Factors associated with anorectal *Chlamydia trachomatis* or *Neisseria gonorrhoea* test positivity in women – a systematic review and meta-analysis.

Authors:

*Andrew Lau[1], Fabian YS Kong[1], Wilhelmina Huston[2], Eric PF Chow[3,4], Christopher K Fairley[3,4], Jane S Hocking[1]

1. Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, University of Melbourne, Level 3, 207 Bouverie St, Carlton, Victoria 3053, Australia
2. School of Life Sciences, The University of Technology Sydney, City Campus, Broadway, Ultimo, New South Wales 2007, Australia
3. Melbourne Sexual Health Centre, Alfred Health, 580 Swanston St, Carlton, Victoria 3053, Australia
4. Central Clinical School, Faculty of Medicine, Monash University, Melbourne, Australia

* Corresponding author:
Andrew Lau
Melbourne School of Population and Global Health
University of Melbourne
Level 3, 207 Bouverie St
Carlton, Victoria 3053, Australia
Email: andrew.lau@unimelb.edu.au

Summary: Anorectal chlamydia in women is more common than anorectal gonorrhea, but anorectal gonorrhea is more associated with urogenital and oropharyngeal detection and anal intercourse. Longitudinal data are needed to understand the etiology and importance of anorectal STIs in women.

Key words: *Chlamydia trachomatis*; *Neisseria gonorrhoeae*; extra-genital sexually transmitted infections; women.

Conflicts of Interests: All authors have none to declare.

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ABSTRACT

Background

There has been considerable discussion about anorectal *Chlamydia trachomatis* (CT) in women, but little about anorectal *Neisseria gonorrhea* (NG). This systematic review and meta-analysis investigates whether anorectal CT in women is associated with positivity at other sites (urogenital and oropharyngeal) and compares these with anorectal NG within the same populations.

Methods

Electronic databases were searched for English-language studies published to October 2018 using the search terms: ("Chlamydia" OR "*Chlamydia trachomatis*") AND (("anal" OR "rect*" OR "anorect*") OR ("extra?genital" OR "multi?site")). Studies were included if anorectal NG data were available. Random effects meta-analyses were used to calculate pooled estimates; heterogeneity was investigated using meta-regression.

Results

25 studies were eligible. Anorectal CT positivity ranged from 0% to 17.5% with a summary estimate of 8.2% (95% CI: 7.2, 9.2; I^2=86.4%). Anorectal NG positivity ranged from 0% to 17.0% with a summary estimate of 2.2% (95% CI: 1.6, 2.8; I^2=92.6%). The association between urogenital and anorectal positivity was stronger for NG than CT (summary prevalence ratio (PR)=82.2 [95% CI: 50.0, 140.9; I^2=80.4%], PR=29.7 [95% CI 23.8, 37.1; I^2=64.6%], respectively). Anal intercourse was associated with anorectal NG (PR=4.3; 95% CI: 2.18, 8.55; I^2=0.0%) but not anorectal CT (PR=1.0; 95% CI: 0.71, 1.4; I^2=0.0%).

Conclusions

Anorectal CT is more common than anorectal NG, but anorectal NG is more strongly associated with anal intercourse, urogenital, and oropharyngeal infection. Longitudinal data are required to further understanding of the etiology of anorectal STIs and to inform whether anorectal screening is needed in women.

INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) are two commonly diagnosed bacterial sexually transmitted infections (STIs), with CT rates usually four to five-fold higher than NG in high income countries [1]. In the last five years, CT rates in the United States and Australia have increased marginally by up to 20% overall but have declined by 6% in England during this time [2-4]. In contrast, NG rates increased between 2013 and 2017 by between 40% and 77% overall in these countries [2-4].

Both CT and NG can cause pelvic inflammatory disease (PID) in women, although data linkage studies suggest that the risk of PID is considerably higher with NG than CT infection [5]. However, there has been much less published research investigating the upper genital tract consequences of NG infection and its role in PID.

In recent years, there has been considerable discussion about the prevalence and role of anorectal CT in women with three reviews reporting high median anorectal CT test positivity estimates ranging from 6.0% to 9.2% [6-8]. Further, there have been calls for anorectal CT screening in women [9]. However, the clinical significance of anorectal CT in women is unclear with some questioning whether it can cause urogenital infection by auto-inoculation, thereby potentially leading to upper genital tract infection in women [10]. Some question whether anorectal CT test positivity in women represents true infection, or a false positive result due to contamination from the genitals, particularly as anal intercourse has not been shown to be associated with anorectal test positivity in women [8]. The possibility that anorectal CT can occur due to ingestion of CT during oral sex has also been raised as a potential contributor to anorectal CT test positivity in women [11].

In contrast, there has been very little published about anorectal NG in women and given that urogenital NG is more strongly associated with PID in women, this is a considerable evidence gap. We conducted a systematic review and meta-analysis to investigate whether anorectal CT positivity in women is associated with positivity at other infection sites (urogenital and oropharyngeal) and to compare these with infection sites associated with anorectal NG in the same populations.

METHODS

Search strategy

The study protocol was registered on PROSPERO (CRD42017080188) and the results are reported according to PRISMA guidelines [12] (Supplementary material 1). We searched for peer-reviewed studies reporting on extra-genital testing for *Chlamydia trachomatis* infection in women published up to the end of October 2018. The search was performed on electronic databases PubMed, EMBASE and MEDLINE. Search terms were ("Chlamydia" OR "*Chlamydia trachomatis*") AND (("anal" OR "rect*" OR "anorect*") OR ("extra?genital" OR "multi?site")) (Supplementary material 2). Medical subject headings were used where possible. Citation lists were hand-searched for additional references.

Eligible studies were those in humans aged 15+ years old, published in English and provided original data on CT and NG anorectal test results for women. Ineligible studies were those reporting exclusively on urogenital infection, those reporting no anorectal NG data, and those conducted in

men only. Review and opinion pieces, or when the sample size was under 10 were excluded, although review articles were hand-searched to identify any other eligible studies. CT positivity was defined as a detection by nucleic acid amplification test (NAAT) or DNA hybridization probe. Studies using only culture or immuno-fluorescent assay for CT were excluded. NG positivity was defined as detection by culture or NAAT.

Data extraction

Data extracted included country of study (North America, United Kingdom, The Netherlands and Africa/South America), study design, final year of data collection (<2010, 2010-2012, 2013+), study population, anal intercourse profiling of participants (recruited only women who reported anal intercourse versus recruited women who did or did not report anal intercourse), and site-specific CT and NG test positivity (anorectal, urogenital or oropharyngeal).

The primary outcomes were anorectal CT and anorectal NG positivity among those tested. Prevalent-only estimates were included. Secondary outcomes were associations between anorectal positivity and positivity at the oropharyngeal and urogenital sites or association with anal intercourse. These secondary outcomes were measured as a prevalence ratios (PR). Where these were not reported, the PR and 95% confidence intervals (CI) were calculated using available data.

One author (AL) extracted data from the included studies and a second author (JSH) checked the extracted data. Disagreement was resolved by discussion between the two authors and consultation with an additional author (FYSK) until consensus was reached.

Analysis

For meta-analysis, random effects methods were used to calculate pooled estimates of test positivity and PR with the assumption that observed heterogeneity was not wholly due to sampling variation. The I^2 test was used to calculate the proportion of total variability attributable to heterogeneity rather than chance alone, and was considered moderate or high if greater than 50% or 75%, respectively [13]. If I^2 test was >25%, factors contributing to heterogeneity were investigated using two methods: i) we calculated stratum-specific summary estimates across several different sub-groups, and ii) we used meta-regression to estimate PRs (for positivity estimates) and ratio of PRs (for association estimates). The relative reduction of between-study variance (τ^2 or "tau-squared") provided an indication of the factor's contribution to heterogeneity.

A sensitivity analysis was conducted excluding two studies in which a considerably larger number of women were tested for NG than CT [14, 15]. All analyses were performed using the 'metaprop', 'metan' and 'metareg' commands in STATA (Stata v13; Stata, Austin, Texas, USA).

Assessment of study quality

Assessment of within-study bias was undertaken using a combination of the evaluation criteria adopted by Sanderson *et al* [16] and the critical appraisal tool for cross-sectional studies, AXIS [17].

RESULTS

Study selection and characteristics:

Overall, 583 references were identified of which 458 were unique papers. Hand-searching reference lists identified a further six papers giving a total of 464 papers. Overall, 25 reporting on both CT and NG anorectal positivity were eligible (Figure 1). Twelve (48%) studies were from North America [18-29], seven (28%) from the Netherlands [14, 15, 30-34], three (12%) from the United Kingdom [35-37], two (8%) from South America [38, 39], and one (4%) from Africa [40].

Table 1 shows that 23 studies were cross-sectional [14, 15, 18-24, 26-38, 40], one was a prospective cohort study [25] and one was a case-control [39]. The cohort study reported both prevalent and incident STI data [25]. A total of 18 (72%) were set in STI/sexual health clinics [14, 15, 18, 19, 21-23, 25, 27, 29-35, 38, 39], five (20%) in hospital, genito-urinary/obstetrics/gynaecology or primary health settings [20, 26, 36, 37, 40], and two (8%) were based on surveillance data from a laboratory [28] or STI clinics [24].

Rectal positivity:

Overall 25 studies reported anorectal CT and NG positivity [14, 15, 18-40]. Anorectal CT positivity among women tested ranged from 0% to 17.5% with a summary estimate of 8.2% with high heterogeneity (95% CI: 7.2, 9.2; I^2=86.4%,) (Figure 2A). Meta-regression identified that whether or not the study population included only women reporting anal intercourse was an important contributor to heterogeneity ($\Delta\tau^2$= -13.6%) (Table 2).

Anorectal NG positivity ranged from 0% to 17.0% with a summary estimate of 2.2% with high heterogeneity (95% CI: 1.6, 2.8; I^2=92.6%) (Figure 2B). Meta-regression identified that region of study was a major contributor to heterogeneity ($\Delta\tau^2$=-46.4%) (Table 3).

Sensitivity analysis showed negligible impact on anorectal CT and NG positivity summary estimates when studies that had a disproportionately larger numbers of women tested for NG were excluded [14, 15] (Supplementary material 3).

Association of between anorectal and urogenital positivity:

A total of 12 studies provided data for the association of urogenital positivity with anorectal positivity for CT and NG [14, 18, 19, 21-24, 26, 31, 33, 36] (Figure 3). The PR for the association of urogenital CT with anorectal CT positivity ranged from 15.4 to 196.2. The summary PR estimate for the association was 29.7 with moderate heterogeneity (95% CI: 23.8, 37.1; I^2=64.6%). Meta-regression identified that the final year of data collection was an important contributor to heterogeneity ($\Delta\tau^2$= -17.9%), although no statistically significant differences were found (Table 2). Of those who tested for concurrent anorectal and urogenital CT, meta-analysis found that 22.6% (95% CI: 90.0, 26.3; I^2=45.1%) tested positive for anorectal CT alone (i.e. concurrent urogenital CT negative) (Supplementary material 4).

The PR for the association or urogenital NG with anorectal NG positivity ranged from 12.5 to 1123.5 and the summary estimate was 80.6 with high heterogeneity (95% CI: 47.6, 137.5, I^2=80.6%). Meta-regression identified that region of study was an important contributor to heterogeneity ($\Delta\tau^2$=-

22.8%) although there was no statistical difference between regions (Table 3). Of those who tested for concurrent anorectal and urogenital NG, meta-analysis found that 19.0% (95% CI: 11.6, 27.3; I^2=59.0%) tested positive for anorectal NG alone (Supplementary material 4).

Sensitivity analysis showed negligible impact on PR summary estimates when the study that had a disproportionately larger number of women tested for NG was excluded [14] (Supplementary material 3).

Association of oropharyngeal positivity with anorectal positivity:

Four studies provided data to calculate PR for the association of oropharyngeal positivity with anorectal positivity [14, 15, 21, 31] (Supplementary material 5). The PR for the association of oropharyngeal CT with anorectal CT ranged from 6.7 to 8.7 and the summary PR estimate was 8.1 (95% CI: 6.4, 10.4; I^2=46.1%) with moderate heterogeneity. The PR for the association for oropharyngeal NG with anorectal NG ranged from 4.1 to 128.3 and the summary PR estimate was 28.2 (95% CI: 6.0 to 133.7; I^2=92.4%) with high heterogeneity. The small number of studies prevented meta-regression for investigating the association between oropharyngeal positivity and anorectal CT or NG.

Association of anal intercourse with anorectal positivity:

Two studies provided data to calculate PR for the association of anal intercourse with anorectal positivity [18, 33] (Supplementary material 6). The summary estimate for CT was 1.0 (95% CI: 0.7, 1.4; I^2=0.0%) and the summary estimate for NG positivity was 4.32 (95% CI: 2.2, 8.6; I^2=0.0%).

Assessment of within-study bias:

All studies were set in at risk populations and vulnerable to selection bias. There were no general population studies. Overall, 14 (56%) studies specified consecutive recruitment of patients attending clinics or inclusion of all data reducing selection bias [14, 18, 19, 22, 23, 26-31, 33, 35, 36]. Inclusion/exclusion criteria were specified for 22 (88%) studies [15, 19-30, 32-40]. Measurement bias was considered low in 21 (84%) studies because the type of NAAT used was described. However, the risk of bias was considered high in 4 (16%) studies where it was not described [14, 24, 28, 39]. None of the studies confirmed an anorectal test result and none used a viability assay or culture to assess whether a positive test result reflected viable infection rather than remnant nucleic acid. Confounding was not investigated, and sample size calculations were not reported in any studies. Overall, 19 (76%) studies reported conflict of interest [15, 18, 19, 22-30, 32-35, 37, 38, 40], 21 (84%) reported funding source [15, 18, 20, 22-35, 37-40] and 19 (76%) reported patient consent and/or ethical approval [15, 19-21, 23-30, 32-34, 37-40] (Table 4) (Supplementary material 7).

DISCUSSION

This novel systematic review and meta-analysis investigated anorectal CT and NG positivity in the same female populations and their association with detection at other sites. We found that anorectal CT estimates were higher than anorectal NG estimates which is expected considering that CT is much more common in women than NG [1]. However, we also found that anorectal NG was

substantially more associated with urogenital and oropharyngeal detection than was anorectal CT. Furthermore, anorectal NG was associated with anal intercourse, but anorectal CT was not. Given that these observations were made in the same populations, these data raise several important questions about the anorectal transmission dynamics for these two organisms.

There are several limitations in this review. Firstly, as our aim was to investigate both CT and NG anorectal in the same population, our systematic search focused on identifying papers that reported both CT and NG anorectal estimates so any papers reporting only one infection were excluded; it is possible that findings in these papers were different from those in our review. However, systematic reviews of anorectal CT in women show similar results to ours providing validity to our results [6-8]. Secondly, not every individual woman was tested for both anorectal CT and NG in each study, potentially introducing some selection bias into the comparison between the two organisms. This was particularly the case with the studies by van Rooijen *et al* [15] and Koedjik *et al* [14]. However, our sensitivity analysis showed that excluding these studies did not have an important impact on summary estimates. Thirdly, our investigation of heterogeneity was limited by available data. Ideally, we would have investigated whether type of NAAT (to account for different test sensitivity) or clinician versus patient collected swab (to investigate potential for contamination due to sampling) played a role in heterogeneity, but it was not always possible to extract these data from the papers.

There are several factors that may cause the detection of CT or NG in anorectal swabs from women including anal intercourse; auto-inoculation where the infectious material from one site (e.g. cervix) is transferred to, and establishes infection, in another anatomic site (e.g. rectum) [41]; contamination from another anatomical site such as for example may occur during toileting causing contamination on an anal swab as it is inserted into the anal canal for sampling, and [42]; oral ingestion causing a reservoir of infection in the rectum [43]. However, establishing which of these is responsible is extremely challenging and would require longitudinal data with frequent specimen collection and the use of an anoscope to collect swabs from higher up the rectal canal where anorectal CT infection is likely to establish.

Of interest, we found that the association between urogenital and anorectal positivity was at least three-fold stronger for NG than for CT. There are several possible explanations for this. Firstly, if we assume that positivity equals infection and that urogenital infection occurred first, then these data suggest that NG is easier to transmit and establish infection than CT through auto-inoculation. CT is a slow-growing organism that replicates intracellularly and has tissue tropism for columnar or squamous-metaplastic epithelium that in the rectum, is found about 4cm above the sphincter [44]. NG is a predominantly intracellular bacteria that replicates more quickly than CT; it commences replication once it adheres to epithelial cells before it invades them [45]. While CT infecting squamous epithelium is rare [46], NG can adhere and invade squamous epithelium of the cervix [47]. Given this, it seems likely that NG would be able to adhere to the squamous epithelium inside the anal canal and establish infection. Secondly, it is possible that differences in test performance may contribute to observed differences with several NAAT tests being more sensitive for NG than CT [48]. Finally, selection bias may have played a role because anorectal symptoms occur more commonly with NG than CT [49] and this may have prompted the women to seek healthcare and be tested. There was considerably more heterogeneity in the NG data but paucity of data for sub-group analysis limited our ability to investigate this.

We found evidence that anorectal NG was associated with anal intercourse but anorectal CT was not. This is not a new finding. The absence of an association between anal intercourse and anorectal CT was reported in early CT studies [50] whereas an association between AI and anorectal NG was reported in the 1950s [51]. It is possible that not all cases of anorectal CT represent a true infection particularly as we have suggested above that anorectal CT infection is more difficult to establish than anorectal NG infection. A previous study found that rectal CT organism load was higher in women reporting anal intercourse than women not reporting it [52], raising the possibility that contamination rather than infection may explain some of the anorectal CT results.

Oropharyngeal detection was associated with both anorectal CT and NG, but this association was three to four times greater for NG than for CT. It is possible that these results may be in part due to oral ingestion causing anorectal detection as has been suggested for CT [43], but there are no data available about whether NG can survive through the gut. The stronger association with NG is more likely to reflect that NG infection is often multi-site; oropharyngeal NG is far more common than oropharyngeal CT [6] and can be readily cultured from oropharyngeal swabs and saliva [53]. Further, there is some evidence that saliva has anti-CT effects, potentially reducing the incidence of oropharyngeal CT [54].

So, given these results, should we worry about anorectal CT or NG in women? Anorectal infections tend to be asymptomatic [49] and are likely to clear over time if left untreated [55, 56]. However, anorectal infections are a concern if they can auto-inoculate, causing urogenital infection and creating the risk that the infection ascends causing PID. If auto-inoculation between the cervix and rectum can occur, then this is an issue for CT because of the potential reduced efficacy of azithromycin for anorectal infection [57]. While anorectal STI screening in women is likely to be acceptable to women and clinicians in specialist STI clinics, it is less likely to be acceptable in other primary health clinics where sexual health is not the core focus.

Although anorectal CT in women has generated considerable recent debate in the literature with calls for anorectal CT screening, our review highlights that anorectal NG also occurs and is more likely to occur with a concurrent urogenital infection. This review highlights the urgent need for quality research involving longitudinal data collection with sufficient sample size and in representative population groups accounting for selection bias and confounding, to further our understanding of anorectal STIs in women, particularly their etiology and whether they cause urogenital infection. This information is urgently needed to inform anorectal STI screening guidelines for women.

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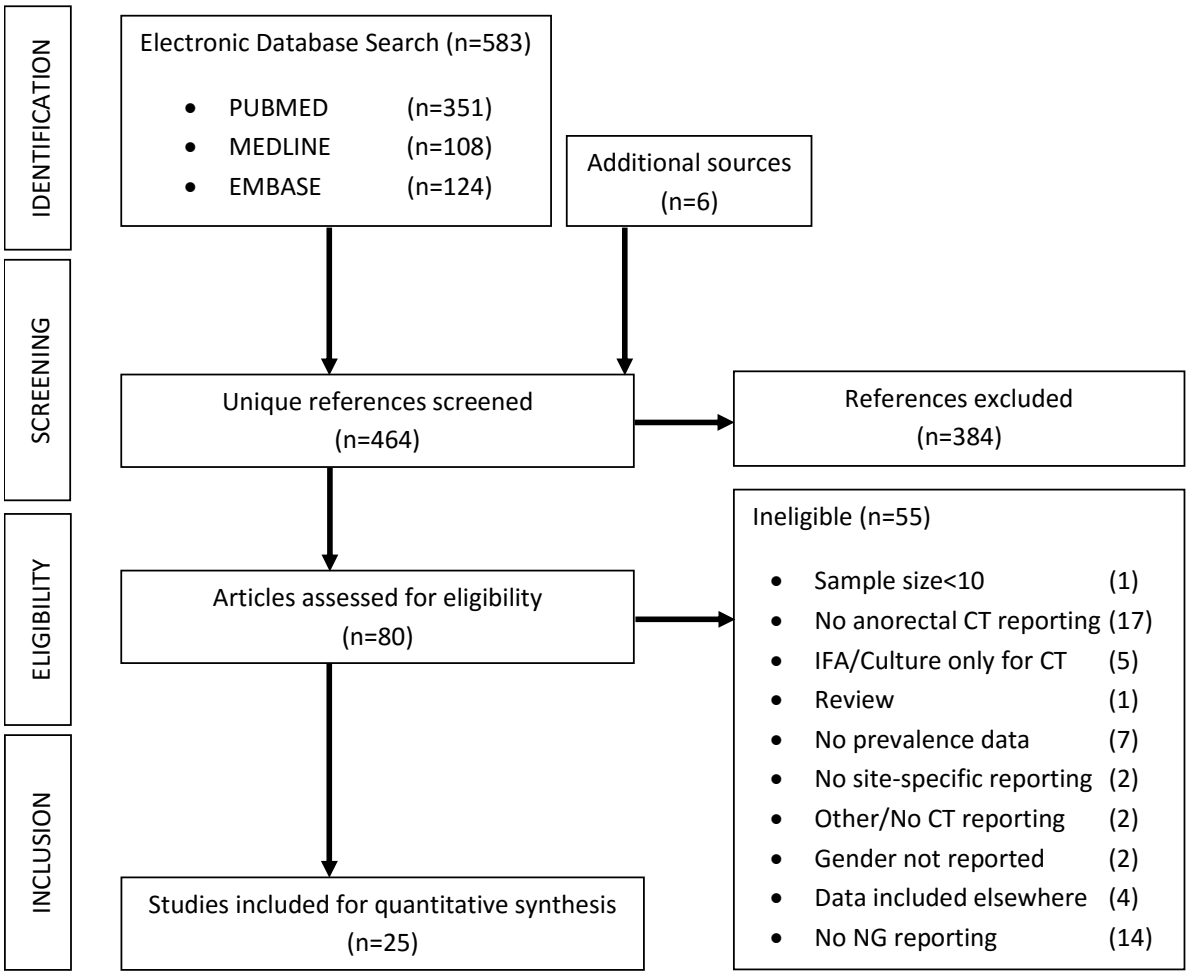

Figure 1: Flowchart of study selection. CT=*Chlamydia trachomatis*, IFA= immuno-fluorescent assay. NG=*Neisseria gonorrhoeae*

Table 1: Characteristics of included studies

Author, publication year	Country	Study design	Study population and setting	Age range (years)	Data collection	Chlamydia		Gonorrhoea	
						No. of women tested, n	Rectal positivity, n (%)	No. of women tested, n	Rectal positivity, n (%)
Barry *et al,* 2010[18]	USA	Cross-sectional	Women receiving pelvic exam, STI clinic	15-65	March 2007-August 2008	1308	62 (4.7%)	1308	22 (1.7%)
Bazan *et al*, 2015[19]	USA	Cross-sectional	Women who reported any anal intercourse in the previous 12 months, STI clinic	16-66	August 2012-June 2013	341	46 (13.5%)	341	22 (6.5%)
Cosentino *et al*, 2012[20]	USA	Cross-sectional	Women reporting lifetime history of anal intercourse, PHC and STI clinic	18-64	May 2009-March 2010	272	21 (7.7%)	272	7 (2.6%)
Danby *et al*, 2016[21]	USA	Cross-sectional	Women reporting lifetime history of anal intercourse, STI clinic	18-49	March 2014-March 2015	175	20 (11.4%)	175	4 (2.3%)
Duker-Muijrers *et al*, 2015[30]	The Netherlands	Cross-sectional	Women screening at STI clinic	20-30	August 2010-October 2013	2092	136 (6.5%)	2143	15 (0.7%)
Garner *et al*, 2015[35]	United Kingdom	Cross-sectional	Women reporting history of anal intercourse, sexual health centre	16-66	March 2010-May 2010	91	6 (6.6%)	91	1 (1.1%)
Hunte *et al*, 2010[22]	USA	Cross-sectional	Women reporting anal intercourse, STI clinic	17-46	May 2007-August 2008	97	17 (17.5%)	97	13 (13.4%)
Javanbakht *et al*, 2012[23]	USA	Cross-sectional	Women reporting anal intercourse in the previous 90 days, STI clinic	14-45+ (*upper range NR)*	January 2008-December 2010	1203	171 (14.2%)	1203	60 (5.0%)
Koedijk *et al*, 2012[14]	The Netherlands	Cross-sectional	Women attending STI clinics	<25 – 35+	2006-2010 *(months NR)*	18238	1695 (9.3%)	37168	442 (1.2%)
Llata *et al*, 2018[24]	USA	Cross-sectional	Surveillance data of women reporting anal intercourse previous 3 months	<24 – 35+	January 2015-December 2016	2878	231 (8.0%)	2878	97 (3.4%)
Mayer *et al*, 2012[25]	USA	Prospective cohort	HIV-positive population attending HIV-specialty clinics	21-69	March 2001-June 2006	119	2 (1.7%)	119	1 (0.8%)

Author, publication year	Country	Study design	Study population and setting	Age range (years)	Data collection	Chlamydia		Gonorrhoea	
						No. of women tested, n	Rectal positivity, n (%)	No. of women tested, n	Rectal positivity, n (%)
Nelson *et al*, 2007[39]	Peru	Case-control study	Heterosexual couples, STI clinic	18-55	February 2001-March 2003	195	6 (3.1%)	195	2 (1.0%)
Peters *et al*, 2011[31]	The Netherlands	Cross-sectional	Women reporting anal intercourse, STI clinic	13-72	January 2007-July 2008	876	76 (8.7%)	876	13 (1.5%)
Peters *et al*, 2014[40]	South Africa	Cross-sectional	Women who report to have been sexually active during last 6 months, PHC	18-49	November 2011-February 2012	603	43 (7.1%)	603	15 (2.5%)
Rodriguez-Hart *et al*, 2012[26]	USA	Cross-sectional	Adult film industry performers, PHC	18-42	May 2010-September 2010	112	4 (3.6%)	112	19 (17.0%)
Sethupathi *et al*, 2010[36]	United Kingdom	Cross-sectional	High risk women, GUM	15-62	September 2006-August 2008	160	20 (12.5%)	160	7 (4.4%)
Shaw *et al*, 2013[37]	United Kingdom	Cross-sectional	Women reporting anal intercourse, GUM	IQR 21-31	5-month period, ending no later than April 2013	312	22 (7.1%)	312	2 (0.6%)
Stephens *et al*, 2014[27]	USA	Cross-sectional	Patients seeking care at an STI clinic	<25-55+	August 2011-December 2012	24	0 (0%)	25	0 (0%)
Tao *et al*, 2018[28]	USA	Cross-sectional	Laboratory surveillance data for all women tested for CT or NG	15-60	November 2012-September 2015	5499	484 (8.8%)	5499	154 (2.8%)
Travassos *et al*, 2016[38]	Brazil	Cross-sectional	HIV-positive patients, STI reference center	Mean 37.7	June 2013-June 2015	305	16 (5.2%)	305	2 (0.7%)
Trebach *et* al, 2015[29]	USA	Cross-sectional	Women reporting extragenital exposures in the last 3 months, STD clinic	<18-30+	June 201-May 2013	502	52 (8.6%)	611	18 (3.0%)

Author, publication year	Country	Study design	Study population and setting	Age range (years)	Data collection	Chlamydia		Gonorrhoea	
						No. of women tested, n	Rectal positivity, n (%)	No. of women tested, n	Rectal positivity, n (%)
van der Helm *et al*, 2009[34]	The Netherlands	Cross-sectional	Women reporting anal intercourse in the past 6 months, STI clinic	IQR 22-31	2006-2007	901	85 (9.4%)	697	13 (1.9%)
van Liere *et al*, 2013[32]	The Netherlands	Cross-sectional	Swingers, STI clinic	IQR 38-49	January 2010-February 2011	461	31 (6.7%)	461	5 (1.1%)
van Liere *et al*, 2017[33]	The Netherlands	Cross-sectional	Women attending STI clinics	IQR 21,28 <21-26+	January 2015—December2015	950	127 (13.4%)	950	12 (1.3%)
van Rooijen *et al*, 2015[15]	The Netherlands	Cross-sectional	High risk women reporting anal intercourse in the past 6 months, STI clinic	IQR 31-47	January 2011-July 2012	1656	154 (9.3%)	6858	57 (0.7%)

NR=not reported; STI=Sexually transmissible infection, PHC= primary health care, GUM= genito-urinary medicine, IQR= interquartile range; n=number of cases/women

Table 2: Anorectal *Chlamydia trachomatis* sub-group analysis and meta-regression.

Anorectal Chlamydia Positivity										
	Sub-group analysis					Meta-regression[a]				
Variable	Number of studies, n	Summary prevalence estimate, %	95%CI	I^2	p	Summary PR	95%CI	p	Residual I^2	τ^2, Δ
All	25	8.2	7.2, 9.2	86.4%	<0.01					0.1254, ref
Region									86.5%	0.1231,
N. America	12	8.2	6.4, 10.2	89.9%	<0.01	ref				-1.8%
U.K.	3	8.5	5.3, 12.4	50.8%	0.13	1.0	0.5, 1.8	0.91		
The Netherlands	7	9.0	7.7, 10.3	85.6%	<0.01	1.0	0.7, 1.5	0.96		
Africa/S. America	3	5.3	3.3, 7.8	58.9%	0.09	0.6	0.3, 1.1	0.09		
Final year of data collection									85.5%	0.1287,
<2010	7	8.7	5.8, 12.0	85.2%	<0.01	ref				+2.6%
2010-2012	9	8.1	6.5, 9.8	83.0%	<0.01	0.86	0.5, 1.5	0.57		
2013+	9	7.9	6.5, 9.5	88.7%	<0.01	0.81	0.5, 1.3	0.39		
Anal intercourse reporting									85.5%	0.1083,
AI only	11	9.9	8.3, 11.6	80.3%	<0.01	ref				-13.6%
Mixed/Unspecified	14	6.9	5.7, 8.2	89.1%	<0.01	0.71	0.5, 1.0	0.05		
Association of Urogenital Chlamydia with Anorectal Chlamydia										
	Sub-group analysis					Meta-regression[b]				
Variable	Number of studies, n	Summary PR estimate	95%CI	I^2	p	Summary ratio of PR	95%CI	p	Residual I^2	τ^2, Δ
All	12	29.7	23.8, 37.01	64.6%	<0.01					0.1099, ref
Region									67.5%	0.1184,
N. America	7	36.3	23.3, 56.5	76.7%	<0.01	ref				+7.7%
U.K.	1	119.2	16.8, 846.0	.	.	3.5	0.3, 41.7	0.28		
The Netherlands	3	27.7	22.8, 33.6	36.5%	0.21	0.8	0.4, 1.6	0.53		
Africa/S. America	1	17.4	10.5, 28.7	0.0%	0.52	0.5	0.2, 1.3	0.14		
Final year of data collection									63.3%	0.0903,
<2010	4	41.7	27.9, 62.2	0.0%	0.68	ref				-17.9%
2010-2012	4	22.2	14.9, 33.1	76.7%	0.01	0.5	0.2, 1.0	0.06		
2013+	4	34.2	22.3, 52.3	72.6%	0.01	0.7	0.3, 1.5	0.32		
Anal intercourse reporting									66.1%	0.1362,
AI only	6	30.1	21.4, 42.3	60.8%	0.03	ref				+23.9%
Mixed/Unspecified	6	30.9	20.8, 45.9	72.6%	<0.01	1.0	0.5, 2.0	0.98		

a=Stephens et al excluded from meta-regression as 0% prevalence for CT and NG. b=Danby at el naturally excluded as PR involved division of 0. CI=confidence interval. PR=Prevalence ratio

Table 3: Anorectal *Neisseria gonorrhoeae* sub-group analysis and meta-regression

Anorectal Gonorrhoea Positivity										
	Sub-group analysis					Meta-regression[a]				
Variable	Number of studies, n	Summary prevalence estimate, %	95%CI	I^2	p	Summary PR	95%CI	p	Residual I^2	τ^2, Δ
All	25	2.2	1.6, 2.8	92.6%	<0.01					0.75, ref
Region									85.2%	0.4022,
N. America	12	3.8	2.5, 5.5	87.4%	<0.01	ref				-46.4%
U.K.	3	1.7	0.1, 4.7	71.5%	0.03	0.4	0.1, 1.5	0.17		
The Netherlands	7	1.1	0.8, 1.3	64.5%	0.02	0.3	0.1, 0.5	<0.01		
Africa/S. America	3	1.4	0.5, 2.8	55.1%	0.11	0.3	0.1, 1.0	0.05		
Final year of data collection									94.0%	0.749,
<2010	7	3.2	1.4, 5.5	85.5%	<0.01	ref				-0.13%
2010-2012	9	2.2	1.3, 3.4	93.8%	<0.01	0.8	0.3, 2.2	0.61		
2013+	9	1.7	1.0, 2.6	89.3%	<0.01	0.5	0.2, 1.4	0.19		
Anal intercourse reporting									94.4%	0.7654,
AI only	11	2.9	1.3, 5.1	94.0%	<0.01	ref				+2.1%
Mixed/Unspecified	14	1.7	1.1, 2.4	90.1%	<0.01	0.7	0.3, 1.7	0.33		
Association of Urogenital Gonorrhoea with Anorectal Gonorrhoea										
	Sub-group analysis					Meta-regression[b]				
Variable	Number of studies, n	Summary PR estimate	95%CI	I^2	p	Summary ratio of PR	95%CI	p	Residual I^2	τ^2, Δ
All	12	80.6	47.3, 137.5	80.6%	<0.01					1.638, ref
Region									91.0%	1.264,
N. America	7	71.6	34.3, 149.8	74.5%	<0.01	ref				-22.8%
U.K.	1	114.0	15.5, 837.4	.	.	1.8	0.0, 187.1	0.76		
The Netherlands	3	142.2	85.0, 237.9	27.7%	0.251	3.0	0.2, 39.5	0.33		
Africa/S. America	1	13.6	5.0, 36.8	.	.	0.2	0.0, 8.5	0.33		
Final year of data collection									97.5%	1.424,
<2010	4	70.5	15.7, 317.4	81.4%	<0.01	ref				-13.1%
2010-2012	4	53.9	20.4, 142.4	88.4%	<0.01	0.6	0.0, 6.6	0.66		
2013+	4	204.1	66.1, 629.8	65.4%	0.03	6.0	0.3, 105.6	0.18		
Anal intercourse reporting										1.819,
AI only	6	65.5	32.0, 134.0	74.9%	<0.01	ref				+11.2%
Mixed/Unspecified	6	117.1	41.3, 331.7	79.4%	<0.01	1.9	0.2, 18.8	0.5	94.6%	

a=Stephens et al excluded from meta-regression as 0% prevalence for CT and NG. b=Danby et al naturally excluded as PR involved division of 0. CI=confidence interval. PR=Prevalence ratio.

Table 4: Assessment of study quality and risk of bias

Author, publication year	Selection	Measurement	Confounding and Bias	Statistical methods	Conflict of interests and ethics
Barry, 2010 [18]	++	+	+	+	++
Bazan, 2015 [19]	++	++	+	+	+
Cosentino, 2012 [20]	+++	+/++	+	+	+/++
Danby, 2016 [21]	++	+/++	++	+	++
Dukers-Muijrers, 2015 [30]	++	+	+	+	+
Garner, 2015 [35]	++	++	++	++	+
Hunte, 2010 [22]	++	+	+	++	++
Javanbakht, 2012 [23]	++	+	+	+	+
Koedijk, 2012 [14]	++	+++	++	+	+++
Llata, 2018 [24]	++	+++	+	+	+
Mayer, 2012 [25]	+++	++	+	+	+
Nelson, 2007 [39]	++	+++	+	+	+
Peters, 2011 [31]	++	+	+	+	++
Peters, 2014 [40]	++	+	+	+	+
Rodriguez-Hart, 2012 [26]	+++	+	++	+	+
Sethupathi, 2010 [36]	++	+	++	+	+++
Shaw, 2013 [37]	+++	++	+++	+	+
Stephens, 2014 [27]	++	+	+	+++	+
Tao, 2018 [28]	+	+++	+	+	+
Travassos, 2016 [38]	+++	++	++	+	+
Trebach, 2015 [29]	++	+	++	+	+
van der Helm, 2009 [34]	++	++	+	+	+
van Liere, 2013 [32]	++	+	+	+	+
van Liere, 2017 [33]	++	+	+	+	+
van Rooijen, 2015 [15]	++	+	+	+	+

Abbreviations: +, low risk of bias, ++, moderate risk of bias, +++, high risk of bias

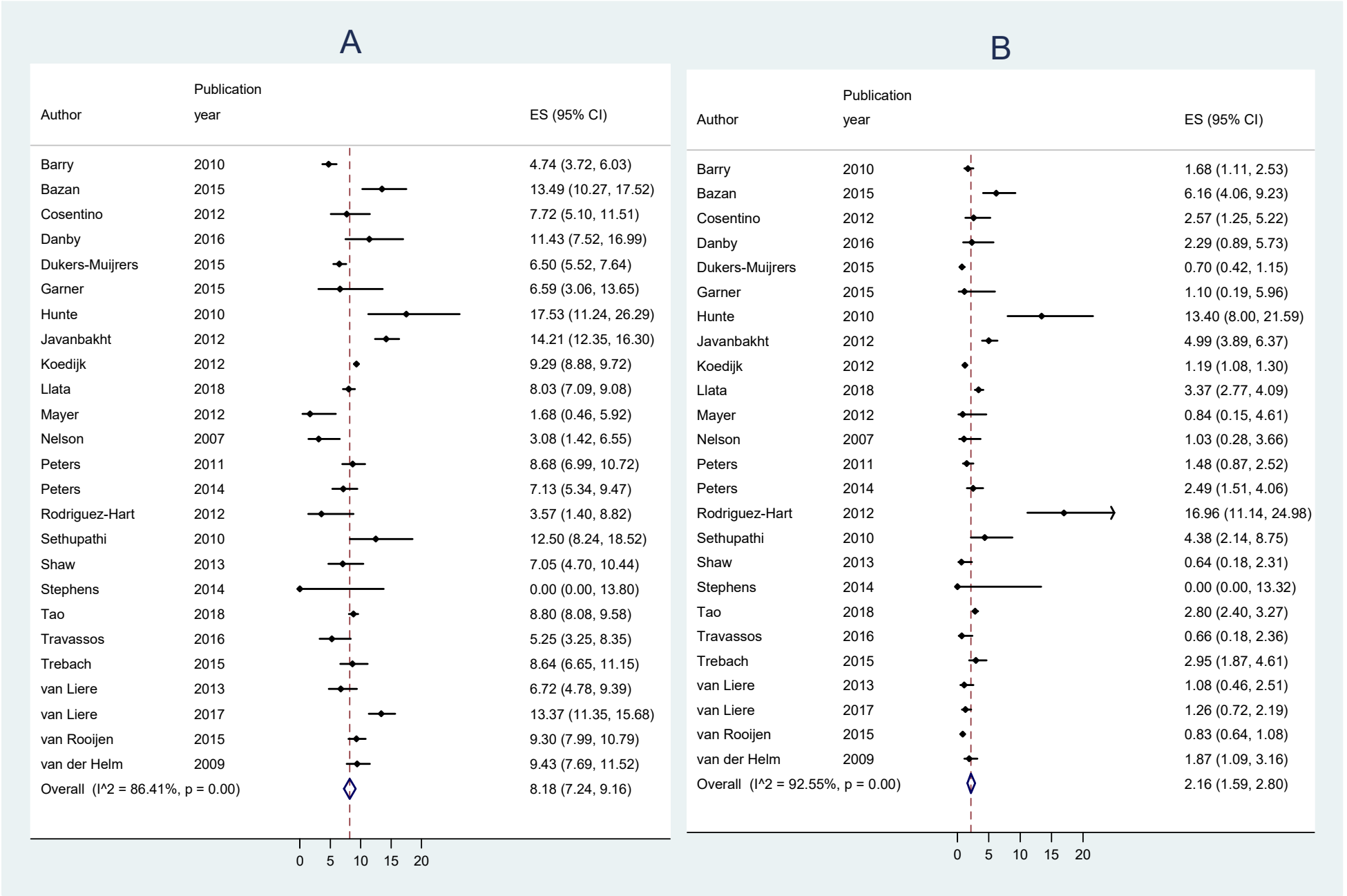

Figure 2: Anorectal positivity among women. A= *Chlamydia trachomatis*. B=*Neisseria gonorrhoea*. ES=Rectal positivity among those tested (%)

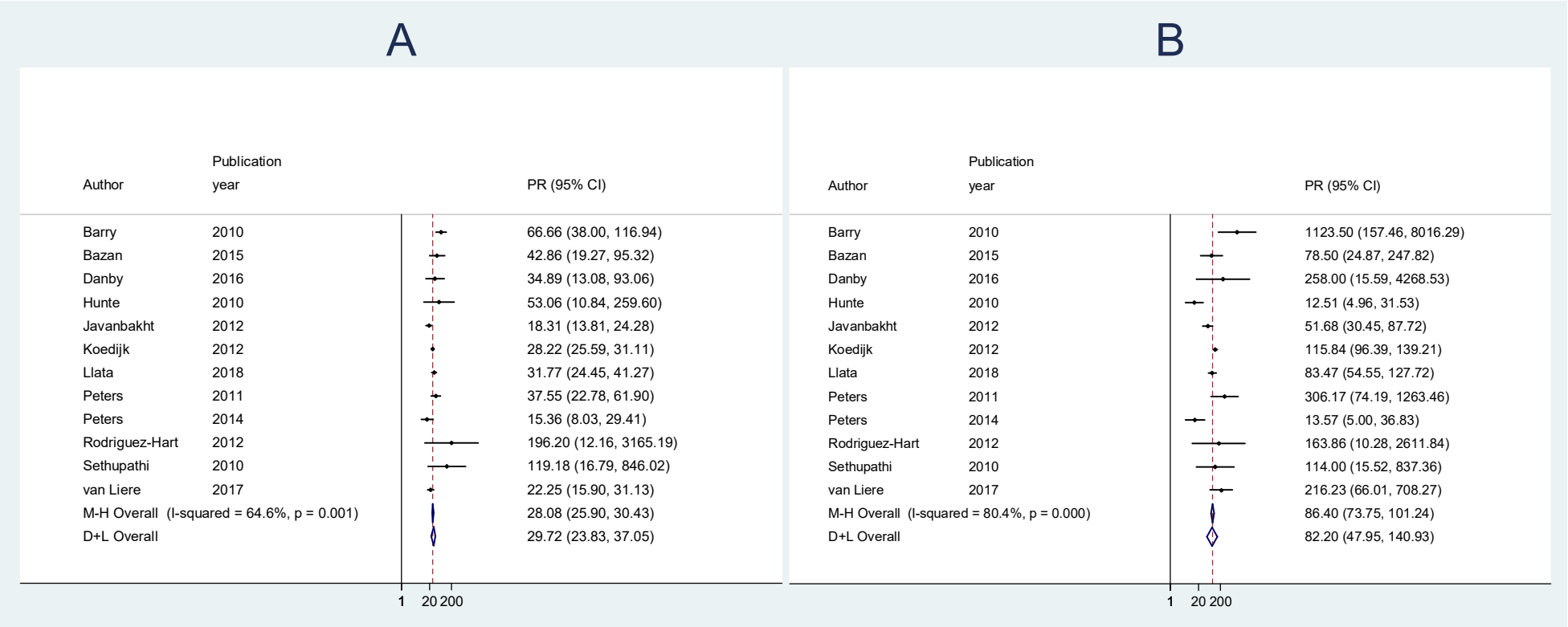

Figure 3: Association of urogenital positivity with anorectal positivity. A= *Chlamydia trachomatis*. B=*Neisseria gonorrhoea*. PR=prevalence ratio; prevalence of testing anorectal positive among those urogenital positive, compared to the prevalence among those urogenital negative