Systematic review of respiratory viral pathogens identified in adults with community-acquired pneumonia in Europe.

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Abstract

Community-acquired pneumonia (CAP) is an important respiratory disease and the fifth leading cause of mortality in Europe. The development of molecular diagnostic tests has highlighted the contributions of respiratory viruses to the aetiology of CAP, suggesting the incidence of viral pneumonia may have been previously underestimated. We performed a systematic review and meta-analysis to describe the overall identification of respiratory viruses in adult patients with CAP in Europe, following PRISMA guidelines (PROSPERO; CRD42016037233). We searched EMBASE, MEDLINE, CINAHL, WHOLIS, COCHRANE library and grey literature sources for relevant studies, and screened these against protocol eligibility criteria. Two researchers performed data extraction and risk of bias assessments, independently, using a piloted form. Results were synthesised narratively, and random effects meta-analyses performed to calculate pooled estimates of effect; heterogeneity was quantified using I^2. Twenty-eight studies met inclusion criteria of which 21 were included in the primary meta-analysis. The pooled proportion of patients with identified respiratory viruses was 22.0% (95% CI: 18.0%-27.0%), rising to 29.0% (25.0%–34.0%) in studies where polymerase chain reaction (PCR) diagnostics were performed. Influenza virus was the most frequently detected virus in 9% (7%-12%) of adults with CAP. Respiratory viruses make a substantial contribution to the aetiology of CAP in adult patients in Europe; one or more respiratory viruses are detected in about one quarter of all cases.

Introduction

Community-acquired pneumonia (CAP) is a principal cause of excess hospitalisation and mortality worldwide[1-3]. Historically, the overriding clinical approach to the management of CAP has been to focus on bacterial aetiologies, with *Streptococcus pneumoniae* the dominant pathogen [4-8]. More recently, coupled to the increasing availability of polymerase chain reaction (PCR) tests, the identification of viral pathogens in the aetiology of CAP has increased. Contemporary studies identify that viruses may be implicated in 15%-30% of all CAP[9-11]; in turn this heightens the possibility that empirical antibiotic treatment of CAP in the absence of adequate testing for viral pathogens may contribute to inappropriate antibiotic usage[12,13].

Given the considerable variation across individual studies in estimating the contribution of respiratory viruses to CAP aetiology, reliable summaries of relevant data are necessary to inform future research and policy initiatives, particularly as new respiratory virus vaccines and antiviral drugs are anticipated in the short to medium term[11,14-17]. Two recent systematic reviews of studies investigating the proportions of viral pathogens in patients with CAP focussed on studies that only used polymerase chain reaction (PCR)-based assays to detect viral pathogens and pooled results from studies conducted across the world.[18,19] We report an additional systematic review of studies conducted within the World Health Organization European Region, which offers additional granularity according to setting, timing of study , viral diagnostic techniques and study quality.

Methods

The study protocol was registered on the National Institute for Health Research International Prospective Register of Systematic Reviews (PROSPERO; CRD42016037233; available at: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016037233) and conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20]

Eligibility criteria

We identified studies which investigated the aetiology of CAP in adults in Europe (defined as those countries covered by the WHO Regional Office for Europe http://www.euro.who.int/en/countries) and reported quantitative data on the identification of respiratory viruses. We searched for original articles describing longitudinal studies or case series, in English, which investigated adults aged ≥16 years diagnosed with CAP. All other study designs were excluded. We included studies that performed either PCR or non-PCR detection techniques.

We excluded studies of paediatric populations and patients residing in nursing homes, residential care homes or rehabilitation facilities. Studies of adults diagnosed with CAP based on clinical signs but without radiologic confirmation, and studies focused on CAP in adults with severe immunosuppression through disease and/or drug treatment were also excluded.

Search strategy and screening

The following electronic databases were systematically searched: EMBASE, MEDLINE, CINAHL, WHOLIS, and Web of Science from January 1999 to April 2016. A comprehensive search strategy was developed for EMBASE **(Supplementary Appendix 1)** and subsequently adjusted as required to suit other databases. The reference lists of all eligible articles were manually searched to identify other eligible studies.

All identified articles were imported to ENDNOTE software X4 (Thomson Reuters, Toronto, CA, USA) and duplicates removed. Two review authors (YA and JSN-V-T) independently screened the retained articles against protocol eligibility criteria, in three stages: by title, abstract and full text. Any disagreements were resolved through discussion between YA and JSN-V-T; and a third author (WSL) adjudicated over any outstanding discrepancies.

Data extraction and Risk of Bias assessment

Data extraction for each eligible study was also performed independently by YA and JSN-V-T using a pre-piloted data extraction form using Microsoft® Office Excel® 2010 (Microsoft Corporation, Richmond, VA, USA). For all included studies, information was extracted on: author(s); year of publication; country; healthcare setting; number of evaluable patients; viral diagnostic techniques employed; samples collected for virus detection; number of respiratory virus pathogens tested for; and number and proportion of respiratory viruses detected. YA and JSN-V-T independently assessed the quality of all included studies, using criteria adapted from the Newcastle – Ottawa scale for observational studies[21], focusing on three key domains: representativeness of patient population; ascertainment of CAP diagnosis; and ascertainment of virus aetiology. We awarded zero or one star in each domain; for representativeness, one star was awarded for studies sampling from the general community (as opposed to more specialised patient subgroups); for ascertainment of CAP diagnosis we awarded one star for independent radiographic confirmation of diagnosis; and for virus aetiology, one star for use of 'gold standard' PCR diagnostic techniques.

Summary measures, and analysis

The proportion of respiratory viruses identified in evaluable CAP patients was pooled using the generic inverse variance approach, based on a random effects model (DerSimonian- Laird weights method)[22], stabilising the variances using the Freeman-Tukey double arcsine transformation so that studies with proportions close to 0% or 100% were appropriately estimated[23]. Exact binomial confidence intervals were computed for outcomes. The primary outcome was the overall contribution of respiratory viruses in the aetiology of CAP, calculated as the total number of patients with respiratory viruses identified (numerator) as a proportion of the total number of evaluable patients (denominator). We report, as secondary outcomes, the contribution of individual viruses calculated as the total number of patients with individual respiratory viruses identified as a proportion of all evaluable patients for each specific pathogen.

Heterogeneity between studies was quantified using the I^2 statistic[23]. We investigated potential sources of heterogeneity by performing subgroup analyses; by study setting (inpatient vs. outpatient), study quality, viral diagnostic methods used (PCR diagnostic techniques vs non-PCR methods) and mixed infections (bacterial and viral infections). All analyses were conducted with the *metaprop* commands within Stata (V.13, Stata Corp, College Station, Texas, USA).

Results

We identified a total of 1106 articles from database searches, reducing to 1083 after the removal of duplicates. Eleven additional papers were identified via citation tracking. After screening, 27 articles remained within protocol eligibility criteria (Figure 1); one of the included articles[25] presented two separate studies and data from both were extracted and presented separately. Thus, 28 studies from 27 articles were included in the systematic review[25-51], and 21 from 20 in the primary meta-analysis[25-44]. When examined as full-text articles, seven studies did not present sufficient quantitative data for inclusion in the primary meta-analysis[45-51] (Figure 1).

Study characteristics

All 28 studies included in the systematic review were prospective or retrospective longitudinal studies or case-series. The patient population size in each ranged from 71 to 1356 (total=8,777). The earliest publications were in 2001[37,40], and the most recent article was published in October 2015[26].

Studies from 11 different European countries were included of which Spain was most frequently represented (9 studies; 32.1%)[27,28,31,33,41,44,47,50,51]. Nineteen studies[*] (67.9%)[25,26,29-32,35,36,39-44,47-50] were carried out among inpatient populations (n=5,515 patients), three[34,38,46] (10.7%) in outpatient/community populations (n=524 patients) and six (21.4%)[27,28,33,37,45,51] in mixed populations (n=2,738 patients). Details of the characteristics of the included studies are summarised in Table 1. Sixteen studies (57.1%) [26,29,30,32,34-36,39,41-45,47,49,50] had used PCR techniques for the detection of respiratory viruses, alone or in combination with other diagnostic methods. 14 studies (50%) obtained upper respiratory samples[26,28,30,35,36,38,39,41-44,46,49,50], 16 (57.1%) lower respiratory[25,31-34,38,42,43,45-51](nine publications), and six (21.4%) both[38,42,43,46,49,50] . In 10 (35.7%) studies (9 publications) respiratory tract sampling was combined with assessment of paired serology[25,31-33,45,46,49-51]; and in four (14.3%) studies, serology alone was performed[27,29,37,40].

[*] Citation #25 describes two studies

Risk of bias assessment

Study population representativeness, diagnostic accuracy of CAP and ascertainment of virus aetiology were assessed with a maximum of three stars per study. Eleven studies[26,30,32,34-36,39,41-43,45](39.3%) were assessed as being at low risk of bias (three stars; one star per domain), 14[†] studies[25,26,29,33,37,38,40,44,46,47,49-51] (53.6%) at moderate risk of bias (2 out of 3 stars), and three[28,31,48] (7.1%) were at high risk of bias (one or zero stars). Six studies[α] (21.4%) reported difficulty in obtaining adequate samples for microbiological testing[25,27,32,37,41]. Within-study variation in viral diagnostic methods across different study years was reported in ten studies (35.7%)[26,29,30,35,36,39,41-44].

Overall identification of respiratory viruses

The percentage of respiratory viruses detected in CAP patients ranged from 6% in a Spanish study comprising both inpatients and outpatients[33], to 45% in a study of hospitalised patients in Israel[42]. By meta-analysis, the pooled proportion of respiratory viruses detected in CAP patients was 22.0% (95% CI 17.0%-27.0%; I^2=94.7%) (Figure 2).

There was a significant trend for the identification of respiratory virus pathogens to be lower in studies (n=8)[α] published from 2001 to 2009[25,31-34,37,40], (pooled estimate=14.0% (95%CI 9.0%-21.0%)) compared with more recent studies (n=13) published after 2010[26-30,35,36,38,39,41-44] (pooled estimate=27.0% (95%CI 20.0%-33.0%)), test for subgroup differences, p=0.007 (Supplementary Appendix 2).

Sub-group analyses

The pooled proportion of respiratory viruses identified among inpatient studies (n=15) [25,26,29-32,35,36,39-44]with CAP was 27.0% (95% CI 23.0%-31.0%; I^2=85.1%); compared with 19.0%

† Citation #25 describes two studies

(95% CI 14.0%-24.0%; I^2=95.3%) for outpatient studies (n=2)[34,38], and 9.0% (95% CI 6.0%-12.0%; I^2 =85.8%) in two studies with mixed populations (n=4)[27,28,33,37] (Figure 3). Each of these populations revealed results that were statistically significantly different from each other (test for subgroup differences, p<0.01). Studies with mixed populations[27,28,33,37], relied exclusively on non-PCR diagnostic methods and were of lower quality compared to other studies.

The pooled proportion of respiratory viral pathogens identified in 12 studies[26,29,30,32,34-36,38,41-44] using PCR (with or without additional testing methods) was 29.0% (95% CI 25.0%–34.0%, I^2=83.5%) compared with 13.0% (95% CI 9.0%–18.0%, I^2=90.7%) in nine studies using other non-PCR methods[25,27,28,31,33,37,38,40], with a significant difference between the two groups, p<0.001 (Figure 4).

In lower risk of bias studies (NOS score=3 stars)[26,30,32,34-36,39,41-43]the pooled proportion for total respiratory viral pathogens was 30%, (95% CI 25%–34%, I^2=77.4%), compared with 11% (95% CI 9%-13%, I^2=99.3%) in higher risk of bias studies (NOS score=1 star)[28,31], explaining the observed heterogeneity between studies, p<0.001 (Figure 5).

Mixed Infections

The pooled proportion of mixed respiratory viruses and bacterial co-infections detected in CAP patients was 10% (95% CI 6%-14%, I^2=94.7%) reported across 14 studies [26-29,32,33,35-37,40-44] (Figure 6).

Individual viruses

Data on the seven most common respiratory viruses identified are presented in Table 2. Influenza viruses were most frequently detected (9%), followed by rhinoviruses (5%) and coronaviruses (4%); together accounting for the majority of respiratory viruses detected (Table 2).

Discussion

This review updates evidence on the microbiological identification of respiratory viral pathogens in adult patients with radiographically confirmed CAP in Europe. Overall our data suggest that respiratory viruses are detectable in at least 22% of radiologically confirmed CAP cases, mostly hospitalised cases. However significantly higher proportions of respiratory viruses were evident in studies conducted after 2010 (27%), studies that included viral PCR techniques (29%), and studies assessed to be at lower risk of bias (29%), suggesting that the true proportion of CAP associated with respiratory viruses is at least one quarter (25%). Our findings accord with recent major studies or reviews conducted in Asia and North America [11,14,52,53]. In the CDC EPIC study[11], viruses were detected in 27.0% of adult patients with CAP, while Qu et al. detected viruses in 27.5% of Asian patients with CAP[51].

Our review suggests that in Europe, as in other parts of the world, a relatively large burden of CAP disease may be attributable to viral infections. However, the clinico-pathological significance of virus detection in patients with CAP remains uncertain. A clear limitation of our approach (and of each of the included studies) is that no proof is offered that the virus or viruses identified were of pathological significance in all cases. There was also heterogeneity between studies in terms of the respiratory sites sampled and/or use of serology. Viruses recovered from upper respiratory tract (URT) sites might have less pathological significance than those recovered from lower respiratory tract (LRT) sites; nevertheless, in the absence of concomitant sampling from URT and LRT it is not possible to disregard viruses identified from URT sites which may have been replicated in the LRT if it had also been sampled. Whilst respiratory viruses are undoubtedly implicated in the aetiology of a substantial proportion of the cases in which they are detected, asymptomatic illness associated with virus shedding is well recognised, especially in children who experience longer periods of shedding than adults[54]. In addition, modern PCR diagnostic techniques are comparatively more sensitive than methods for the detection of bacteria and capable of detecting small quantities of nucleic acid which may not in all cases represent culturable virus; therefore, some patients with 'viral-only' pathogens identified may also have a microbiologically unrecognised bacterial infection; and some detections of viral pathogens may represent previous or resolved virus infection. In a recent study, Gadsby et al. employed PCR techniques to identify bacteria as well as viruses from lower respiratory tract samples, viruses were detected in 30% of 323 adults admitted to hospital with CAP and a co-bacterial pathogen was detected in 82% of these[55]. In contrast we noted only 10% of cases with a bacterial co-pathogen; this might reflect the use of PCR testing for bacteria by Gadsby and colleagues, whereas the studies we included used standard approaches for the identification of bacteria. The detection of respiratory viruses in healthy asymptomatic individuals is not as extensively described as in symptomatic patients; nevertheless Jartti and colleagues summarised data from 51 studies, noting maximum baseline prevalences of respiratory viruses as follows: rhinoviruses, 15%; adenoviruses, 5.3%; influenza, 4.3%; RSV, 2.6%; coronaviruses, 2.5%; eneteroviruses 1.2%; human bocavirus, 1.1%; parainfluenza, 0.9%; and hMPV, 0.6%[54]. Jansen and colleagues have observed that rhinovirus is extremely common in asymptomatic children (28%), but that if other viruses are identified, notably RSV, adenoviruses and hMPV, these are much more likely to be clinically relevant[56]; this may be different in adults. We lacked direct comparison with any such 'asymptomatic control' group in the included studies, nor did we have access to data on the host response to viruses in individual subjects. However separate studies in asymptomatic patients[54,56] offer important contextualization for our findings; and inclusion of an asymptomatic comparator group would be likely to add granularity in future studies.

Since previous work identified high heterogeneity in the extant literature from other parts of the world,[18,19] we expected this and decided, *a priori*, that high heterogeneity would not preclude meta-analysis. We were unable to identify a single clear reason for the observed high heterogeneity which we attribute to multiple factors including study quality (Figure 5), variable settings, patient populations, sampling sites, and diagnostic methods; disease severity and co-infections with other pathogens. Since rhinovirus and Respiratory syncytial virus (RSV) RSV infections have a predilection for asthmatic patients[57,58], underlying comorbidities may have influenced our findings.

Influenza (9%) viruses, rhinoviruses (5%) and coronaviruses (4%) accounted for the majority of virus detections; these proportions are similar to the estimates reported previously by Burk et al and Wu et al[18,19]. However, RSV was identified in only 2% of adult CAP which may be relevant to the potential role of future RSV vaccines targeted at the elderly.

These findings highlight the importance of respiratory viruses in the aetiology of adult CAP, and the potential relevance of our findings towards improving clinical outcomes, and reducing inappropriate antibiotic use. Influenza appears to be the most significant virus pathogen, followed by rhinoviruses and coronaviruses. Notwithstanding, different included studies looked for between 4-11 separate respiratory viruses (Table 1); if all included studies had tested for all 11 viruses the overall proportion of virus detection may well have been considerably higher, although, as discussed above, not all detections necessarily have clinical relevance to CAP. This potential source of bias will not have affected the estimates for individual viruses (Table 2) because these analyses were organism-specific and based on all available data by organism. Viral diagnostic evaluation of CAP facilitates greater precision in the assessment of illness severity, and the tailoring of therapy, in particular the rapid use of neuraminidase inhibitors for cases of influenza and more judicious use of antibiotics. Since there are realistic near-term prospects for novel antiviral treatments for several respiratory virus infections and RSV vaccines[59-61], there is a need to establish baseline data on the incidence of viral CAP and develop a wider culture of testing for respiratory virus pathogens without which it will be difficult to assess the impact of advances in therapy.

We included only articles reported in English. An analysis including country-specific data reported in other languages may reveal regional variations in the contribution of respiratory viruses to the microbiology of CAP. Although, the effect of age was considered as an important source of heterogeneity, a sub-analysis by age could not be performed due to the lack of detailed reporting of study results by age groups; this may have influenced our results. Similarly, subgroup analyses could not be performed according to patient illness severity, patient comorbidities, type of respiratory sample or the presence of specific bacterial co-pathogens due to lack of data. Publication bias applies when studies reporting 'positive' findings are more likely to be published than those reporting 'negative' findings and is an important consideration in meta-analyses evaluating treatment effects. However, in the context of studies examining the proportion of CAP patients in whom viruses were detected, well-conducted 'negative' studies are as 'surprising' as 'positive' studies and both would be expected to be published. The first study to examine the use of standard publication bias tests for proportional meta-analyses (such as this one) found that funnel plots and statistical tests potentially yield misleading results, especially where the proportions within the studies are either very high or very low[62]. These researchers describe an alternative method that can be used to explore the potential for publication bias, where the sample size is used instead of the standard error for each study; however, the reliability and accuracy of this method has yet to be fully explored and independently validated. Therefore, we elected not to analyses publication bias.

Conclusion

This systematic review suggests that, in Europe, respiratory viruses are identifiable in at about one quarter of all adults presenting with CAP. Of these, the most frequently identified pathogens are influenza viruses, rhinoviruses and coronaviruses, accounting for over one half of all identified viral pathogens. Further study to determine the importance of identifying viral pathogens in relation to treatment with antibiotics or antivirals is warranted.

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Contributorship

All of the authors designed and contributed to the systematic review. Y.A. and J.S. N-V-T. performed study selection independently. Y.A. and JS. N-V-T. performed paired data extraction, data synthesis and quantitative analyses. Y.A. and JS. N-V-T drafted the article, and all other authors critically reviewed the article before submission.

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Potential conflicts of interest

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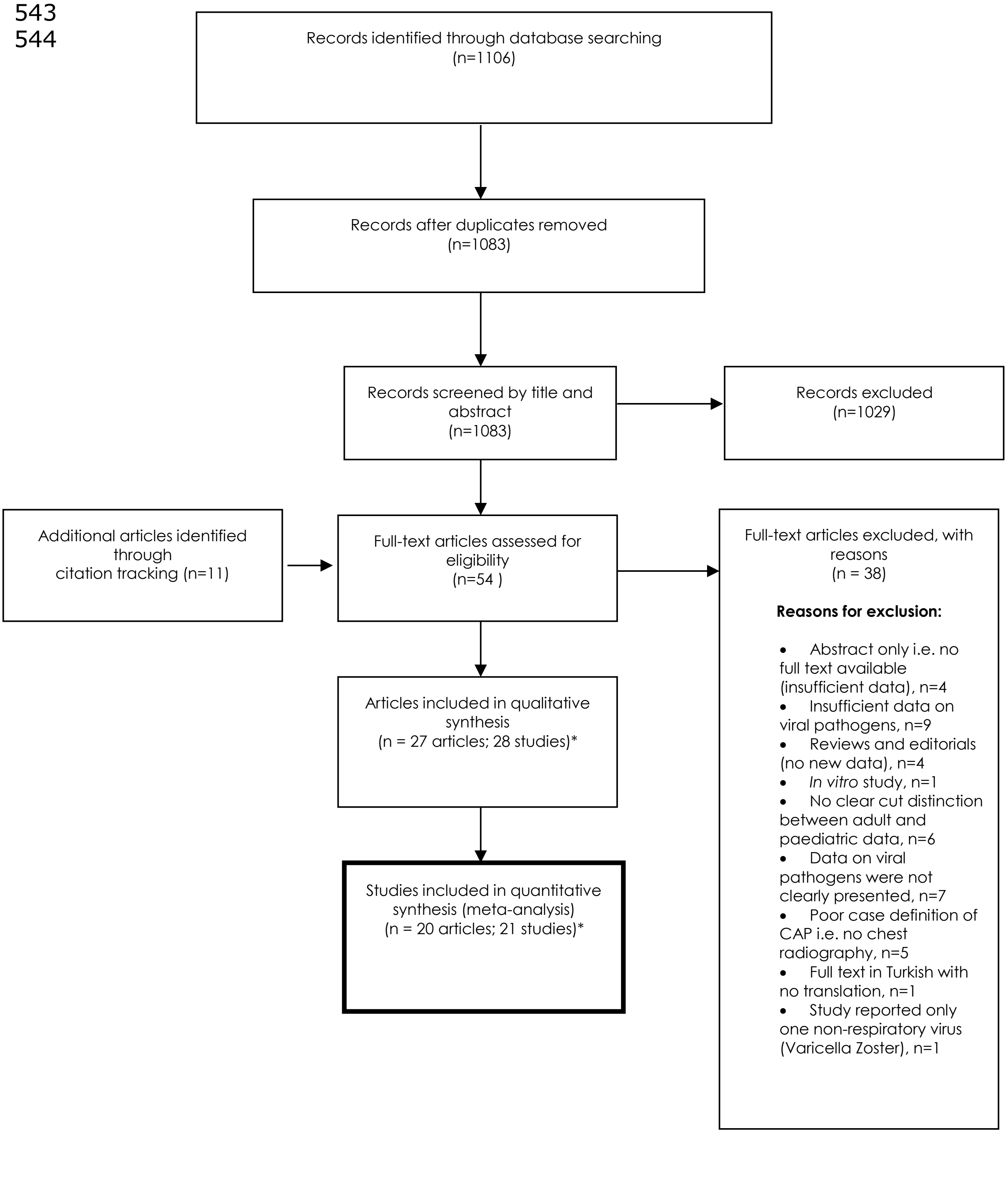
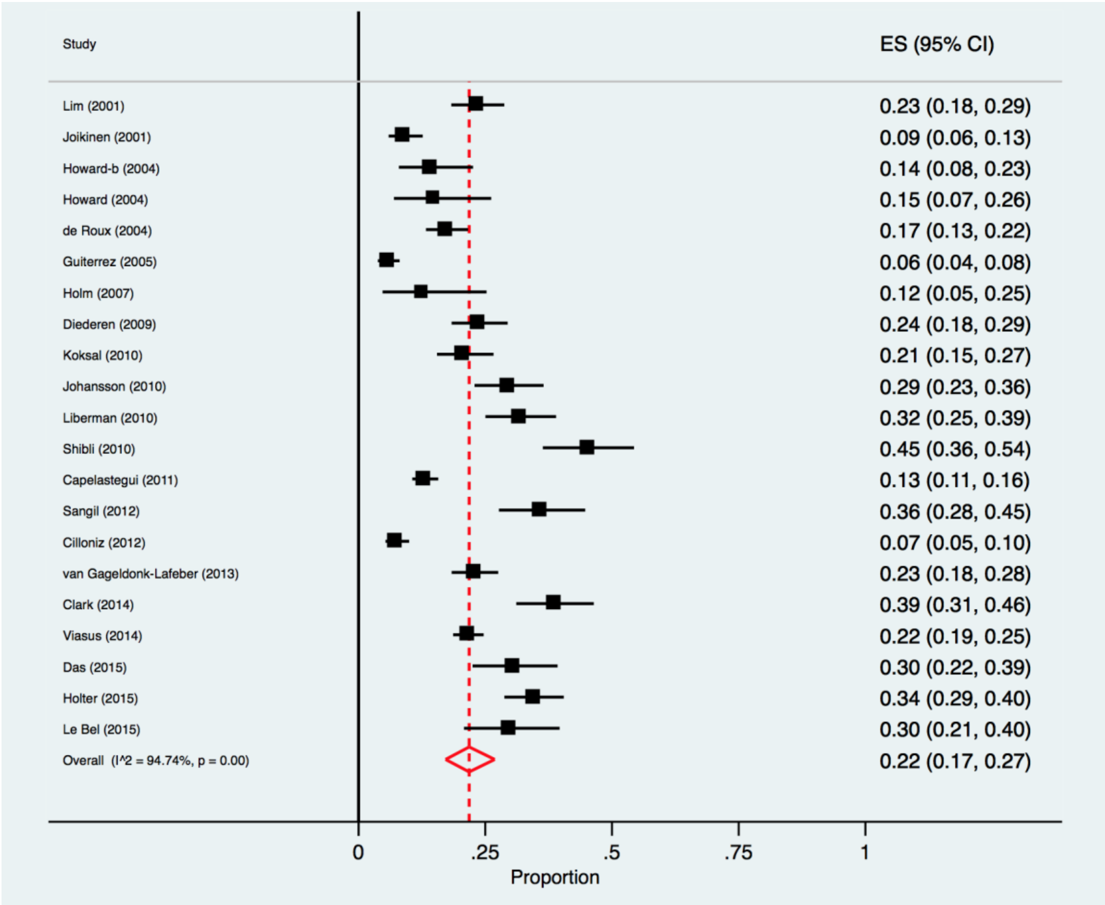
Figure 1: PRISMA flowchart.‡

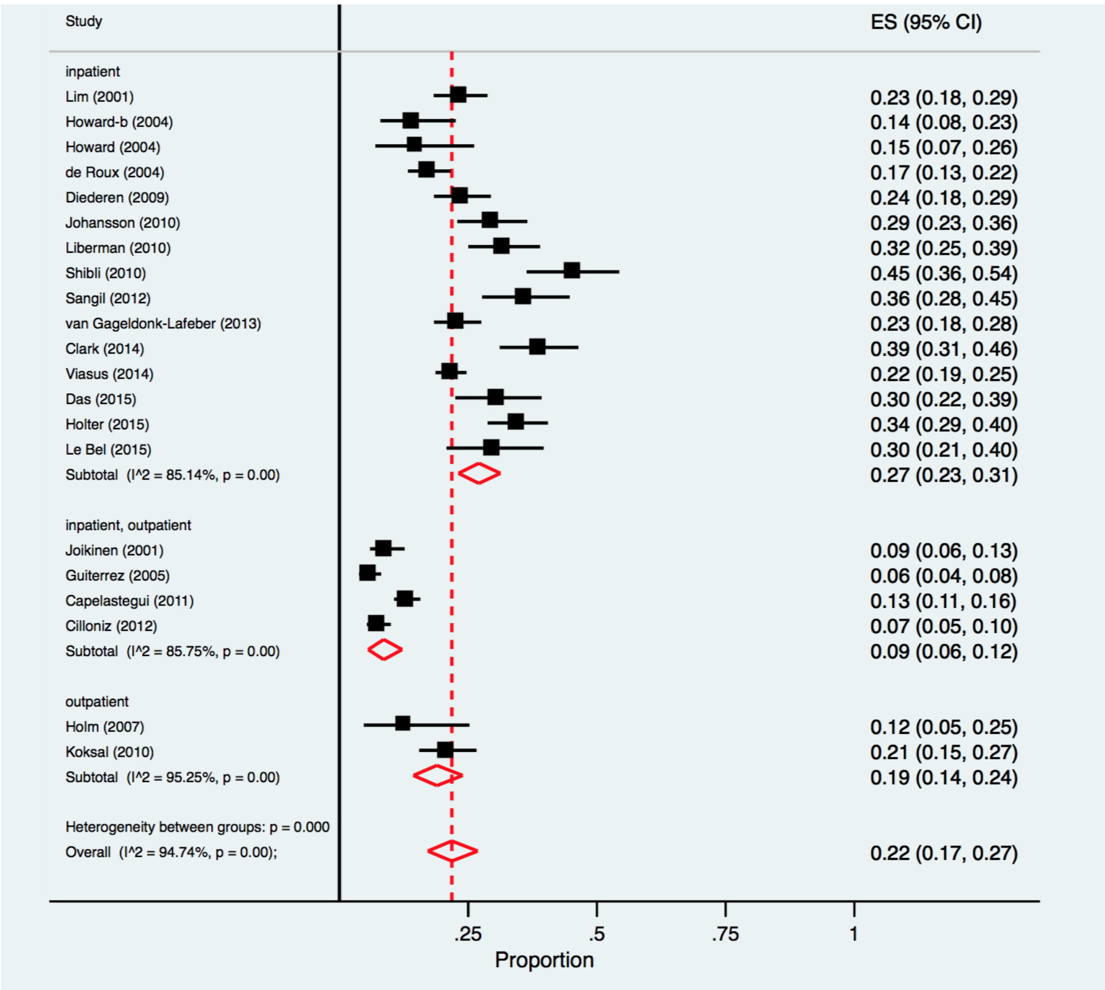


‡ One article presented data on two separate studies[25]



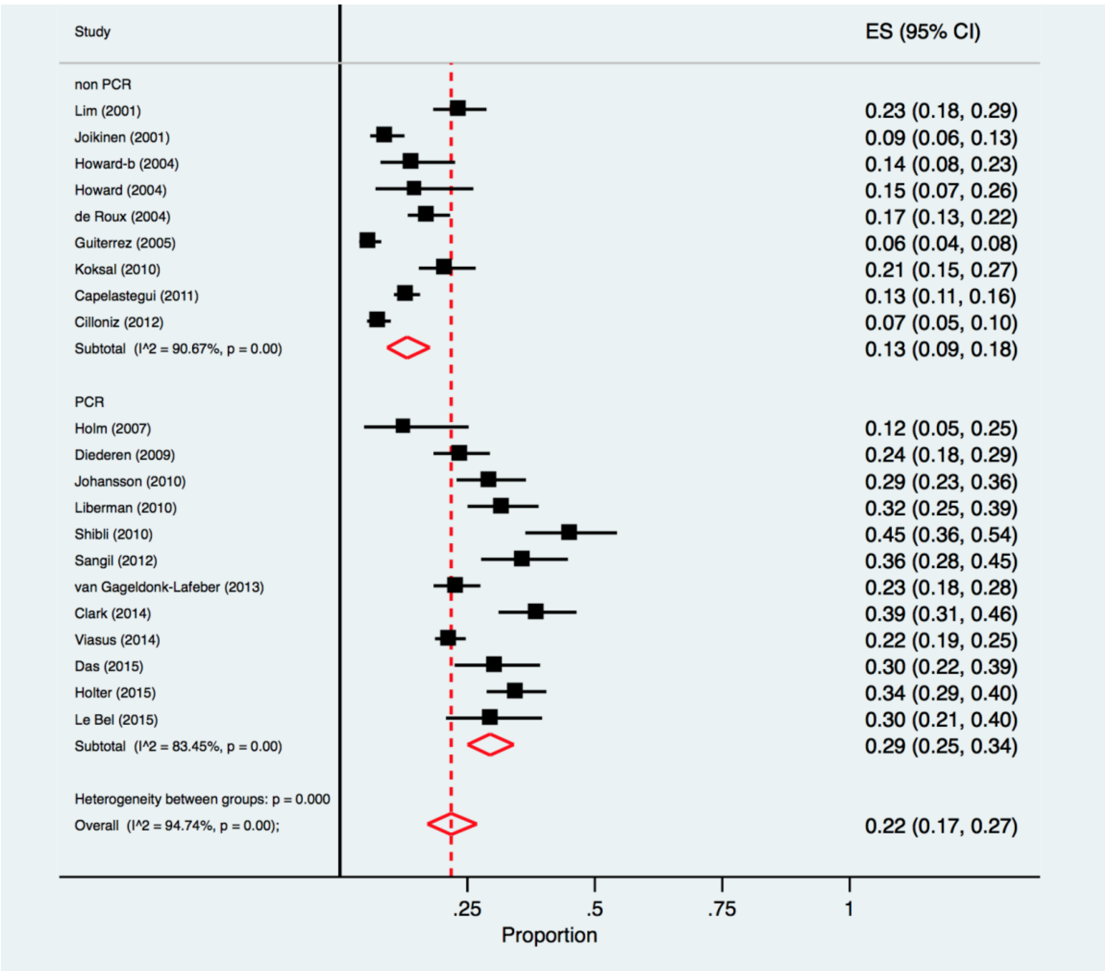
ES = effect size for pooled identification of respiratory viruses

Figure 2: Forest plot: overall identification of respiratory viruses in European adult patients with CAP.



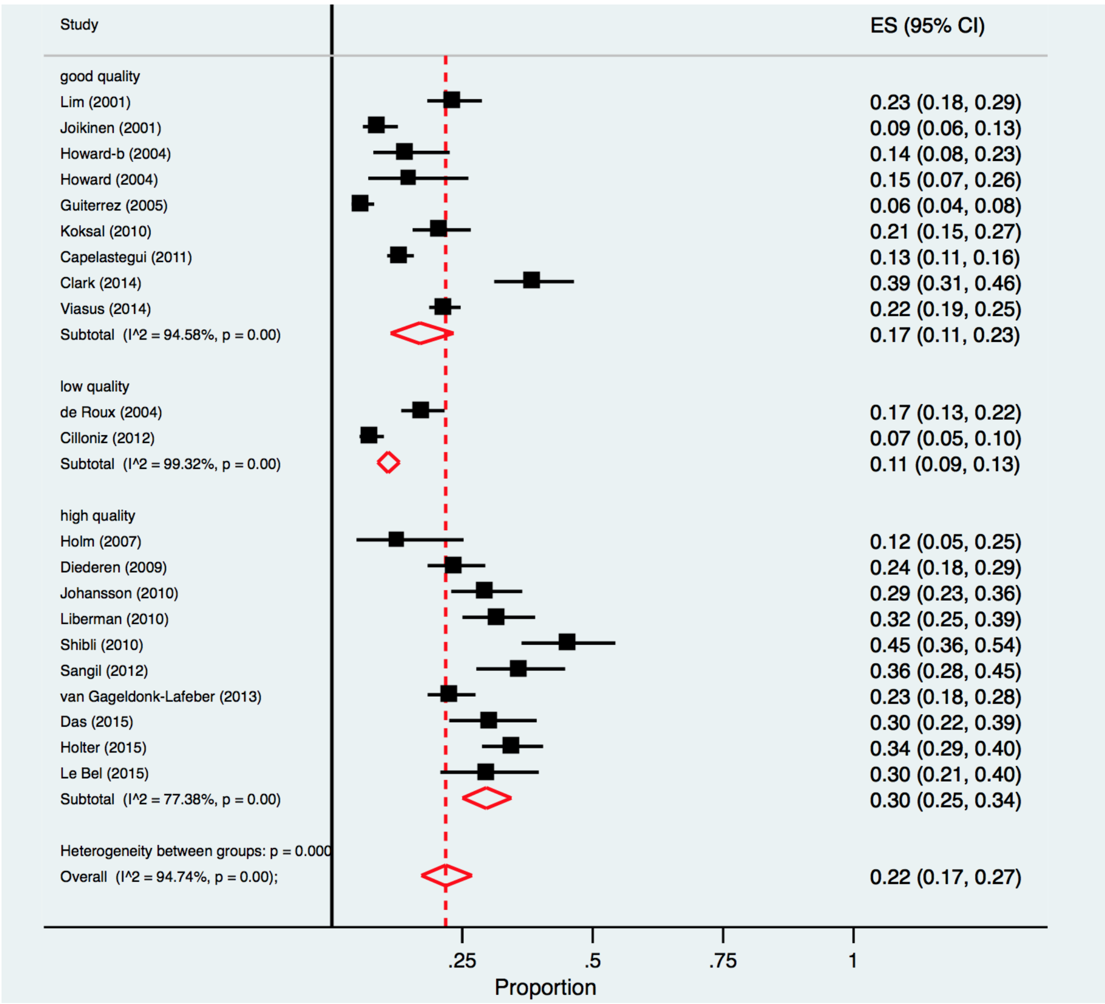
ES = effect size for pooled identification of respiratory viruses

Figure 3: Forest plot: overall identification of respiratory viruses in European patients with CAP, stratified by study setting.



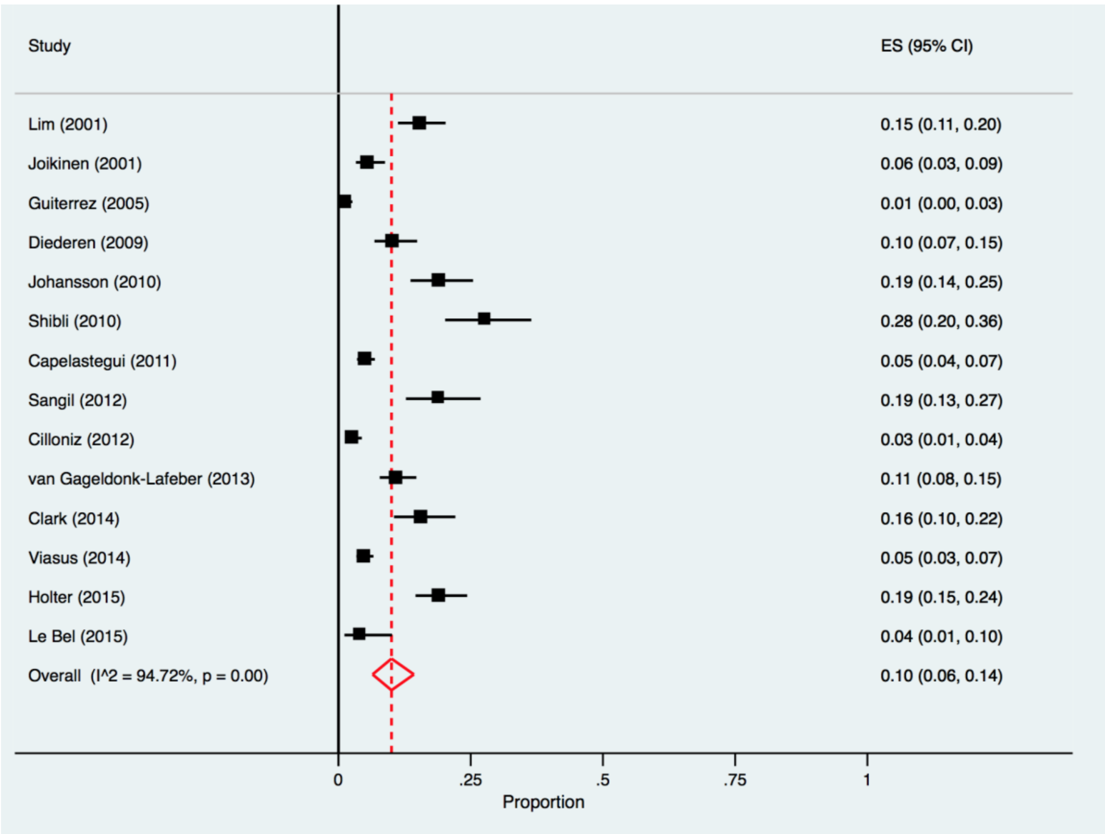
ES = effect size for pooled identification of respiratory viruses

Figure 4: Forest Plot: overall identification of respiratory viruses in European patients with CAP, by diagnostic method employed



ES = effect size for pooled identification of respiratory viruses

Figure 5: Forest plot: overall identification of respiratory viruses in European patients with CAP, according to study quality



ES = effect size for pooled identification of respiratory viruses

Figure 6: Forest plot: mixed respiratory virus and bacterial co-infections in European patients with CAP.

Table 1: Characteristics of included studies.

First author	Study setting	Study design	Patient characteristics	Total number of patients with CAP	Number of viruses tested for	Male %	Diagnostic methods	Principal study focus	Specimen sites*
Le Bel [2015] [26]	France, inpatients	Prospective cohort	Patients aged >18 years presented to Emergency dept.	319	8	101 (31.7%)	PCR	Inflammatory biomarkers in CAP patients	UR
Capelastegui [2011] [27]	Spain, inpatients and outpatients	Prospective cohort	Patients aged >18 years in the community and hospital	700	5	Not reported	Blood cultures, urinary antigen tests, serology, direct immunofluorescence antibody assay	Aetiology of CAP	S
Cilloniz [2012] [28]	Spain, inpatients and outpatients	Prospective cohort	Patients aged >16 years admitted to the Emergency wards and outpatients.	568	5	301 (53.0%)	Serology, blood culture, antigen tests.	Aetiology of CAP	UR
Clark [2014][29]	UK, inpatients	Prospective cohort	Patients aged >18 years admitted to hospital with acute respiratory infection but	166	9	87(52.4%)	Blood and Sputum culture, PCR	Aetiology of ARI in adults	S

			subset with CAP patients						
Das [2015][30]	France, inpatients	Prospective cohort	Patients aged >18 years admitted to the Emergency dept.	125	7	Not reported	PCR	Aetiology of CAP	UR
de Roux [2004][31]	Spain, inpatients	Prospective cohort	Patients aged >18 years admitted to hospital	1356	5	893(65.8%)	Serology, complement fixation kit tests for viruses.	Viral CAP in non-immunocompromised adults	LR, S
Diederen [2001][32]	Netherlands, Inpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	242	8	7(2.9%)	PCR, serology, ELISA	Detection of respiratory pathogens using PCR	LR, S
Guiterrez [2005] [33]	Spain, inpatients and outpatients	Prospective cohort	Patients aged >15 years admitted to the hospital	493	5	308(62.5%)	Blood and sputum cultures, complement fixation tests.	Investigating the influence of age and gender on the incidence of CAP	LR, S
Holm [2007][34]	Denmark, outpatients	Prospective cohort	Patients aged >18 years with CAP presenting to the GP	48	6	28(58.3%)	PCR	Aetiology of CAP	LR
Holter [2015][35]	Norway , inpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	267	8	140(52.4%)	Culture, serology, PCR	Aetiology of CAP in Norway	UR

Howard-a [2004] [25]	UK, inpatients	Prospective cohort	Patients aged >15 years	69	Not reported	6(8.7%)	Complement fixation tests, blood culture	Not reported	LR, S
Howard-b [2004] [25]	UK, inpatients	Prospective cohort	Patients aged >16 years	99	Not reported	6(6.1%)	Complement fixation tests, blood culture	Aetiology of CAP	LR,S
Johansson [2015][36]	Sweden, inpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	184	9	94(51.1%)	Culture, PCR, Serology	Aetiology of CAP	UR
Joikinen [2001] [37]	Finland, inpatients and outpatients	Prospective cohort	Patients aged >15 years admitted to the hospital and patients in the community	345	7	176(51.0%)	Serology	Aetiology of CAP in adults in Eastern Finland	S
Koksal [2010] [38]	Turkey, outpatients	Cross – sectional	Patients aged >17 years with CAP in outpatient settings	292	6	147(50.3%)	Culture, direct immunofluorescence, serology	Aetiology of CAP in adults in Turkey	UR, LR
Liberman [2010] [39]	Israel, inpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	183	8	105(57.4%)	PCR	Evaluate the role of respiratory viruses in adult CAP	UR
Lim [2001] [40]	UK, inpatients	Prospective cohort	Patients aged >16 years admitted to the hospital	267	5	135(50.6%)	Other conventional methods	Investigate the aetiology of CAP and implication for CAP management	S
Sangil [2012][41]	Spain, inpatients	Prospective cohort	Patients aged >18 years	169	9	Not reported	PCR, serology	Aetiology of CAP using PCR and other	UR

			admitted to the hospital					conventional methods.	
Shibli [2010] [42]	Israel, inpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	127	6	73(57.5%)	PCR, DNA & RNA extraction, Serology	Investigate the aetiology of CAP in hospitalised patients .	UR, LR
van Gageldonk-Lafeber [2013][43]	Netherland, inpatients	Prospective cohort	Patients aged >18 years presented to the Emergency dept.	339	9	212(62.5%)	Culture , serology, antigen tests, PCR	Aetiology of CAP	UR, LR
Viasus [2014] [44]	Spain, inpatients	Prospective cohort	Adult patients admitted to the hospital	747	8		PCR, Serology	Aetiology of CAP	UR
Templeton [2005] [45]	Netherlands, inpatients and outpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	136	Not reported	75(55.1%)	Culture, PCR, Serology	Aetiology of CAP	LR, S
Bochud [2001][46]	Switzerland, outpatients	Prospective cohort	Patients aged >15 years	184	4	82(44.6%)	Serology	Aetiology of CAP in outpatients	UR, LR, S
Marcos [2006] [47]	Spain, inpatients	Prospective cohort	Patients aged >14 years admitted to the hospital	198	7	Not reported	PCR, immunofluorescence and culture	Aetiology of CAP	LR
Hohenthal [2004] [48]	Finland, inpatients	Prospective cohort	Patients aged >18 years admitted to the hospital	71	7	48(67.6%)	Culture	Diagnostic value of BAL	LR
Huijskens [2013][49]	Netherland , inpatients	Prospective	Patients aged >18 years	408	11	250(61.3%)	PCR , Culture and serology	to differentiate pure bacterial, pure viral	UR, LR, S

			presented to the emergency dept.					and mixed viral and bacterial aetiologies based on clinical signs admission	
Cilloniz [2011] [50]	Spain, Inpatients		>18 years with CAP admitted to ICU within 24hrs	362	5	232(64.1%)	Immunofluorescence, PCR	polymicrobial pneumonia	UR, LR, S
Almirall [2007] [51]	Spain, inpatients and outpatients		>14 years, 216 patients were managed at home and 280 patients were admitted to hosp.	496	7		Culture, serology, Immunofluorescence	Differences in aetiology of CAP	LR, S

*Specimen sites: UR=upper respiratory tract; LR= lower respiratory tract; S=serological assessment (using paired sera). *In studies which sampled from >1 site, not all patients will have undergone sampling at all sites*

Virus type	Pooled %	95% CI	No. of studies (and patients) included in pathogen-specific meta-analysis	I^2(%)
Influenza (A or B)	9	7-12	17 (6,487)	93.45
Rhinovirus	5	4-7	12 (3,324)	88.22
Coronavirus	4	2-7	7 (1,343)	80.37
Parainfluenza	3	2-5	14 (5,600)	88.35
Human metapneumovirus (hMPV)	2	1-2	10 (1,779)	7.49
Respiratory syncytial virus (RSV)	2.	1-3	17 (5,968)	82.42
Adenovirus	1	0-1	13 (3,166)	32.88

Enterovirus, poliovirus, cytomegalovirus, coxsackie virus, varicella-zoster virus, human bocavirus and herpes simplex virus were detected in <1% of adult patients with CAP.

Table 2: Summary of individual pathogen-specific meta-analyses for respiratory viruses most commonly identified in European adult patients with CAP

APPENDIX 1: EMBASE SEARCH STRATEGY

1. virus.mp. or virus/

2. exp virus pneumonia/et [Etiology]

3. exp Adenoviridae/

4. exp Coronavirus/ or exp SARS coronavirus/

5. exp Influenzavirus B/ or exp Influenzavirus A/ or exp Influenzavirus C/

6. exp influenza/ or exp influenza B/ or exp Influenza B virus/ or exp influenza C/ or exp Influenza C virus/ or exp influenza A/ or exp Influenza A virus/

7. exp Parainfluenza virus infection/

8. exp Human respiratory syncytial virus/

9. exp Rhinovirus/ or exp Human rhinovirus/ or exp Rhinovirus infection/

10. exp Human metapneumovirus/

11. exp Human metapneumovirus/ or exp Metapneumovirus infection/ or exp Metapneumovirus/ or exp Human metapneumovirus infection/

12. Nipah virus/ or exp Paramyxovirinae/

13. (virus* or viral or (influenza or flu) or (parainfluenza or paraflu) or (metapneumovirus or hmpv) or adenovirus or (respiratory and syn* and virus*) or rsv or rhinovirus or coronavirus).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13

15. exp community acquired pneumonia/ep, et [Epidemiology, Etiology]

16. exp infectious pneumonia/ep, et [Epidemiology, Etiology]

17. (community and acquired and pneumonia).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

18. (community-acquired and pneumonia).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

19. 15 or 16 or 17 or 18

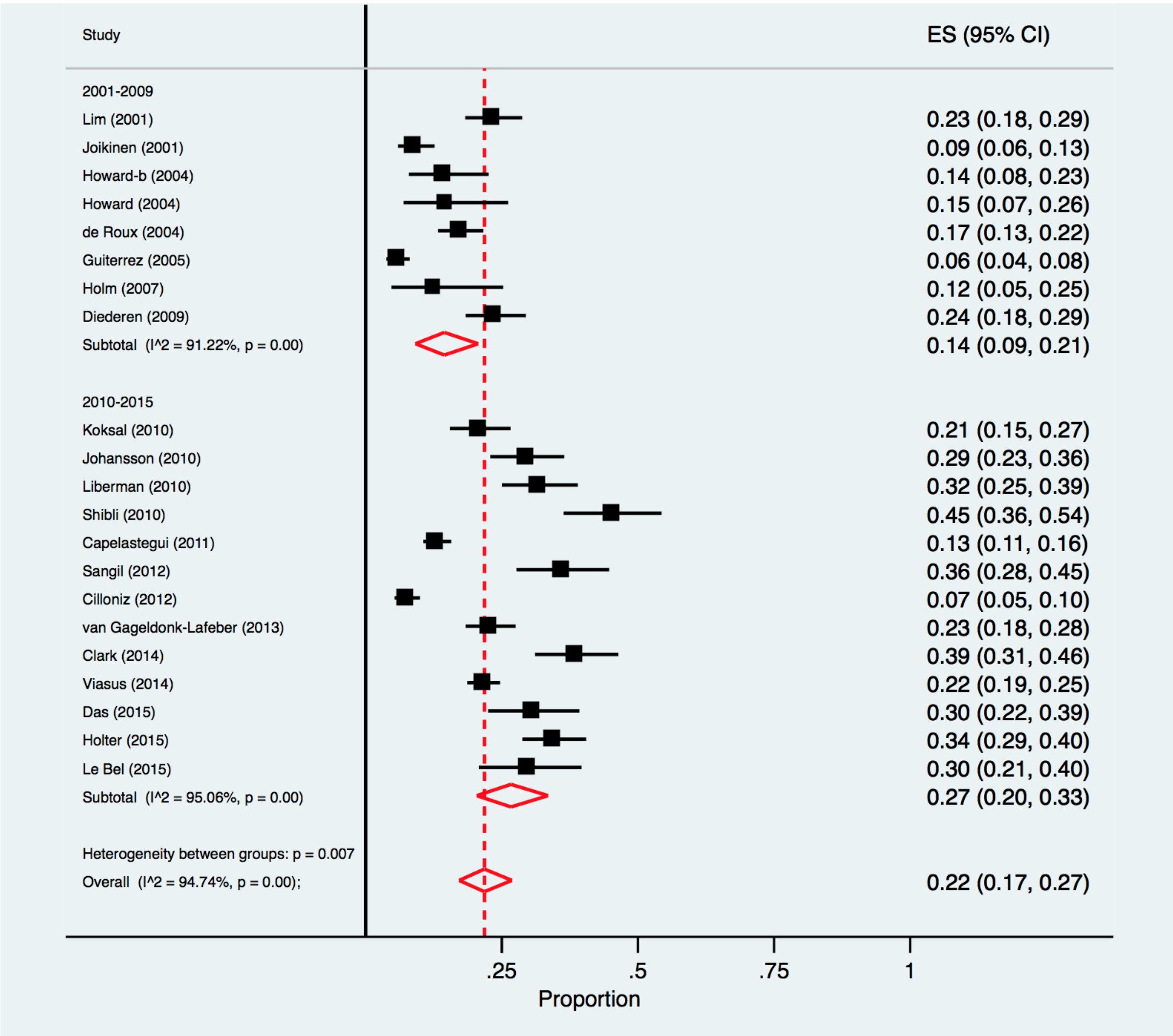
20. exp Europe/ or exp Eastern Europe/ or exp Western Europe/ or exp Southern Europe/

21. exp Spain/ or exp Eastern Europe/ or exp Europe/ or exp France/ or exp Italy/ or exp United Kingdom/ or exp Germany/

22. exp United Kingdom/ or exp France/ or exp Europe/ or exp Netherlands/ or exp European/ or exp Italy/ or exp Germany/

23. (albania or andorra or armenia or austria or azebaijan or belarus or belgium or herzegovina or bulgaria or croatia or cyrus or czech or denmark or estonia or finland or france or georgia or germany or greece or hungary or iceland or ireland or israel or italy or kazakhstan or kyrgyzstan or latvia or lithuania or luxembourg or malta or monaco or montenegro or netherlands or norway or poland or portugal or moldova or romania or russia * or san marino or serbia or slovakia or spain or sweden or switzeland or tajikistan or macedonia or turkey or turkmenistan or ukraine or england or wchartales or scotland or united kingdom or Uk or uzbekistan).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

APPENDIX 2



ES = effect size for pooled identification of respiratory viruses

Appendix 2: Forest plot: overall identification of respiratory viruses in European patients with CAP, by study year