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The Association between Histamine 2 Receptor Antagonist Use and *Clostridium difficile* Infection: A Systematic Review and Meta-analysis

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Abstract

Background: *Clostridium difficile* infection (CDI) is a major health problem. Epidemiological evidence suggests that there is an association between acid suppression therapy and development of CDI.

Purpose: We sought to systematically review the literature that examined the association between histamine 2 receptor antagonists (H_2RAs) and CDI.

Data source: We searched Medline, Current Contents, Embase, ISI Web of Science and Elsevier Scopus from 1990 to 2012 for all analytical studies that examined the association between H_2RAs and CDI.

Study selection: Two authors independently reviewed the studies for eligibility.

Data extraction: Data about studies characteristics, adjusted effect estimates and quality were extracted.

Data synthesis: Thirty-five observations from 33 eligible studies that included 201834 participants were analyzed. Studies were performed in 6 countries and nine of them were multicenter. Most studies did not specify the type or duration of H_2RAs therapy. The pooled effect estimate was 1.44, 95% CI (1.22–1.7), $I^2 = 70.5\%$. This association was consistent across different subgroups (by study design and country) and there was no evidence of publication bias. The pooled effect estimate for high quality studies was 1.39 (1.15–1.68), I2 = 72.3%. Meta-regression analysis of 10 study-level variables did not identify sources of heterogeneity. In a speculative analysis, the number needed to harm (NNH) with H_2RAs at 14 days after hospital admission in patients receiving antibiotics or not was 58, 95% CI (37, 115) and 425, 95% CI (267, 848), respectively. For the general population, the NNH at 1 year was 4549, 95% CI (2860, 9097).

Conclusion: In this rigorous systematic review and meta-analysis, we observed an association between H_2RAs and CDI. The absolute risk of CDI associated with H_2RAs is highest in hospitalized patients receiving antibiotics.

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Introduction

Clostridium difficile infection (CDI) is considered a major health problem with a point prevalence of 13.1/1000 in-patient [1] and is increasing in incidence and mortality [2–5]. The CDI cost in the United States of America (USA) alone was conservatively estimated to exceed $1.1 billion annually [6]. Risk factors associated with CDI acquisition are numerous and traditionally have included exposure to antibiotics, advanced age, comorbidities, enteral feeding, prolonged hospitalization, endoscopy and antineoplastic medications [7–10].

The role of gastric acid suppression therapy has gained interest recently as a risk factor for CDI. Four recently published meta-analyses have suggested an association between gastric acid suppression therapy with proton pump inhibitors (PPI) and CDI [11–14]. The United States Food and Drug Administration (FDA)

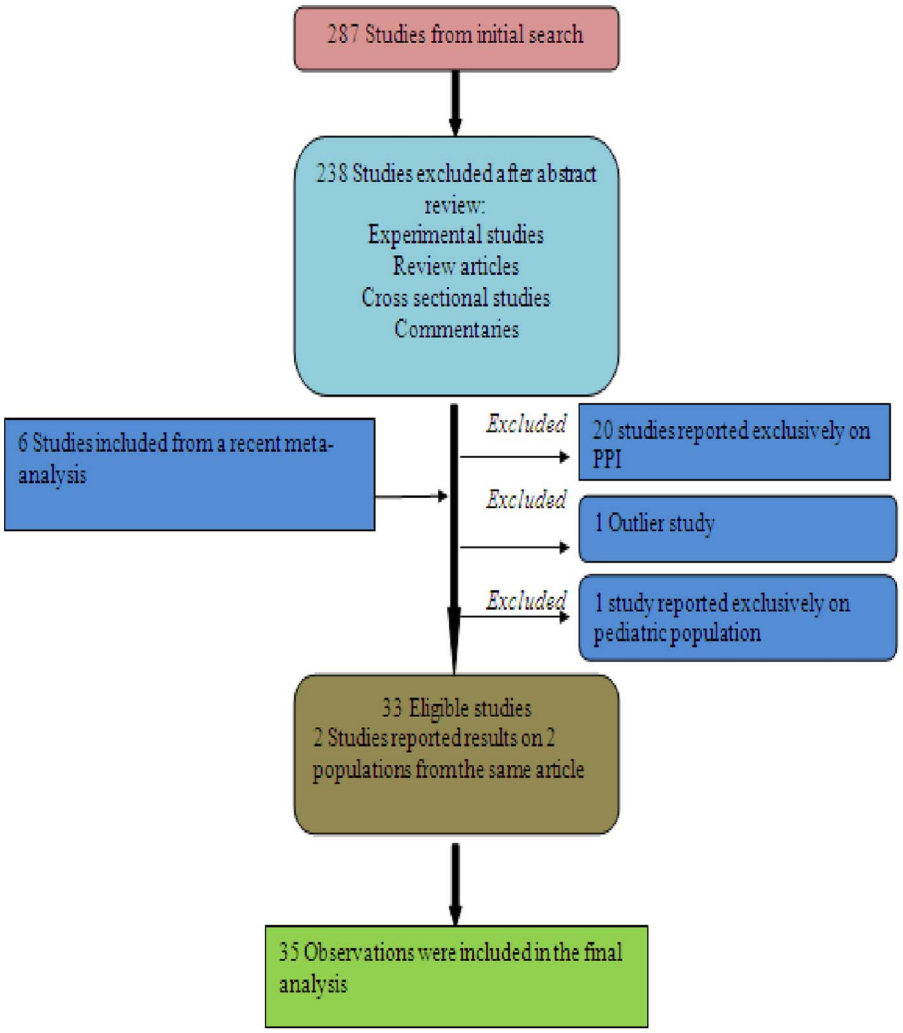

Figure 1. Flow diagram of eligible studies.
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recently warned the public about a possible association between CDI and PPI use [15]. However, to date; there is no systematic review dedicated to evaluate the potential association between histamine $_2$ receptors antagonists (H_2RAs) use and risk of CDI.

H_2RAs are popular over-the-counter (OTC) drugs worldwide [16]. Off -label use of H_2RAs and substitution for physician care were reported in 46 % and 34% of the adult consumer, respectively [15]. Masking serious conditions, missed diagnosis, and the potential for inappropriate use by patients are concerns about OTC use of H_2RAs [17]. Nonetheless, the implications of OTC H_2RAs use are not yet well defined.

Given the high prevalence of prescription use and OTC use of H_2RAs and the increasing incidence and severity of CDI, we sought to systematically review the published literature that examined the association between H_2RAs use and development of CDI following the MOOSE [18] and PRISMA [19] guidelines. We use the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) framework [20] to interpret our findings.

Methods

Search strategy

The search strategy and subsequent literature searches were performed by a medical reference librarian (PJE) with 37 years of experience. The initial strategy was developed in Ovid MEDLINE (1990 through January 2012), using MeSH (Medical Subject Headings) controlled vocabulary, and then modified for Ovid EMBASE (1990 through January 2012). Primary terms were: enterocolitis, pseudomembranous/ AND the therapeutic agents of interest: explode omeprazole, explode proton pump inhibitors, anti-ulcer agents, and explode histamine H_2 antagonists (Explode allows including all of the specific drugs, without having to use all of the various terms, synonyms, brands and generic names.) Articles were limited to randomized controlled trials, cohort studies, and or case-control studies. The same process was used with Ovid EMBASE with alterations as necessary to accommodate EMBASE's more granular subject headings. ISI Web of Science and Elsevier Scopus use text words: (difficile OR pseudomembranous OR pseudo-membranous) AND (omeprazole OR "proton pump" OR ranitidine OR h2 OR h-2 OR "acid suppression" OR antacid*)) AND (random* OR trial* OR blind* OR cohort* OR controlled OR prospective). Moreover, bibliographic references of

Table 1. Characteristics of the included studies.

Source	Country	Centers	Setting	Condition	Study Design	Inclusion Criteria	Acid Suppression Therapy
Kutty et al (VA),[26]2010	US	Multicenter	Community	Gen Pop	Case-control	Age: ≥18 yr; Community onset CDAD	**H_2RAs: exposure 3mo prior to test**
Kutty et al (D),[26] 2010	US	Multicenter	Community	Gen Pop	Case-control	Age: ≥18 yr; Community onset CDAD	**H_2RAs: exposure 3mo prior to test**
Nath et al, [28]1994	CA	Single	Hospital	Hem-onco pts	Case-control	Adult; In-patient >3d	**Acid suppression therapy**
Jayatilaka et al, [27] 2007	US	Single	Hospital	Gen In-patient	Case-control	Age >18	**H_2RAs: pre admission**
Jayatilaka et al,[27]2007	US	Single	Hospital	Gen In-patient	Case-control	Age >18	**H_2RAs: post admission**
Shah et al (D),[29] 2000	UK	Single	Hospital	Gen In-patient	Case-control	Age >65 yr; Gen medical/ elderly care wards	**H_2RAs: upto 16 wk before diarrhea**
Dial et al, [30] 2005	UK	GPRD	Community	Gen Pop	Case-control	Age ≥18 yr; At least 2 yrs of records in the GPR; first occurrence of CDAD	**H_2RAs: 90 d prior to the index date**
Debast et al, [31] 2009	NL	Single	Hospital	Gen In-patient	Case control	Age:≥18 yr; CDAD	**H_2RAs: exposure**
Lowe et al, [32] 2006	CA	Single	Community	Gen Pop	Case-control (R)	1 hospital admission for CDAD; Age ≥ 66yr; CDAD diagnosis within 60d of ABX therapy	**H_2RAs: exposure**
Dial et al, [33] 2006	UK	GPRD	Community	General pop	Case-control	First prescription oral Vancomycin; No previous admission 1yr before index date	**H_2RAs: 90d prior to index date**
Aseeri et al, [34] 2008	US	Single	Hospital	Gen In-patient	Case-control	Age ≥18 Yr; Inpt for ≥3 d	**H_2RAs: 3d before CDAD**
Dubberke et al,[35] 2007	US	Single	Hospital	Gen In-patient	Case- Control	Pts admitted for >48 hr between study period	**H_2RAs**
Loo et al, [36] 2005	UK	Single	Hospital	Gen In-patient	Case-control	Hospital Acquired CDAD;	**H_2RAs: 6wk before diagnosis**
Sundram et al,[37] 2009	UK	Single	Hospital	Gen In-patient	Case-control	Adult Hospital Acquired CDAD	**H_2RAs: 6wk prior to onset**
Howell et al,[38] 2010	US	Single	Hospital	Gen In-patient	Cohort	Age ≥18 yr; LOS ≥3 d; Only first diagnosis	**H_2RAs**
Dalton et al, [39] 2009	CA	Multicenter	Hospital	Med/Surgical Subspecialty	Cohort, (R)	Age: ≥18 yr; Minimum 7-d LOS; ABX exposure	**H_2RAs**
Dubberk et al,[40]2007	US	Single	Hospital	Gen In-patient	Cohort, (R)	All pts admitted to BJH for more than 48 hours	**H_2RAs**
Pepin et al,[41] 2005	CA	Single	Hospital	Gen In-patient	Cohort, (R)	Adult In-patient	**H_2RAs**
Beaulieu et al, [42] 2007	CA	Single	Hospital	Medical ICU	Cohort	ICU LOS>24hr; Diarrhea >24 hr and positive CD toxin (2d to 2months post discharge)	**H_2RAs**
Peled et al, [43] 2007	IL	Single	Hospital	Gen In-patient	Cohort, (P)	CD testing during 4m period; ABX within 40d prior to diarrhea	**H_2RAs**
Dial et al, [44] 2004	CA	Single	Hospital	Med/CT/Surgical wards	Cohort	Pharmacy database; ABX during study period; positive toxin in the infection control registry	**H_2RAs**
Novell et al, [45], 2010	US	Single	Hospital	Gen Inpatients	Case-control, (R)	Age ≥18 yr; CDAD	**H_2RAs**
Netland et al, [46] 2011	US	Single	Both	Gen Pop	Cohort, (R)	Recurrent CDI	**H_2RAs**

Table 1. Cont.

Source	Country	Centers	Setting	Condition	Study Design	Inclusion Criteria	Acid Suppression Therapy
Jung et al, [47] 2010	Korea	Single	Hospital	Gen Inpatients	Cohort study, (R)	Recurrent CDAD or treatment failure cases	**H_2RAs**
Loo et al, [48] 2011	CA	Multicenter	Hospital	Gen Inpatients	Cohort study(P)	Age ≥18, Health Care Associated CDAD	**H_2RAs**
Manges et al, [49] 2010	CA	Single	Hospital	Gen Inpatients	Case control	Nosocomial CDAD	**H_2RAs**
Kuntz et a,l[50] 2011	US	Single	Community	Gen Pop	Case control, (R)	Community Associated CDAD	**Acid suppression therapy**
Naggie et al,[51] 2011	US	Multicenter	Community	Gen Pop	Case control	Age≥18 yr	**Acid suppression therapy**
Stevens et al,[52], 2011	US	Single	Hospital	Gen Inpatients	Cohort, (R)	Age ≥18 yr, Hospital acquired	**H_2RAs**
Dial et al, [53] 2008	CA	Multicenter	Community	Elderly patients	Case control	Age ≥65, Community Associated CDAD	**H_2RAs**
McFarland et al,[54] 2007	US	Multicenter	Both	Gen Pop	Case control	CDAD Diagnosis	**H_2RAs**
Kazakova et al,[55] 2012	US	Single	Both	Gen Pop	Case control	CDAD Diagnosis, onset during the pre-outbreak or outbreak periods, hospitalization	**H_2RAs**
Modena et al,[56] 2005	US	Single	Both	Gen Pop	Case control	Received at least 5 days of antibiotics prior to diagnosis of CDAD	**H_2RAs**
Muto et al,[57] 2005	US	Single	Hospital	Gen Inpatients	Case control	Nosocomial CDAD	**H_2RAs: During the 4 weeks before detection of CDAD**
Yip et al, [58] 2001	**CA**	**Single**	**Hospital**	**Gen Inpatients**	**Case control**	**Nosocomial CDAD**	**H_2RAs**

Abbreviations: US, United States;UK, United Kingdom; BMT, Bone Marrow Transplant; ESRD, End Stage Renal Disease; GPRD – general practice research database; IBD, Inflammatory Bowel Disease; CD, Clostridium Difficile; CDAD, Clostridium difficile associated diarrhea; LOS, Length of Stay; LTCF, Long Term Care Facility; Gen, General.; Pop, Population; d, day/days; mo, month/months; yr, year/year; wk, week/week; Pts, Patients; Pt, Patient; Med, Medical; CT, Cardio-thoracic; NL, Netherland; CA, Canada; IL, Israel; Abd, Abdominal; (P), prospective; (R), Retrospective.*, Mostly hospital.

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all articles and previous meta-analyses were searched for eligible studies. We have designed the search strategy to capture any association between gastric acid suppression therapy and development of CDI.

There was no restriction to language. All results were downloaded into EndNote 7.0 (Thompson ISI Research soft, Philadelphia, Pennsylvania), a bibliographic database manager, and duplicate citations were identified and removed. Two authors (A.B.A. and F.A.) independently assessed the eligibility of identified studies.

Study selection

To be included, a study had to: (1) be an analytical study; and (2) examine the association between H_2RAs use and incidence of CDI in adult population.

Data collection

A data collection form was developed and used to retrieve information on relevant features and results of pertinent studies. Two reviewers (A.B.A. and F.A.) independently extracted and recorded data in a predefined checklist. Disagreements among reviewers were discussed with two other reviewers (I.M.T. and M.A.A.), and agreement was reached by consensus. We collected adjusted effect estimates and 95% confidence intervals (CI) based on the multivariable regression model used in each study.

We used the Newcastle-Ottawa Quality Assessment Scale for cohort and case-control studies [21] which is intended to rate selection bias, comparability of the exposed and unexposed groups of each cohort, outcome assessment, and attrition bias. Two reviewers (M.A.G and F.A.) independently assessed the methodological quality of selected. Disagreement among reviewers was discussed with 2 other reviewers (I.M.T. and M.A.A.), and agreement was reached by consensus.

We used the GRADE framework to interpret our findings. The Cochrane Collaboration has adopted the principles of the GRADE system [20] for evaluating the quality of evidence for outcomes reported in systematic reviews.

For purposes of systematic reviews, the GRADE approach defines the quality of a body of evidence as the extent to which one can be confident that an estimate of effect or association is close to the quantity of specific interest. Quality of a body of evidence involves consideration of within-study risk of bias (methodological quality), directness of evidence, heterogeneity, precision of effect estimates and risk of publication bias.

Statistical Analyses

Meta-analyses. The primary effect measures used in the meta-analysis were Odds Ratios (OR), Hazard Ratios (HR) and Relative Risks (RR) which were assumed to reasonably estimate the same association between CDI and H_2RAs given the low

Table 2. The Association between H_2RAs use and development of *Clostridium difficile* infection from case-control studies.

Source	Case Ascertainment	Selection of Controls	Sample size	Adjusted Effect Estimates
Kutty et al,[26] (VA)	Non-formed stool, Positive CD toxin	Randomly selected from the same geographical outpatients territory	Exposed group; cases: 7, controls: 13	Crude OR, 1.8 (0.6–4.8)
			Non-exposed group; cases: 29, controls: 95	
Kutty et al,[26] (D)	Non-formed stool, Positive CD toxin	Randomly selected from the same geographical outpatients territory	Exposed group; cases: 6, controls: 3	Crude OR, 1.3 (0.3–5.6)
			Non-exposed group; cases: 67, controls: 45	
Sundram et al,[37] 2009	Diarrhea, Positive stool for CD toxin, ribotyped	Inpatients, No diarrhea, Never tested positive for CD	Exposed group; cases: 65, controls: 52	Crude OR for PPI/ H_2RAs : 1.7, P 0.456
			Non-exposed group; cases: 32, controls: 45	
Jayatilaka et al,[27] 2007	Diarrhea, Positive toxin	Age and sex matched, Same period of time	H_2RAs use pre and during admission	H_2RAs use pre and during admission
			Exposed group; cases: 9, controls: 17	OR: 0.95 (0.39-2.34)
			Non-exposed group; cases: 6, controls: 14	
Jayatilaka et al,[27] 2007	Diarrhea, Positive toxin	Age and sex matched, Same period of time	H_2RAs use post admission	H_2RAs use post admission
			Exposed group; cases: 133, controls: 227	OR: 0.73 (0.26-2.06)
			Non-exposed group; cases: 116, controls: 230	
Loo et al,[36] 2005	Diarrhea/positive CD, Endoscopic diagnosis, histological evidence	Matched to Age, Charlson index, date of admission, ward, LOS	Exposed group; cases: 47, controls: 47	Diarrhea/positive CD, Endoscopic diagnosis, histological evidence
			Non-exposed group; cases: 190, controls: 190	
Shah et al,[29] 2000	Diarrhea Positive stool for CD toxin	Negative stool toxins, Similar age, Hospital ward, Same time	Exposed group; cases: 22, controls: 22	Diarrhea
			Non-exposed group; cases: 104, controls: 104	Positive stool for CD toxin
Dial et al,[33] 2006	Patients with first prescription of oral Vancomycin	Age matched, Same ward	Exposed group; cases: 23, controls: 112	Patients with first prescription of oral Vancomycin
			Non-exposed group; cases: 294, controls: 2055	
Asseri et al,[34] 2008	Diarrhea	Matched to date of admission, antibiotic use, gender, age group, patient location, room type	Exposed group; cases: 17, controls: 9	Diarrhea
	Positive stool for CD toxin		Non-exposed group; cases: 77, controls: 85	Positive stool for CD toxin
Dial et al,[30] 2005	Positive CD toxin	Same general practice, Not hospitalized in the year prior to index date, Negative CD toxin, No diagnosis of CDI	Exposed group; cases: 83, controls: 367	Positive CD toxin
	Clinical diagnosis made by GP		Non-exposed gp; cases: 1150, controls: 11963	Clinical diagnosis made by GP
Lowe et al,[32] 2006	CDAD	Matched to age, sex, and antibiotic use	Exposed group; cases: 213, controls: 1846 Non-exposed gp; cases: 1176, controls: 10457	Exposed group; cases: 213, controls: 1846 Non-exposed gp; cases: 1176, controls: 10457

Table 2. Cont.

Source	Case Ascertainment	Selection of Controls	Sample size	Adjusted Effect Estimates
Debast et al,[31] 2009	Diarrhea	Randomly selected fro the same time and same wards as CDI cases	Exposed group; cases: 2, controls: 2	Exposed group; cases: 2, controls: 2
	Positive stool for CD toxin		Non-exposed group; cases: 43, controls: 88	Non-exposed group; cases: 43, controls: 88
Nath et al,[28] 1994	Diarrhea	Age matched, Same hospital unit	Exposed group; cases: 51, controls: 32	Exposed group; cases: 51, controls: 32
	Positive stool for CD toxin		Non-exposed group; cases:29, controls: 48	Non-exposed group; cases:29, controls: 48
Dubberke et al,[35] 2007	Diarrhea	Randomly selected During the study period	Exposed group; cases: 206, controls: 426	Exposed group; cases: 206, controls: 426
	Positive stool for CD toxin		Non-exposed group; cases: 176, controls: 1102	Non-exposed group; cases: 176, controls: 1102
Novell et al,[45] 2010	New diarrhea	Matched to in-patient unit, age, gender, date of admission	Exposed group; cases: 12, controls: 07	Exposed group; cases: 12, controls: 07
	Positive stool for CD toxin		Non-exposed group; cases: 162, controls: 167	Non-exposed group; cases: 162, controls: 167
Manges et al,[49] 2010	Diarrhea/positive CD, Endoscopic diagnosis, histological evidence	Matched to Age, gender, date of hospitalization	Exposed group; cases: 09, controls: 12	Exposed group; cases: 09, controls: 12
			Non-exposed group; cases: 16, controls: 38	Non-exposed group; cases: 16, controls: 38
Kuntz et al,[50] 2011	ICD-9 code, CDAD	Randomly selected	Exposed group; cases: 55, controls: 157	Exposed group; cases: 55, controls: 157
			Non-exposed group; cases: 249, controls: 2883	Non-exposed group; cases: 249, controls: 2883
Naggie et al,[51] 2011	Diarrhea	Matched by geographic location	Exposed group; cases: 22, controls: 44	Exposed group; cases: 22, controls: 44
	Positive stool for CD toxin		Non-exposed group; cases: 44, controls: 70	Non-exposed group; cases: 44, controls: 70
Dial et al,[53] 2008	ICD-9 code 008.45, CDAD	Randomly selected, matched to index date and date of first hospital admission	NR	RR:1.60 (0.90-2.20)
McFarland et al,[54] 2007	Acute diarrhea Culture positive or positive C.D toxins	Matched to time of CDAD, Age, Ward	Exposed group; cases: 24, controls: 160	NR
	No other cause for the diarrhea		Non-exposed group; cases: 23, controls: 161	
Kazakova et al,[55] 2012	Diarrhea, positive CD toxin A	Matched to Sex, Age, admission date	Exposed group; cases:19, controls: 49	OR:2.69 (1.22-5.97)
			Non-exposed group; cases: 18, controls: 109	
Modena et al,[56] 2005	Diarrhea	Inpatients, Received antibiotics for at least 5 days	Exposed group; cases:32, controls:18	NR
	Positive stool for CD toxins		Non-exposed group; cases: 98, controls: 102	
Muto et al,[57] 2005	Diarrhea	Matched to admission date, Type of medical service, Length of hospital stay	Exposed group; cases:159, controls:44	OR:2.00 (1.10-3.50)
	Positive stool for CD toxin		Non-exposed group; cases: 141, controls: 62	
Yip et al,[58] 2001	Diarrhea	Matched to Age, Gender, admission date	Exposed group; cases:14, controls:13	OR:2.70 (0.71–10.10)
	Positive stool for CD toxin		Non-exposed group; cases: 9, controls: 18	

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Table 3. The Association between H2RAs use and development of *Clostridium difficile* infection from cohort studies.

Source	Case Ascertainment	Selection of Controls	Sample size	Adjusted Effect Estimates
Howell et al,[38] 2010	Positive CD toxin	A nearest-neighbor–matching algorithm was applied	Exposed group; cases: 66, controls: 10619	OR : 1.53 (1.12–2.10)
			Non-exposed group; cases:599, controls: 90512	
Dalton et al,[39] 2009	Positive stool toxins or colonoscopy-confirmed psudomembraneous colitis	Age, ≥ 18 years, Minimum 7d LOS, Antibiotic exposure	Exposed group; cases: 28 controls: 2135	OR, 1.70 (1.09 2.64)
			Non-exposed group; cases:121, controls: 12435	
Dubberk et al,[40] 2007	Positive stool for CD	In-patient, No positive stool toxin assay during the period (60d before start of study to the end)	Exposed group; cases: 206, controls: 998 Non-exposed group; cases: 176, controls: 25716	OR, 2.0 (1.6-2.6)
Pepin et al, [41] 2005	Diarrhea, Positive toxin, proven pseudomembranous colitis	Unclear	Exposed group; cases: 1199, controls: NR	HR, 1.07 (0.8-1.43)
			Non-exposed gp; cases: 6222, controls: NR	
Beaulieu et al,[42] 2007	Diarrhea Positive stool for CD toxin	Unclear	Exposed group; cases: 470, controls: NR	HR, 0.78 (0.5 – 1.23)
			Non-exposed group; cases: 357, controls: NR	
Peled et al,[43] 2007	Diarrhea Positive stool for CD toxin	Diarrhea with negative stool for CD, same institution	Exposed group; cases: 22, controls: 45	OR, 3.1 P value : 0.024
			Non-exposed group; cases: 30, controls: 120	
Dial et al,[44] 2004	Positive stool for CD toxins	Unclear	Exposed group; cases: NR, controls: NR	OR : 1.1 (0.4-3.4)
			Non-exposed group; cases: NR, controls: NR	
Netland et al, [46] 2011	Diarrhea between 5–60 days after antibiotic therapy for CDAD	Patients with CDAD in the same institution	Exposed group; cases: 05, controls: 50	OR, 0.49 P value : 0.33
			Non-exposed group; cases: 50, controls: 99	
Jung et al,[47] 2010	Diarrhea or pseudomembranous colitis, Positive toxin	Same institution	Exposed group; cases: 06, controls: 31	OR, 1.59 P value : 0.367
			Non-exposed group; cases: 08, controls: 66	
Loo et al,[48] 2011	Diarrhea and: positive CD, histological evidence or pseudomembranous colitis	Frequency matching approach	Exposed group; cases: NR, controls: NR	OR : 0.55 (0.21 – 1.49)
			Non-exposed group; cases: 190, controls: 190	
Stevens et al,[52] 2011	Diarrhea Positive stool for CD toxin	Same institution	Exposed group; cases: 23, controls: 1060	HR, 1.7 (0.7 – 3.9), P value 0.25
			Non-exposed group; cases: 218, controls: 8853	

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incidence of CDI and thus were pooled together. Adjusted effect estimates were primarily used for this analysis. Unadjusted effect estimates were used as alternatives if studies did not pursue adjustment because of absence of association on univariate comparison.

Effect estimates from all included studies were pooled in a meta-analysis weighing individual studies according to their log-transformed inverse variance. The DerSimonian and Laird random effects model [22] was used to calculate the pooled effect estimates.

We extracted data on the proportion of CDI cases that were exposed to antibiotics from all studies that reported these data. We then performed a meta-analysis for the proportion on logit scale using random effects model weighing the individual studies according to their log-transformed inverse variance.

Exploring heterogeneity. Homogeneity among studies was tested by means of Cochran's Q test and calculation of the variation across studies attributable to heterogeneity rather than chance (I^2). The influence of a range of a-priori selected study-level and aggregated individual-level parameters on the observed effect

Table 4. Modified Newcastle-Ottawa quality assessment scale for case-control studies included in the meta-analysis.

	Selection*					Exposure[o]			
Included Studies	Adequacy of Case Definition	Represent-ativeness of the Cases	Selection of Controls	Definition of Controls	Compara-bility[•]	Ascertainment of Exposure	Same Method of Ascertainment for Cases and Controls	Non-Response-Rate	Total No. of stars
Kutty et al,[26] 2010.	A*	A*	A*	A*	A*	A*	A*	C	7
Nath et al,[28] 1994	A*	A*	B	A*	A**	A*	A*	C	7
Jayatilaka et al,[27] 2007	B	A*	B	A*	A**	A*	A*	C	6
Shah et al,[29] 2000	A*	A*	B	A*	A*	A*	A*	C	6
Lowe et al,[32] 2006	A*	A*	A*	A*	A*	A*	A*	C	7
Dial et al,[30] 2005	A*	A*	A*	A*	A*	A**	A*	C	8
Dial et al,[33] 2006	B	A	A*	A	A*	A**	A*	C	5
Aseeri et al,[34] 2008	A*	A*	B	A*	A**	E	B*	C	6
Dubberke et al,[35] 2007	A*	B	B	A*	A**	A*	A*	C	6
Loo et al,[36] 2005	A*	A*	B	A*	A*	E	A*	C	5
Sundram et al,[37] 2009	A*	A*	B	A*	A*	A*	A*	C	6
Novell et al,[45] 2010	A*	A*	B	A*	A**	A*	A*	C	7
Debast et al,[31] 2009	A*	A*	B	A*	A*	A*	A*	C	6
Kuntz et al,[50] 2011	A*	A*	A*	A*	A*	A*	A*	C	7
Manges et al,[49] 2010	A*	A*	B	B	A*	A*	A*	C	5
Naggie et al,[51] 2011	A*	A*	A*	A*	A*	C	A*	C	6
McFarland et al,[54] 2007	B	A*	C	A*	A*	A*	A*	C	6
Modena et al,[56] 2005	B	A*	B	A*	A**	A*	A*	C	5
Muto et al,[57] 2005	B	A*	B	A*	A**	A*	A*	C	6
Yip, et al,[58] 2001	B	A*	B	A*	A**	A*	A*	C	6
Dial et al,[53] 2008	B	A*	B	A*	A*	A*	A*	C	5
Kazakova et al,[55] 2006	A*	A*	B	A*	A**	D	A*	C	6

*Selection:
(1)Is this case definition adequate? A, yes, with independent validation; B, yes, eg record linkage or based on self reports C, no description.
(2) Representativeness of the cases: A, Consecutive or obviously representative series of cases; B, Potential for selection biases or not stated.
(3) Selection of controls: A, Community controls; B, Hospital controls; C, No description.
(4) Definition of controls: A, No history of disease; B, No description of source.
[•]Comparability: Comparability of cases and controls on the basis of the design or analysis: A, study controls for co-morbidities; B, study controls for any additional factor (e.g., age and severity of illness).
[o]Exposure:
Ascertainment of exposure: A, Secured records; B, Structured interview where blind to case/control status; C, Interview not blinded to case/control status; D, written self report or medical record only.
Same method of ascertainment for cases and controls; A, yes; B, no.
Non-response rate: A, Same for both groups; B, Non-respondents described; C, Rate different and no designation.
doi:10.1371/journal.pone.0056498.t004

Table 5. Modified Newcastle-Ottawa Quality Assessment Scale for Cohort studies included in the Meta-analysis

	Selection*					Outcome[0]			
Included Studies	**Representativeness of the exposed cohort**	**Selection of the Non-exposed Cohort**	**Ascertainment of Exposure**	**Incident Disease**	**Comparability[•]**	**Assessment of Outcome**	**Length of Follow-up**	**Adequacy of Follow-up**	**Total number of stars**
Howell et al,2010	A*	A*	A*	A*	A**	B*	A*	A*	9
Dalton et al, 2009	A*	A*	A*	A*	A**	B*	A*	A*	9
Dubberke et al, 2007	A*	A*	A*	A*	A**	B*	A*	A*	9
Pepin et al, 2005	A*	A*	A*	A*	A*	B*	A*	A*	8
Beaulieu et al, 2007	B	A*	A*	A*	A*	A*	A*	A*	7
Peled et al, 2007	A*	A*	B	A*	A**	A*	A*	A*	8
Loo et al 2011	A*	A*	B	A*	A**	A*	A*	A*	8
Netland et al, 2011	A*	A*	A*	A*	A**	B*	A*	A*	9
Jung et al, 2010	A*	A*	A*	A*	A**	B*	A*	A*	9
Stevens et al, 2011	A*	A*	A*	A*	A**	B*	A*	A*	9
Dial et al 2004	A*	A*	A*	A*	A*	A*	A*	A*	8

*Selection:
(1) Representativeness of the exposed cohort: A, truly representative; B, somewhat representative; C, selected group; D, no description of the derivation of the cohort.
(2) Selection of the non-exposed cohort: A, drawn from the same community as the exposed cohort; B, drawn from a different source; C, no description of the derivation of the non-exposed cohort.
(3) Ascertainment of exposure: A, secure record; B, structured interview; C, written self-report; D, no description.
(4) For demonstration that the outcome of interest was not present at start of study: A, yes; B, no.
[•]Comparability: For comparability of cohorts on the basis of the design or analysis: A, study controls for co-morbidities; B, study controls for any additional factor (e.g., age and severity of illness); C, not done.
[0]Outcome:
(1) Assessment of outcome: A, independent blind assessment; B, record linkage; C, self-report; D, no description.
(2) Was follow-up long enough for outcomes to occur? A, yes, (i.e. in-hospital or up to 30 d); B, no.
(3) Adequacy of follow-up of cohorts: A, complete follow-up and all subjects accounted for; B, subjects lost to follow-up was unlikely to introduce bias, because a small number were lost or a description was provided of those lost; C, follow-up rate 90% or lower (select an adequate percentage) and no description of those lost; D, no statement.
doi:10.1371/journal.pone.0056498.t005

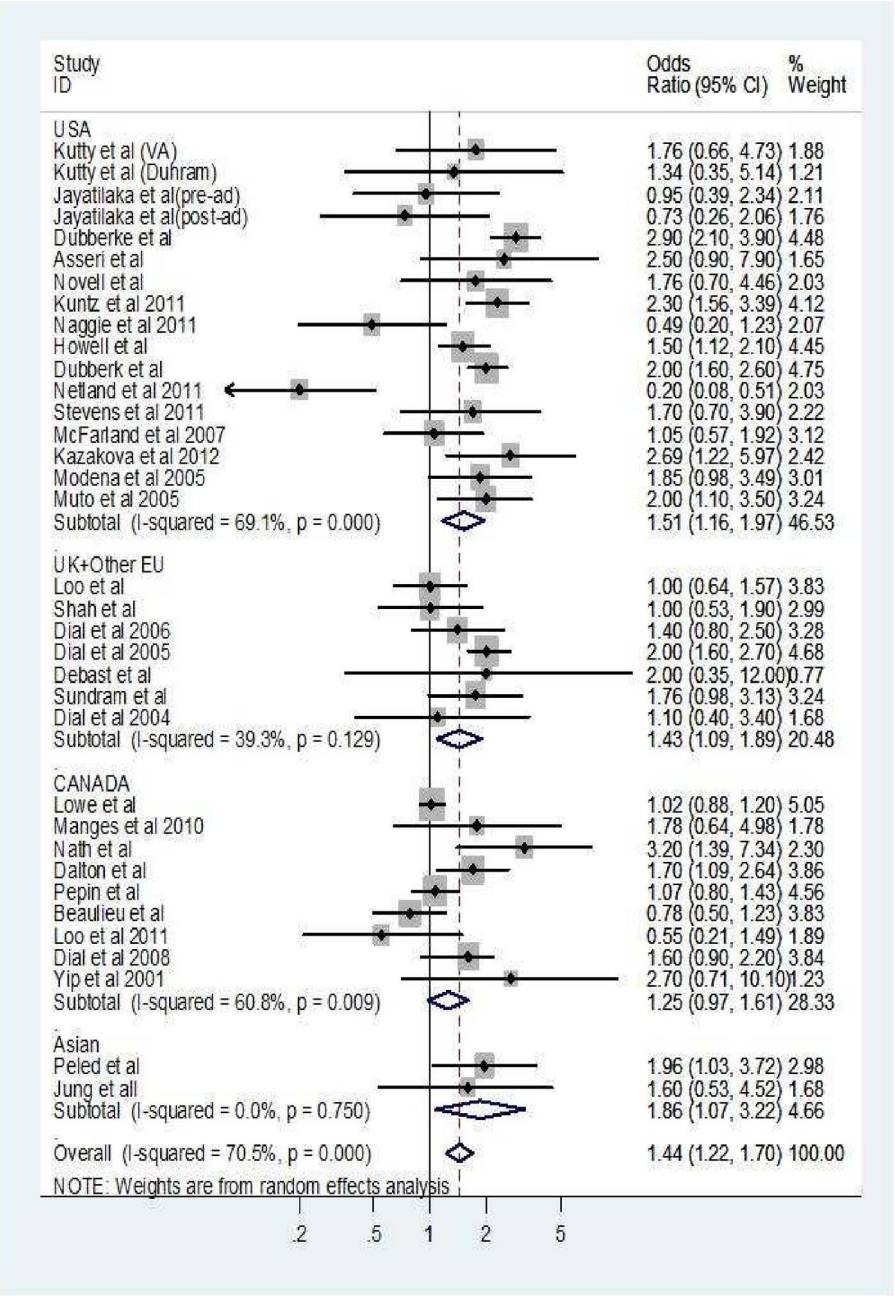

Figure 2. Forest plot-random effect model meta-analysis of the association between CDI and H2RAs based on 35 observations stratified by country. Error bars indicate confidence interval.
doi:10.1371/journal.pone.0056498.g002

estimate was investigated by means of meta-regressions. In these analyses, the log odds ratio from each study was regressed on the potential confounders in univariate and multivariate weighted linear regressions, weighted according to the inverse standard error and the residual between-study variance. Ten potential confounders were considered. Seven variables were categorical: design of the study (case-control vs. cohort), country of publication, setting (single center vs. multicenter), method of ascertainment of antibiotic use, method of effect measure (OR vs. RR/HR), effect estimate (adjusted vs. unadjusted) and quality of included studies (high score vs. low score). Three continuous variables were: the impact factor of the journal where the study was published, number of variables the effect measure was adjusted for and proportion of cases that were exposed to antibiotics.

Publication bias. The possible influence of publication bias was graphically assessed with the novel method of contour-enhanced funnel plot where log-transformed odds ratios were plotted against standard errors. This method examines whether any funnel plot asymmetry is likely to be due to publication bias compared with other underlying causes of funnel plot asymmetry. The contours help to indicate whether areas of the plot, where studies are perceived to be missing, are where studies would have statistically significant effect sizes or not and thus decrease or increase the evidence that the asymmetry is due to publication

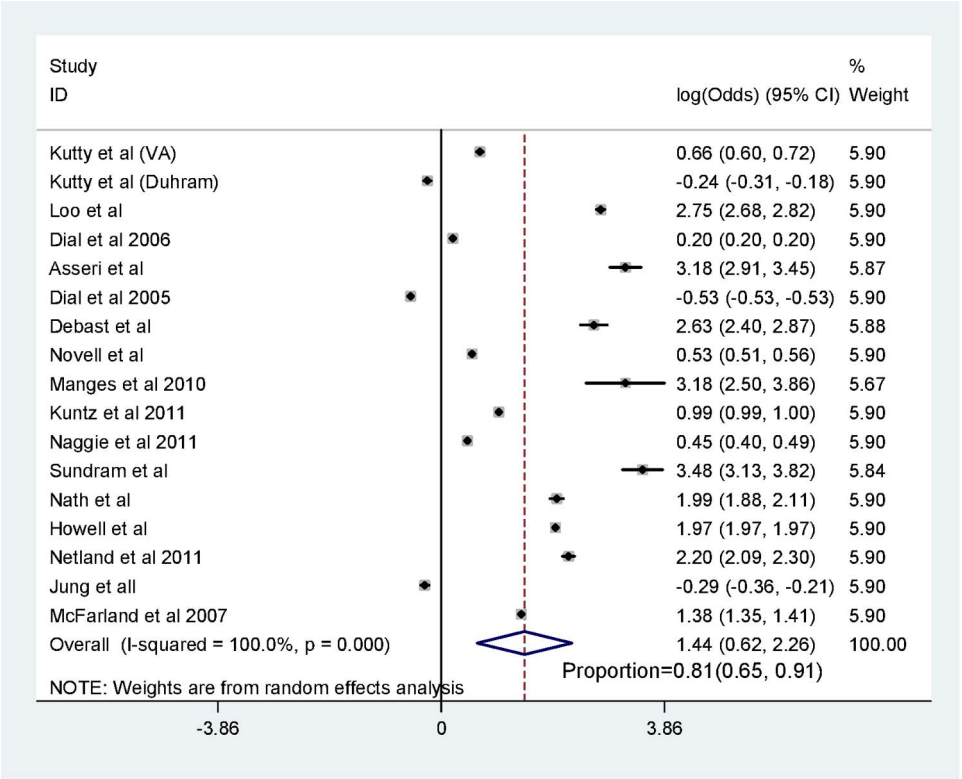

Figure 3. Forest plot of the pooled proportion of *Clostridium difficile* cases that were exposed to antibiotics.
doi:10.1371/journal.pone.0056498.g003

bias. The presence of funnel plot asymmetry was also assessed using Egger's test [23].

Residual confounding. Finally, the possible influence of unknown confounders (residual confounding) was investigated with a rule-out approach described by Schneeweiss [24]. This approach stipulates the influence of a hypothetical confounder and determines what characteristics this confounder must have to fully account for the observed association between use of H_2RAs and occurrence of CDI. The hypothetical confounder is characterized by its association to H_2RAs use (OR_{EC}, odds ratio of exposure to the confounder) and its association to the outcome (RR_{CO}, relative risk of outcome in individuals exposed to the confounder vs. non-exposed). For this analysis, the absolute risk in the pooled non-exposed group was used for conversion of odds ratio to relative risk using the method described by Zhang and Yu [25]. Separate analyses were performed to demonstrate what levels of OR_{EC} and RR_{CO} would be required to fully explain the observed association between H_2RAs and CDI for different hypothetical prevalence of the unknown confounder (i.e. $P_C = 0.2$, $P_C = 0.4$) before and after adjustment for publication bias as described above.

In all analyses, results associated with p-values <0.05 (two-sided test) were considered statistically significant. All statistical analyses were performed using Stata version 12 statistical software (StataCorp, College Station, Texas).

Results

Search results

The search yielded 27 eligible studies after excluding 260 citations. Six more studies were retrieved from recent review articles and added to the total eligible studies. Kutty [26] et al and Jayatilaka [27] et al, each reported 2 different observations for

Table 6. Influence of study type and country on the pooled effect estimate and its associated heterogeneity.

Group	Pooled Effect Estimate (95 % CI)	I^2 %	Number of Observations
All studies	1.44 (1.22, 1.70)	70.5	35
Case-control studies	1.58 (1.28, 1.95)	68.9	24
Cohort studies	1.19 (0.87, 1.62)	75.6	11
Asia	1.86 (1.07, 3.22)	0	2
Canada	1.25 (0.97, 1.61)	60.8	9
Europe	1.43 (1.09, 1.89)	39.3	7
USA	1.51 (1.16, 1.95)	65.1	17

doi:10.1371/journal.pone.0056498.t006

Table 7. Meta-regression analysis to explore sources of heterogeneity.

	Univariate Analyses	
Study Characteristics	**Coefficient**	**p-values**
Study Design	−.27729	0.137
Low score study	.194575	0.389
Country where the study is conducted		
United States	Reference	
Canada	−.1738854	0.431
European countries	−.0849204	0.726
Asian Countries	.1809134	0.686
Setting	−.0286546	0.893
No of variables adjusted for	.0251339	0.175
Method of measuring effect estimate	−.2540725	0.325
Impact factor of the journal	−.0067289	0.380
Method of ascertainment of antibiotic		
Patient chart	Reference	
Pharmacy record	−.0139199	0.955
Interview	.3666586	0.517
Questionnaire	.2703275	0.703
Combined	.0368821	0.905
Not reported	.2469137	0.381
Proportion of antibiotic use	−.0023797	0.588

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different participants. Thus, a total of 33 articles met our inclusion criteria representing 35 observations that included 201834 participants. There was excellent agreement for the inclusion of the studies, data abstraction and quality assessment between the reviewers (kappa statistic being 1.0, 1.0 and 0.91 respectively).

The study selection process is illustrated in Figure 1 and the main characteristics of the included studies are summarized in Table 1. Twenty-four case control studies [26–37,45,49–51,53–58] and 11 cohort studies [38–44,46–48,52] reported data on both community-acquired and hospital-acquired CDI (8 observations were from community-acquired CDI, 23 from hospital-acquired CDI and 4 representing both type of CDI). Six studies [26,39,47,51,53,54] were from multiple centers; two from UK general practice research database [28,30], and the remaining were from single centers. The included studies were performed in 6 countries (17 studies from USA, 9 from Canada, 6 from United Kingdom, 1 from Netherlands, 1 from Israel, and one from Korea). Most studies did not specify the type or duration of therapy with H_2RAs. Tables 2 and 3 summarized the case ascertainment, control or non-exposed group selection method for case control and cohort studies, respectively. Among all citations, seventeen studies reported the proportion of cases exposed to antibiotics. Eight studies used antibiotics exposure as inclusion criteria. Three studies did not provide either the absolute number of exposed or unexposed groups thus were not included in this pooled proportion analysis.

Quality assessment

Quality assessment of all included studies was done using the validated Newcastle-Ottawa Quality Assessment Scale [21] for cohort and case control studies (Tables 4 and 5). Included studies were scored based on the sum number of the stars given to each study. Among case-control studies, Loo et al 2011, Manges et al 2010, McFarland et al 2007, Modena et al 2005 and Dial et al 2008 scored the lowest. While Beaulieu et al 2005 scored the lowest among cohort studies. Most studies were of good quality with no evidence of selection bias, and with good comparability of the exposed and unexposed groups of each cohort, and outcome assessment.

Meta-analysis

Thirty-five observations from 33 eligible studies were pooled using a random effect model meta-analysis. We excluded the study by Jenkins et al. as an outlier due to its large standard error. The pooled effect estimate was 1.44, 95% CI (1.22–1.7), $I^2 = 70.5\%$. The pooled effect estimate for high quality studies was 1.39 (1.15–1.68), $I^2 = 72.3\%$.

Although the heterogeneity between the analyzed studies was moderate, the majority of studies pointed towards a positive association. Figure 2 shows the forest plot and the pooled effect estimate for all studies stratified by country. Table 6 summarizes the pooled estimates and associated heterogeneity across different subgroups. The pooled proportion of CDI cases that were exposed to antibiotics was 0.81, 95% CI (0.65–0.91) as shown in Figure 3.

Exploring heterogeneity

The influence of a range of a-priori selected study-level and aggregated individual-level parameters on the observed effect estimate was investigated by means of meta-regressions. Table 7 summarizes the meta-regression analyses for all 35 results. Heterogeneity could not be explained by any of the 10 considered variables.

Publication bias

Figure 4 displays the contour enhanced funnel plot which showed no evidence of publication bias. This was confirmed by the Egger's test ($P = 0.905$).

Residual confounding

The results of the residual confounding analysis are presented in Figure 5. Panel A refers to a confounder with a prevalence of 0.20; at this prevalence level, a strong confounder causing a two-fold increased risk of CDI would have to be severely imbalanced between H_2 blockers users and non users (OREC = 8.87) in order to fully account for the observed adjusted RR of 1.40. For a very common confounder with a prevalence of 0.40, stronger associations with acid-suppression use and/or CDI would be needed to explain the observed association between acid-suppression use and CDI. At this prevalence level, the confounder would have to be both imbalanced (OREC = 5.87) and increase the CDI risk (2.5-fold) to account for the observed OR, after taking publication bias into account.

Number needed to harm

The number needed to harm (NNH) was estimated by using the pooled OR from the meta-analysis [59]. A recent large prospective hospital cohort [48] reported the incidence of CDI at 14 days after hospital admission in patients receiving antibiotics or not: which was 42/1,000 and 5.4/1000, respectively. Based on these reported baseline risks, the number needed to harm (NNH) was 58, 95% CI (37, 115) and 425, 95% CI (267, 848), respectively. For the general population, the NNH at 1 year was 4549, 95% CI (2860, 9097) at 1 year, based on a baseline incidence of CDI of 48/100,000 person-years [60].

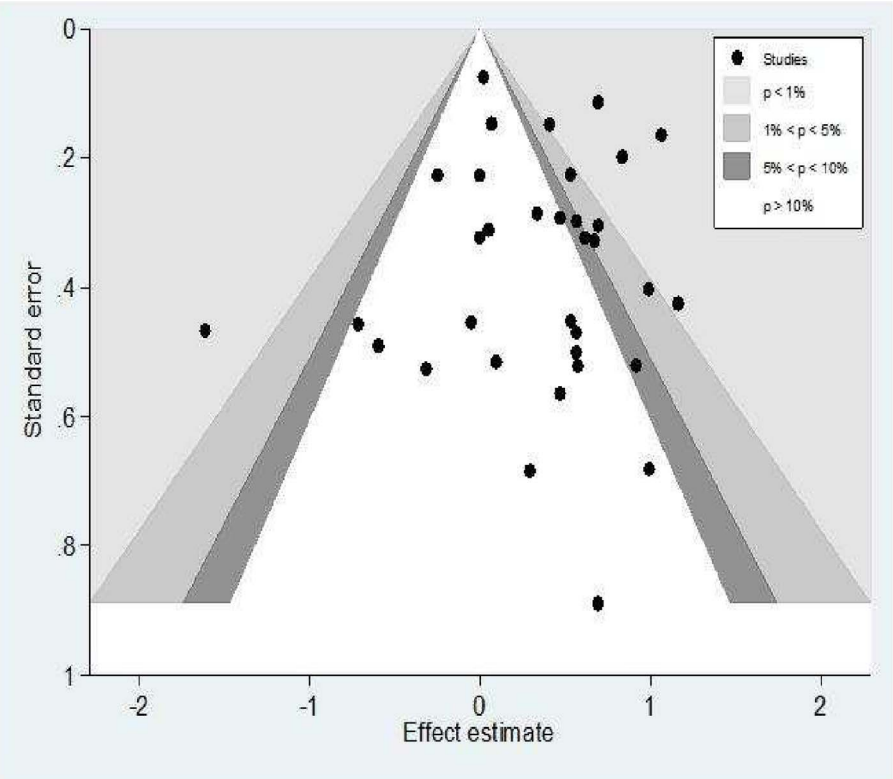

Figure 4. Contour-enhanced funnel plot of the association between the estimated effect size and its standard error in all studies comparing those exposed and unexposed to H2RA displays areas of statistical significance on a funnel plot. Contours represent conventional "milestone" levels of statistical significance (e.g., <0.01, <0.05, <0.1). This funnel plot is symmetrical as it is not missing studies in the white area excluding the possibility of publication bias (Egger's test, p = 0.905).
doi:10.1371/journal.pone.0056498.g004

Discussion

Findings

In this rigorously conducted systematic review and meta-analysis, we observed an association between H_2RAs use and development of CDI. Using the GRADE framework, the evidence supporting this association is considered of moderate quality. Although evidence from observational studies is considered of weak quality, we have ruled out a strong effect of an unmeasured confounder and, therefore, have upgraded its quality to moderate evidence in favor of this association.

The absolute risk of CDI was highest in hospitalized patients receiving antibiotics with an estimated NNH of 58 at 2 weeks. In contrast, the risk was very low (4549) in the general population. We also observed that, on average, 19% of CDI cases had not been recently exposed to antibiotics.

These findings add to previous subgroup analyses of a limited number of H_2RA studies performed in a recent systematic review of the association between PPI and CDI. In this review, Kwok [11] et al conducted a subgroup analysis of 15 H_2RA studies and reported a pooled effect estimate of 1.50, 95% CI (1.23–1.83). Similarly, Leonard et al [61] reported in 2007 an analysis based on 12 studies that showed H_2RAs use was also associated with risk of CDI with a pooled OR 1.40, 95% CI (0.85–2.29).

Biologic plausibility

The pathogenic mechanisms operative in H_2RAs therapy causing an increased risk of CDI acquisition are unclear, because gastric acid does not kill gastric *C. difficile* spores. One potential explanation for the association between CDI and gastric acid suppression therapies could be that the vegetative form of *C. difficile*, which is killed by acid, plays a role in pathogenesis. Vegetative forms survive on surfaces and could be ingested by patients [62]. Survival of acid-sensitive vegetative forms in the stomach could be facilitated by two primary factors: (1) suppression of gastric acid production by acid-suppressive medications; and (2) presence of bile salts in gastric contents of patients on acid-suppressive therapy. Bile salts, which are mainly found in the small intestine, are present in gastric contents, particularly among patients with gastro-esophageal reflux disease (GERD).

The extent of gastric acid suppression could play an important role in potentiating the risk of infection. Kwok [11] et at compared the risk of CDI with gastric acid suppression from 15 studies that reported on estimates of both PPI and H_2RAs independently on their sample of participants and found that PPI is associated with higher risk of infection in comparison to H_2RAs though both increase the risk.

Implications

Our findings have global implications both on the inappropriate use of acid-suppression therapy and on the increasing incidence of CDI.

Given the relatively low NNH (58 patients) needed to cause a case of CDI in hospitalized patients receiving antibiotics it becomes necessary to judiciously use H_2RAs in these patients. In addition, reducing the inappropriate use of acid-suppression

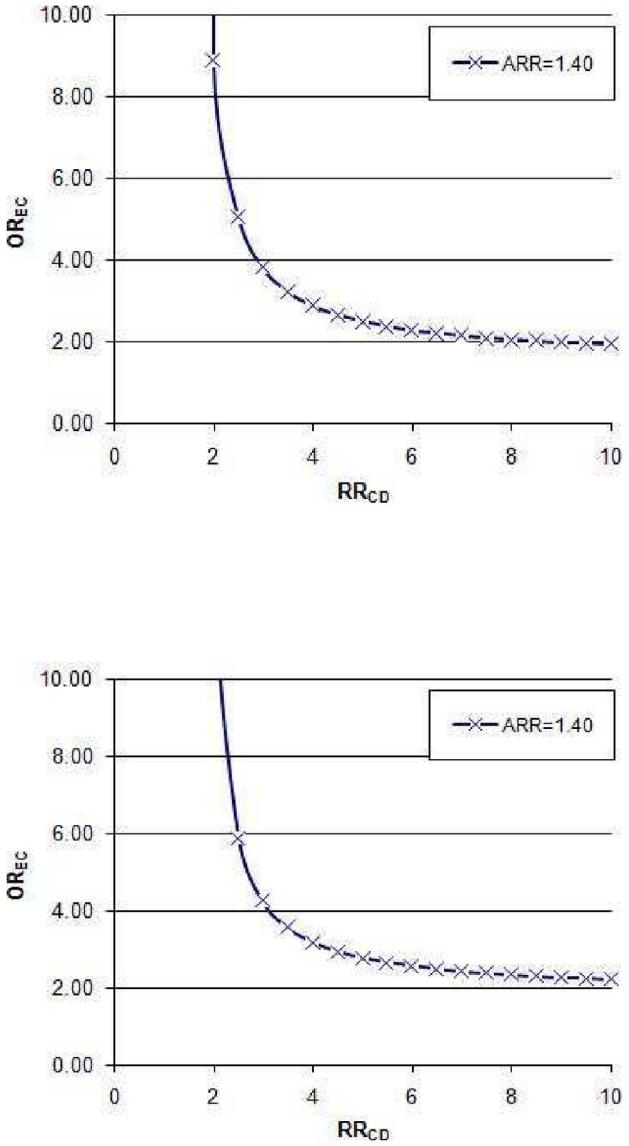

Figure 5. Influence of a hypothetical dichotomous confounder present in 20% (panel A) and 40% (panel B) of the study population, unaccounted for in prior adjustments performed in individual studies. The graphs depict what combinations of OREC and RR would be necessary for the confounder to fully account for the observed association between H2RA use and CDI acquisition. Abbreviations: OR_{EC}, odds ratio of exposure to the confounder in H2RA non-users vs. H2RA users; RR_{CD}, relative risk of CDAD in individuals exposed to the confounder vs. non-exposed.
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medications in this patient population could lead to a significant reduction in the incidence of CDI.

On the other hand, our findings are re-assuring to the public that H_2RAs use in the general population as over-the-counter medications do not pose significant CDI risk and is associated with a high NNH.

Strengths

Our study has several important strengths. This review is the first systematic evaluation dedicated to examine the association between H_2RAs and risk of CDI. It includes a comprehensive, up-to-date literature search and formal assessment of the methodological quality of pertinent studies with the largest number of relevant studies as compared to previous reviews [11,61]. In addition, our pooled estimates are based on multivariate ORs of studies adjusting for several important CDI risk factors. We also performed subgroup analyses and sensitivity analyses that confirmed the robustness of our main results. There was no statistical evidence of publication bias and the effect of residual confounding on the observed association was examined. Finally, the NNH in different risk groups was calculated to aid physicians and patients in making a decision to use H_2RA or not.

Limitations

Our review has certain limitations. There was moderate between-study heterogeneity; however, this is often the case in meta-analyses of large observational studies [63–65]. Moreover the majority of studies pointed towards a positive association. There was virtually no qualitative heterogeneity, and subgroup and sensitivity analyses showed results consistent with the main analysis. There are many patient level parameters which may have led to substantial heterogeneity. Nevertheless, investigating these variables is only possible with individual patient data meta-analysis.

Conclusions

In this rigorous systematic review and meta-analysis, we observed an association between H_2RAs and CDI. The absolute risk of CDI associated with H_2RAs was highest in hospitalized patients receiving antibiotics. On the other hand, our findings are re-assuring that H_2RAs use in the general population as over-the-counter medications do not pose a significant CDI risk.

Supporting Information

Figure S1 PRISMA 2009 flow diagram.
(DOC)

Table S1 PRISMA checklist.
(DOC)

Author Contributions

Conceived and designed the experiments: IMT ABA AS MR LMB. Analyzed the data: IMT AS MR ABA. Wrote the paper: IMT ABA AS MR FAA MAA MAT MAG ARK PJE LMB.

References

1. Jarvis WR, Schlosser J, Jarvis AA, Chinn RY (2009) National point prevalence of *Clostridium difficile* in US health care facility inpatients, 2008. Am J Infect Control 37: 263–270.
2. Archibald LK, Banerjee SN, Jarvis WR (2004) Secular trends in hospital-acquired *Clostridium difficile* disease in the United States, 1987–2001. J Infect Dis 189: 1585–1589.
3. McDonald LC, Owings M, Jernigan DB (2006) *Clostridium difficile* infection in Patients discharged from US short-stay hospitals, 1996–2003. Emerg Infect Dis 12: 409–415.
4. Elixhauser A, Jhung M. *Clostridium difficile*-associated disease in US hospitals, 1993–2005. Statistical Brief #50: *Clostridium difficile*-Associated Disease in U.S. Hospitals, 1993–2005.Available at: http://www.hcupus.ahrq.gov/reports/statbriefs/sb50.jsp. Last accessed Oct 21/2012.
5. Redelings MD, Sorvillo F, Mascola L (2007) Increase in *Clostridium difficile*-related mortality rates, United States, 1999–2004. Emerg Infect Dis 13: 1417–1419.
6. Kyne L, Hamel MB, Polavaram R, Kelly CP (2002) Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. Clin Infect Dis 34: 346–353.

7. Schuller I, Saha V, Lin L, Kingston J, Eden T, et al. (1995) Investigation and Management of *Clostridium difficile* colonisation in a paediatric oncology unit. Arch Dis Child 72: 219–222.
8. Emoto M, Kawarabayashi T, Hachisuga MD, Eguchi F, Shirakawa K (1996) *Clostridium difficile* colitis associated with cisplatin-based chemotherapy in ovarian cancer patients.Gynecol Oncol 61: 369–372.
9. Bliss DZ, Johnson S, Savik K, Clabots CR, Willard K, et al. (1998) Acquisition of *Clostridium difficile* and *Clostridium difficile*-associated diarrhea in hospitalized Patients receiving tube feeding. Ann Intern Med 129: 1012–1019.
10. Brown E, Talbot GH, Axelrod P, Provencher M, Hoegg C (1990) Risk factors for *Clostridium difficile* toxin-associated diarrhea. Infect Control Hosp Epidemiol 11: 283–290.
11. Kwok CS, Arthur AK, Anibueze CI, Singh S, Cavallazzi R, et al. (2012) Risk of *Clostridium difficile* infection with acid suppressing drugs and antibiotics: meta-analysis. Am J Gastroenterol 107: 1011–1019.
12. Deshpande A, Pant C, Pasupuleti V, Rolston DD, Jain A, et al. (2012) Association between proton pump inhibitor therapy and *Clostridium difficile* infection in a meta-analysis. Clin Gastroenterol Hepatol 10: 225–233.
13. Bavishi C, Dupont HL (2011) Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. Aliment Pharmacol Ther 34: 1269–1281.
14. Tleyjeh IM, Bin Abdulhak AA, Riaz M, Alasmari FA, Garbati MA, et al. (2012) Association between Proton Pump Inhibitor Therapy and *Clostridium difficile* Infection: A Contemporary Systematic Review and Meta-Analysis. PLoS ONE 7(12): e50836. doi:10.1371/journal.pone.0050836.
15. U.S food and drug administration. Proton Pump Inhibitors (PPIs) – Drug Safety Communication: *Clostridium difficile*-Associated Diarrhea (CDAD) Can be Associated With Stomach Acid Drugs. Available: http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm290838.htm. Accessed May, 31, 2012.
16. Shi CW, Gralnek IM, Dulai GS, Towfigh A, Asch S (2004) Consumer usage patterns of nonprescription histamine2-receptor antagonists. Am J Gastroenterol 99 (4):606–10.
17. Erwin J, Britten N, Jones R (1997) General practitioners' views on the over-the-counter availability of H2-antagonists. Br J Gen Pract 47: 99–102.
18. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, et al. (2000) Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 283: 2008–2012.
19. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, et al. (2009) The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLoS Med 6(7): e1000100.
20. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, et al. (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336: 924–926.
21. Wells G, Shea B, O'Connell D, et al. The Newcastle- Ottawa scale (NOS) for assessing the quality of non-randomized studies in meta-analysis. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Last accessed May31.2012.
22. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7: 177–188.
23. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629–634.
24. Schneeweiss S (2006) Sensitivity analysis and external adjustment for unmeasured confounders in epidemiologic database studies of therapeutics. Pharmacoepidemiol Drug Saf 15: 291–303.
25. Zhang J, Yu KF (1998) What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA 280: 1690–1691.
26. Kutty PK, Woods CW, Sena AC, Benoit SR, Naggie S, et al. (2010) Risk factors for and estimated incidence of community-associated *Clostridium difficile* infection, North Carolina, USA. Emerg Infect Dis 16: 197 204.
27. Jayatilaka S, Shakov R, Eddi R, Bakaj G, Baddoura WJ, et al. (2007) *Clostridium difficile* infection in an urban medical center: five-year analysis of infection rates among adult admissions and association with the use of proton pump inhibitors. Ann Clin Lab Sci 37: 241–247.
28. Nath SK, Salama S, Persaud D, Thornley JH, Smith I, et al. (1994) Drug risk factors associated with a sustained outbreak of Clostridium difficile diarrhea in a teaching hospital. Can J Infect Dis 5: 270–275.
29. Shah S, Lewis A, Leopold D, Dunstan F, Woodhouse K (2000) Gastric acid suppression does not promote clostridial diarrhoea in the elderly. QJM 93: 175–181.
30. Dial S, Delaney JA, Barkun AN, Suissa S (2005) Use of gastric acid-suppressive agents and the risk of community-acquired *Clostridium difficile*-associated disease. JAMA 294: 2989–2995.
31. Debast SB, Vaessen N, Choudry A, Wiegers-Ligtvoet EA, van den Berg RJ, et al. (2009) Successful combat of an outbreak due to *Clostridium difficile* PCR ribotype 027 and recognition of specific risk factors. Clin Microbiol Infect 15: 427–434.
32. Lowe DO, Mamdani MM, Kopp A, Low DE, Juurlink DN (2006) Proton pump inhibitors and hospitalization for *Clostridium difficile*-associated disease: a population-based study. Clin Infect Dis 43:1272–1276.
33. Dial S, Delaney JA, Schneider V, Suissa S (2006) Proton pump inhibitor use and risk of community acquired *Clostridium difficile*-associated disease defined by prescription for oral vancomycin therapy.CMAJ 175: 745–748.
34. Aseeri M, Schroeder T, Kramer J, Zackula R (2008) Gastric acid suppression by proton pump inhibitors as a risk factor for *Clostridium difficile*-associated diarrhea in hospitalized patients. Am J Gastroenterol 103: 2308–2313.
35. Dubberke ER, Reske KA, Olsen MA, McMullen KM, Mayfield JL, et al. (2007) Evaluation of *Clostridium difficile*-associated disease pressure as a risk factor for *C difficile*-associated disease. Arch Intern Med.167: 1092–1097.
36. Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, et al. (2005) A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med 353: 2442–2449.
37. Sundram F, Guyot A, Carboo I, Green S, Lilaonitkul M, et al. (2009) *Clostridium difficile* ribotypes 027 and 106: clinical outcomes and risk factors. J Hosp Infect 72: 111–118.
38. Howell MD, Novack V, Grgurich P, Soulliard D, Novack L, et al. (2010) Iatrogenic gastric acid suppression and the risk of nosocomial *Clostridium difficile* infection. Arch Intern Med 170: 784–790.
39. Dalton BR, Lye-Maccannell T, Henderson EA, Maccannell DR, Louie TJ (2009) Proton pump inhibitors increase significantly the risk of *Clostridium difficile* infection in a low-endemicity, non-outbreak hospital setting. Aliment Pharmacol Ther 29: 626–634.
40. Dubberke ER, Reske KA, Yan Y, Olsen MA, McDonald LC, et al. (2007) *Clostridium difficile* – associated disease in a setting of endemicity: identification of novel risk factors. Clin Infect Dis 45: 1543–1549.
41. Pepin J, Saheb N, Coulombe MA, Alary ME, Corriveau MP, et al. (2005) Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. Clin Infect Dis 41: 1254–1260.
42. Beaulieu M, Williamson D, Pichette G, Lachaine J (2007) Risk of *Clostridium difficile*-associated disease among patients receiving proton-pump inhibitors in a Quebec medical intensive care unit. Infect Control Hosp Epidemiol 28: 1305–1307.
43. Peled N, Pitlik S, Samra Z, Kazakov A, Bloch Y, et al. (2007) Predicting *Clostridium difficile* toxin in hospitalized patients with antibiotic-associated diarrhea. Infect Control Hosp Epidemiol 28: 377–381.
44. Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D (2004) Risk of *Clostridium difficile* diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. CMAJ 171: 33–38.
45. Novell MJ, Morreale CA (2010) The relationship between inpatient fluoroquinolone use and *Clostridium difficile*-associated diarrhea. Ann Pharmacother : 826–831.
46. Netland PJ, Brochardt SM, Koo JM, Lo TS, Krenz T (2011) A Retrospective Analysis of Risk factors and Therapeutic Outcomes for *Clostridium difficile* Infection. Hosp Pham 46(5): 336–340.
47. Jung KS, Park JJ, Chon YE, Jung ES, Lee HJ, et al. (2010) Risk Factors for Treatment Failure and Recurrence after Metronidazole Treatment for *Clostridium difficile*-associated Diarrhea. Gut Liver 4: 332–337.
48. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, et al. (2011) Host and pathogen factors for *Clostridium difficile* infection and colonization. N Engl J Med 365: 1693–1703.
49. Manges AR, Labbe A, Loo VG, Atherton JK, Behr MA, et al. (2010) Comparative metagenomic study of alterations to the intestinal microbiota and risk of nosocomial *Clostridum difficile*-associated disease. J Infect Dis 202: 1877–1884.
50. Kuntz JL, Chrischilles EA, Pendergast JF, Herwaldt LA, Polgreen PM (2011) Incidence of and risk factors for community-associated *Clostridium difficile* infection: a nested case-control study. BMC Infect Dis 11:194.
51. Naggie S, Miller BA, Zuzak KB, Pence BW, Mayo AJ, et al. (2011) A case control study of community associated *Clostridium difficile* infection: no role for proton pump inhibitors. Am J Med 124: 276 e271–277.
52. Stevens V, Dumyati G, Brown J, Wijngaarden E (2011) Differential risk of *Clostridium difficile* infection with proton pump inhibitor use by level of antibiotic exposure. Pharmacoepidemiol Drug Saf 20: 1035–1042.
53. Dial S, Kezouh A, Dascal A, Barkun A, Suissa S (2008) Patterns of antibiotic use and risk of hospital admission because of *Clostridium difficile* infection. CMAJ 179: 767–772.
54. McFarland LV, Clarridge JE, Beneda HW, Raugi GJ (2007) Fluoroquinolone use and risk factors for *Clostridium difficile*-associated disease within a Veterans Administration health care system. Clin Infect Dis 45: 1141–1151.
55. Kazakova SV, Ware K, Baughman B, Bilukha O, Paradis A, et al. (2006) A hospital outbreak of diarrhea due to an emerging epidemic strain of *Clostridium difficile*. Arch Intern Med 166: 2518–2524.
56. Modena S, Bearelly D, Swartz K, Friedenberg FK (2005) *Clostridium difficile* among hospitalized patients receiving antibiotics: a case-control study. Infect Control Hosp Epidemiol 26: 685–690.
57. Muto CA, Pokrywka M, Shutt K, Mendelsohn AB, Nouri K, et al. (2005) A large outbreak of *Clostridium difficile*-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol 26: 273–280.
58. Yip C, Loeb M, Salama S, Moss L, Olde J (2001) Quinolone use as a risk factor for nosocomial *Clostridium difficile*-associated diarrhea. Infect Control Hosp Epidemiol 22: 572–575.

59. Cates CJ (2002) Simpson's paradox and calculation of number needed to treat from meta-analysis. BMC Med Res Methodol 2: 1.
60. Khanna S, Pardi DS, Aronson SL, Kammer PP, Orenstein R, et al. (2012) The epidemiology of community-acquired *Clostridium difficile* infection: a population based study. Am J Gastroenterol 107: 89–95.
61. Leonard J, Marshall JK, Moayyedi P (2007) Systematic review of the risk of enteric infection in patients taking acid suppression. Am J Gastroenterol 102: 2047–2056
62. Jump RL, Pultz MJ, Donskey CJ (2007) Vegetative Clostridium difficile survives in room air on moist surfaces and in gastric contents with reduced acidity: a potential mechanism to explain the association between proton pump inhibitors and *C. difficile* associated diarrhea? Antimicrob Agents Chemother 51: 2883–2887.
63. Coory MD (2010) Comment on: Heterogeneity in meta-analysis should be expected and appropriately quantified. Int J Epidemiol 39: 932; author reply 933.
64. Higgins JP (2008) Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. Int J Epidemiol 37: 1158–1160.
65. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta analyses. BMJ 327: 557–560.