Proportion of laboratory-confirmed seasonal influenza in people with medically-attended, acute respiratory illness in Europe: A meta-analysis.

Abstract

Background: Across the WHO European Region, there are few estimates of the proportion of people seeking medical care for influenza-like illness or acute respiratory infections and who have confirmed seasonal influenza infection when tested for respiratory viruses.

Methods: We conducted meta-analyses of data extracted from a) literature review of studies published between 2004 and 2017; b) sentinel data from the European surveillance system (TESSy), pooling within-season estimates by influenza type/subtype, setting (outpatient/inpatient) and age-group, to estimate the proportion of people tested who have laboratory-confirmed medically-attended seasonal influenza in Europe.

Results: In the literature review, the pooled proportion for all influenza was 33% (95% CI, 30%-36%), and higher among outpatients (36% [33-40%]) than inpatients (24% [20-29%]). Pooled estimates for all influenza by age group were: 0-17 years, 26% (22-31); 18-64 years, 41% (32-50); ≥65 years, 33% (27-40%). From TESSY data, 33% (31-24%) of outpatients and 24% (21-27%) of inpatients were positive. The highest proportion of influenza A overall was in people aged 18-64 years (22% [16-29%]). By subtype, influenza A(H1N1)pdm09 was highest in 18-64 year-olds (16% [11-21%]) whereas influenza A(H3N2) was highest in those ≥65 years (10% [2-22%]). For influenza B, the highest proportion of infections was those aged 18-64 years (15% [9-24%]). Estimated proportions of confirmed influenza varied across seasons and across countries.

Conclusions: Both the literature review and TESSy analyses showed a higher proportion of laboratory-confirmed influenza in non-hospitalised patients, with further variation by influenza type, age-group, country and season.

Introduction

Seasonal and pandemic influenza are global public health problems associated with significant clinical burden [1]. However, seasonal influenza causes higher cumulative morbidity and mortality, as it affects populations on an annual basis [1, 2]. The epidemiological impact of influenza varies from season to season, between geographic areas and according to the circulating antigenic variants of the main influenza types, A and B [2-6]. Within a population, the clinical impact in subgroups considered at-risk for the development of serious influenza-related complications (the very young and the elderly, pregnant women and people with underlying co-morbidities) is greater than in those not in risk groups [2].

Influenza infection is very common; asymptomatic infections may account for between 16% and 85% of seasonal influenza infections, depending upon study design and testing method [1, 7], and most symptomatic individuals infected experience only mild and self-limiting illness [2, 3]. In people seeking medical care, accurately diagnosing influenza A or B solely on the basis of clinical criteria is difficult as the signs and symptoms of influenza overlap with those of many other respiratory viral pathogens which co-circulate with influenza every winter in temperate regions of the world. Laboratory-testing identifies the specific causative virus but in clinical practice few patients presenting with signs and symptoms suggestive of influenza are tested due to timeliness and cost; thus, the actual contribution of influenza viruses to total respiratory illness in the population remains relatively uncertain, especially in primary care settings where laboratory testing is extremely rarely undertaken.

Since the early 2000s, large investments by the United States and others have supported development of the global capacity for influenza surveillance, and many networks for influenza surveillance now exist globally and in the European region specifically [8-11], where the WHO Regional Office for Europe (WHO/Europe) and European Centre for Disease Prevention and Control (ECDC) co-ordinate the collection and analysis of surveillance data provided by Member States (European Surveillance System (TESSy). These data provide a source for estimating the frequency of influenza within countries. In 2015, we conducted a scoping literature review on the burden of influenza within the WHO European Region which provided an overview of the general burden caused by seasonal influenza and highlighted the lack of data from eastern European countries (*author*, unpublished data). However, some of the estimates provided were derived from symptom-based endpoints (e.g., influenza-like illness [ILI] or severe acute respiratory infection [SARI]) which made it impossible to compare the clinical burden of laboratory-confirmed influenza between countries.

In this study we aim to estimate the proportion of laboratory-confirmed influenza in the WHO European region in people seeking medical care with clinically diagnosed acute respiratory infection (ARI) or ILI and who are tested for respiratory viruses, including influenza. Two methods are presented: a review and meta-analysis of data published in the literature from 2004 to 2017; a meta-analysis of seasonal influenza data from the European Surveillance System (TESSy).

Methods

Literature Review

The study was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12]. We identified articles which reported quantitative data on laboratory-confirmed influenza infections in people seeking medical attention for ILI/ARI, as defined by individual studies, in the WHO European Region. MEDLINE was searched in September 2017 using a search strategy devised by one author (Appendix A, Supplementary material). The strategy sought studies of any design, published in English between 2004 and 2017 (influenza seasons up until 2016–2017 but excluding data from the pandemic year, 2009-2010) which were conducted in countries within the WHO European Region [13]. Studies were included which offered within-season influenza positivity data for at least one full influenza season (from October through May of the following year) on symptomatic and medically-attended acute respiratory illness in patients of any age, and in whom influenza virus infection was confirmed by culture or reverse transcription polymerase chain reaction (RT-PCR). Additionally, we searched the reference lists of included studies and relevant systematic reviews [14-18] and the references included in a literature review we conducted previously that looked at studies conducted between the years 2004-2015 (*author*, personal communication). We included study populations of all ages or in stratified age groups, and all healthcare settings including primary care/ambulatory outpatients (OP) and hospitalised in-patients (IP).

Studies were excluded if the total number of specimens tested each year was less than 50, or were outbreak reports from closed or semi-closed communities (e.g., nursing homes, army bases, or religious groups), in which the results would not be representative of the

wider population. Studies were also excluded if data presented were combined for more than one season or more than one pathogen with no separate influenza data. We also excluded studies in which there was no clear sampling strategy or in which participants were sampled at the discretion of the treating clinician, which could introduce bias.

One of the authors screened the titles and abstracts of all the identified literature for relevance, then conducted a full review of papers. We extracted the following variables: influenza season, country, age group, laboratory testing method, healthcare setting, case definition, total number of symptomatic subjects tested and number of subjects positive for influenza. If data for separate influenza types and subtypes were presented, the number of positive subjects for each were also extracted. The percentage of positive subjects was calculated based on number of subjects tested as a denominator, and number of positive subjects as a numerator (aggregated influenza, types and subtypes according to the data presented in each study).

For quality assessment, we used a modified version of the Newcastle-Ottawa assessment scale for cohort studies [19]. Indicators used to assess quality were the following: representativeness (geographic, age and general representativeness) of the subjects tested, assessment of the outcome (sensitivity of symptoms prompting laboratory testing such as number of symptoms and having a clear case definition), and laboratory method. Some indicators were not applicable and excluded (i.e., representativeness of exposed cohort, ascertainment of exposure and demonstration that outcome was not present at the start of the study). Other indicators (comparability of cohorts and adequacy and length of follow up)

were also excluded since we only included studies that had at least one complete season of data.

The European Surveillance System (TESSy) data

We retrospectively analysed laboratory-confirmed influenza detection data reported to the European Surveillance System (TESSy; hosted by the European Centre for Disease Prevention and Control [ECDC]) as a part of the surveillance of influenza in the WHO European Region, which is jointly coordinated by WHO/Europe and ECDC. Sentinel influenza surveillance is conducted in a representative subset of sites and co-ordinated by national or sub-national networks, with systematic sampling of patients meeting pre-defined case definitions. Data were provided by Albania, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Israel, Italy, Latvia, Lithuania, Luxembourg, North Macedonia, Malta, Republic of Moldova, Netherlands, Norway, Poland, Portugal, Kazakhstan, Kyrgyzstan, Portugal, Romania, Russian Federation, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Turkey, United Kingdom, Ukraine and Uzbekistan, and released by ECDC. Data on the duration of participation of these countries are listed in Appendix B (Supplementary Data). Data submitted by week and country for the period 2004 to 2018 (weeks 40 to 20) were extracted from TESSy on 29 August 2018. Separately, the total number of specimens collected from patients presenting to sentinel primary care sites and meeting the influenza-like illness (ILI) or ARI case definitions and hospitalised patients meeting the case definition for SARI were calculated by country and influenza season (International Organization for Standardization [ISO] week 40 in a given year to week 20 in

the following year) for the 2004-2005 to 2017-2018 seasons, as were the corresponding total number of detections by influenza virus type and subtype (for influenza A) . Country-seasons were excluded if there were fewer than 50 specimens or less than 20 weeks of data submitted to TESSy. The proportion of sampled patients that tested positive for any influenza virus, influenza A virus and influenza B virus were calculated by country-season.

Data extraction and analysis

Data from the literature review and those derived from the TESSy dataset were extracted to a Microsoft® Office Excel® 2013 spreadsheet (Microsoft Corporation, Richmond, VA, USA). As we anticipated a degree of heterogeneity due to the observational nature of the included studies, we used a generic variance approach based on a random effects model (DerSimonian-Laird weights method)[20] to estimate the pooled proportion of laboratory-confirmed influenza virus identified in tested patients,, stabilising the variances using the Freeman-Tukey double arcsine transformation so that studies with proportions close to 0% or 100% were appropriately estimated [21]. Exact binomial confidence intervals (CIs) were computed for outcomes. The main outcome was the proportion (and 95% CI) of laboratory-confirmed influenza in people with ARI or ILI symptoms who sought medical care and were tested for influenza (the denominator). Data from the literature review and TESSY were analysed separately. For analysis of the TESSy data, people with ARI/ILI were classified as outpatients as the data were derived from primary care surveillance of mild influenza disease due to influenza, and those with SARI were classified as hospitalised as data originated from sentinel surveillance of hospitalised cases presenting with severe disease [22]. The denominator in the latter was the number of people with SARI who were tested for influenza. Heterogeneity between the studies was assessed using the I^2 statistic.

The initial analysis was for all types of influenza (aggregated influenza) and for all ages. To investigate potential sources of heterogeneity, we performed subgroup analyses by influenza virus type and subtype [influenza A, influenza A(H1N1), influenza A (H3N2) and influenza B], age group, healthcare setting (OP versus IP), and requirement for fever in the case definition. To further investigate high heterogeneity, we undertook an I^2sensitivity analysis by excluding datasets in which the estimated proportion was furthest from the overall estimated pooled proportion. We excluded data collected during the 2009 pandemic; influenza A(H1N1) was stratified by pre-pandemic seasons [A(H1N1), up to and including the 2008-2009 season] and post-pandemic seasons [A(H1N1)pdm09, 2010-2011 onwards] and analysed separately because population susceptibility to these two viruses will have been markedly different. Age groups in studies included were not uniform, so for the purpose of this analysis we created the following categories to best fit the majority of the data: 0-17 years, 18-65 years, and $\geq$65 years. All analyses were conducted using the *metaprop* command in Stata (SE 16, StataCorp, College Station, Texas, USA).

Ethical Statement

Ethical approval was not required; the review of the literature is based on secondary data with no personally identifiable information. ECDC has a legal basis to collect surveillance data (DECISION No 1082/2013/EU).

Results

Literature Review

The Medline database search identified 9316 manuscripts. In total, 9496 were screened by title and abstract, 176 were fully reviewed, and 38 met the inclusion criteria Figure 1 and Appendix C [Supplementary Material]).

Figure 1 Flow chart displaying number of articles identified and screened at each stage of the literature review

Study Characteristics

Studies reported data from 25 European countries (Albania, Armenia, Austria, Belarus, Bulgaria, Czech Republic, Finland, France, Georgia, Germany, Greece, Italy, Kazakhstan, Kyrgyzstan, Netherlands, Portugal, Romania, Russian Federation, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom and Ukraine). The greatest number of studies were six each from Italy and the United Kingdom, the lowest was one (from each of Albania, Armenia, Georgia, Kazakhstan, Romania, Belarus, Ukraine and Kyrgyzstan). Five studies reported data from multiple countries.

The studies included covered influenza seasons from 1996-1997 to 2014-2015. Of the 38, all study designs were cross sectional except for two (case-control and randomized control trial). Detection of influenza viruses was achieved by RT-PCR with or without culture in 32 studies and by culture alone in six. A sensitivity analysis was conducted excluding the culture-only studies (Supplemental Table 1), however this had a negligible effect on the pooled estimates. Ten studies were conducted among IPs while the remainder were among OPs.

Risk of Bias assessment

Of the 38 studies, the quality assessment was high or intermediate in 24 (63%) for geographical representativeness, in 29 (76%) for age representativeness, in 38 (100%) for general representativeness, in 31 (82%) for sensitivity of symptoms, and in 38 (100%) for laboratory methods (Supplementary tables 2 and 3). Age representation was rated as low in 10 (26%) because data were presented for all ages, and sensitivity of symptoms was rated low in seven (18%) as they required two or more specific symptoms for inclusion of subjects in study.

Meta-analysis

Over the influenza seasons included, the pooled estimates of the proportion of people of any age who were tested and who were positive for any type of influenza were 36% (95% CI 33%-40%, I^2=99.5%, 9 studies, 47 datasets) for OPs, and 24% (95% CI 20-29, I^2=98.4%, 5 studies, 16 datasets) for IPs (Figure 2). Proportions by influenza types and subtypes are presented in Table 1. I-squared sensitivity analysis in which 10 datasets where the proportion of positivity was <5% or >50% were excluded slightly reduced the estimated estimates but did not significantly decrease the observed heterogeneity (33% [95% CI 30-37, I^2=99.34%] and 23% [95% CI 19-27, I^2=97.76%] for OPs and IPs respectively).

Figure 2: Forest plot (studies from literature review) showing proportion of all influenza viruses grouped by healthcare setting (outpatient or inpatient) in Europe
Key: ES effect size; CI confidence intervals; IP inpatient; OP outpatient
Note: Many studies reported findings for more than one season and more than one country.

Table 1. Pooled estimates of the proportion of medically-attended people with ARI/ILI testing positive for influenza viruses, overall and by age group from literature review meta-analysis.

Influenza virus	Proportion positive %	95% CI, number of studies	Proportion positive by age group (%) (95% CI, number of studies)		
			0-17yrs	18-64yrs	≥65yrs
Any influenza virus					
Overall	**33**	**30-36, 14**	**26 (22-31), 21**	**41 (32-50),4**	**33 (27-40),5**
Outpatient	36	33-40, 9	26 (21-31), 18	41 (32-50), 4	33 (27-40), 5
Inpatient	24	20-29, 5	9 (6-12), 3	-	-
Influenza A					
Overall	**24**	**21-26, 11**	**12 (9-15), 12**	**22 (16-29), 4**	**18 (8-31), 4**
Outpatient	24	21-28, 8	14 (11-18), 9	22 (16-29), 4	18 (14-31), 4
Inpatient	26	16-26, 3	6(4-9), 3	-	-
Influenza B					
Overall	**7**	**6-10, 16**	**6 (3-8), 17**	**15 (9-24), 4**	**10 (3-19), 5**
Outpatient	9	6-12, 10	9 (5-14), 13	15 (9-24),4	10 (3-19) ,5
Inpatient	5	3-8, 6	3(1-5), 4	-	-
Influenza A(H1N1): pre 2009 pandemic					
Outpatient	3	1-5, 2	3 (1-6),4	-	-
Inpatient	-			-	-
Influenza A(H1N1): post 2009 pandemic					
Overall	**12**	**8-16, 7**	**6 (4-8), 4**	**16 (11-22), 2**	**4 (1-8), 2**
Outpatient	11	7-15, 3	8 (5-10), 2	16 (10-22), 2	4 (1-8), 2
Inpatient	14	5-26 , 4	2 (1-5), 1	-	-
Influenza A(H3N2)					
Overall	**11**	**8-14, 11**	**7 (4-9), 11**	**8 (4-12), 3**	**10 (2-22), 4**
Outpatient	13	9-17, 6	10 (7-13), 10	8 (4-12), 3	10 (2-22), 4
Inpatient	9	5-14, 5	4(1-8) 1	-	-

For OPs, the following pooled estimates of proportion influenza positive patients were noted from studies which reported by age groups: 0-17 years, 26% (95% CI 21-31, 18 studies, 32 datasets); 18-64 years 41% (32-50, 4 studies, 14 datasets); and ≥ 65 years, 33% (95% CI 27-40, 5 studies, 16 datasets)(Supplemental figure 1). For IPs, data were only available for the age group 0-17 years, 9% (95% CI 6-12, 3 studies, 3 studies, 9 datasets) (Supplemental figure 2).

Table 1 shows the proportions of influenza A, subdivided into pre-pandemic A(H1N1), post-pandemic A(H1N1)pdm09, and A(H3N2) viruses and influenza B in OPs and IPs across the different age groups. The highest proportion of influenza A infections was seen in the 18-65 year age group with a pooled proportion estimate of 22% (95% CI 16-29, 4 studies, 12 datasets), followed by the ≥65 years age group (18% [95% CI 14-31, 4 studies,13 datasets]). Similar to influenza A overall, the proportion infected with A(H1N1)pdm09 virus was highest in the age group 18-64-65 years at 16% (95% CI 11-22, 2 studies, 10 datasets) in OPs. The highest proportion of influenza A(H3N2) virus was noted in the ≥65 years age group (10% [95% CI 2-22, 4 studies]). For influenza B, the highest proportion of confirmed patients were in the 18-64 year group (15% [95% CI 9-24, 4 studies]), 12 datasets (Supplemental Figures 3-14).

Pooled estimates of the proportion of confirmed influenza stratified according to influenza season ranged from 19% in 2011-12 (95% CI 9-31, 2 studies) and in 2013-14 (95% CI 14-24, 2 studies, 5 datasets) to 48% (95% CI 46 to 49, 2 studies) in 2002-03. When stratified by country, pooled estimates of confirmed influenza ranged from 9% (2 studies) to 65% (1

study). Stratification by the requirement for fever in the case definition of ILI/ARI in individual studies, did not reveal a significant difference in the pooled proportion of influenza positivity between studies specifying the presence of fever and those in which it was not mandatory (32% [95% CI 25-39, 8 studies] versus 31% [95% CI 28-35, 3 studies respectively], p=0.82). Heterogeneity was high (I^2>90%) in all the meta-analyses.

The European Surveillance System (TESSy) data

ILI/ARI data were collected from 44 countries and SARI data from 15 countries. Both datasets presented findings from persons of all ages only. The pooled estimate for all influenza in tested patients seeking medical attention from the ILI/ARI dataset was 33% (95% CI 31-34, I^2=99.35%), and for SARI the proportion was 24% (95% CI 21-27, I^2=98.75%). Table 2 shows the proportions of detections of influenza A, B, A(H1N1), A(H1N1)pdm09 and A(H3N2) viruses by case definition in TESSy.

Pooled estimates of the proportion of confirmed influenza in tested ARI patients for seasons between 2004-05 and 2017-18, ranged from 22% (95% CI 18-27) in 2013-14 to 39% (95% CI 34-44) in 2012-13 and in 2017-18. The highest estimated proportions of confirmed infections were noted in 2016-2017 for influenza A (32% [95% CI 28-37]), in 2015-2016 for influenza A(H1N1)pdm09, excluding 2009-2010 (17% [95% CI 14-20], in 2016-2017 for influenza A(H3N2) (27% [95% CI 22-32]) and in 2012-2013 for influenza B (17% [95% CI 13-21]) (Figure 3).

Figure 3: Influenza positivity as a proportion of all ILI/ARI tested by influenza season: all influenza and by type and subtype (TESSy data)

The pooled estimated proportion of confirmed influenza varied across countries, ranging from 6% (95% CI 2-10, 8 seasons) to 78% (1 season) for ARI, and from 8% (1 season) to 76% (1 season) for SARI.

Table 2 . Pooled proportions of medically-attending patients with ARI/ILI/SARI testing positive for influenza from TESSy data; by all influenza types and by influenza subtypes

	Pooled proportion % of influenza positive (95% CI)	
	ILI/ARI [OP:Total tested 609,368][a]	**SARI [IP: Total tested 61,182)[b]**
All influenza	33 (31-34)	24 (21-27)
Influenza A	21 (20-22)	16 (13-18)
Influenza B	8 (7-9)	6 (4-8)
Influenza A(H1N1) **Pre-pandemic** **Post-pandemic**	 3 (2-3) 6 (5-7)	 - 5 (3-7)
Influenza A(H3N2)	8 (7-9)	5 (3-7)

[a]44 countries, 558 country-years
[b]15 countries, 87 country-years
Denominator = number of specimens tested from patients with ARI/ILI/SARI
ARI acute respiratory infection
CI Confidence intervals
ILI influenza-like illness
IP inpatients
OP outpatients
SARI severe acute respiratory infection

Discussion

To our knowledge, these are the most comprehensive data assembled to date on the proportion of laboratory-confirmed influenza in people across the WHO European Region presenting for medical care and with clinically-diagnosed ARI or ILI. We used two approaches: a literature review and a review of surveillance data reported to WHO, to estimate the prevalence of within-season influenza in Europe. Both methods produced findings in all ages and for inpatients and hospitalised patients. The proportion of seasonal

influenza from the literature review data in outpatients was 36% and 24% for inpatients. From the TESSy data ILI/ARI proportion for all influenza was 33%, while the SARI proportion of all influenza was 24%. The 95% CIs for the TESSy ARI and SARI data were narrower reflecting the larger number of samples. The lower proportion of laboratory-confirmed influenza in hospitalised patients compared to patients seeking outpatient care has been found in other parts of the world [23-26]. Diagnostic tests for influenza perform best when specimens are collected as close to the onset of symptoms as possible, ideally within 72 hours [27]; hospitalised patients may experience symptoms for some time prior to admission and being swabbed which may decrease detection rate. The observation may also be a reflection of different characteristics of the outpatient and inpatient populations, and neither the ILI nor the SARI case definition is specific to influenza.

Our pooled analyses of the proportion of confirmed influenza using both the literature review data and the TESSy data are somewhat higher than those that have been estimated in the United States. A study pooling data from the North American literature estimated an influenza incidence in children under 18 years of 12% (95% CI 4.6 to 14.7%) and 6.1% (95% CI 4.3 to 7.9%) in adults over several seasons when influenza severity was moderate [28]. However, there is some evidence that the situation in Europe may be different. In a meta-analysis examining the contribution of influenza to medically-attended ARI in children over several seasons in high-income countries, the proportion of influenza positive patients ranged from 18% (95% CI 12 to 25%) in the United States to 29% (95% CI 21 to 37%) in Europe, which is closer to the results we obtained [14]. This may be partly explained by differences in influenza vaccine recommendations in Europe and the United States. Although most countries in the European Union and European Economic Area have policies

in place for seasonal influenza vaccination of people in high-risk groups, not all countries target children and vaccination coverage rates vary widely across the groups recommended for vaccination [29]. In the United States however, seasonal influenza vaccination is recommended for everyone aged 6 months and over unless contraindicated [30]. Additionally, there may be differences between healthcare-seeking behaviour for ILI in different parts of the world, with some countries having a higher threshold than others [31].

The proportion of positive influenza tests varied from season to season in both the literature review and analysis of the TESSy data. Over the totality of included seasons, influenza A accounted for a greater proportion of laboratory-confirmed influenza infections than influenza B. Since the 2009 pandemic, influenza A viruses have been dominant or co-dominant in seven of eight seasons across the European Region with influenza B dominant seasons occurring only infrequently, most recently in 2017-2018 [32]. Our literature review data of five seasons since the 2009 pandemic mirror these findings, with influenza A virus accounting for the greatest proportion of confirmed influenza in 2010-11, 2011-2012, 2013-2014 and 2014-2015, and similar proportions of confirmed influenza A and influenza B in 2012-2013, a season when the influenza A and B were co-dominant. Over the ten year period between 1999 and the 2009 pandemic, seven influenza seasons in Europe were dominated by influenza A(H3N2), with or without co-circulation of influenza B, with significant circulation of pre-pandemic H1N1 in only two of these seasons in 2000-2001 and 2007-2008 [33-36], which is in accordance with the data from both our literature review and the TESSY analysis.

We acknowledge that our study has a number of important limitations. Differences in estimated proportions between age groups may be genuine, but such differences may also arise through variation in health-care seeking behaviour according to age. There is evidence that younger working age adults are less likely to seek healthcare than children and even then only when they are very unwell, so the denominator may be smaller in this age group which could lead to increased overall positivity in accordance with our findings [[37]. Observed differences in the proportions of influenza positive patients between the age groups should be interpreted with caution. We were only able to collect age group data from the literature review, and the papers identified varied in how age was categorised. There were very few studies that reported on children who were under 3 years old; the largest number of the papers reported the age group ranging from 0-17 years, and hence it is possible that older children have been over-represented in our meta-analysis. Furthermore, although we did not include data for the 2009 pandemic, it is possible that health-seeking behaviour may have increased in some age groups in the seasons immediately following the pandemic and this may have affected our findings.

We defined influenza burden as the percentage of patients with an ARI or ILI seeking medical care and tested for respiratory viruses, who are positive for laboratory-confirmed influenza. We are unable to comment on the burden of laboratory-confirmed influenza in people who get ARI or ILI and who seek care but are not tested, nor in those who develop symptoms but do not seek medical care. The proportion of true influenza in each of these populations may well be lower than our estimates. A study from the United States estimated that 38% of people with influenza present for medical care, less than the proportion of people with RSV or adenovirus infections but greater than in those with

rhinovirus, coronavirus, parainfluenza virus and other respiratory viral infections [38]. A FluWatch cohort study over five seasons from the UK found that only 17% of those with PCR-confirmed influenza had medically-attended illness [1]. Other studies from Europe have shown that the majority of people with ARI or ILI do not seek medical care, but with much variation between countries and between northern and southern Europe, suggesting regional cultural differences [31]. Healthcare seeking behaviour and clinician behaviour are complex issues and decisions made at different points of the clinical interaction may affect the overall composition of the sampled population in terms of severity of illness. Even if influenza attack-rates and healthcare seeking behaviour are similar between countries, if the propensity of clinicians to test patients, refer them to hospital and to admit them differs across countries, the resulting hospitalised populations will vary in the severity of their illness. Our estimates from the literature review and from TESSy were within-season estimates as influenza shows clear seasonality in temperate regions, so the positivity estimates will not apply to respiratory illnesses occurring outside the influenza season. This also limits direct comparability to existing influenza burden estimates, including multiplier-based burden analyses and global burden estimates, which use annualised estimates and may thus have lower influenza positivity [39-41]. However, within-season estimates have more relevance for public health planning in countries with defined influenza seasons.

In the literature review, one person reviewed the references and extracted the data, so it is possible that some studies were missed. Additionally, there was greater representation of countries in the western part of Europe in the published literature and although we did not exclude non-English language studies, it is likely that the database searched was less likely to include studies from countries in Eastern Europe. We did not search Russian language

databases in our literature review which may have added more data from eastern European countries.

Heterogeneity was very high in the meta-analyses but as our outcomes were absolute measures rather than ratio measures which tend to be more stable across studies, this was not unexpected [42]. Multiple factors are also likely to cause such heterogeneity, including differences in healthcare systems, case definitions, age groups, climate, vaccination coverage and general health, which makes comparisons challenging. It is also likely that there are cultural differences between countries in terms of the healthcare seeking behaviour of citizens. We included papers reporting ARI or ILI as defined by the individual studies rather than standardised definitions so this is a potential source of heterogeneity. In 2011 the WHO revised the clinical case definition of ILI to enhance its specificity without greatly compromising its sensitivity, such that the requirement for 'sore throat' and 'absence of another diagnosis' were omitted and 'sudden onset of fever' was replaced by 'acute respiratory illness'. The case definition of an ARI does not require fever to be present so is less specific for detecting influenza than the revised ILI definition [43]. We explored potential sources of heterogeneity through stratification and sensitivity analyses, yet considerable heterogeneity remained and the results should be interpreted taking this unexplained heterogeneity into consideration.

Notwithstanding, we believe that this study adds to the knowledge base on the contribution of seasonal influenza virus infections to respiratory illness across the region. Estimates of influenza positivity can help with appropriate allocation of limited health resources among competing disease priorities, establish epidemic thresholds for comparison of disease

severity between seasons and localities, and provide a platform for the evaluation of the effectiveness of vaccines and other intervention [44]. Particular strengths of this study include the use of viral culture or RT-PCR, which is the gold standard for influenza diagnosis because of its superior analytic and clinical sensitivity [45]. In the literature review we used a comprehensive search strategy, careful inclusion criteria and used an adaptation of an accepted quality assessment scale. Additionally, we relied on data collected individually within a full season which strengthens the validity of our results.

In conclusion, this analysis estimated the proportion of laboratory-confirmed seasonal influenza in symptomatic people who presented for health care with ARI and were subsequently tested for influenza viruses in the European region across the influenza seasons between 1996 and 2017. The estimated proportion of positive tests was shown to be greater in outpatients than in hospitalised patients by both methods, with differences according to influenza subtype and across different age groups. Overall, in Europe laboratory-confirmed influenza accounts for around one third of all acute respiratory infections for which medical care is sought during the influenza season and where laboratory testing for influenza is undertaken. The effect of the ongoing COVID-19 pandemic on healthcare-seeking behaviour for ILI, and changes in countries' testing priorities and capacities may potentially affect estimates of influenza positivity in future seasons and this should be taken into account when comparing our results and will require further investigation in forthcoming influenza seasons.

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