people continue to be diagnosed with HIV at an advanced stage of illness, particularly PWID and heterosexually acquired cases. These data suggest ongoing problems with access to, and uptake of, HIV testing in many countries [13].

Limitations to this analysis include incomplete data for some variables, particularly CD4+ T-cell count at diagnosis, transmission mode and country of birth. We addressed this by limiting the analysis to countries with more than 50% completeness of data for transmission mode and country of birth, and by performing sensitivity analysis for CD4+ T-cell trends over time by excluding countries that did not report more than 40%

Figure 5

Trends in median CD4⁺ T-cell count/mm³ at HIV diagnosis, by year of diagnosis, transmission group and migration status, EU/EEA, 2005–2014 (n = 115,149)



EU/EEA: European Union and European Economic Area; HIV: human immunodeficiency virus; MSM: men who have sex with men; PWID: people who inject drugs.

Logarithmic scale. Data from countries not reporting CD4⁺ T-cell count at diagnosis were excluded (Croatia, Germany, Hungary, Iceland, Lithuania, Malta, Norway, Poland, and Sweden).

CD4⁺ T-cell data in each transmission group for the entire period.

HIV infection and AIDS can be eliminated in Europe with more resolute prevention measures, early HIV diagnosis and access to effective treatment [14]. The elimination of AIDS and of the high associated healthcare costs and mortality is achievable in Europe, but this will require greater investment in evidence-based HIV prevention measures, tailored to the local HIV epidemic scenario. It will further require greater attention to HIV testing, using new approaches to reach those at risk more effectively and more often.

*Authors' correction

Upon request of the authors, reference [6] was added on 10 December 2015 and all the references were renumbered.

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Conflict of interest

None declared.

Authors' contributions

The ECDC HIV/AIDS Surveillance network supplied the data and provided comments on the manuscript. AP developed the concept of the manuscript, analysed the data, wrote the first draft and responded to reviewers' comments. CQ conducted reporting delay adjustments and statistical tests for trend. CQ, LT, GS and AJAG contributed to the concept of the manuscript and analysis and revised the article to ensure important intellectual content. All authors have read and approved the final manuscript.

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Research article

Late presentation for HIV care across Europe: update from the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study, 2010 to 2013

The late presenters working group in COHERE in EuroCoord [1]
1. The members of the group are listed at the end of the article

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Late presentation (LP) for HIV care across Europe remains a significant issue. We provide a cross-European update from 34 countries on the prevalence and risk factors of LP for 2010–2013. People aged ≥16 presenting for HIV care (earliest of HIV-diagnosis, first clinic visit or cohort enrolment) after 1 January 2010 with available CD4 count within six months of presentation were included. LP was defined as presentation with a CD4 count <350/mm³ or an AIDS defining event (at any CD4), in the six months following HIV diagnosis. Logistic regression investigated changes in LP over time. A total of 30,454 people were included. The median CD4 count at presentation was 368/mm³ (interquartile range (IQR) 193–555/mm³), with no change over time (p=0.70). In 2010, 4,775/10,766 (47.5%) were LP whereas in 2013, 1,642/3,375 (48.7%) were LP (p=0.63). LP was most common in central Europe (4,791/9,625, 49.8%), followed by northern (5,704/11,692; 48.8%), southern (3,550/7,760; 45.8%) and eastern Europe (541/1,377; 38.3%; p<0.0001). There was a significant increase in LP in male and female people who inject drugs (PWID) (adjusted odds ratio (aOR)/year later 1.16; 95% confidence interval (CI): 1.02–1.32), and a significant decline in LP in northern Europe (aOR/year later 0.89; 95% CI: 0.85–0.94). Further improvements in effective HIV testing strategies, with a focus on vulnerable groups, are required across the European continent.

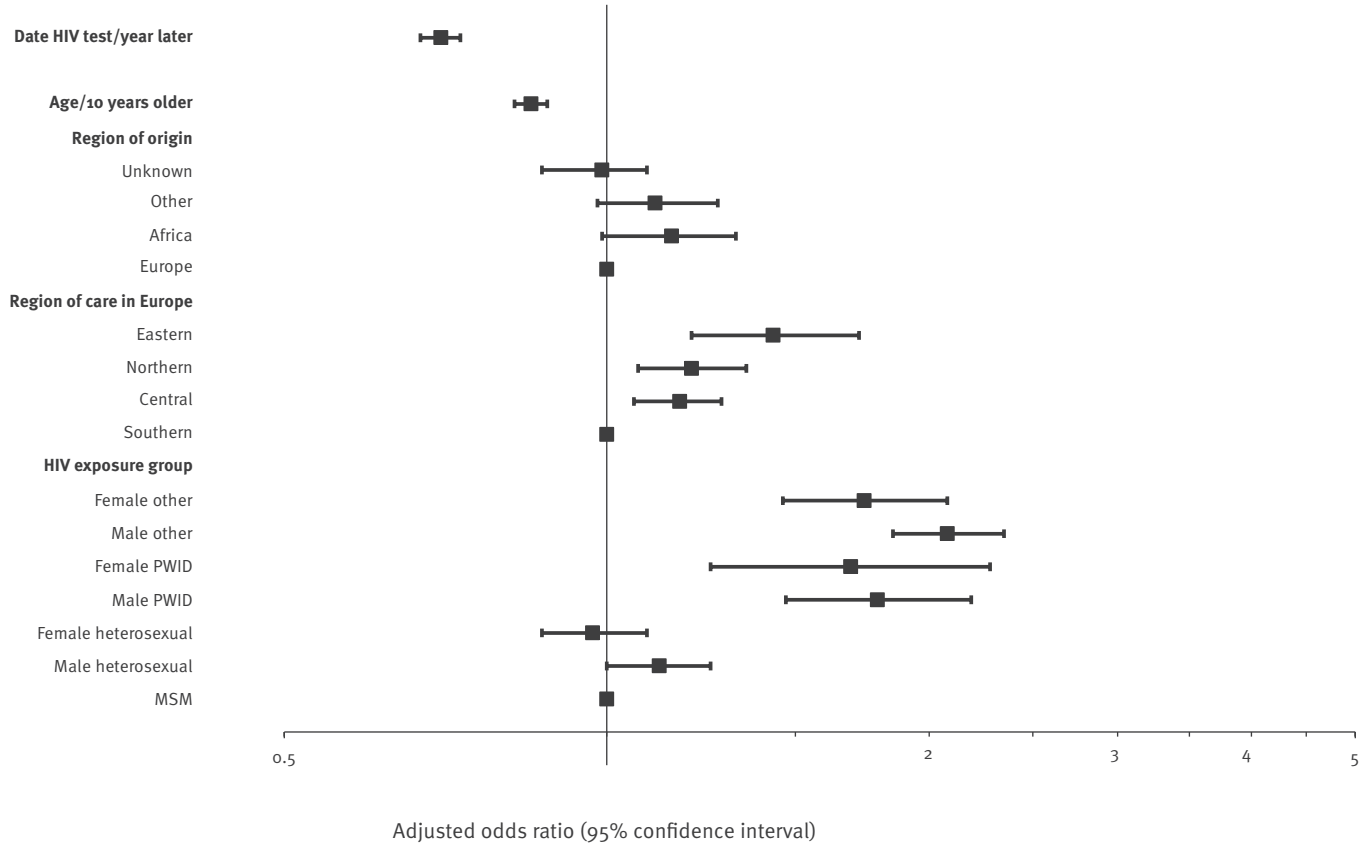
Introduction

The United Nations Programme on HIV/AIDS (UNAIDS) recently released an ambitious strategy that calls for 90% of HIV infections to be diagnosed by 2020 [1]. Many people however remain unaware of their HIV status and cascades for care for HIV vary widely from country to country within Europe, with 20–70% of people infected with HIV remaining undiagnosed [2-4]. These estimates rely on estimates of the population with HIV, which itself is estimated using a variety of different methods [5]. In addition, ca 40–60% of HIV-positive people are diagnosed with HIV at a late stage of infection [6,7], defined as people presenting for HIV care with a CD4 count of less than 350/mm³ or an AIDS defining illness [8]. Individuals at greatest risk of late diagnosis and/or late entry into care have poorer outcomes and higher resource use once diagnosed. Those who are unware of their HIV status are also less likely to take steps to prevent onward transmission to others [9-11]. However, many who present late, do so because they perceive their risk for HIV as low, as they have few sex partners for example.

The rates of late presentation (LP) among newly diagnosed HIV positive people in any setting serves as a proxy of effective HIV testing strategies. Such strategies should ensure people enter appropriate care to start antiretroviral therapy (ART) [12]. However, the extent to which these recommendations are implemented across Europe is variable [13]. Following the recent publication of findings from the Strategic Timing of Antiretroviral Treatment (START) [14], treatment is now recommended for all people infected with HIV [13]. The World Health Organization (WHO) recognises early HIV diagnosis as a crucial first step in the successful care of HIV [15]. There are a number of programmes and initiatives to increase HIV testing; these include indicator-condition-guided HIV testing and national HIV testing strategies, linkage and retention in care of those already diagnosed. Furthermore, there are initiatives specifically aimed at reducing HIV transmission, such as harm-reduction, condom use, initiation of antiretroviral therapy and pre-exposure prophylaxis [16-18].

Figure 1

Adjusted odds of having no CD4 count information within six months following HIV diagnosis, COHERE study, 2010–2013



COHERE: Collaboration of Observational HIV Epidemiological Research Europe; MSM: men who have sex with men; PWID: people who inject drugs.

Logarithmic scale.

The Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study provides a unique opportunity to describe the epidemiology of those diagnosed HIV-positive at a late stage of HIV infection compared with those diagnosed earlier, and to look at geographical differences within HIV exposure groups. COHERE is a collaboration of 39 cohorts across Europe and is part of the EuroCoord network (www.EuroCoord.net). COHERE was established in 2005 with the aim of conducting epidemiological research on the prognosis and outcome of HIV-positive people, which the individual contributing cohorts cannot address themselves because of insufficient sample size or heterogeneity of specific subgroups of HIV-positive people. Local ethics committee and/or other regulatory approvals were obtained as applicable according to local and/or national regulations in all participating cohorts unless no such requirement applied to observational studies. Each cohort submits data using the standardised HIV Collaboration Data Exchange Protocol (HICDEP) [19], including information on patient demographics, use of combination antiretroviral therapy (cART), CD4 counts, AIDS, and deaths. Further details can be found on EuroCoord website [20].

Previous work showed an overall 4% decrease in LP per year of late presentation between 2000 and 2010 across Europe, albeit with an increase over time in people who inject drugs (PWID) [7]. The aims of this update were to determine if the downward trend in LP observed between 2000 and 2010 continued, and whether there were any groups of individuals in which LP continues to increase.

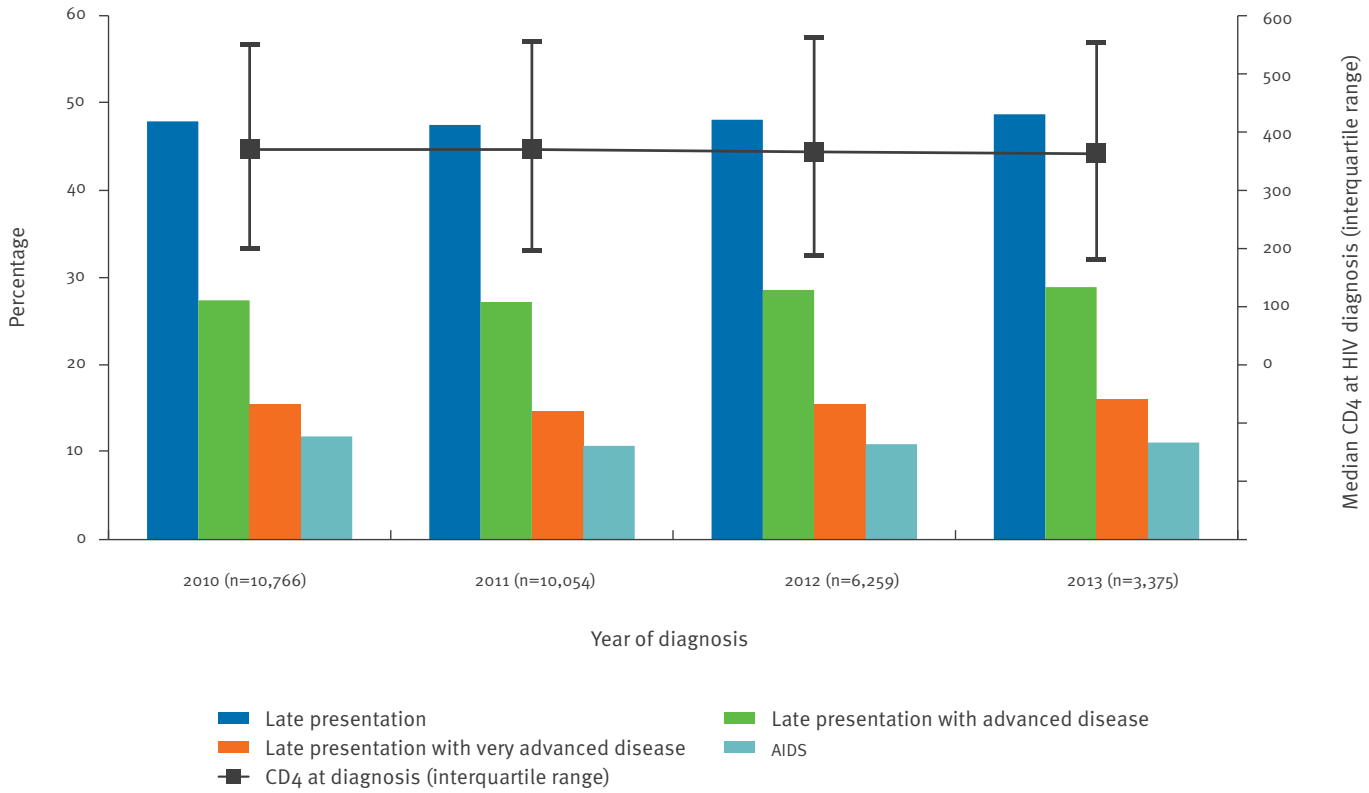
Methods

Patients

Twenty-four cohorts including data from 34 European countries provided data for the present analysis. All people aged ≥ 16 years, who presented for care (defined as earliest date of HIV diagnosis, first clinic visit, or enrolment into the participating cohort, referred to as 'baseline') for the first time after 1 January 2010 were included to provide an update to the report from 2013 which included people diagnosed to the end of 2010 [7]. People were excluded if information on sex or date of HIV diagnosis was missing, or where there was evidence of an earlier HIV diagnosis (CD4 count, AIDS diagnosis, or having started antiretroviral therapy

Figure 2

Changes over time in stages of late presentation and CD4 count at HIV diagnosis, COHERE study, 2010–2013 (N=30,454)



COHERE: Collaboration of Observational HIV Epidemiological Research Europe.

Late presentation: diagnosed with HIV with a CD4 count below 350/mm³ or an AIDS defining event regardless of the CD4 count, in the six months following HIV diagnosis.

Late presentation with advanced disease: diagnosed with HIV with a CD4 count below 200/mm³ or an AIDS defining event, regardless of CD4 cell count, in the six months following HIV diagnosis.

Late presentation with very advanced disease: diagnosed with HIV with a CD4 count below 50/mm³ or an AIDS defining event, regardless of CD4 cell count, in the six months following HIV diagnosis

(ART)) more than one month before first clinic visit, as were people from Argentinean centres in EuroSIDA [21]. People from the seroconverter cohorts in COHERE were also excluded, as in our previous work [7]. By definition, such people are diagnosed soon after HIV infection, even if they have a low CD4 count at HIV-1 diagnosis.

Definitions of late presentation

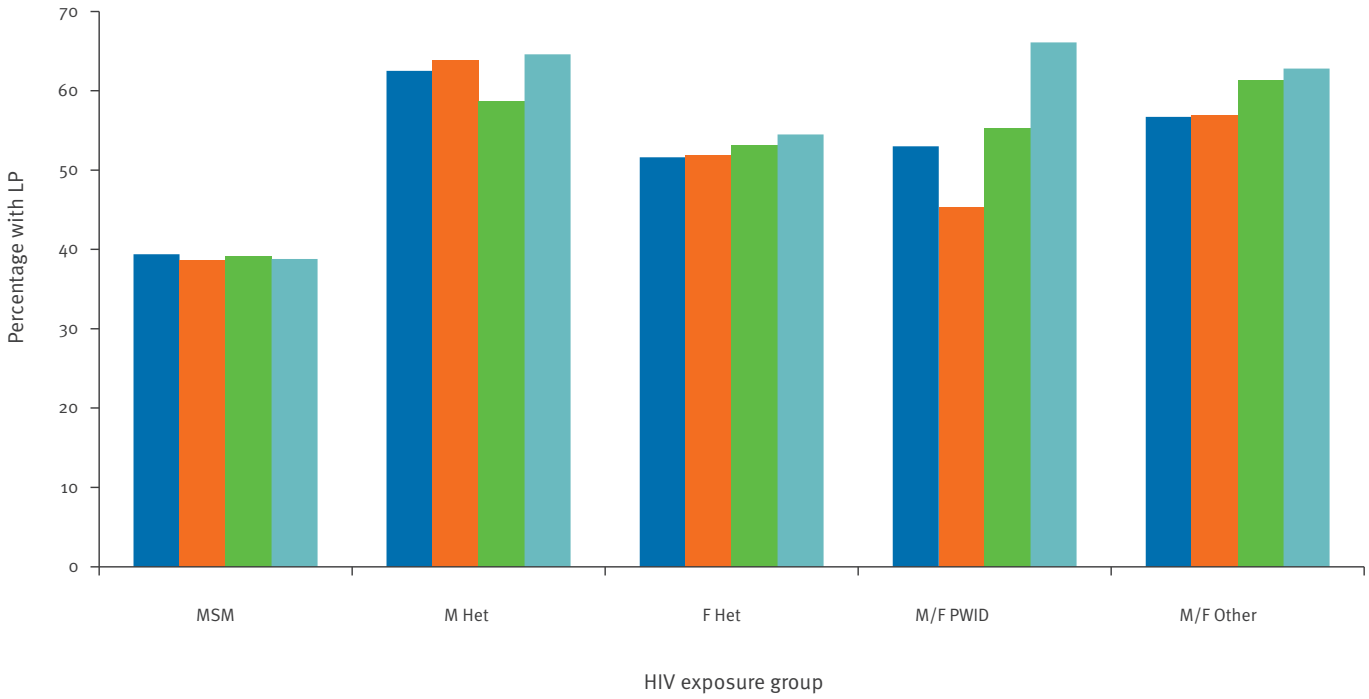
LP was defined as an individual diagnosed with HIV with a CD4 count below 350/mm³ or an AIDS-defining event regardless of the CD4 count, in the six months following HIV diagnosis. LP with advanced disease was defined as an individual diagnosed with HIV with a CD4 count below 200/mm³ or an AIDS defining event, regardless of CD4 cell count, in the six months following HIV diagnosis. LP with very advanced disease was defined as an individual diagnosed with HIV with a CD4 count below 50/mm³ or an AIDS defining event, regardless of CD4 cell count, in the six months following HIV diagnosis. The proportion presenting with AIDS, regardless of the CD4 count at which it occurred, was also presented. Delayed entry into care was defined as more than three months between HIV diagnosis and first clinic visit, in those where both dates were recorded. All people were required to have at least one CD4 count measured in the six months following diagnosis.

Statistical methods

Baseline characteristics of late presenters were compared with those of non-late presenters and logistic regression was used to identify factors associated with late presentation and late presentation with advanced disease. Factors investigated were age, HIV exposure group (men who have sex with men (MSM), heterosexual men, heterosexual female, male PWID, female PWID, other (including patients with unknown HIV exposure group)), continent of origin (Europe, Africa, other (including patients from Central/Southern

Figure 3

Changes over time in late presentation by HIV exposure groups, COHERE study, 2010–2013 (n=30,454)



Odds of late presentation: per year later of presentation.

Univariate	0.99 (0.96–1.03)	0.99 (0.93–1.05)	1.04 (0.98–1.09)	1.16 (1.03–1.31)	1.10 (1.02–1.17)
Multivariate[a]	0.98 (0.95 – 1.01)	0.97 (0.91 – 1.03)	0.99 (0.93 – 1.05)	1.16 (1.02 – 1.32)	1.08 (1.00 – 1.16)

2010 (n=10,766) 2011 (n=10,054) 2012 (n=6,259) 2013 (n=3,375)

COHERE: Collaboration of Observational HIV Epidemiological Research Europe; F: female; Het: heterosexual; LP: late presentation; M: male; MSM: men who have sex with men; PWID: people who inject drugs.

[a]Adjusted for age, region of care in Europe, continent of origin, delayed access to care.

Late presentation: diagnosed with HIV with a CD4 count below 350/mm^3 or an AIDS defining event regardless of the CD4 count, in the six months following HIV diagnosis.

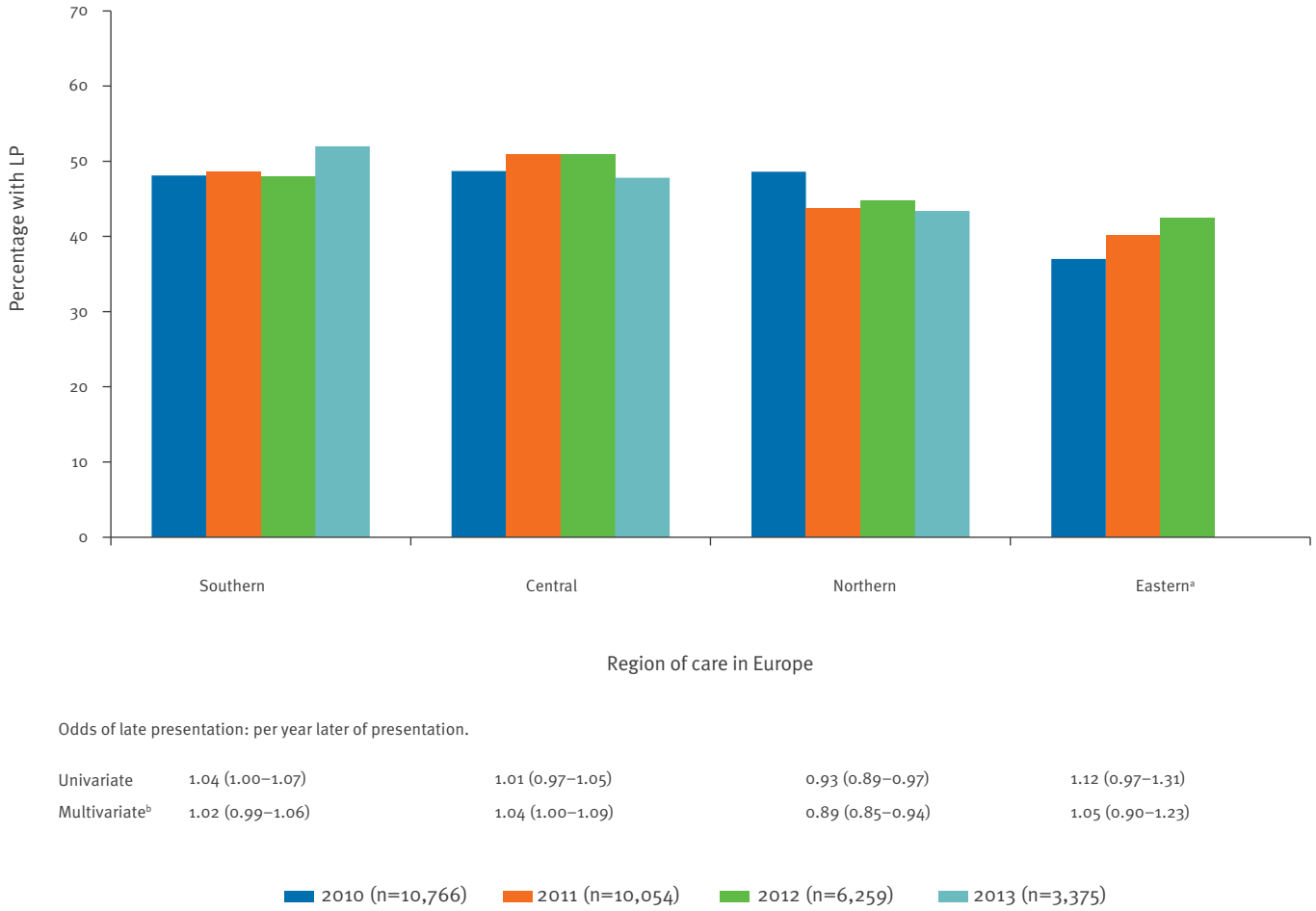
America and Asia), unknown), region of HIV diagnosis in Europe, based on the cohort location and defined similarly to the EuroSIDA study [22] (Table 1), calendar year of diagnosis, and delayed entry into care.

A priori, we were interested in comparing changes over time within region of HIV diagnosis in Europe and HIV exposure groups. Simple descriptive data were used to present the proportions of LP, advanced LP, very advanced LP and presentation with AIDS by country; countries were grouped into regions and anonymised, those countries with less than 50 people included were combined. Linear regression was used to assess change over time in CD4 counts overall and among LP, and Cox proportional hazards models were used to compare the risk of development of a new clinical event i.e. a new AIDS defining illness occurring more than one month after the first if the person had AIDS within six months of baseline or death), whether this has changed over time, and at a similar rate for LP and non-LP.

Descriptive analyses were used to investigate whether someone classified as a LP based on a CD4 count›350/mm^3 (but no AIDS diagnosis) would not be a LP if the next CD4 count was used (misclassification). This analysis was limited to the small subset with CD4 counts measured after HIV diagnosis and before starting ART. Logistic regression was used to determine whether potential misclassification of people as LP had changed over time, after adjusting for age, HIV exposure group, region of origin, region of HIV diagnosis in Europe,

Figure 4

Changes over time in late presentation by region of care in Europe, COHERE study, 2010–2013 (N=30,454)



Odds of late presentation: per year later of presentation.

Univariate	1.04 (1.00–1.07)	1.01 (0.97–1.05)	0.93 (0.89–0.97)	1.12 (0.97–1.31)
Multivariate[b]	1.02 (0.99–1.06)	1.04 (1.00–1.09)	0.89 (0.85–0.94)	1.05 (0.90–1.23)

COHERE: Collaboration of Observational HIV Epidemiological Research Europe; LP: late presentation.

[a]Adjusted for age, HIV exposure group, region of origin, delayed access to care.

[b]No data available for 2013.

Late presentation: diagnosed with HIV with a CD4 count below 350/mm^3 or an AIDS defining event regardless of the CD4 count, in the six months following HIV diagnosis.

calendar year of diagnosis, delayed entry into care, and CD4 count and HIV viral load at HIV diagnosis.

All analyses were performed using Statistical Analysis Software Version 9.3 (Statistical Analysis Software).

Results

Of 37,859 people with a HIV-1 test after 1 January 2010, 4,197 were excluded because they were aged‹16, from seroconverter cohorts, or where there was evidence that the person had started ART, had a CD4 count or an AIDS diagnosis more than 28 days before the first reported HIV-1 test. A further 3,208 of 33,662 (9.5%) were excluded due to missing CD4 counts, 9.6% from south Europe and 8.6%, 9.8% and 13.5% from central, northern and eastern Europe respectively. Compared with the 30,454 included, those excluded due to missing CD4 counts were more likely to be PWID, from other (including unknown) HIV exposure groups, and to be in care in northern, central or eastern Europe compared with southern Europe (Figure 1). Older people were less likely to be excluded, as were those with a more recent test for HIV.

Late presentation and changes over time

Table 2 summarises the characteristics of the 30,454 people included, stratified by LP status; 14,586 (47.9%) were LP, ranging from›60% of heterosexual men or people originating from Africa to ca 39% MSM and female PWID.

Table 1

Geographical categorisation of European countries to regions, COHERE study, 2010–2013[a]

Region in Europe	Countries included
Northern	Denmark, Finland, Ireland, the Netherlands, Norway, Sweden, United Kingdom
Southern	Greece, Israel, Italy, Portugal, Spain
Central	Austria, Belgium, France, Germany, Luxemburg, Switzerland
Eastern	Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Ukraine

COHERE: Collaboration of Observational HIV Epidemiological Research Europe.

[a] Defined similarly to the EuroSIDA study [22].

Among people in whom both first visit and HIV test date were known, 1,247/27,818 (4.5%) had delayed entry into care. Figure 2 shows the annual proportion of people with LP, LP with advanced or very advanced disease, and with an AIDS diagnosis, regardless of the CD4 count at presentation.

In 2010 4,775/10,766 (47.5%) were LP, compared with 1,642/3,375 (48.7%) in 2013 or later (p = 0.63). The proportion of people with LP, advanced disease, very advanced disease, or AIDS did not change significantly over time (p = 0.63, 0.090, 0.16, and 0.075 respectively). The proportion of those presenting who would be eligible for starting cART with a CD4 count of ‹500/mm³ was 69.0% in 2010, 68.8% in 2011, 68.3% in 2012 and 69.0 in 2013 or later (p = 0.77).

In multivariate analyses, there was no evidence of a change over time in LP (adjusted odds ratio (aOR) 0.99/year later; 95% confidence interval (CI): 0.97–1.02; p = 0.60), or in LP with advanced disease (aOR 0.99/year later; 95% CI: 0.97–1.02; p = 0.65). This finding was consistent across a wide range of sensitivity analyses, such as including those with AIDS but without a CD4 count measured as LP, including deaths within the first six months as LP, when the window required for a CD4 count after HIV diagnosis to three months, and defining LP as a CD4 count ‹350/mm³ or an AIDS diagnosis within three months of HIV diagnosis. There was some evidence that presentation with very advanced disease had decreased over time by 3% per year later (aOR 0.97/year later; 95% CI: 0.93–1.00; p = 0.035), and that LP based on an AIDS diagnosis alone, regardless of the CD4 count at which it was diagnosed, had decreased by 7% per year (aOR 0.93; 95% CI: 0.89–0.96; p = 0.0001).

Changes in CD4 count at presentation

The median CD4 count at presentation was 368/mm³ (interquartile range (IQR) 193–555/mm³). There was no evidence of a change over time in the median CD4 count at presentation (p=0.70), suggesting that overall, the level of immunodeficiency at which HIV was diagnosed has not changed over time (adjusted change/year 1.2/mm³; 95% CI: -0.8 to 3.3/mm³; p = 0.89). Similar results were seen in an analysis limited to LP (adjusted change/year -1.1/mm³; 95% CI: -3.1 to 0.8/mm³, p = 0.31), demonstrating that LP are diagnosed with HIV at a similar level of immunodeficiency between 2010 and 2013.

Of 14,586 LP, 3,984 (27.3%) did not have AIDS as part of the LP definition and had at least one CD4 count during follow-up before starting ART. Among these, for 1,067 (26.8%) the next CD4 count was ›350/mm³, suggesting they may not be LP or they may be seroconverters; this proportion was highest for MSM (698/2,154; 32.4%), and was ca 20% in all other HIV exposure groups (p ‹0.0001). There was some evidence that the proportion that may be incorrectly classified as LP had increased over time (aOR 1.14/year later; 95% CI: 1.04–1.28; p = 0.0050). This proportion of potentially misclassified LP was lower using a confirmed CD4 count ›350/mm³ (317/1,279 (24.8%) with more than two CD4 counts after HIV diagnosis and before ART started).

Changes in late presentation in HIV exposure groups and regions of Europe

Figure 3 summarises the change over time in LP among HIV exposure groups. Male and female PWID were combined due to smaller numbers as were men and women belonging to the ‘other’ risk groups. There was strong evidence to suggest that the rate of change in LP differed between HIV exposure groups (p ‹0.0001, test for interaction). After adjustment, there was no change over time in LP among MSM, or male or female heterosexuals, but there was a significant increase in LP among PWID (both men and women combined) (aOR 1.16/year later; 95% CI: 1.02–1.32; p = 0.024) and in the other exposure groups (aOR 1.08/year later; 95% CI: 1.00– 1.16, p = 0.040).

LP was most common in central Europe (4,791/9,625, 49.8%), followed by northern (5,704/11,692; 48.8%), southern (3,550/7,760; 45.8%) and eastern Europe (541/1,377; 38.3%; p‹0.0001). There were considerable differences in LP in countries within regions of care in Europe (Table 3), particularly within eastern Europe. Figure 4 presents similar data to Table 3, stratified by region of care in Europe, with evidence to suggest the rate of change in LP differed between regions (p ‹0.0001; test for interaction). There was a marginally significant increase in LP over time in central Europe

Table 2

Characteristics of included patients, COHERE study, 2010–2013 (n=30,454)

Patients' characteristics		All		Late presenters	
		N	Percentage (%)[a]	N	Percentage of late presenters (%)[b]
All		30,454	100	14,586	47.9
Delayed entry to care[c]	No	26,751	95.5	12,818	47.9
	Yes	1,247	4.5	494	39.6
HIV exposure group	MSM	15,371	50.5	5,993	39.0
	Heterosexual men	4,826	15.8	3,011	62.4
	Heterosexual females	5,487	18.0	2,864	52.2
	PWID (male)	843	2.8	481	57.1
	PWID (female)	321	1.1	126	39.3
	Male other	2,551	8.8	1,495	58.6
	Female other	1,055	3.5	616	58.4
Region of care in Europe	Southern	11,692	38.4	5,704	48.8
	Central	9,625	31.6	4,791	49.8
	Northern	7,760	25.5	3,550	45.8
	Eastern	1,377	4.5	541	38.3
Continent of origin	Europe	20,701	68.0	9,495	45.9
	Africa	2,651	8.7	1,696	64.0
	Other	2,685	8.8	1,428	53.2
	Unknown	4,417	14.5	1,967	44.5
		Median	IQR	Median	IQR
Age	Years	36	29–45	39	31–48
CD4	/mm³	368	193–555	184	73–276
Baseline[d]	Month/year	6/2011	9/2010–4/2012	6/2011	9/2010–4/2012

COHERE: Collaboration of Observational HIV Epidemiological Research Europe; IQR: interquartile range; MSM: men who have sex with men; PWID: people who inject drugs.

[a]% represents percentage of total; for example, 15,371/30,454 (50.5%) of the population included were MSM.

[b] % represents the percentage of late presenters; for example, 5,993/15,371 (39.0%) of MSM were late presenters.

[c] Delayed entry into care was defined as more than three months between HIV diagnosis and first visit to clinic, in people with both dates recorded (n=27,998).

[d] Baseline was defined as the earliest of HIV test, first study visit or cohort enrolment.

Late presentation: diagnosed with HIV with a CD4 count below 350/mm³ or an AIDS defining event regardless of the CD4 count, in the six months following HIV-diagnosis [8].

(aOR 1.04/year later; 95% CI: 1.00–1.09, p=0.084), and a significant decrease in LP over time in northern Europe (aOR 0.89; 95% CI: 0.85–0.94; p<0.0001).

Clinical disease progression

During 39,790 person-years of follow-up (PYFU) 886 (2.9%) people developed a new AIDS defining illness or died, giving an incidence of clinical progression of 22.3/1,000 PYFU (95% CI: 20.8–23.7). A total of 409 disease progression events were death, 486 were a new AIDS event, and nine patients had both types of events on the same date. There were no differences in the proportion of events that were attributable to AIDS (63/125 (50.4%) vs 423/761 (55.6%); p=0.28), deaths (63/125 (50.4%) vs 346/761 (45.5%); p=0.31), or in the specific AIDS events diagnosed (p=0.053) comparing LP and non-LP. The incidence of clinical progression was 6.5-fold higher among LP (761 events, 39.6/1,000 PYFU; 95% CI: 36.8–42.4) compared with non-LP (125 events, 6.1/1,000 PYFU; 95% CI: 5.0–7.1).

There was no evidence of any change over time in the risk of developing a new AIDS event or death within the first six months of presentation or after this time (Table 4). For example, after adjustment, there was no change over time in the risk of developing a new clinical event per year later of presentation after six months of follow-up (adjusted hazard ratio (aHR) 1.04/year later; 95% CI: 0.91–1.19, p=0.40). There was no evidence that this relationship differed in LP vs non-LP (test for interaction>0.2), and the results are also shown in Table 4. These findings were also consistent for

Table 3

Late presentation, late presentation with advanced disease, late presentation with very advanced disease and presentation with an AIDS defining event at any CD4 count stratified by country, COHERE study, 2010–2013

			Late presentation		Late presentation with advanced disease		Late presentation with very advanced disease		AIDS	
Region of care in Europe	Country	N	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Southern	[a]	40	55.0	39.6–70.4	27.5	13.7–41.3	7.5	0.0–15.7	2.5	0.0–7.3
Southern	1	1,097	45.9	43.0–48.9	25.9	23.3–28.5	12.5	10.5–14.4	8.1	6.5–9.7
Southern	2	4,782	53.9	52.5–55.3	33.9	32.6–35.2	17.1	16.1–18.2	9.6	8.7–10.4
Southern	3	5,773	45.0	43.8–46.3	25.8	24.7–26.9	14.3	13.4–15.2	11.0	10.2–11.8
Central	[a]	19	31.6	10.7–52.5	15.8	0.0–32.2	10.5	0.0–24.3	5.3	0.0–15.3
Central	1	568	43.7	39.6–47.7	24.6	21.1–28.2	13.4	10.6–16.2	11.3	8.7–13.9
Central	2	778	47.6	44.0–51.1	27.9	24.7–31.0	17.4	14.7–20.0	14.1	11.7–16.6
Central	3	810	54.9	51.5–58.4	30.9	27.7–34.0	15.1	12.6–17.5	11.6	9.4–13.8
Central	4	2,675	56.2	54.3–58.1	37.2	35.3–39.0	24.6	23.0–26.2	20.0	18.5–21.6
Central	5	4,775	46.5	45.1–47.9	25.1	23.8–26.3	13.5	12.5–14.5	10.5	9.6–11.3
Northern	[a]	21	47.6	26.3–69.0	33.3	13.2–53.5	19.0	0.0–35.8	14.3	0.0–29.3
Northern	1	600	52.7	48.7–56.7	33.7	29.9–37.4	19.3	16.2–22.5	13.2	10.5–15.9
Northern	2	3,419	44.7	43.1–46.4	24.2	22.7–25.6	11.7	10.7–12.8	7.6	6.7–8.5
Northern	3	3,720	45.5	43.9–47.1	26.7	25.3–28.1	17.4	16.2–18.7	14.2	13.1–15.4
Eastern	1	74	63.5	52.5–74.5	18.9	10.0–27.8	8.1	1.9–14.3	6.8	1.0–12.5
Eastern	2	84	50.0	39.3–60.7	26.2	16.8–35.6	19.0	10.7–27.4	19.0	10.7–27.4
Eastern	[a]	98	58.2	48.4–67.9	39.8	30.1–49.5	24.5	16.0–33.0	19.4	11.6–27.2
Eastern	3	1,121	35.2	32.4–38.0	12.1	10.2–14.0	1.4	0.7–2.1	0.7	0.2–1.2

COHERE: Collaboration of Observational HIV Epidemiological Research Europe.

[a] Represents >1 country within specific regions where countries with <50 people have been combined.

Late presentation: diagnosed with HIV with a CD4 count below 350/mm3 or an AIDS defining event regardless of the CD4 count, in the six months following HIV diagnosis.

Late presentation with advanced disease: diagnosed with HIV with a CD4 count below 200/mm3 or an AIDS defining event, regardless of CD4 cell count, in the six months following HIV diagnosis.

Late presentation with very advanced disease: diagnosed with HIV with a CD4 count below 50/mm3 or an AIDS defining event, regardless of CD4 cell count, in the six months following HIV diagnosis.

AIDS: presentation with AIDS regardless of CD4 count.

different HIV exposure categories and across regions of care in Europe and when using death alone as the clinical endpoint.

Discussion

This study, which included more than 30,000 people from across 34 European countries, demonstrated no overall change in the proportion of LP across Europe since 2010. LP increased significantly in PWID as presumed HIV exposure. This lack of improvement in diagnosing HIV earlier was consistent across a wide range of analyses; there was no change over time in LP with advanced disease, in the average CD4 count at presentation, or in progression to a new AIDS event/death.

The proportion of LP decreased significantly across Europe between 2000 and 2010 [7], but despite numerous interventions and initiatives in recent years to optimise testing for HIV, we found no overall change between 2010 and 2013 in the proportion of LP across Europe. LP is impacted by the underlying incidence of HIV which itself is difficult to estimate [5]. If HIV incidence increases and HIV testing does not change, the proportion of LP will decline as more are diagnosed early. Conversely, if incidence is declining and HIV testing does not change, the proportion of LP increases. Assuming the overall incidence of HIV is not decreasing in Europe, as there appears to have been no decline in HIV diagnoses per 100,000 population over the last decade [4], our findings of no decrease in LP overall suggests there are areas for further interventions for reducing LP on a European level. A more detailed analysis by region showed a small decrease over time in LP from northern Europe, but not from other regions. Combining countries into these regions was decided a priori and used the stratification previously used by EuroSIDA [23]. Such a broad grouping may not be ideal for a number of reasons, including history, politics and economy and the rates of LP within regions varied considerably, reflecting this heterogeneity. HIV surveillance in Europe for the European Union (EU)/European Economic Area (EEA) is coordinated by the European

Table 4

Relative hazard of a new AIDS defining event or death following HIV diagnosis per year later of presentation, COHERE study, 2010–2013

		In first six months after presentation			More than six months after presentation		
		HR	95% CI	P	HR	95% CI	p
All	Univariate	1.02	0.92–1.11	0.83	1.01	0.89–1.15	0.87
	Multivariate [a]	0.98	0.89–1.08	0.73	1.04	0.91–1.19	0.40
Non-LP	Univariate	0.99	0.64–4.53	0.95	1.04	0.77–1.40	0.79
	Multivariate [a]	0.92	0.59–1.45	0.73	0.98	0.89–1.09	0.72
LP	Univariate	1.01	0.92–1.12	0.83	1.00	0.87–1.16	0.98
	Multivariate [a]	1.06	0.78–1.44	0.71	1.05	0.90–1.22	0.54

CI: confidence interval; COHERE: Collaboration of Observational HIV Epidemiological Research Europe; HR: hazard ratio; LP: late presentation.

[a] Multivariate models were adjusted for age, HIV exposure group, region of care in Europe, continent of origin, and whether an AIDS diagnosis was present at baseline. The model in all patients adjusts additionally for late presentation.

All HR are per year later of presenting for care.

Centre for Disease Prevention and Control (ECDC) in collaboration with the WHO Regional Office for Europe and the ECDC report displays LP in central-east and eastern Europe separately [4]; in this study, they were combined due to small numbers from the central-east region. Further, combining southern, central and northern Europe into one region in our study to compare to ECDC data may hide important findings within these regions. Despite these differences in classification, the proportion with LP in this study was very similar to that recently reported by the ECDC [4], who reported eight countries with›50% as LP, including Greece and Italy, both of which contributed significant numbers to our analyses.

Possible action points for increasing HIV awareness and HIV testing, and therefore minimising LP include a combination of both community-based and provider-initiated models for HIV testing, removal of stigmatisation, as well as working towards acceptance of verbal informed consent for testing [16,18,24,25]. Community-based testing should be a priority, as should targeting key populations.

Previous analyses from COHERE showed an increase in LP among PWID from southern and eastern Europe [7], a trend that continued in these analyses. It is worth noting again that these data are difficult to interpret; the number of new diagnoses of HIV is declining in PWID in Europe [4] and if HIV testing is stable this could lead to an increase in the proportion of PIWD presenting late. Although PWID account for a comparatively small proportion of new HIV infections in western Europe, this route of transmission is more common in eastern Europe [4,7], and issues continue to exist around needle exchange, opiate substitution therapy, as well as access to ART and retention in care once HIV has been diagnosed [26,27]. Recent data suggest that those in prison and migrants were among those least likely to be targeted for HIV testing; with challenges being providing HIV services and support, although the ECDC-funded report by Deblonde et al. acknowledges excellence in some countries [28]. Further, PWID are more likely to face greater barriers to accessing healthcare and to belong to lower socioeconomic groups and have lower levels of education, all factors known to be associated with poorer medical outcomes [29,30]. Thus while there is evidence for barriers for PWID to access care and be retained in care, there is much less evidence that HIV is not diagnosed, or that diagnosis of HIV or access to care is even worsening.

We found a small decline in the proportion of LP with very advanced disease or in the proportion presenting with AIDS over time. The fact that the CD4 count at presentation has remained stable over time may suggest that health systems are better able to recognise and capture people with symptomatic HIV disease occurring at higher CD4 counts and that asymptomatic patients are not routinely diagnosed with HIV, especially in groups at low risk of HIV infection. Evidence from other studies concerning changes over time in CD4 count at presentation in recent times have shown mixed results [31-34]; some have limited data from 2011 and others have not been able to adjust for confounding variables. Other studies have described a decrease in the proportion presenting with AIDS [35,36], although the extent to which this is due to the under-reporting of AIDS is unknown.

We found no evidence of a change over time in short-term clinical progression (within six months) or after that time in all people or in LP and non-LP considered separately, in different regions of Europe or HIV exposure categories, although median follow-up was limited by only including people diagnosed with HIV-1 since 2010. The greatest risk in clinical progression for LP has been observed in the years immediately following LP [7,10,37,38], and in this study, LP had approximately a six-fold higher incidence of clinical progression. A lack of change in clinical outcomes in LP over calendar time suggests that, once people have accessed care, treatment and outcomes are uniform across a variety of settings. Given the poor outcomes after LP, work is needed to reduce the proportion of those presenting

late to reduce morbidity and mortality associated with HIV, as well as to reduce the financial impact on health systems and onward transmission of HIV [9-11].

It is possible that some people presenting with symptomatic seroconversion for HIV are misclassified as LP due to the transient drop in CD4 count occurring at this time [39]. Misclassification of LP may be highest in MSM [40]. In our study, there was a small subset with CD4 counts after HIV diagnosis but before starting antiretroviral therapy. Approximately 20% did not have a CD4 count of ‹350/mm^3 at the next CD4 count, and this proportion was highest for MSM. This proportion was similar when using a confirmed CD4 count of ›350/mm^3, suggesting this is not largely due to laboratory variation, although it is worth noting that only a small subset of people had one or two CD4 counts after HIV diagnosis and before starting ART. In addition, only 5% had a CD4 count ›500/mm^3 at the second measurement, which is higher than the currently recommended threshold for initiation of antiretroviral therapy [12]. In addition, we found no changes over time in the proportion of LP, presentation with advanced or very advanced disease, suggesting that an increasing proportion of primary HIV infections is unlikely to explain the lack of change in LP in recent years.

There are a number of limitations which should be considered. We are likely underestimating LP as people who do not survive long enough to have a CD4 count measured were excluded [41]. Our data suggest this was more likely in PWID, other HIV exposure groups, those under care in northern and eastern Europe, and affected ca 10% of those in the COHERE cohorts. This is considerably lower than reported by surveillance studies [42], highlighting that cohort studies such as COHERE can supplement information available from the WHO or ECDC. Furthermore, cohorts participating in COHERE tend to be receiving healthcare at centres of excellence and clinic-based cohorts rather than non-clinic outpatient settings, where LP may be higher. We excluded seroconverter cohorts participating in COHERE as in our previous work, where inclusion of these cohorts did not alter our findings [7]. Even in a collaboration as large as COHERE we were not able to consider LP for male and female PWID separately, although it is worth noting that there was no statistically significant rise in LP in any one region, suggesting that the problem of LP in IDUs is not limited to one region of Europe, but a potential problem on a wider scale.

In conclusion, LP across Europe account for almost 50% of HIV diagnoses with no evidence of a change since 2010. Increased HIV testing, with a focus on vulnerable groups, will reduce the harm for the individual and it may as well reduce onward transmission. Earlier diagnosis for HIV is an important component of achieving the UNAIDS target of ending the AIDS epidemic by 2030 [1].

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Conflict of interest

None declared.

Authors' contributions

Amanda Mocroft, Ole Kirk and Jens Lundgren proposed and developed the project. Amanda Mocroft performed the analyses and wrote the first draft of the manuscript. All other authors contributed to discussions of results, interpretation of data and contributed to writing the manuscript.

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Research article

The utility of multiple molecular methods including whole genome sequencing as tools to differentiate *Escherichia coli* O157:H7 outbreaks

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A standardised method for determining *Escherichia coli* O157:H7 strain relatedness using whole genome sequencing or virulence gene profiling is not yet established. We sought to assess the capacity of either high-throughput polymerase chain reaction (PCR) of 49 virulence genes, core-genome single nt variants (SNVs) or *k*-mer clustering to discriminate between outbreak-associated and sporadic *E. coli* O157:H7 isolates. Three outbreaks and multiple sporadic isolates from the province of Alberta, Canada were included in the study. Two of the outbreaks occurred concurrently in 2014 and one occurred in 2012. Pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat analysis (MLVA) were employed as comparator typing methods. The virulence gene profiles of isolates from the 2012 and 2014 Alberta outbreak events and contemporary sporadic isolates were mostly identical; therefore the set of virulence genes chosen in this study were not discriminatory enough to distinguish between outbreak clusters. Concordant with PFGE and MLVA results, core genome SNV and k-mer phylogenies clustered isolates from the 2012 and 2014 outbreaks as distinct events. *k*-mer phylogenies demonstrated increased discriminatory power compared with core SNV phylogenies. Prior to the widespread implementation of whole genome sequencing for routine public health use, issues surrounding cost, technical expertise, software standardisation, and data sharing/comparisons must be addressed.

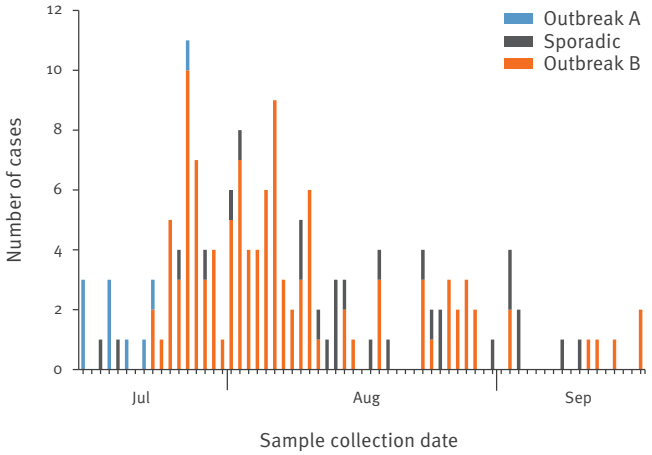
Introduction

Shiga toxin-producing *Escherichia coli* (STEC) consisting of O157 and non-O157 serogroups are a major public health concern. Cattle and other ruminants are natural reservoirs for STEC organisms, shedding the organisms in their faeces, which can cause food and/or water contamination [1]. Consumption of contaminated meat, dairy products, vegetables/fruit, water, contact with animals [1] and person-to-person transmission [2] have all been associated with STEC infections. Infection may be asymptomatic or can cause gastrointestinal symptoms, including mild diarrhoea to haemorrhagic colitis [3]. In five to 20% of the infected patients post-diarrhoea haemolytic uraemic syndrome (HUS) occurs, which is characterised by haemolytic anaemia, thrombocytopenia and kidney injury or failure [2,3]. Paediatric and elderly patients are at greatest risk for developing systemic STEC complications, which are not limited to HUS and can include cardiac, central nervous system, pancreatic, and pulmonary complications [3-5]. Shiga toxins (Stx1 and Stx2) are the major virulence determinants responsible for symptoms associated with both haemorrhagic colitis and systemic infections [5].

Due to the public health importance of STEC infections, epidemiological and molecular surveillance systems are essential for early outbreak detection. In recent years, rapid advancements in the use of molecular typing methods have improved STEC surveillance and outbreak detection. The application of these tools helps to identify disease clusters, refine outbreak case definitions, facilitate case finding, and link human cases to environmental sources. In order to achieve these outcomes, molecular typing assays must possess the discriminatory power required to distinguish between related and nonrelated bacterial isolates, have high reproducibility, and be easy to perform. Furthermore,

Figure 1

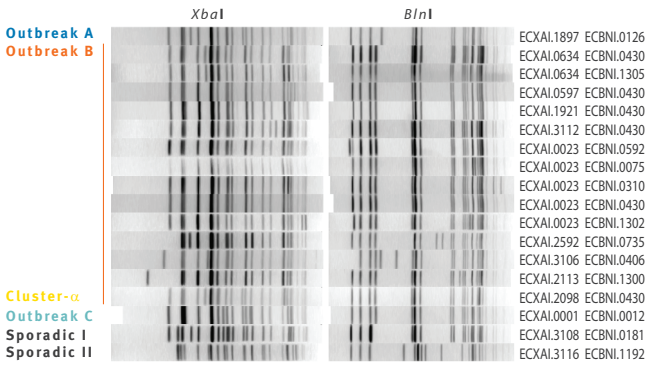
Distribution of *Escherichia coli* O157:H7 cases in residents of Alberta according to time and outbreak, Canada, 14 July–17 September 2014 (n=149)



During the time period covered by the Figure, the Alberta Provincial Laboratory for Public Health (ProvLab) received a total of 149 clinical isolates from individual human cases, 10 belonged to outbreak A and 111 to outbreak B. Sporadic cases (n=28) had no epidemiological or pulsed-field gel electrophoresis (PFGE)/ multilocus variable-number tandem repeat analysis (MLVA) profile link to outbreaks.

Figure 2

All pulsed-field gel electrophoresis (PFGE) patterns found in outbreaks A, B, and C, Alberta, Canada, 14 July–17 September 2014 (n=16)



Restriction enzyme digestion was done using *Xba*I and *Bln*I. Cluster-α pattern was found in a clinical and an environmental isolate associated with outbreak B (see results for detail). Two sporadic isolates are also included for a reference (sporadic I and sporadic II). Pulsed-field gel electrophoresis (PFGE) national pattern designation is represented by the following: for *Xba*I restriction pattern as ECXAI (4 numerals) and for *Bln*I restriction pattern as ECBNI (4 numerals).

the results generated need to be easy to interpret, portable and allow inter-laboratory comparison. All typing results, especially during an outbreak, must be able to correlate with epidemiological data for accurate interpretation [6-8].

In Alberta, Canada, all *E. coli* O157:H7 are routinely typed by pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat analysis (MLVA) under surveillance practices. In July 2014, a unique cluster of *E. coli* O157:H7 was identified by PFGE with concordant MLVA analysis. The subsequent molecular and epidemiological investigation revealed that the cluster was associated with one of the largest human *E. coli* O157:H7 outbreaks in Canada since the implementation of PulseNet Canada (PNC; a national molecular subtyping network for food-borne disease surveillance) in 2000 (Linda Chui, PNC internal communications). To investigate the relatedness of isolates, whole genome sequencing (WGS) and virulence gene profiling were performed separately in real-time with concomitant analysis. The objectives of this study were twofold: (i) We sought to determine the relatedness of this large outbreak event to a concurrent, albeit smaller outbreak as well as to all sporadic cases occurring in the summer of 2014 in Alberta. In addition, a representative panel of isolates from a socially and economically significant, 2012 beef-associated outbreak was included for comparison. (ii) Using combined PFGE and MLVA profiles as a molecular typing standard, we assessed the individual ability of WGS-based methods (core single nt variants (SNV) and *k*-mer analysis) or virulence gene profiling to differentiate sporadic cases from simultaneously occurring *E. coli* O157:H7 outbreak clusters.

Methods

Molecular detection of *Escherichia coli* O157:H7 outbreaks in Alberta

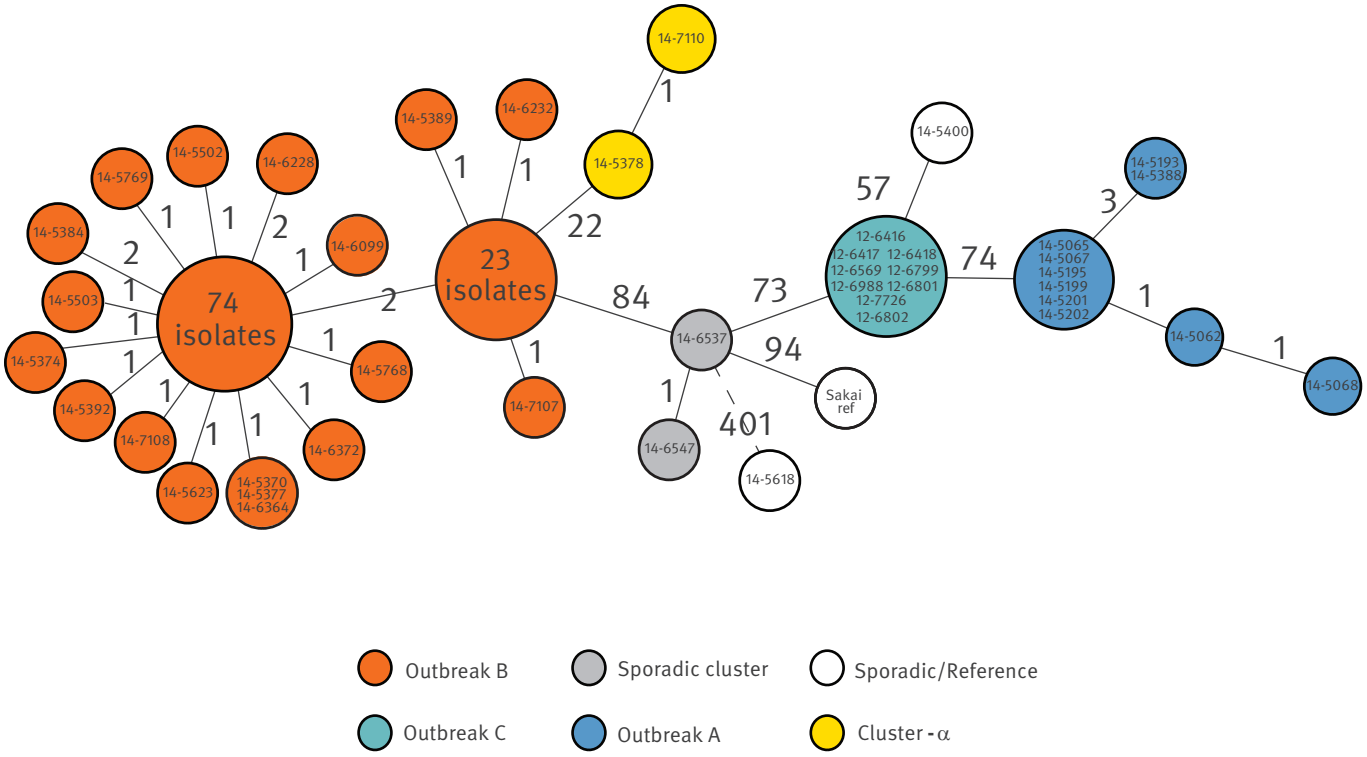
Following established protocols from frontline microbiology diagnostic laboratories for enteric bacteria isolation, all presumptive *E. coli* O157:H7 isolates are forwarded to the Alberta Provincial Laboratory for Public Health (ProvLab) for serotype confirmation and molecular typing. Routinely, all *E. coli* O157:H7 isolates are subjected to PFGE and MLVA using standardised PulseNet protocols (www.pulsenetinternational.org). For *E. coli*, *Xba*I endonuclease is the primary restriction enzyme used for chromosomal DNA digestion and is followed by secondary enzyme digestion with *Bln*I. Images (tagged image file format) of the PFGE profiles for all isolates are uploaded to the PNC Public Health Agency of Canada National Microbiology Laboratory in Winnipeg, Manitoba (PHAC-NML) secure national database for national pattern designation. Participating PNC public health laboratories across Canada are alerted of clusters (n=2 indistinguishable patterns) through the PNC web discussion board. In Alberta, the identification of STEC PFGE clusters triggers a public health investigation, involving the local public health authority and the Alberta ProvLab. MLVA analysis is performed at PHAC-NML on all *E. coli* O157:H7-confirmed isolates. A PFGE and MLVA cluster is defined as isolates with indistinguishable PFGE and MLVA patterns.

Outbreak identification

In Alberta, the Medical Officer of Health is notified of each case of *E. coli* O157:H7 in the province, which prompts an investigation into the case by an Alberta

Figure 3

Core single nucleotide variant analysis, represented as a minimum spanning tree, of *Escherichia coli* O157:H7 sequences from isolates submitted to the Alberta Provincial Laboratory for Public Health, Canada, 14 July–17 September 2014 (n=140 sequences)



Outbreak A, B (including cluster-α), C, and sporadic isolates are indicated by specified colour. Numbers on lines indicate core SNVs differences between each adjacent node. The PFGE/MLVA patterns for outbreak B are indicated in the Table.

Health Services Environmental Health Officer who uses a standard questionnaire to identify potential sources of exposure. An outbreak investigation is initiated when a group of *E. coli* O157:H7 cases is identified with a common source or the aforementioned molecular typing criteria are met. ProvLab and public health officials are updated on the epidemiological and laboratory investigations through teleconferences, an online portal, and the distribution of line lists.

Confirmation of *Escherichia coli* O157:H7 identification

Tests performed on all isolates to identify and confirm the presumptive *E. coli* O157:H7 or to characterise the isolates further included: mauve colour colony on BBL CHROMagar O157 (Becton, Dickinson and Co., Mississauga, ON, Canada), haemolysis on blood agar, biochemical profiles using API20E (bioMérieux, Marcy l'Étoile, France), beta-glucuronidase expression, motility, sorbitol fermentation, citrate utilisation, malonate fermentation, tryptophanase production, serotyping using O157 direct antibody agglutination (BD Difco, Burlington, ON, Canada), and H7 antiserum (BD Difco) by tube flocculation.

Genomic DNA isolation for virulence factor detection

A single colony pick of *E. coli* O157:H7 grown overnight on 5% sheep blood agar (Dalynn Biologicals, Calgary, AB, Canada) at 35 to 37°C in ambient air was used for DNA extraction using the MagaZorb DNA Mini-Prep Kit (Promega Corporation, Madison, WI, US) on the KingFisher Magnetic Particle Processor (Thermo Scientific, Mississauga, ON, Canada). Purified DNA was used as template for the Stx typing and virulence gene dynamic array.

Shiga toxin typing

Detection of Stx genes, stx_1 and stx_2 was determined using a real-time multiplex polymerase chain reaction (PCR) assay consisting of two separate reactions run on a ABI Prism 7500FAST Sequence Detection System (Life Technologies, Inc., Burlington, ON, Canada) as previously described [9,10]. Conventional PCR was used to subtype stx_1 and stx_2 using primers from the World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* [11].

Virulence gene dynamic array

The BioMark real-time PCR system (Fluidigm, San Francisco, CA, US) was used for real-time PCR

amplification of 49 genetic markers using 48.48 dynamic arrays. Amplifications were performed using the dyes, 6-carboxyfluorescein (FAM)- and 6-carboxy-2', 4, 4', 5', 7, 7'-hexachlorofluorescein succinimidyl ester (HEX)-labelled TaqMan probes as previously described [12]. All amplification assays included both positive and negative controls. Primers and probes used in this study have been described previously and target genetic markers derived from different plasmids and chromosomal loci [12-16]. Genetic markers included genes coding for the O157 antigen (*rfbE*$_{O157}$), the flagellar antigen H7 (*fliC*$_{H7}$), the long polar fimbriae (*lpfA*$_{O157}$), the protein fimbriae of sorbitol-fermenting STEC O157 (*sfpA*), Stx 1 and 2 (*stx*$_1$ and *stx*$_2$), the pO157 markers (*ehxA*, *katP*, *espP*, *toxB*, *etpD*, *ecf1*), the heat-stable enterotoxin EAST1 (*astA*), adhesion factors (*eae*, *eae*-gamma, *iha*), effectors translocated by the type III secretion system derived from OI-122 (*pagC*, *ent/espL2*, *nleB*, *nleE*, *efa1*, *efa2*, *Z4331*), OI-50 (*espK*, *espN*, *espO1–1*, *espX7*), OI-71 (*ecs1822, espM1*, *nleF*, *nleA*, *nleH1–2*), OI-37 (*espX2*), OI-44 (*espV*), OI-108 (*espM2*, *espW*), OI-153 (*espY4–2*), OI-174 (*espX6*), bacterial tellurite resistance (*terE*), urease (*ureD*), as well as open reading frames of the OI-57 (*ecs1763*, *Z2096*, *Z2098*, *Z2099*, *Z2121*), clustered regularly interspaced short palindromic repeats (CRISPR)$_{O157}$-associated markers: SP_O157-A, SP_O157-B, and SP_O157-C, and the reference genetic marker for *E. coli* (*wecA*).

Genome sequencing and assembly

WGS of isolates was performed at the PHAC-NML Core Genomics facility. Sample libraries were prepared using Nextera XT library preparation kit (Illumina, Inc., San Diego, CA, US). Sequencing was performed on the Illumina MiSeq platform with the MiSeq Reagent Kit V2 to achieve an average genome coverage greater than 50x for all isolates. Raw sequence reads are available under National Center for Biotechnology Information (NCBI) Bioproject PRJNA291542.

Sequencing reads were de novo assembled into contigs using SPAdes [17] and annotated with Prokka [18]. SPAdes-assembled contigs smaller than 1 kb were removed from the analysis.

Core single nucleotide variant data pre-processing, quality control and data reduction

All read data available for each genome were processed using the following steps: (i) FFastq files were converted to Sanger quality encoding Fastq format, (ii) all Fastq files for each isolate were concatenated into one Fastq file per isolate. The concatenated Fastq files were next subjected to a quality control step using a custom Perl script that trims the reads up to a maximum of 10 bases on either end of the reads if the average base call quality in that region was below 25. Next, reads less than 36 bp in length and reads with an average base quality call below 25 were discarded from the analysis. Following the quality control step, all data for each isolate were reduced to a maximum of 200 x coverage (estimated based on the total bp length of the *E. coli* O157:H7 strain Sakai genome, including a 92,721-bp plasmid, pO157) by random selection of reads.

Core single nucleotide variant calling

Core genome analysis was performed using the PHAC-NML bioinformatics custom Single Nt Variant Phylogenomic pipeline (SNVPhyl) [19] consisting of open-source software and custom Perl scripts. Briefly, sequencing reads were mapped against the complete reference genome, *E. coli* O157:H7 strain Sakai using SMALT v.0.7.0.1 [20] with a *k*-mer size of 13, a step size 6, and a minimum alignment fraction of 0.5. Variants were called using FreeBayes v0.9.8 [21] with variant reporting for all variants, no complex variants, minimum mapping quality of 30, minimum base quality of 30, minimum alternate fraction of variant bases in agreement of 75%, and minimum coverage of 20 reads at every position in the reference sequence.

Core single nucleotide variant phylogeny

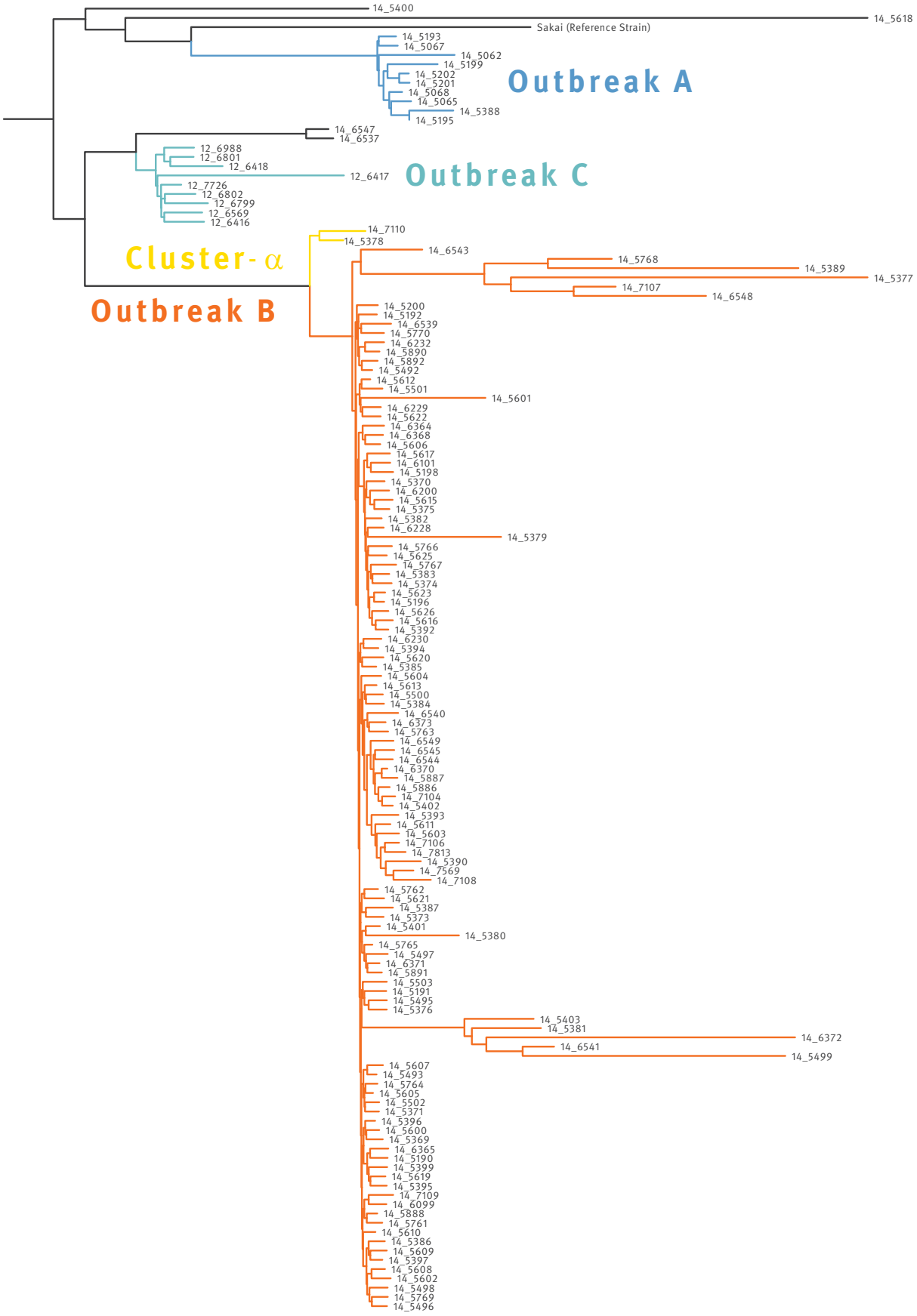
For each of the variant call format files created by FreeBayes, complex variant calls were split into single variant calls to create new variant call format files. All variant calls were merged into a single alignment file. SAMtools v 0.1.1.18 [22] was used to investigate positions where not every genome had a variant call. Positions where no variant was called with SAMtools mpileup and with at least a minimum coverage of 20 were assigned the reference base in the alignment. Positions where a variant was called with SAMtools mpileup and with a minimum coverage of 20 were excluded from the alignment. Other positions were excluded. The alignment files were used to generate a phylogenetic tree with PhyML v3.0 [23] using a generalised time reversible (GTR) model and the best of both nearest neighbour interchange (NNI) and Subtree Pruning and Regrafting (SPR) tree topology searching strategies. Horizontally transferred elements arising from recombination events were identified using Progressive Mauve [24], PHAge Search Tool (PHAST) [25] and Island Viewer [26]. These coordinates were then masked from the SNV PHYLogenomics (SNVPhyl) analysis as were repetitive regions identified using the nucmer programme in the Mummer sequence alignment package.

Core single nucleotide variant minimum spanning trees

The high-quality (hq) core SNV for each isolate, identified in the SNVPhyl pipeline and used to generate the core SNV phylogenies were also visualised using minimum spanning trees (MST) generated with the open source Phyloviz goeBurst algorithm [27]. Each unique set of hq core SNVs and corresponding isolates were assigned a unique identifier or sequence type, and a table of core SNV positions and the unique sequence types were entered into the Phyloviz goeBURST algorithm. Additional metadata, including outbreak event, MLVA and PFGE patterns were annotated onto the MST.

Figure 4

k-mer phylogeny, represented as a minimum spanning tree, of *Escherichia coli* O157:H7 isolates submitted to the Alberta Provincial Laboratory for Public Health, 14 July–17 September 2014 (n=140 isolates)



Outbreaks A, B (including cluster-α), and C are labelled by a specified colour. Sporadic and *E. coli* O157:H7 str. Sakai branches are in black. 14–6547 and 14–6537 (in black) are a cluster of two isolates unrelated to other isolates with exposure to ground beef.

k-mer clustering

The frequencies of all nt sequences of predefined lengths (*k*-mers) in the entire genome of each isolate was compared with the frequency of *k*-mers in all other isolates to determine a *k*-mer phylogeny tree. The alignment-free feature frequency profile (FFP) method [28] was used at a *k*-mer length of 25 nt and the SPAdes assembled contigs for each isolate. The optimal *k*-mer length was determined using the ffpvoacb utility part of the FFP package and the ffpreprof –e32 to determine the upper bound and the ffpvprof –f 2 to determine the lower bound. Phylogenetic trees were inferred from the resulting divergence distance matrix using the neighbour-joining method installed in the Phylip [29] package.

Results

Description of the outbreaks

From 14 July to 17 September 2014, 149 clinical isolates of *E. coli* O157:H7 were received by the Alberta ProvLab for molecular typing (Figure 1). Two outbreaks (designated as A and B) were identified in Alberta during this time period along with multiple sporadic isolates that were unrelated to either outbreak based on definitions involving epidemiological and PFGE/MLVA profiles. For comparison to an outbreak not temporally associated with outbreak A and B, a 2012 outbreak (outbreak C) was also included in this study.

The first outbreak (outbreak A), involved ten patients (four of whom developed HUS) with isolates collected from 14 to 22 July. Epidemiological investigations revealed a common exposure of visiting the same summer fair. There was one unique PFGE pattern combination and a total of three MLVA patterns among outbreak A isolates (Table and Figure 2). No food or environmental isolates were recovered in regards to this outbreak.

The second outbreak (outbreak B) was of particular interest because it was the largest in Alberta since PNC's inception in the year 2000. In 2014, as of 17 September, 182 clinical isolates of *E. coli* O157:H7 were collected and referred to the Alberta ProvLab, whereas the mean annual case number for the previous five years was 87.6 (95% confidence interval: +/–10.99). One hundred and eleven of these cases were associated with outbreak B during this time period, in which five developed HUS. Epidemiological investigations revealed a common exposure to contaminated pork products that were produced and distributed in Alberta [30]. This finding was confirmed by matching PFGE and MLVA profiles in human and food/environmental outbreak isolates (five food and one environmental isolates were received by Alberta ProvLab in regards to this outbreak).

PulseNet Canada routine surveillance using PFGE analysis revealed the presence of two concurrent clusters with closely related PFGE patterns (ECXAI.0023, ECBNI.0430 and ECXAI.0634, ECBNI.430) (Figure 2). As more isolates were received at the ProvLab, PFGE and MLVA subtyping revealed that the outbreak consisted of several isolates with variant, yet closely related PFGE and MLVA patterns (Figure 2).

The two most common PFGE/MLVA profiles were ECXAI.0023, ECBNI.0430/ 13_9_18_2_8_6_11_8 with 70 isolates and ECXAI.0634, ECBNI.430/ 13_9_19_2_8_6_11_8 with 14 isolates (Table). The most predominant difference in MLVA profiles was at the third locus, which varied from 17 to 20 repeats (Table). Further complicating the analysis were isolates collected from household members that were found to possess variant PFGE/MLVA profiles. For instance, isolates from one family with a HUS case all had the PFGE pattern ECXAI.0230, ECBNI.0430, however two MLVA patterns were observed, 13_9_19_2_8_6_11_8 (n=1) and 13_9_20_2_8_6_11_8 (n=2, including the HUS case). Another family with two infected members had their isolates collected on the same day with different PFGE/MLVA profiles (ECXAI.0230, ECBNI.0430/13_9_18_2_8_6_11_8 and ECXAI.0634, ECBNI.0430/ 13_9_19_2_8_6_11_8). No temporal associations were observed with the variant PFGE/MLVA profiles. There were also no distinct PFGE or MLVA patterns among HUS cases, which had the PFGE patterns ECXAI.0230 or ECXAI.0634, ECBNI.0430, and MLVA profile 13_9_19_2_8_6_11_8 except for one HUS isolate with the MLVA profile 13_9_20_2_8_6_11_8. Overall, 23 different PFGE and MLVA profiles and five clusters (two or more isolates with the same profile) were identified in outbreak B.

Outbreak B included a cluster of two isolates that was designated cluster-α. One isolate, 14–7110, recovered from a swab of a food tray at a distributor under investigation for a possible link with outbreak B was PFGE typed with an indistinguishable *Bln*I DNA restriction pattern as the primary outbreak B pattern (ECBNI.0430), but a variant, though closely related *Xba*I pattern (ECXAI.2098), and a variant MLVA pattern from the other outbreak B isolates (15_9_18_3_8_7_6_8) (Table and Figure 2). This isolate (14–7110) was collected one month after an isolate (14–5378) was recovered from an individual with an indistinguishable PFGE/MLVA profile. An epidemiological linkage between cluster-α and outbreak B was demonstrated in part by the recovery of an isolate (14–5369) with the most predominant PFGE/MLVA profile in outbreak B from an individual who dined at the same restaurant within three days of the individual from whom 14–5378 was isolated.

In 2012, there was a beef-associated *E. coli* O157:H7 outbreak with great social and economic impact involving 18 human-clinical cases across Canada and no cases of HUS (outbreak C) [31]. All human-clinical and beef isolates in this outbreak had one indistinguishable PFGE/MLVA profile (Table and Figure 2).

Table

Number of samples for each pulsed-field gel electrophoresis (PFGE) and multilocus variable-number tandem repeat analysis (MLVA) profile found in outbreaks A, B, (14 July–17 September 2014) and C (2012), Alberta, Canada (n=136)

Outbreak/ Type of isolate	*XbaI*[a]	*BlnI*[a]	MLVA	N	Location on core SNV tree[b]
A					
Clinical	1897	126	8_10_13_4_7_6_7_14	6	Main outbreak A cluster
	1897	126	8_10_14_4_7_6_7_14	2	3 SNV from main outbreak A cluster
	1897	126	8_10_14_4_6_6_7_14	2	1–2 SNV from main outbreak A cluster
B					
Clinical	23	430	13_9_18_2_8_6_11_8	70	Cluster 74 (n=59), cluster 23 (n=1), 1–2 SNV from cluster 74: 14–6228, 14–5502, 14–5769, 14–5384, 14–5503, 14–5374, 14–5392, 14–7108, 14–5623, 14–6099
	634	430	13_9_19_2_8_6_11_8	14	Cluster 23 (n=13), 1 SNV from cluster 23: 14-6232
	634	430	13_9_18_2_8_6_11_8	3	Cluster 74 (n=2), cluster 23 (n=1)
	634	1305	13_9_19_2_8_6_11_8	3	Cluster 23
	634	430	13_9_20_2_8_6_11_8	3	Cluster 23
	23	75	13_9_18_2_8_6_11_8	1	1 SNV from cluster 74: 14–5768
	23	310	13_9_18_2_8_6_11_8	1	1 SNV from cluster 74: 14–6372
	23	430	13_10_18_2_8_6_11_8	1	Cluster 74
	23	430	13_9_17_2_8_6_11_8	1	Cluster 74
	23	430	14_9_18_2_8_6_11_8	1	Cluster 74
	23	592	13_9_18_2_8_6_11_8	1	Cluster 74
	23	1302	13_9_18_2_8_6_11_8	1	1 SNV from cluster 74: 14–5377
	597	430	13_9_18_2_8_6_11_8	1	Cluster 74
	634	430	13_9_17_2_8_6_11_8	1	Cluster 74
	634	430	13_9_18_2_8_6_12_8	1	Cluster 74
	634	430	13_9_19_2_8_6_0_9.5	1	1SNV from cluster 23: 14–5389
	634	430	13_9_19_2_8_6_12_8	1	Cluster 23
	2113	1300	13_9_18_2_8_6_11_8	1	Cluster 74
	2592	735	13_9_18_2_8_6_11_8	1	Cluster 74
	3106	406	13_9_18_2_8_6_11_8	1	1 SNV from cluster 74: 14–5370
	3112	430	13_9_18_2_8_6_11_8	1	Cluster 74
	1921	430	13_9_19_2_8_6_11_8	1	1 SNV from cluster 23: 14–7107
	2098	430	15_9_18_3_8_7_6_8	1	Cluster-α
Pork	23	430	13_9_18_2_8_6_11_8	4	Cluster 74 (n=3), 1 SNV from cluster 74: 14–6364
	634	430	13_9_19_2_8_6_11_8	1	Cluster 23
Environmental	2098	430	15_9_18_3_8_7_6_8	1	Cluster-α
C					
Clinical	1	12	8_10_13_7_7_6_3_7	7	Outbreak C cluster
Beef	1	12	8_10_13_7_7_6_3_7	2	Outbreak C cluster

MLVA: multilocus variable-number tandem repeat analysis; PFGE: pulsed-field gel electrophoresis; SNV: single nt variant.

The MLVA and PFGE profiles for the food isolates (pork or beef) and the one environmental isolate from outbreak B (14–7110) are shown separately from the clinical clusters.

[a] PFGE pattern designation without the preceding zero (s).

[b] Location of the PFGE/MVA profiles in the core SNV minimum spanning tree (Figure 3). Of the 111 clinical, five food and one environmental outbreak B-associated isolates sequenced, there were two major clusters in the core SNV minimum spanning tree, one with 74 isolates (cluster 74) and the other with 23 isolates (cluster 23), whereby no core SNV differences between the isolates occurred within the clusters. Outbreak B isolates that were not part of a core SNV cluster, but differed by 1–2 core SNVs from a core SNV cluster are designated as 1 or 2 SNV from cluster 74 or 1 SNV from cluster 23 with the isolate number(s). Cluster 74 included three pork isolates (14–6540, 14–6549 and 14–7569) and 71 clinical isolates with the following laboratory accession numbers: 14–5190 to 91, 14–5196, 14–5369, 14–5371, 14–5373, 14–5375 to 76, 14–5379 to 83, 14–5385 to 87, 14–5393 to 5399, 14–5401 to 03, 14–5493, 14–5495 to 500, 14–5600, 14–5603 to 07, 14–5609 to 11, 14–5613, 14–5615 and 16, 14–5619 to 21, 14–5625 to 26, 14–5761 to 67, 14–5886 to 88, 14–5891, 14–6200, 14–6370 to 71, 14–6373, 14–6541, 14–6543–45, 14–6548, 14–7104, 14–7109. Cluster 23 included one pork isolate (14–6539) and 22 clinical isolates: 14–5192, 14–5198, 14–5200, 14–5390, 14–5492, 14–5501, 14–5601 to 02, 14–5608, 14–5612, 14–5617, 14–5622, 14–5770, 14–5890, 14–5892, 14–6101, 14–6229 to 30, 14–6365, 14–6368, 14–7106, 14–7813.

Whole genome sequencing

The genetic relationships among outbreak-associated isolates were also determined by WGS using the PHAC-NML bioinformatics SNVphyl pipeline and by *k*-mer tree analysis. All aforementioned clinical, food, and environmental isolates were sequenced for each outbreak. For outbreak C, isolates from eight respective human cases that had occurred in Alberta and two food isolates were available for sequencing. One clinical isolate was excluded because it did not meet the sequence quality threshold. The sporadic isolates (no epidemiological or PFGE/MLVA link to an outbreak) that were sequenced included a cluster of two isolates unrelated to any outbreak with suspected exposure to ground beef (14–6537 and 6547), one isolate (14–5400) that came from an individual who worked with pork and one isolate (14–5618) that was randomly chosen.

Core single nucleotide variant phylogenetic analysis

The interrogation of hq core SNVs revealed that clinical human isolates within an outbreak event varied by 0–5 SNVs from one another other (0–5 SNVs for outbreak A and 0–5 SNVs for outbreak B) (Figure 3). Food/environmental isolates from outbreak B also clustered within 0–5 SNVs from the clinical human isolates. Outbreak A and outbreak B isolates clustered into distinct and well-defined branches, separated by a distance of 231–257 SNVs (Figure 3). Both outbreaks also clustered away from the sporadic isolates and the reference strain, *E. coli* O157:H7 str. Sakai.

Within outbreak B, the major PFGE/MLVA clusters ECXAI.0634, ECBNI.0430/13_9_19_2_8_6_11_8 and ECXAI.0023, ECBNI.0430/ 13_9_18_2_8_6_11_8 were separated by 2–4 SNV (Table and Figure 3). The HUS-associated isolates from outbreak B clustered with the other outbreak isolates with four in cluster 74 and one in cluster 23 (Figure 3). Upon further examination of the core SNV phylogenetic trees, the two isolates composing cluster-α were separated by 22–27 hq core SNVs from all other outbreak B isolates and were distanced by only one core SNV from each other (Figure 3). The isolate that was not part of cluster-α, but isolated from a diner at the same restaurant (14–5369) was found in the cluster of 74 isolates.

Performing core SNV analysis on the seven Alberta clinical human isolates involved in outbreak C (the 2012 beef-related outbreak) and two food isolates, revealed no core SNV differences between these nine outbreak-associated isolates. Moreover, this method differentiated the branch corresponding to the outbreak C isolates from the outbreak B branch by a distance of 157–161 core SNVs and outbreak A by 74–77 core SNVs

Several sporadic isolates were identified concurrent with outbreaks A and B, and possessed variant PFGE/MLVA profiles. These isolates have a minimum genetic distance of 57 hq core SNVs from all three outbreak clusters. Notably, one sporadic case (14–5400) recovered from an individual who worked directly with pork had a similar, but distinguishable *Bln*I pattern to outbreak A and a distinct MLVA profile (ECXAI.3108, ECBNI.0181 /9_10_12_7_7_6_3_6) (denoted as Sporadic I in Figure 2). Core SNV phylogenetic analysis demonstrated that this isolate was distant from outbreak B isolates by a high number of core SNVs, 214–237.

k-mer clustering

The *k*-mer method also delineated outbreak A from outbreak B with each group of outbreak isolates located on distantly related nodes (Figure 4). There were isolates found within the outbreak B node (orange in Figure 4) with greater horizontal branch distances than the average branch lengths for outbreak B isolates, demonstrating additional resolution of certain isolates from other outbreak B isolates. These more distant isolates included ones with the most and least frequently observed MLVA and PFGE profiles. Cluster-α was also distinguished from the main outbreak B branch and was identified in a separate node, but originated from the same node as the outbreak B branch and thus, share a most recent common ancestor (Figure 4). In concordance with core SNV analysis, the isolate that was not part of cluster-α, but isolated from a diner at the same restaurant (14–5369) was not differentiated from other outbreak B isolates by *k*-mer clustering. *k*-mer analysis also did not indicate a close relationship between sporadic isolates and outbreak-associated isolates.

Shiga toxin genes typing

The presence of stx_1 and stx_2 was determined for all isolates received during the sampling period. For stx_1 and stx_2 subtyping, 36 isolates were selected from the 2014 sampling period comprising of outbreak-related food isolates (n = 3), HUS-associated isolates (n = 2 for outbreak A and n = 5 for outbreak B), all isolates from household members of HUS cases (based on address) (n = 4, all outbreak B), both the environmental and clinical isolate from cluster-α, a representative isolate for each outbreak B variant PFGE and MLVA profile not covered by other selection criteria (n = 19), and one isolate (14–6547) from the sporadic cluster of two individuals. In addition, all clinical and food isolates from outbreak C were subtyped (n = 10). All outbreak A isolates (n = 3) were stx_1 negative and stx_2 positive (subtype stx_{2a}). Most outbreak B isolates (27 of 31), including HUS-associated isolates were positive for both *stx* genes and subtyped as stx_{1a} and $stx_{2a.}$ The clinical human isolate from cluster-α (14–5378) tested positive for stx_{1a}, stx_{2a}*and* stx_{2c} and the environmental isolate in this cluster (14–7110) was stx_{1a} and stx_2 positive, but no subtype was identified for stx_2. The other variant *stx* subtypes in outbreak B included two clinical isolates, one stx_{1a}/stx_2 untypeable and one stx_1 negative $/stx_2$ untypeable. All outbreak C isolates and isolate 14-6547 from the sporadic cluster were stx_{1a} and stx_{2a} positive.

Virulence gene profiling

Virulence gene profiling was performed on all isolates (n = 155 received by ProvLab for molecular typing from

14 July to 17 September 2014. Outbreak A, outbreak B and sporadic isolates were indistinguishable based on gene profiling of 49 STEC virulence genes. All isolates were negative for the pilin subunit gene found in sorbitol fermenting STEC, *sfpA* and all but six isolates were positive for all other genes tested. Two outbreak B isolates, 14–6543 and 14–5377, tested negative for the putative proteins ZZ2096 and Z2098. In the *k*-mer analysis result, these isolates clustered within outbreak B, but both were distinguishable from each other and all other outbreak B isolates (Figure 4). A single outbreak B isolate, 14–5380, tested negative for the secreted effector proteins, *espK*, *espN*, *espX7*, and *esp*O1–1 and was found to be related to outbreak B, but distinguishable from all other outbreak B isolates by *k*-mer analysis (Figure 4). All of the outbreak B isolates with genes not detected on the virulence gene array were indistinguishable from other outbreak B isolates using SNV analysis. The other three isolates that typed negative for virulence genes were sporadic isolates with the first testing negative for *espX7*, the second negative for *espP*, and the third testing negative for *efa1, efa2*, *ent, nleB, nleE, pagC,* and Z4331. None of the HUS cases were negative for any virulence genes other than *sfpA*.

Discussion

Technologies such as high throughput screening of virulence genes [32,33] and WGS have the potential to be used for early outbreak detection and characterisation. To be used as such, these methods must be compared with current international standards of bacterial typing. For *E. coli* O157:H7, the currently validated and widely employed standard typing methods are MLVA and PFGE. Furthermore, a bacterial typing method must have the following characteristics: accuracy, inter and intra-laboratory reproducibility (including with multiple passaging of isolates), high discriminatory power, concordance with epidemiological data, rapid and ease-of use, cost effectiveness, and amenability to computerised analysis [34]. This work demonstrates that for typing of *E. coli* O157:H7, WGS is a suitable typing method already meeting many of these criteria, but not virulence gene profiling involving the genetic targets used in this study.

No virulence factors tested in this work could reliably distinguish between outbreak and non-outbreak strains. The high prevalence of the genetic markers used in this study in *E. coli* O157:H7 (reference 13 and this study), prevents the use of any assay that detects the presence or absence of these genes for the purpose of accurate discrimination between isolates. Shiga toxin subtyping was able to differentiate isolates only in cases where the subtypes differed from the most prevalent subtypes (stx_{1a} and stx_{2a}), a result similarly observed in an analysis of Albertan *E. coli* O157:H7 isolates collected from 2004 to 2012 [15].

Phylogenetic analysis using whole genome or core SNVs derived by a variety of methods is the most frequently published method to determine relatedness between isolates of *E. coli* or other Enterobacteriaceae such as *Salmonella* species [33-38]. To the best of our knowledge, this is the first example of a real-time, large-scale study comparing virulence gene profiling, hq core SNV or *k*-mer analysis to MLVA and PFGE profiling.

Using different bioinformatics pipelines and groups of *E. coli* isolates, four previously published studies have demonstrated the ability of WGS to discriminate between isolates of *E. coli* [35-38]. Core SNV phylogeny cannot only differentiate STEC isolates from other STEC isolates, but also uropathogenic *E. coli* from one another [35]. Two other studies have used MLVA as the 'standard' reference method and demonstrated core SNV phylogenies to be equivalent or better than MLVA at identifying outbreaks [36,37]. One of these two studies used *E. coli* O157:H7 isolates from the United Kingdom and identified outbreaks using core SNV analysis that were missed by epidemiological investigations and MLVA analysis [37]. Another study analysing isolates from a beef-associated outbreak of *E. coli* O157:H7 in Denmark demonstrated that core SNV phylogenetic analysis and nt-distance based trees (built using 17-base *k*-mers) methods were each capable of differentiating between outbreak isolates and concurrently occurring, non-outbreak sporadic *E. coli* O157:H7 and non-O157 STEC isolates. In the Danish study, PFGE was also performed on selected isolates, but its utility for outbreak detection was not compared with the two types of WGS approaches [38]. The study herein adds to this knowledge by demonstrating that core SNV or *k*-mer phylogenies alone are concordant with combined PFGE and MLVA data when used to differentiate between concurrently occurring outbreak and non-outbreak isolates of *E. coli* O157:H7.

k-mer analysis showed concordance with PFGE and MLVA profiling while advantageously revealing increased discriminatory power when compared with PFGE and MLVA profiling or core SNV. Feature frequency profiling generates *k*-mers-based profiles using the entire genomic sequence of test organisms to determine relatedness, whereas core SNV analysis uses only the 'conserved' portion of the genome; therefore one may hypothesise that *k*-mer analysis would provide additional discriminatory power. With this increased discriminatory power, there is the concomitant risk that *k*-mer analysis may be too discriminatory by including not only 'core' conserved features, but also genetic elements resulting from horizontal gene transfer. These elements can be easily lost and gained as isolates undergo natural and passaging in the laboratory. Therefore, the inclusion of these elements in the analysis has the potential to mask core phylogenetic inference, but no evidence of this was observed in our study.

Cluster-α consisted of two isolates that differed from all other outbreak B isolates by at least 22 SNV, but differed from each other by only one core SNV. This cluster was included in outbreak B based on PFGE and epidemiological links (an outbreak B implicated restaurant),

but was differentiated from other outbreak B isolates by MLVA, *k*-mers and core SNV analysis. Furthermore, the stx_2 subtype for these two isolates differed from each other and the other subtyped outbreak B isolates. This cluster likely represents different strains contributing to the outbreak. Similar observations have been made previously in our laboratory as well as by Gilmour et al. [39]. These observations emphasise the need to consider careful correlation of epidemiological together with molecular subtyping data.

The core SNV genetic distances observed between cluster-α and outbreak B exemplifies the utility of predefining a SNV threshold for isolate relatedness. Cluster-α differed from outbreak B by 22–27 SNVs and other outbreak B isolates differed by 0–5 SNVs whereas outbreak-unrelated, sporadic isolates differed by ≥ 58 SNVs. Despite using different SNV calling and phylogeny methods, other studies have also identified ≤ 5 SNVs as a potential threshold for genetic relatedness among *E. coli* O157 [36,37]. In one study of extended-spectrum beta-lactamase-*E. coli* outbreak-associated isolates, 0 SNVs were identified between outbreak-associated isolates [35]. It is more difficult to assign a numerical 'threshold' or pattern for relatedness with *k*-mer analysis, which produces trees based on a distance matrix generated from *k*-mer profiles and their presence or absence. Unlike MLVA and/or PFGE using PulseNet standardised methods, both *k*-mer and core SNV methods do not generate a numerical 'barcode' for the organisms, which makes it difficult to compare isolates from different laboratories. Therefore, a large shared and curated database will likely be required to generate cluster identifiers that could act as the organism's 'barcode'.

Currently, PulseNet networks worldwide conduct timely surveillance and facilitate outbreak detection. For WGS to be used in this manner, standardised protocols, quality validation metrics and a robust method to determine isolate relatedness will need to be established. Published reports use different sequencing platforms and bioinformatics pipelines to assemble and analyse WGS data, prohibiting direct comparisons between studies. Head-to-head comparison of these different methods will help determine the appropriate standard(s). The inter-laboratory comparison of WGS data also requires communal database of isolates, which requires adequate computing infrastructure and secure electronic networks capable of transmitting large datasets. Other considerations encompass the ethical, legal or political barriers to sharing complete genomics data between various health authorities.

In conclusion, WGS holds significant potential to replace current gold-standard typing methods such as PFGE for the routine surveillance and detection of enteric outbreaks. This shift will be driven by the advantages offered by WGS such as increased discriminatory power and genetic resolution. However, before this technology can be widely implemented, certain barriers remain to be addressed such as initial capital expense, computing infrastructure, and validated automated, user-friendly WGS analysis software. Moreover, quality metrics and standardised protocols including standardised definitions for isolate relatedness are required before routine application of WGS within public health laboratories.

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Conflict of interest

None declared.

Authors' contributions

All authors reviewed the manuscript drafts and approved the final version. BMB designed the study, performed Shiga toxin subtyping and genomic DNA isolation for virulence gene profiling, analysed the data, prepared the figures and wrote the manuscript. TP and CB performed comparative genomics, the phylogenetic and minimum spanning tree assembly. CB contributed writing to sections of the manuscript. SD and PF designed the virulence gene profile, performed the assays and revised the manuscript. VL prepared the PFGE figure and revised the manuscript. CN and LT Performed PFGE pattern designation and MLVA analysis. LH reviewed the epidemiological data and revised the manuscript. ML reviewed and revised the manuscript. LC designed the study, coordinated data collection, analysed the data and wrote the manuscript.

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International travel and acquisition of multidrug-resistant *Enterobacteriaceae*: a systematic review

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International travel is considered to be an important risk factor for acquisition of multidrug-resistant *Enterobacteriaceae* (MRE). The aim of this systematic review was to determine the effect of international travel on the risk of post-travel faecal carriage of MRE. Secondary outcomes were risk factors for acquisition of MRE. A systematic search for relevant literature in seven international databases was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Articles needed to report on (i) foreign travel, (ii) screening of asymptomatic participants, (iii) antimicrobial susceptibility data and (iv) faecal *Enterobacteriaceae* carriage. Two researchers independently screened the abstracts, assessed the full article texts for eligibility and selected or rejected them for inclusion in the systematic review. In case of disagreement, a third researcher decided on inclusion. Eleven studies were identified. In all studies, a high prevalence (> 20%) of carriage of MRE after international travel was found. The highest prevalence was observed in travellers returning from southern Asia. Foreign travel was associated with an increased risk of carriage of MRE. Further research is needed to assess if this leads to an increase in the number of infections with MRE. Systematic review registration number: PROSPERO CRD42015024973.

Introduction

Rationale

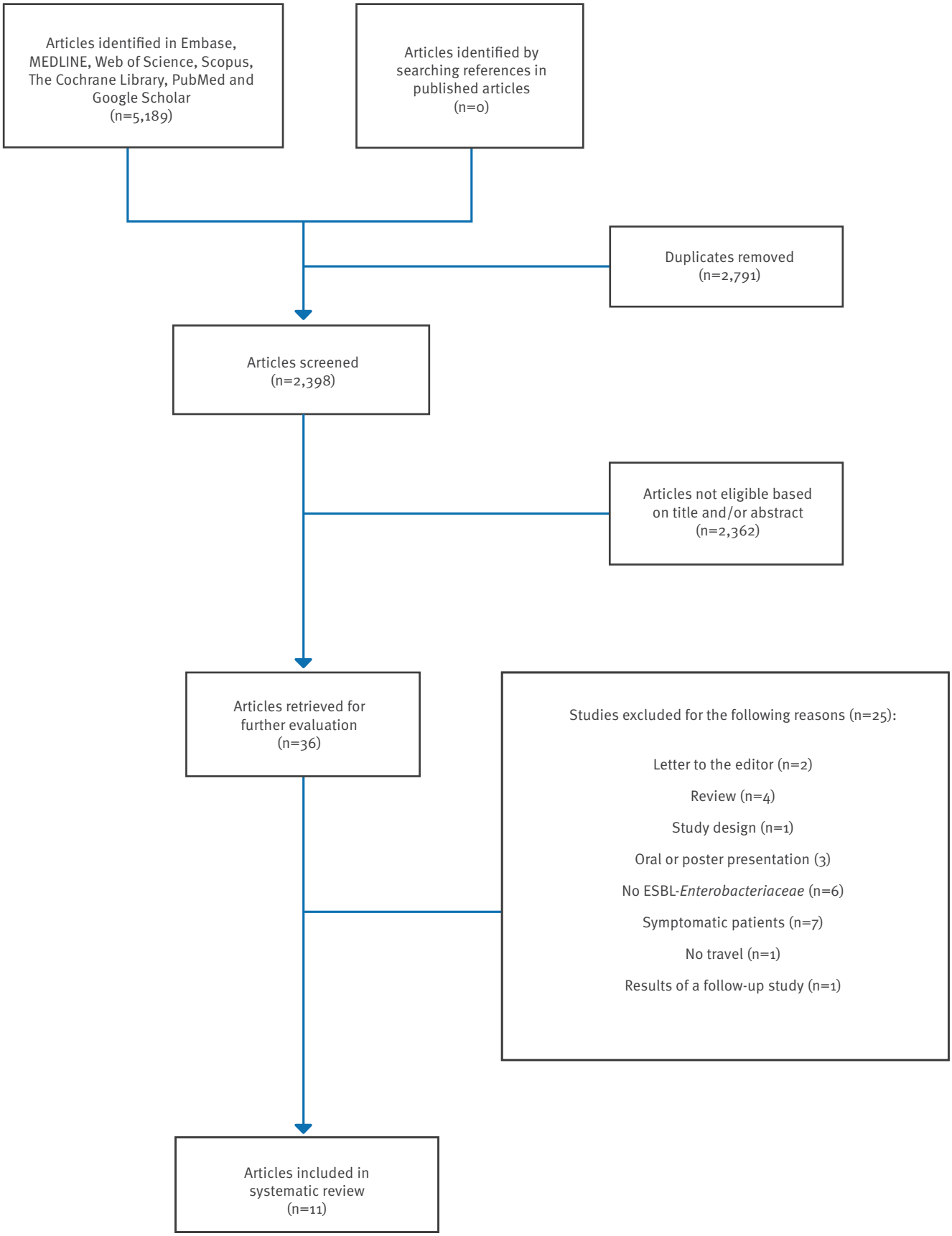
Worldwide, the number of international travellers has grown from 25 million in 1950 to 1,087 million in 2013 [1]. According to the World Tourism Organization, this number is expected to increase by an average of 3.3% a year [1]. Of the international travellers visiting developing countries, 22–64% have self-reported health problems and about 8% require medical care during or after travel [2,3]. Healthy travellers may be exposed to a broad range of microorganisms while travelling, including drug-resistant *Enterobacteriaceae,* which may subsequently be introduced into their home country [4,5].

Enterobacteriaceae are Gram-negative bacteria that are part of the human body's normal commensal flora, called microbiota. *Enterobacteriaceae*, such as *Escherichia coli* and *Klebsiella* species, are capable of causing both healthcare-associated and community-acquired infections [6]. Multidrug-resistant *Enterobacteriaceae* (MRE), including extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) and plasmid-mediated Amp C-producing *Enterobacteriaceae* (pAmp C-E) are emerging worldwide [7]. Cases of carbapenemase-producing *Enterobacteriaceae* (CPE) are also reported more frequently [8].

Since 2003, community carriage rates of MRE have increased dramatically in various regions, such as South-East Asia, the Western Pacific and the Eastern Mediterranean [7]. During visits to such areas, travellers might acquire MRE and become asymptomatic carriers of MRE. In their home country, they may cause spread in the community and contribute to worldwide emerging antimicrobial resistance [6,9,10]. Acquired MRE in the digestive tract are considered apathogenic, however carriage of such *Enterobacteriaceae* have resulted in clinically relevant infections [8]. International travel has been reported as a risk factor for urinary tract infections caused by ESBL-E [11,12]. The question arises if these observations warrant clinicians being aware of MRE in recently returned otherwise healthy, international travellers who seek medical attention even for unrelated conditions.

Figure

Flowchart for literature search on the acquisition of multidrug-resistant *Enterobacteriaceae* in international travel (n = 4,989)



ESBL: extended-spectrum beta-lactamase.

Box

Search strategy for systematic review of the acquisition of multidrug-resistant *Enterobacteriaceae* in international travel

Embase.com ('Gram negative bacterium'/exp OR 'Gram negative infection'/de OR Enterobacteriaceae/de OR Escherichia/exp OR Klebsiella/exp OR Salmonella/exp OR Shigella/exp OR Yersinia/exp OR 'Enterobacteriaceae infection'/exp OR ('Gram negative' OR Enterobacteri* OR (Enter* NEXT/1 bacteria*) OR Enterobacter* OR Escherichia* OR 'e coli' OR Klebsiella* OR Salmonell* OR Shigell* OR Yersinia*):ab,ti) AND (travel/de OR 'traveller diarrhoea'/de OR aviation/exp OR (travel* OR touris* OR turista OR aviation OR 'air transport' OR airport*):ab,ti) AND ('antibiotic resistance'/exp OR 'multidrug resistance'/de OR 'drug resistance'/de OR 'antibiotic sensitivity'/de OR 'bacterial colonization'/exp OR 'bacterium carrier'/de OR (resistan* OR coloni* OR ((antibiotic* OR antimicrob*) NEAR/3 sensitivit*) OR susceptib* OR carriage* OR carrier*):ab,ti) NOT ([animals]/lim NOT [humans]/lim)

MEDLINE (OvidSP) (exp "Gram-Negative Bacteria"/ OR exp "Gram-Negative Bacterial Infections"/ OR Enterobacteriaceae/ OR exp Escherichia/ OR exp Klebsiella/ OR exp Salmonella/ OR exp Shigella/ OR exp Yersinia/ OR exp "Enterobacteriaceae infections"/ OR ("Gram negative" OR Enterobacteri* OR (Enter* ADJ bacteria*) OR Enterobacter* OR Escherichia* OR "e coli" OR Klebsiella* OR Salmonell* OR Shigell* OR Yersinia*).ab,ti.) AND (travel/ OR "Travel Medicine"/ OR exp aviation/ OR (travel* OR touris* OR turista OR aviation OR "air transport" OR airport*).ab,ti.) AND (exp "Drug Resistance, Microbial"/ OR exp "Drug Resistance, Multiple"/ OR "drug resistance"/ OR "bacterium carrier"/ OR (resistan* OR coloni* OR ((antibiotic* OR antimicrob*) ADJ3 sensitivit*) OR susceptib* OR carriage* OR carrier*).ab,ti.) NOT (exp animals/ NOT humans/)

Cochrane Library (('Gram negative' OR Enterobacteri* OR (Enter* NEXT/1 bacteria*) OR Enterobacter* OR Escherichia* OR 'e coli' OR Klebsiella* OR Salmonell* OR Shigell* OR Yersinia*):ab,ti) AND ((travel* OR touris* OR turista OR aviation OR 'air transport' OR airport*):ab,ti) AND ((resistan* OR coloni* OR ((antibiotic* OR antimicrob*) NEAR/3 sensitivit*) OR susceptib* OR carriage* OR carrier*):ab,ti)

Web of Science TS=((("Gram negative" OR Enterobacteri* OR (Enter* NEAR/1 bacteria*) OR Enterobacter* OR Escherichia* OR "e coli" OR Klebsiella* OR Salmonell* OR Shigell* OR Yersinia*)) AND ((travel* OR touris* OR turista OR aviation OR "air transport" OR airport*)) AND ((resistan* OR coloni* OR ((antibiotic* OR antimicrob*) NEAR/3 sensitivit*) OR susceptib* OR carriage* OR carrier*)))

Scopus TITLE-ABS-KEY((("Gram negative" OR Enterobacteri* OR (Enter* W/1 bacteria*) OR Enterobacter* OR Escherichia* OR "e coli" OR Klebsiella* OR Salmonell* OR Shigell* OR Yersinia*)) AND ((travel* OR touris* OR turista OR aviation OR "air transport" OR airport*)) AND ((resistan* OR coloni* OR ((antibiotic* OR antimicrob*) W/3 sensitivit*) OR susceptib* OR carriage* OR carrier*)))

PubMed ((Gram negative[tiab] OR Enterobacteri*[tiab] OR Entero bacteria*[tiab] OR Enteric bacteria*[tiab] OR Enterobacter*[tiab] OR Escherichia*[tiab] OR e coli[tiab] OR Klebsiella*[tiab] OR Salmonell*[tiab] OR Shigell*[tiab] OR Yersinia*[tiab])) AND ((travel*[tiab] OR touris*[tiab] OR turista[tiab] OR aviation[tiab] OR air transport*[tiab] OR airport*[tiab])) AND ((resistan*[tiab] OR coloni*[tiab] OR ((antibiotic*[tiab] OR antimicrob*[tiab]) AND sensitivit*[tiab]) OR susceptib*[tiab] OR carriage*[tiab] OR carrier*[tiab])) AND publisher[sb]

Google Scholar "Gram negative"|Enterobacteriaceae|Escherichia|Klebsiella|Salmonella|Shigella|Yersinia travel|traveller|tourist|tourism resistance|resistant|colonization|colonisation|susceptibility|carriage|carrier

Objectives

The aim of this systematic review was to determine the effect of international travel on the risk of acquisition of faecal carriage of MRE. A secondary objective was to determine risk factors for acquisition of drug resistance.

Methods

Protocol and registration

A specific protocol was designed and used to conduct the study. The study is registered in the international prospective register of systematic reviews (PROSPERO) under registration number CRD42015024973.

Search strategy and selection criteria

The systematic review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13]. The following databases were searched, attempting to identify all relevant studies: Embase, MEDLINE, Web of Science, Scopus, Cochrane Library, PubMed and Google Scholar. The latest search was conducted on 17 August 2015.

The topic search terms used for searching the databases were 'Gram negative bacteria', 'Gram negative bacterial infections', 'Enterobacteriaceae', 'Escherichia', 'Klebsiella', 'Campylobacter', 'Salmonella', 'Shigella', 'Yersinia', 'travel', 'traveller', 'tourist', 'tourism', 'turista', 'aviation', 'air transport', 'airport', 'colonisation', 'carriage', 'carrier', 'susceptibility' and '(multiple) drug resistance'.

The queries differed per database searched and were developed with help of a biomedical information specialist (Box). Articles written in English, German, French and Dutch were included.

For inclusion the article needed to fulfil the following criteria [1]: It needed to be related to foreign travel [2], report on screening in asymptomatic participants [3], present antimicrobial susceptibility data and [4] report on faecal *Enterobacteriaceae* carriage. We used the following exclusion criteria: case reports, reviews, meta-analyses, veterinary medicine, in vitro studies and studies regarding symptomatic patients. The reference lists of reviews were screened to identify studies possibly missed by the search.

Two researchers (R.H. and J.A.) independently performed the screening of the abstracts. Any discordant result was discussed in consensus meetings. After

screening the abstracts, the full text of the articles was assessed for eligibility by the same two researchers and selected or rejected for inclusion in the systematic review. In case of disagreement a third researcher (A.V.) decided on inclusion.

Data collection process

The following data (if available) were extracted from each article: year of publication, country of the study, study period, study design, microorganism studied, study population, study size, age, sex, sample time before and after travel, duration of travel, travelling in pairs or groups, symptoms during travel, countries visited, MRE prevalence before travel, MRE prevalence after travel, MRE resistance acquired during travel, resistance to other antibiotic drugs of acquired MRE, risk factors for acquisition (among which travel to predefined United Nations geographical regions: southern Asia, Asia except southern Asia, Africa, South and Central America, North America, Europe and Oceania [14]), method of MRE susceptibility determination, phenotypic approaches, genotypic characterisation of post-travel MRE isolates, molecular typing of post-travel MRE isolates, duration of MRE colonisation and MRE transmission to household contacts. To obtain missing data, authors of the articles were contacted.

Quality assessment

We assessed the methodological quality and the risk of bias in individual studies that may affect the cumulative evidence, using tools for assessing quality and susceptibility to bias in observational studies as recommended in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement [15,16].

Data synthesis and analysis

As a result of the design of the studies (cohort studies) and the heterogeneity in patient populations (e.g. travellers, healthcare workers and healthcare students), a formal meta-analysis was not possible. Therefore, the study results were summarised to describe the main outcomes of interest. The principle summary measure was percentage of MRE acquisition during travel, defined as ESBL-E or pAmp C-E. Furthermore, risk factors for acquisition of drug resistance were assessed. If possible, percentages not presented in the articles were calculated from the available data.

Results

Study selection

A total of 2,398 studies were identified through database searching after duplicates had been removed (Figure). After screening of titles and summaries, 36 articles were selected for full-text assessment. Eleven articles were included in the qualitative synthesis of the systematic review (see Figure for reasons for exclusion) [17-27].

Study characteristics

Eleven prospective cohort studies, conducted in northern and western Europe, Australia and the United States (US) were included [17-27]. The characteristics of these studies are shown in Table 1. Nine studies investigated travellers visiting a travel or vaccination clinic, one study hospital staff and contacts, and one study healthcare students working or studying abroad. The number of study participants ranged from 28 to 574. The median age of travellers in the individual studies varied between 25 and 66 years, with the youngest group being healthcare students. In all studies, the majority of travellers were female (range: 55–78%). The proportion of participants who were lost to follow up varied from 3.8% (4/106) [18] to 30% (12/40) [21]. The mean duration of travel was similar in all studies (14–21 days). In the study by Angelin et al. on healthcare students, median length of stay was 45 days (range: 13–365 days) [22]. In four studies, follow-up samples of MRE carriers were collected at six months after returning from travel, and in one of these studies, samples were collected monthly in the first three months with further follow-up until 12 months after return [25]. Ten studies used a phenotypic method for susceptibility testing, with genotypic confirmation of ESBL positivity by PCR [17-22,24-27]. One study used a PCR-based approach [23]. In one study, only isolated *E. coli* were included, whereas the other studies included all isolated *Enterobacteriaceae*, which mainly consisted of *E. coli* [17-27].

Acquisition of multidrug-resistant *Enterobacteriaceae*

Faecal carriage of MRE varied from 1 to 12% before travel and acquisition of MRE from 21% to 51% (Table 2 [17-21,23-27].

In the study by Kuenzli et al. on travellers to the Indian subcontinent only, a much higher MRE acquisition rate of 69% was demonstrated [26]. The risk of acquisition of MRE varied with the geographical region (Table 3) [17-21,23-27]. Travel to southern Asia posed the highest risk (range: 29–88%), followed by other Asian countries (18–67%) and Northern Africa (range: 31–57%). Acquisition of MRE after travelling to sub-Saharan Africa (range: 0–49%) or South and Central America (range: 0–33%) was less frequent, and three studies did not observe any acquisition of MRE after travel to South or Central America (Table 3). Acquisition of MRE after travel to North America, Europe and Oceania was rare. Results of the genotypic characterisation of MRE isolated after travel are presented in Table 2, the majority of the genes belonged to the CTX-M type.

Risk factors for acquisition of multidrug-resistant *Enterobacteriaceae*

Besides travel destinations, other risk factors for acquiring MRE were age, use of antibiotics during travel (beta-lactam use) and gastroenteritis or other gastrointestinal symptoms (Table 2). The study of Kantele et al., designed to study these risk factors as primary

Table 1

Characteristics of prospective cohort studies included for systematic review of the acquisition of multidrug-resistant *Enterobacteriaceae* in international travel (n = 11)

Study	Country	Study period	Population characteristics	Study sizea	Median age in years (range or SD)	Proportion of women in %	Identification of MRE-positive organisms in post-travel isolates	Sample method used	Sample time (range) before/after travel	Mean duration of travel in days (range)	Total number of co-travellers participating in study	Follow-up of resistant isolates
Tängdén [17]	Sweden	November 2007–31 January 2009	Travel clinic	100	43 (2–84)	55	*Enterobacteriaceae* 100% (24/24) *E.coli*	Stool	Unknown	14 (1–26)	23	6 months
Kennedy [18]	Australia	January 2008–April 2009	Hospital staff and contacts	102	45 (17–77)	62	*E.coli*	Rectal or perianal swab	Within 2 weeks before and after	21 (9–135)	Unknown	6 months
Östholm-Balkhed [19]	Sweden	September 2008–April 2009	Vaccination clinic	231	54 (18–76)	59	*Enterobacteriaceae* 90% (104/116) *E.coli*[b]	Stool sample	15 (1–114) days/ 3 (0–191) days	16 (4–119)	Unknown	None
Kantele [20]	Finland	March 2009–February 2010	Travel clinic	430	40 (0–77)	61	*Enterobacteriaceae* 97% (94/97) *E. coli*[b]	Stool sample	Before and first (or second) stool after	19 (4–133)	83	None
Weisenberg [21]	United States	July 2009–February 2010	Travel clinic	28	66 (41–83)	68	*Enterobacteriaceae* 100% (7/7) *E. coli*[b]	Stool sample	1 week before/ 1 week after	16 (8–24)	Unknown	None
Angelin [22]	Sweden	April 2010–January 2014	Healthcare students	99	25 (15–20)	78	*Enterobacteriaceae* 100% (36/36) *E. coli*[c]	Stool sample	Close to departure/ 1 to 2 weeks after returning	45 (13–365)[d]	Unknown	None
von Wintersdorff [23]	The Netherlands	November 2010–August 2012	Travel clinic	122	43 (18–72)	58	Not done	Stool sample	Before and immediately after	21 (5–240)	Unknown	None
Paltansing [24]	The Netherlands	March 2011–September 2011	Travel clinic	370	33 (19–82)	63	*Enterobacteriaceae* 92% (146/158) *E. coli*[c]	Rectal swab	Immediately before and after	21 (6–90)	None	6 months
Ruppé [25]	France	February 2012–April 2013	Vaccination centres	574	36 (SD 13)	61	*Enterobacteriaceae* 93% (491/526) *E. coli*[b]	Stool sample	Within 1 week before and after	20 (15–30)	None	12 months
Kuenzli [26]	Switzerland	December 2012–October 2013	Travel clinic	170	41 (30–53)	56	*Enterobacteriaceae* 98% (157/161) *E. coli*[b]	Rectal swab	1 week before/ directly after	18 (5–35)	Unknown	None
Lübbert [27]	Germany	May 2013–April 2014	Travel clinic	205	34 (3–76)	57	*Enterobacteriaceae* 92% (58/63) *E. coli*[b]	Stool sample	Before/within 1 week after	21 (3–218)	22	6 months

E.coli: *Escherichia coli*; MRE: multidrug-resistant *Enterobacteriaceae*; SD: standard deviation.

[a] Number of travellers who provided pre- and post-travel swab.

[b] Data of MRE-positive isolates newly acquired during travel.

[c] Data of MRE-positive isolates post-travel.

[d] Healthcare students, median duration of stay.

outcome, showed that travel diarrhoea (adjusted odds ratio (AOR) = 31.0; 95% confidence interval (CI): 2.7–358.1)) and antibiotic therapy for travel diarrhoea (AOR = 3.0; 95% CI: 1.4–6.7) proved to be the most important risk factors for acquiring MRE [20]. In the study of Kuenzli et al. in which only travellers to southern Asia were included, risk factors for MRE acquisition were length of stay, visit to family or friend and consumption of ice cream or pastry (Table 2) [26]. Angelin et al. found a significant association for travel to the South-East Asia region (OR = 30; 95% CI: 6.3–147.2), and antibiotic treatment during travel (OR = 5; 95% CI: 1.1–26.2), but found no association with travellers' diarrhoea or patient-related healthcare work [22].

Resistance of multidrug-resistant *Enterobacteriaceae* to other antibiotic drugs

Resistance of post-travel MRE isolates to various antibiotics was determined in nine studies (Table 4) [17-19,21-24,26,27]. In the study by Wintersdorff et al., a PCR-based approach was used, therefore it was not possible to determine which microorganism carried the resistance genes [23]. The resistance data to other antibiotic drugs in the study by Kennedy et al. were not part of the publication, but were provided on request [18]. Antimicrobial resistance was high for ciprofloxacin, varying from 31% to 57%, and for cotrimoxazole, varying from 49% to 86% [17-19,21-24,26,27]. Aminoglycoside resistance was high for gentamicin (range: 17–50%) and tobramycin (range: 18–59%) and low for amikacin (range: 2–5%) [17-19,21-24,26,27]. Carbapenemase-producing *Enterobacteriaceae* were observed in four travellers who had all visited India (in the study by Ruppé et al., two OXA-181 and one New Delhi metallo-beta-lactamase 1 (NDM-1), and in the study by Kuenzli et al., one NDM-1 but this strain was not included in the resistance results) [25,26]. Resistance to nitrofurantoin, colistin and fosfomycin was only analysed in some of the studies (Table 4) [18,19,21-23,26].

Duration of multidrug-resistant *Enterobacteriaceae* carriage after return, risk factors for a long duration and rate of infection after travel

Five studies analysed MRE carriage six months after travel, and the persistence rate of acquired MRE after six months was 6–24% of travellers (Table 2) [17,18,24,25,27]. Ruppé et al. analysed MRE carriage one, two, three, six and twelve months after travel, showing persistence of carriage of an acquired MRE in 34, 19, 10, 5 and 2%, respectively [25]. Travellers to Asia showed longer carriage of MRE compared with other travel destinations. Carriage of multidrug-resistant *E. coli* had a lower risk for prolonged carriage than other multidrug-resistant species. No other risk factors were found for prolonged carriage of MRE. Eight travellers in this study reported an episode of urinary tract infection after their return, but no microbiological data were available [25]. In the study by Tängdén et al., five of 21 travellers remained carriers of MRE after six months. However, none of these participants reported clinical infections [17]. In the study of Kennedy et al., one person developed a urinary tract infection with a travel-related organism [18]. Kantele et al. performed a one-year laboratory-based follow-up and did not find any clinical samples with MRE [20].

Rate of transmission to household members

Only one study screened household contacts for MRE after return of the index traveller. Household contacts were defined as persons who shared the same household with a participant on a regular basis. Two of 11 contacts were found MRE-positive [24]. Both carried a different ESBL-producing *E. coli* based on multilocus sequence typing (MLST) than the associated traveller.

Limitations of the studies

The quality of the studies and the susceptibility of bias between the studies were assessed. In all but one study, participants constituted a non-random sample of the general travelling population [17-21,23-27]. However, Angelin et al. studied healthcare students working or studying abroad [22]. Studies were performed on three different continents. Travel destinations and travel behaviour may differ considerably between different nationalities and age groups. Including co-travellers, as done in all studies except Paltansing et al. and Ruppé et al., can result in similar travel behaviour and therefore, similar risk factors. Overall, the main outcome was not influenced by recall or interviewer bias. For other outcomes such as risk factors, the risk of recall bias or interviewer bias was low because of the use of self-administered questionnaires.

Every study had participants lost to follow-up for post-travel stool samples and follow-up stool samples. Asymptomatic faecal carriage of MRE is probably not related to loss to follow-up, therefore, the risk of information bias is small. Ruppé et al. calculated post-travel MRE carriage as those travellers with persisting MRE carriage divided by all travellers with MRE acquisition plus all travellers without MRE post-travel [25]. However, travellers without MRE were not included in the follow-up. As a result, local MRE acquisition was not included in the calculated post-travel MRE carriage prevalence. Therefore the true prevalence can be assumed to be higher.

In five studies, travellers visited multiple regions or even continents during their trip [17-20,27]. In these travellers, it was not possible to attribute MRE prevalence or MRE acquisition to a certain geographical region. However, travellers in these studies were included in the MRE prevalence or MRE acquisition rates of more than one geographical region, which may have introduced information bias.

Seven studies used stool samples for detection of MRE [17,19-21,23,25,27] and three studies used rectal or perianal swabs for detection of MRE [18,24,26]. This might have influenced detection of MRE carriage.

Table 2A

Risk of multidrug-resistant *Enterobacteriaceae* in travellers (n = 11 studies)

Study	Method of MRE determination	Phenotypic approaches	Results genotypic characterisation post-travel MRE isolates	Results molecular typing of post-travel MRE isolates	MRE prevalence pre-travel *% (ratio)*	MRE prevalence post-travel *% (ratio)*	New MRE acquisition during travel *% (ratio)*[a]	Persistent newly acquired MRE carriage 6 months after travel *% (ratio)*	Results univariate/ multivariate risk factor analysis for MRE acquisition	MRE in non-travelling household contacts *% (ratio)*
Tängdén [17]	Phenotypic approach with genotypic confirmation by PCR	Enrichment broth, selective media, AST: Etest, MRE confirmation: disc diffusion	TEM (n = 11), SHV (n = 3), CTX-M group 1 (n = 14) of which CTX-M-15 (n = 13), CTX-M-1 (n=1), CTX-M group 4 (n = 10) of which CTX-M-9 (n = 3), CTX-M-14 (n = 5), CTX-M-27 (n = 2)[b]	No data	1 (1/105)	No data	24 (24/100)	24 (5/21)	Gastroenteritis; travel to India[c]	No data
Kennedy [18]	Phenotypic approach with genotypic confirmation by PCR	Enrichment broth, selective media, AST: Vitek2, MRE confirmation: disc diffusion	TEM or SHV (n = 4), CTX-M group 1 (n = 12), CTX-M group 9 (n = 6), and pAmp C genes(n =4)[d]	No data	2 (2/106)	22 (22/102)	21 (21/100)	6 (1/18)	Gastroenteritis; use of antibiotics; travelling to Asia, South America and/or Middle East/ Africa[e,c]	No data
Östholm-Balkhed [19]	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: Etest, MRE confirmation: Etest	TEM-19 (n = 1), SHV (n = 6), CTX-M-15-like (n = 36), CTX-M-14-like (n = 36), CTX-M-27-like (n = 5), CTX-M-53-like (n=5), CTX-M-1/61 like (n = 3), CTX-M-2 like (n = 2), CTX-M-3-like (n = 1), pAmpC genes (n = 15), no genes detected (n = 13)[b]	No data	2 (6/251)	31 (72/231)	30 (68/226)	No data	Age; diarrhoea or other gastrointestinal symptoms; travel to Asia, Africa (north of equator), Indian subcontinent[f]	No data
Kantele [20]	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: Vitek2, MRE confirmation: disc diffusion	79% CTX-M-type (CTX-M-1 and CTX-M-9 most prevalent), other common strains TEM and OXA (data not published)[b]	No data	1 (5/430)	22 (93/430)	21 (90/430)	No data	Traveller's diarrhoea; age; use of antibiotics for traveller's diarrhoea[f]	No data
Weisenberg [21]	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: Vitek2, MRE confirmation: disc diffusion	SHV-12 (n = 1), CTX-M-14 (n = 3), CTX-M-15 (n = 2), no gene detected (n = 1)[b]	MLST typing 7 multidrug-resistant *E. coli* isolates: ST 39, 8 (n = 2), 37, 399, 437, 83	4 (1/28)	25 (7/28)	26 (7/27)	No data	No data	No data

AST: antibiotic susceptibility testing; bla: beta-lactamase; CTX-M: cefotaximase; *E. coli*: *Escherichia coli*; ESBL: extended-spectrum beta-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; MLST: Multilocus sequence typing; MRE: multidrug-resistant *Enterobacteriaceae*; NDM: New Delhi metallo-beta-lactamase; OXA: oxacillinase; pAmp C: plasmid-borne AmpC; PCR: polymerase chain reaction; PFGE: pulsed-field gel electrophoresis; rep-PCR: repetitive extragenic palindromic PCR; SHV: Sulphydryl variable; TEM: Temoniera.

[a] Percentage of MRE-positive post-travel samples in those travellers whose pre-travel sample was MRE-negative.

[b] Acquired genes detected in post-travel MRE isolates.

[c] Univariate statistics.

[d] Prevalent genes detected in post-travel MRE isolates.

[e] Risk factors for resistance to gentamicin, ciprofloxacin and/or third generation cephalosporins.

[f] Multivariable logistic regression analysis; participants ESBL-positive before travel were excluded.

[g] Binary regression analysis.

[h] Carbapenemase-positive isolates were included in the definition MRE.

Table 2B

Risk of multidrug-resistant *Enterobacteriaceae* in travellers (n = 11 studies)

Study	Method of MRE determination	Phenotypic approaches	Results genotypic characterisation post-travel MRE isolates	Results molecular typing of post-travel MRE isolates	MRE prevalence pre-travel % *(ratio)*	MRE prevalence post-travel % *(ratio)*	New MRE acquisition during travel % *(ratio)*[a]	Persistent newly acquired MRE carriage 6 months after travel % *(ratio)*	Results univariate/ multivariate risk factor analysis for MRE acquisition	MRE in non-travelling household contacts % *(ratio)*
Angelin [22]	Phenotypic approach for detection of ESBL, pAmp C and phenotypic approach with genotypic characterisation for detection of OXA-48/ OXA-181	Selective media, AST: disc diffusion, MRE confirmation: Etest (ESBL), disc diffusion (pAmpC)	No data	No data	7 (7/99)	36 (36/99)	35 (35/99)	No data	Travel to the South-East Asia region (India, Nepal, Vietnam, Indonesia, Sri Lanka); antibiotic treatment during travel[g]	No data
von Wintersdorff [23]	Metagenomic approach (detection bla_{CTX-M})	No data	bla_{CTX-M} (n = 41)[d]	No data	9 (11/122)	34 (41/122)	32 (36/111)	No data	Travel to Indian subcontinent[f]	No data
Paltansing [24]	Phenotypic approach with genotypic characterisation by microarray	Enrichment broth, selective media, AST: Vitek2, MRE confirmation: disc diffusion	SHV (n = 1), CTX-M group 1 (n = 110) of which CTX-M-1-like (n = 4), CTX-M-3-like (n = 1), CTX-M-15-like (n = 85),CTX-M-32-like (n = 20), CTX-M-group 9 (n = 42), CTX-M-group 2 (n=2), CTX-M-group 8/25 (n = 1), pAmpC genes (n = 3)[d]	MLST typing: 146 multidrug-resistant *E. coli* isolates: most prevalent ST 38 (n = 17), ST10 (n = 10), ST131 (n = 9)	9 (32/370)	36 (133/370)	33 (113/338)	17 (19/113)	Travel to South or East Asia[f]	18 (2/11)
Ruppé [25][h]	Phenotypic approach with genotypic confirmation by PCR	Enrichment broth, selective media, AST: disc diffusion	Predominant CTX-M-type (95.4%) among which CTX-M-group 1 predominated (83.7% of all CTX-M), OXA-181 (n = 2), NDM-1 (n = 1)[b]	No data	12 (81/700)	No data	51 (292/574)	After 1 month 34 (83/245); after 2 months 19 (45/236); after 3 months 10 (24/233); after 6 months 5 (11/230); after 12 months 2 (5/227)	Travel to Asia or sub-Saharan Africa; beta-lactam use during travel; diarrhoea during travel; type of travel[f]	No data

AST: antibiotic susceptibility testing; bla: beta-lactamase; CTX-M: cefotaximase; *E. coli*: *Escherichia coli*; ESBL: extended-spectrum beta-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; MLST: Multilocus sequence typing; MRE: multidrug-resistant *Enterobacteriaceae*; NDM: New Delhi metallo-beta-lactamase; OXA: oxacillinase; pAmp C: plasmid-borne AmpC; PCR: polymerase chain reaction; PFGE: pulsed-field gel electrophoresis; rep-PCR: repetitive extragenic palindromic PCR; SHV: Sulphydryl variable; TEM: Temoniera.

[a] Percentage of MRE-positive post-travel samples in those travellers whose pre-travel sample was MRE-negative.

[b] Acquired genes detected in post-travel MRE isolates.

[c] Univariate statistics.

[d] Prevalent genes detected in post-travel MRE isolates.

[e] Risk factors for resistance to gentamicin, ciprofloxacin and/or third generation cephalosporins.

[f] Multivariable logistic regression analysis; participants ESBL-positive before travel were excluded.

[g] Binary regression analysis.

[h] Carbapenemase-positive isolates were included in the definition MRE.

Table 2C

Risk of multidrug-resistant *Enterobacteriaceae* in travellers (n = 11 studies)

Study	Method of MRE determination	Phenotypic approaches	Results genotypic characterisation post-travel MRE isolates	Results molecular typing of post-travel MRE isolates	MRE prevalence pre-travel *% (ratio)*	MRE prevalence post-travel *% (ratio)*	New MRE acquisition during travel *% (ratio)*[a]	Persistent newly acquired MRE carriage 6 months after travel *% (ratio)*	Results univariate/ multivariate risk factor analysis for MRE acquisition	MRE in non-travelling household contacts *% (ratio)*
Kuenzli [26]	Phenotypic approach with genotypic screening by microarray and confirmation by PCR/DNA sequence analysis	Enrichment broth, selective media, AST: Vitek2, MIC for meropenem and ertapenem: Etest, MRE confirmation: disc diffusion, modified Hodge test	TEM-1-like (n = 33), SHV238S/240K (n = 7), SHV238S (n = 1), SHV-5/12-like (n = 1), SHV-2/3-like (n = 1),CTX-M-15-like (n = 48), CTX-M group 9 (n = 1), CTX-M group 1 (n = 24), predominant ESBL gene was CTX-M-15 (80 representative *E. coli* isolates analysed)[b], NDM-1 (n = 1)	80 representative *E. coli* isolates analysed by rep-PCR: not clonally related. MLST performed on 34 randomly selected *E. coli* isolates: only 3 pandemic strains found (ST131 n = 2; ST648 n = 1)	3 (5/175)	No data	70 (118/170)	No data	Travel to India, Bhutan or Nepal; visiting friends and relatives; consumption of ice cream and pastry;, length of stay[f]	No data
Lübbert [27]	Phenotypic approach with genotypic confirmation by PCR	Selective media, AST: microbroth dilution method, MRE confirmation: Etest	SHV-12 (n = 1), CTX-M group 1 (n = 37) of which CTX-M-15 (n = 33), CTX-M-55 (n = 4), CTX-M group 9 (n = 19) of which CTX-M-14 (n = 9), CTX-M-27 (n = 1), CTX-M-65 (n = 1)[b]		7 (14/205)	31 (63/205)	30 (58/191)	9 (3/35)	Travel to India or South-East Asia; gastroenteritis[c]	No data

AST: antibiotic susceptibility testing; bla: beta-lactamase; CTX-M: cefotaximase; *E. coli*: *Escherichia coli*; ESBL: extended-spectrum beta-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; MLST: Multilocus sequence typing; MRE: multidrug-resistant *Enterobacteriaceae*; NDM: New Delhi metallo-beta-lactamase; OXA: oxacillinase; pAmp C: plasmid-borne AmpC; PCR: polymerase chain reaction; PFGE: pulsed-field gel electrophoresis; rep-PCR: repetitive extragenic palindromic PCR; SHV: Sulphydryl variable; TEM: Temoniera.

[a] Percentage of MRE-positive post-travel samples in those travellers whose pre-travel sample was MRE-negative.

[b] Acquired genes detected in post-travel MRE isolates.

[c] Univariate statistics.

[d] Prevalent genes detected in post-travel MRE isolates.

[e] Risk factors for resistance to gentamicin, ciprofloxacin and/or third generation cephalosporins.

[f] Multivariable logistic regression analysis; participants ESBL-positive before travel were excluded.

[g] Binary regression analysis.

[h] Carbapenemase-positive isolates were included in the definition MRE.

Table 3

Proportion of travellers who acquired multidrug-resistant Enterobacteriaceae, by travel destination (n = 11 studies)

Study	Southern Asia % (ratio)	Asia except southern Asia % (ratio)	Northern Africa % (ratio)	Sub-Saharan Africa % (ratio)	South and Central America % (ratio)	North America % (ratio)	Europe % (ratio)	Oceania % (ratio)
Tängdén [17][a,b]	78 (7/9)	29 (10/34)	33 (4/12)	4 (1/23)	0 (0/7)	0 (0/2)	13 (2/16)	-
Kennedy [18][a,c]	57 (8/14)	25 (21/85)	33 (1/3)	0 (0/2)	20 (1/5)	20 (2/10)	14 (3/21)	0 (0/2)
Östholm-Balkhed [19][a,b]	71 (10/14)	43 (26/60)	57 (17/30)	21 (15/71)	16 (5/31)	0 (0/15)	0 (0/15)	No data
Kantele [20][b,d]	46 (28/61)	32 (37/116)	67 (2/3)	12 (23/193)	0 (0/40)	0 (0/2)	0 (0/15)	No data
Weisenberg [21][b]	29 (2/7)	25 (1/4)	33 (1/3)	13 (1/8)	33 (2/6)	No data	No data	No data
Angelin [22]	63 (25/40)	67 (6/9)	No data	10 (4/40)	0 (0/5)	0 (0/4)	No data	No data
von Wintersdorff [23][c]	58 (18/31)	20 (6/29)	31 (5/16)	29 (5/17)	0 (0/10)	No data	17 (1/6)	No data
Paltansing [24][b,e]	72 (18/25)	41 (60/146)	40 (4/10)	24 (20/82)	15 (9/60)	No data	No data	No data
Ruppé [25][f]	88 (53/60)	66 (61/93)	No data	49 (89/182)	31 (48/155)	No data	No data	0 (0/2)
Kuenzli [26][b]	69	No data	No data	No data	No data	No data	No data	No data
Lübbert [27][a,b]	72 (13/18)[g]	33 (24/73)[g]	No data	24 (19/78)	8 (6/78)	0 (0/2)	20 (2/10)	No data

MRE: multidrug-resistant *Enterobacteriaceae*.

[a] Travellers visiting more than one region are categorised in all the visited geographical regions.

[b] Study reports data on MRE acquisition in travellers.

[c] Study reports data on MRE prevalence in travellers.

[d] Travellers visiting more than one region are categorised in the geographical region with the longest stay for this study.

[e] One traveller who visited Iran is categorised in Asia instead of Southern Asia.

[f] 42 travellers visited more than one country in Asia and may be represented in more than one column in the Table; 28 of them acquired MRE.

[g] Exact numbers unpublished.

Southern Asia: Afghanistan, Bangladesh, Bhutan, India, Iran, Maldives, Nepal, Pakistan, Sri Lanka.

Asia (without southern Asia): Armenia, Azerbaijan, Bahrain, Brunei, Cambodia, China, Cyprus, Georgia, Hong Kong, Indonesia, Iraq, Israel, Jordan, Japan, Kazakhstan, Kuwait, Kyrgyzstan, Laos, Lebanon, Mongolia, Malaysia, Myanmar, North Korea, Oman, Philippines, Qatar, Saudi Arabia, South Korea, Singapore, Palestine, Syria, Tajikistan, Thailand, Timor-Leste, Turkey, Turkmenistan, United Arab Emirates, Uzbekistan, Viet Nam, Yemen.

Northern Africa: Algeria, Egypt, Libya, Morocco, Sudan, Tunisia, Western Sahara.

Sub-Saharan Africa: Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo (Brazzaville), Côte d'Ivoire, Democratic Republic of the Congo, Djibouti, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Ghana, Guinea, Guinea-Bissau, Kenya, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Mauritius, Mozambique, Namibia, Niger, Nigeria, Réunion, Rwanda, São Tomé and Príncipe, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Sudan, Swaziland, Tanzania, The Gambia, Togo, Uganda, Zambia, Zimbabwe.

South and Central America: Anguilla, Antigua and Barbuda, Argentina, Aruba, Bahamas, Barbados, Belize, Bolivia, Bonaire, Sint Eustatius and Saba, Brazil, British Virgin Islands, Cayman Islands, Chile, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Dominican Republic, Ecuador, El Salvador, Falkland Islands, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Montserrat, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Saint Kitts and Nevis, Saint Lucia, Saint Martin, Saint Vincent and the Grenadines, Saint-Barthélemy, Sint Maarten, Suriname, Trinidad and Tobago, Turks and Caicos Islands, US Virgin Islands, Uruguay, Venezuela.

North America: Bermuda, Canada, Greenland, Saint Pierre and Miquelon, United States.

Europe: Åland Islands, Albania, Andorra, Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Channel Islands, Croatia, Czech Republic, Denmark, Estonia, Faeroe Islands, Finland, the former Yugoslav Republic of Macedonia, France, Germany, Gibraltar, Greece, the Holy See, Hungary, Iceland, Ireland, Isle of Man, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Monaco, Montenegro, Netherlands, Norway, Poland, Portugal, Moldova, Romania, Russia, San Marino, Serbia, Slovakia, Slovenia, Spain, Svalbard and Jan Mayen, Sweden, Switzerland, Ukraine, United Kingdom.

Oceania: American Samoa, Australia, Cook Islands, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Micronesia, Nauru, New Caledonia, New Zealand, Niue, Norfolk Island, Northern Mariana Islands, Palau, Papua New Guinea, Pitcairn Islands, Samoa, Solomon Islands, Tokelau, Tonga, Tuvalu, Vanuatu, Wallis and Futuna.

Table 4

Antibiotic drug resistance of newly acquired multidrug-resistant Enterobacteriaceae in travellers (n = 11 studies)

Study	Ciprofloxacin % (ratio)	Cotrimoxazole % (ratio)	Gentamicin % (ratio)	Amikacin % (ratio)	Tobramycin % (ratio)	Carbapenem % (ratio)	Nitrofurantoin % (ratio)	Colistin % (ratio)	Fosfomycin % (ratio)
Tängdén [17][a]	50[b]	79 (19/24)	45[b]	No data	38[b]	0[b]	0[b]	No data	8.0[b]
Kennedy [18][c]	55 (12/22)	No data	50 (11/22)	No data	59 (13/22)	No data	No data	No data	No data
Östholm-Balkhed [19][a]	31 (36/116)	70 (81/116)	41 (48/116)	2 (2/116)	46 (53/116)	0 (0/116)	7 (8/116)	No data	3 (3/116)
Kantele [20]	No data	No data	No data	No data	No data	No data	No data	No data	No data
Weisenberg [21][a]	43 (3/7)[d]	86 (6/7)	43 (3/7)	No data	No data	0 (0/7)	No data	No data	No data
Angelin [22]	57 (28/49)	75[b]	30[b]	No data	No data	0 (0/49)	2[b]	No data	No data
von Wintersdorff [23][e]	37 (45/122) *qnrB* 56 (68/122) *qnrS*	No data	71 (86/122) *aac(6')-aph(2'')*	71 (86/122) *aac(6')-aph(2'')*	71 (86/122) *aac(6')-aph(2'')*	0 (0/122) bla_{NDM}	No data	No data	No data
Paltansing [24][f]	36	67	35	No data	37	0	29	0	No data
Ruppé [25]	No data	No data	No data	No data	No data	0.6 (3/526)[g]	No data	No data	No data
Kuenzli [26][a]	41 (64/157)	49 (77/157)	No data	5 (7/157)	18 (28/157)	0 (0/157)	2 (3/157)	0 (0/157)	0.6 (1/157)
Lübbert [27][a]	43 (25/58)	83 (48/58)	17 (10/58)	2[b]	22[b]	0[b]	No data	0[b]	16[b]

bla: beta-lactamase; CPE: carbapenemase-producing *Enterobacteriaceae*; ESBL: extended-spectrum beta-lactamase.

[a] Resistance among acquired ESBL-positive isolates detected in post-travel samples.

[b] Data extracted from bar chart, exact numbers unpublished.

[c] Resistance among prevalent ESBL-positive isolates detected in post-travel samples.

[d] Percentage of susceptibility to levofloxacin.

[e] Prevalent resistance genes in faecal samples post-travel.

[f] Resistance among prevalent ESBL-positive isolates detected in pre- and post-travel samples.

[g] Three acquired CPE detected in post-travel samples.

Discussion

In this systematic review we found a high prevalence of faecal carriage of MRE after international travel. The highest prevalence of MRE was observed in isolates from travellers returning from southern Asia, with up to 88% acquisition of MRE. In addition to the antibiotics not effective against MRE, an alarmingly high prevalence of resistance to other commonly used antibiotics such as cotrimoxazole (49–86%), ciprofloxacin (31–57%) and aminoglycosides (gentamicin 17–71%) was observed in ESBL-positive isolates in travellers in all studies [17-27].

Returning international travellers with MRE may introduce these microorganisms in their home countries. This may cause community-onset infections with MRE in patients without obvious risk factors transmitted by healthy carriers through food or person-to-person contact [9]. Infections caused by MRE are associated with poorer outcome and a higher overall mortality rate than infections caused by susceptible bacteria [28]. In this review, all studies showed an increased prevalence of faecal carriage of ESBL after international travel. It is not possible to evaluate the proportion of travellers who will develop infection with these resistant bacteria. However, studies have demonstrated that international travel is a risk factor associated with developing an infection with an MRE [11,12,29].

Many countries have infection prevention and control guidelines to detect and treat multidrug-resistant organisms (MDROs) including MRE [30]. In countries with low prevalence of MRE, infection prevention and control guidelines mainly include strategies for early identification and isolation of patients recently hospitalised in foreign hospitals [30,31]. Patients with a recent history of travel to MRE-endemic areas but not admitted to healthcare facilities abroad are not normally considered at risk for carriage of MDROs. However, in hospitalised patients with a recent history of travel, increased rates of carriage of MRE have been observed [10,29,30]. Physicians should be aware of the risk that patients with recent travel to areas with high faecal carriage of MRE, as presented in this review, may introduce MRE to the hospital. Routine screening

for MRE seems indicated in such patients. Furthermore, empiric antibiotic therapy may fail when an infection by MRE is not taken into account. Therefore, careful recording of travel history needs to be incorporated in each patient evaluation. As shown in this review, there is also an increased risk of resistance against other antibiotics in travellers with MRE carriage. It is likely that this is caused by multiple genes, each encoding resistance to different classes of antibiotics, which are often found on the same bacterial mobile genetic element (e.g. a plasmid) [32]. As a result, other antibiotics, such as aminoglycosides, will also fail in many MRE-positive patients.

Of all MDROs, emergence of CPE is most worrisome because of the limited treatment options for these infections. NDM-1-producing *Enterobacteriaceae* have been found in environmental samples in endemic regions [33]. CPE (NDM-1) in patients from the United Kingdom with a recent history of travelling or medical tourism to India are already an important public health problem [8]. Case reports have also demonstrated acquisition of CPE in travellers without contact with medical healthcare facilities [34,35]. In this review, four travellers from India were carrying a carbapenemase-producing *E. coli* [25,26]. Preliminary results of the Carriage Of Multiresistant Bacteria After Travel (COMBAT) study, a large-scale multicentre longitudinal cohort study conducted in the Netherlands among 2001 travellers, show acquisition of CPE in four travellers [36].

There are, besides the destination of travel, additional risk factors for acquiring MRE during travel. Antibiotic therapy was found to increase the risk [20,22]. In five studies, traveller's diarrhoea or gastroenteritis were associated with an increased risk of MRE acquisition during travel [17-20,25]. Also, in one study, meticulous hand hygiene or strict consumption of bottled water did not lower the risk of acquiring MRE [22]. Therefore, it is not clear whether hygiene-related travel advice will decrease faecal carriage of MRE. Surprisingly, healthcare-related activities did not pose an increased risk of acquiring MRE in one study [22].

MRE and CPE could also be carried by food. International spread of these bacteria by food supply has been reported [37]. In this review, only one study showed that food consumption (ice cream and pastry) was associated with MRE carriage in travellers to southern Asia, whereas most of the studies did not focus on dietary patterns during travel.

One limitation of this review is the recruitment of travellers from travel clinics only, resulting in inclusion of very few travellers with European destinations. Some European countries such as Greece and Cyprus are also endemic for MRE and popular travel destinations [34]. In addition, travellers visiting their country of origin, especially Morocco and Turkey usually do not ask for a pre-travel consultation, although these countries are endemic for MRE and CPE [34]. It is not clear whether not including these patients may have led to an under- or overestimation of MRE acquisition.

Another limitation is the lack of sufficient data regarding the duration of carriage and the transmission among non-travelling household members. The study by Ruppé et al. suggests that three months after return, MRE carriage is comparable with the baseline prevalence before travelling. However, the study did not include baseline prevalence in the follow-up. The COMBAT study will address some of these questions [38].

Conclusion

International travel is a major risk factor for acquisition of MRE. This risk is particularly high after travelling to (southern) Asia and in persons with travel-related diarrhoea and antibiotic use. Carriage of MRE-positive isolates is also associated with a high risk of resistance to ciprofloxacin, cotrimoxazole and aminoglycosides. Further research is needed to assess duration of carriage, spread to household contacts and whether introduction of MRE results in an increase of MRE infections. Our results, combined with the worldwide emergence of CPE, further stress the importance of infection prevention and control guidelines.

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Conflict of interest

None declared.

Authors' contributions

Robert-Jan Hassing: This author made substantial contributions to conception and design, acquisition of data and analysis and interpretation of data; this author participated in drafting the article; this authors gave final approval of the version to be submitted and any revised version. Jelmer Alsma: This author made substantial contributions to conception and design, acquisition of data and analysis and interpretation of data; this author participated in drafting the article; this authors gave final approval of the version to be submitted and any revised version. Maris S. Arcilla: This author made substantial contributions to acquisition of data and analysis and interpretation of data; this author participated in drafting the article; this author gave final approval of the version to be submitted and any revised version. Bruno H. Stricker: This author made substantial contributions to conception and design and analysis and interpretation of data; this author participated in revising the article critically for important intellectual content; this authors gave final approval of the version to be submitted and any revised version. Perry J. van Genderen: This author made substantial contributions to conception and design; this author participated in revising the article critically for important intellectual content; this authors gave final approval of the version

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New European HIV Test Finder launched during European HIV-Hepatitis Testing Week

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On the occasion of the 20–27 November European HIV-Hepatitis Testing Week, the European HIV Test Finder, an online tool supporting people to find HIV-testing sites across Europe has been launched [1].

The European HIV Test Finder lists HIV testing centres and clinics across the 28 European Union (EU) member states. It was developed by Aidsmap.com with funding from the European Centre for Disease Prevention and Control (ECDC). Mobile friendly, it enables people to search for a nearby HIV testing centre by country, town and area code [2].

During the European HIV-Hepatitis Testing Week [3], the European Test Finder has been actively promoted via smartphone dating apps for men who have sex with men (MSM), thereby further disseminating the message that it is important to be tested. A newly published report [4] shows that sex between men is still the most common way HIV is transmitted in the EU/European Economic Area. HIV diagnoses among MSM have been rising at a significant rate from 30% of cases diagnosed in 2005 to 42% in 2014.

The European HIV Testing Week was first organised in 2013. Its aim is to increase HIV status awareness and to communicate the benefits of HIV testing with an aim to reduce late diagnosis. Current data shows that 47% of newly reported HIV cases are diagnosed late. Those tested and diagnosed with HIV early are less likely to pass on the virus [4].

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