Proton pump inhibitors and risk of Clostridium difficile infection: a multi-country study using sequence symmetry analysis.

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Running title: PPIs and *C. difficile* multinational results

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Abstract

Objective: To determine the association between incident proton pump inhibitor (PPI) use and *Clostridium difficile* infections across multiple countries

Method: National data covering the total population in Australia and Korea, the Canadian population over 65 years and a 3 million person random sample data set from Taiwan were assessed, as were data from a worker insurance population and a hospital inpatient/ outpatient population in Japan. Sequence symmetry analysis was used to assess the association with oral vancomycin dispensing as the outcome of interest.

Results: 54,957 patients were included. Positive associations were observed in Australia; adjusted sequence ratio (ASR) 2.48 (95% CI 1.90, 3.12), Korea ASR 2.15 (95%CI 2.11, 2.19), Canada ASR 1.45 (95% CI 1.16, 1.79), Japan hospital dataset ASR 3.21 (95%CI 2.12, 4.55) and Japan worker insurance dataset ASR 5.40 (95% CI 2.73, 8.75). The pooled result was ASR 2.40 (95%CI 1.88, 3.05) and 3.16 (95%CI 1.95, 5.10) when limited to Japan, Korean and Taiwan. Results did not vary by individual PPI. The temporal analysis showed effects within the first two weeks of PPI initiation.

Conclusion: Our study confirms the association between PPI initiation and *C. difficile* infections across countries in the Asia-Pacific region.

1. Introduction

There is a significant body of research showing an association between use of proton pump inhibitors and *Clostridium difficile* infections,[1, 2, 3, 4, 5] however, limited research has been undertaken in Asia-Pacific populations. A 2012 meta-analysis assessing the association between *C. difficile* infections and proton pump inhibitor use included 51 studies and reported a pooled odds ratio of 1.65 (95% CI 1.47, 1.85), however, only 3 observations were from the Asia region.[1] When analysis was limited to the Asian observations, and the pooled result was higher at OR 3.26, (95%CI 1.91 -5.58.).[1]

The prevalence of different strains of *C. difficile* varies substantially by region. Ribotyping has shown that the most frequent ribotypes in Australia are 014 and 002,[6] in China it is 017, 046, and 012; in Japan 018, 014, 002, 001, Korea 018, 017, Taiwan 017, and Hong Kong it is 002.[7] This compares with Europe where the ribotypes are 027, 014,001/072, and 078.[8] This may have implications for the risk of *C. difficile* infections from proton pump inhibitors. In some *C. difficile* ribotypes the level of expression of toxin genes and their regulators was greater at higher pH levels and elevated even further in the presence of PPIs.[9] For ribotype 001, PPI exposure was associated with a 120-fold higher expression of tcdA at higher pH levels, while for ribotypes 027 and 028, more than 50 fold increases were observed at higher pH levels. For tcdB and cdtB expression the relationship was less clear.[9] Whether the differing prevalence of different strains of *C. difficile* and the potential for PPIs to affect toxin gene expression differently according to strain means that differences in risk of PPI induced *C. difficile* can be observed across countries is unclear.

There have been only limited studies of the association between proton pump inhibitors and risk of *C. difficile* infection in the Asia Pacific region, with the majority of studies undertaken to date being from North America or Europe (48 of 51).[1] No multi-country studies have

been undertaken to date. Further many studies have been single centre sites (40 of 51), have been undertaken in the hospital setting (37 of 51, 6 involved both hospital and community and 8 involved community only) and have been case-control studies (37 of 51).[1] The majority of studies assessed any proton pump inhibitor exposure with limited analysis of the effect of incident use.

This research aimed to establish the risk of *C. difficile* infections associated with initiation of PPIs in countries in the Asia-Pacific region. We undertook a multi-country study, which included 4 countries (6 databases) from the Asia Pacific region. We also included data from Canada which provided a contemporary reference point, as much of the previous research had been undertaken in North America or Europe.[1] We used sequence symmetry analysis,[10] which has the advantage of inherently controlling for measured and unmeasured confounders that are stable over time. All prior research has used cohort or case-control study designs which may have residual confounding due to unmeasured confounders. We had national data for four of the countries involved, thus providing the largest study sample to date to assess the association between proton pump inhibitor use and *C. difficile* infection.

2. Methods

2.1 Data sources

This multi country study was initiated by Health Canada, with participation from the member groups of the Asian PharmacoEpidemiology Network (AsPEN).[11] AsPEN provides a mechanism to support the conduct of cross-country pharmacoepidemiological research to facilitate prompt detection and communication of emerging safety issues between countries. The data sources used are listed in table 1, with full details of the databases published elsewhere.[12] All datasets held patient level dispensing data which included: a patient identifier, patient demographics, date of medicine supply, medicine dispensed, quantity and

strength. Medicines were mapped from individual country specific codes to the WHO Anatomical Therapeutic Chemical (ATC) classification codes.[13]

2.2 Study design

We used sequence symmetry analysis,[10] that compares the sequence by which people initiate the same two medicines within a defined period of time.[10] The method has been validated as an adverse event signal detection tool against adverse events identified in randomised controlled trials and against negative controls from product information of unrelated products, as well as in a simulation study.[14] [15] It's performance has been shown to be similar to disproportionality methods.[14]

We assessed the association between proton pump inhibitor use and *C. difficile* using oral vancomycin as the marker medicine for *C. difficile*. Oral vancomycin use would be expected to rise after PPI initiation if there was an association with *C. difficile* infection as oral vancomycin is only indicated for *C. difficile* infections and staphylococcal enterocolitis.[16] Oral vancomycin has been used as a marker of *C. difficile* infections in previous research.[17] Vancomycin's poor absorption from the gastrointestinal tract makes it unsuitable for other types of infection, so risk of misclassification bias was low.

A distributive network model [18] was employed. The co-ordinating centre for this study, the University of South Australia, developed the statistical analysis code as a stand-alone SAS program for execution by each participant in their home institution. The SAS program used global macro variables. Participants executed the SAS code and a standardised file of summary results was returned to the co-ordinating centre for collation. These standardised files included graphics of the number of people dispensed the study medicines each month (prevalent population), the number of people starting study medicines each month (incident

population), and the results of the sequence symmetry analyses (SSA) results including the graphics showing temporal sequences.

The primary outcome was the first dispensing of oral vancomycin (ATC code C03CA01) as an indicator medicine for *C.difficile* infection. Oral vancomycin was only used in Taiwan at the beginning of the study period, with injectable formulations of vancomycin used orally at both the beginning of the study and in subsequent years. Hong Kong used injectable forms of vancomycin orally throughout the study period, thus, Hong Kong data were not included in the results. Proton pump inhibitors assessed included omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole. Proton pump inhibitors dispensed as part of a helicobacter eradication therapy co-pack were excluded. We included a sensitivity analysis, where metronidazole was used as the indicator medicine. Metronidazole is used first line in many settings for treatment of *C.difficile* infections, however, it is also used for conditions other than *C.difficile* infection, so is not as specific an indicator.

In the SSA method, the date of incident dispensing of proton pump inhibitors and oral vancomycin was determined for each individual patient. All incident dispensings that occurred within one year of each other for the same person were included in the analysis. We excluded patients who initiated any of the study medicines in the first year of data coverage in any dataset to ensure we limited the analyses to incident users. The SSA method[10] is sensitive to prescribing trends over time and adjustment was made for temporal trends using the null-effect sequence ratio as described by Tsiropolous.[19] Bootstrapped 95% confidence intervals (CI) were obtained using 500 replicates. The SSA analyses were restricted to sequences of incident dispensings within 12 months of each other to limit the effect of age and other potential time-varying covariates on the probability of exposure and outcome. Moreover, a 12-month period has better specificity and positive predictive value compared

with shorter periods.[14] Pooled estimates were obtained with a random effects model, using the generic inverse variance method.[20]

3. Results

In total, 54,957 patients who received oral vancomycin and proton pump inhibitors were included in the study. Positive associations were see in all countries apart from Taiwan, where due to low use of oral vancomycin, insufficient numbers were available to determine effect (Table 2). The pooled estimate showed a 2.4 fold (95% CI 1.88-3.05) increased risk (figure 1). When limited to the Asian countries (Japan, Korea and Taiwan) the pooled estimate was 3.16 (95%CI 1.95, 5.10). The temporal relationship was apparent in all countries, with effects being observed within the first two weeks of treatment (See figure 2 shows results for Australia (national) and Japan Medical Data Centre (JMDC), data for other countries not shown). Results for the individual PPIs are presented in table 3 for Australia (national) and Korea, the only countries with sufficient numbers to undertake individual agent analysis, and show similar risk for each agent. Our sensitivity analysis with metronidazole as the outcome indicator showed similar results (Figure 3).

4. Discussion

This multi-country study found an elevated risk of *C. difficile* infections after initiation of proton pump inhibitors. Our pooled estimate of 2.4 (95% CI 1.88-3.05) is slightly higher but consistent with prior research, where meta-analysis evidence reported an increased odds of 1.65 (95% CI 1.47-1.85). [1] When our pooled analysis was limited to Korea, Japan and Taiwan, we found a three-fold increased risk which is also consistent with previous meta-analysis results from Asian countries OR 3.26, (95%CI 1.91 -5.58.).[1] The risk was present and was of similar magnitude for each individual proton pump inhibitor.

We observed the highest risk in the Japanese data sets, with risk estimates increased three and five fold respectively in the two Japanese data sets, for which confidence intervals overlapped. These results are higher risk estimates than have been reported previously in Japan. The largest previous Japanese study, which was a multi-institution study involving 1025 cases and 878 controls that assessed hospital acquired *C. difficile* infections, did not find proton pump inhibitors were a risk factor for *C. difficile* infections, however, assessment of PPI use was limited to that prescribed during the hospital stay.[21] By comparison our results include outpatient proton pump inhibitor use up to one year prior to *C. difficile* infections. A small Japanese study, limited to 26 cases and 52 controls, assessed risk factors for community acquired *C. difficile* infections, finding no association with proton pump inhibitor use.[22] Another small study of risk of recurrent infection, involving 14 patients with recurrent infection and 62 without also did not find a significant effect of proton pump inhibitors on recurrence.[23] These latter two studies may have had insufficient sample size to demonstrate effect. Finally, a Japanese study amongst a cohort of people who had been prescribed injectable antibiotics did find proton pump inhibitors were associated with increased risk of subsequent *C. difficile* infections.[24] Previous research in Korea has assessed the relationship between recurrent *C. difficile* infections and PPIs, demonstrating an association.[25, 26] No prior studies from Korea examined the effect on incident infection. One small study involving 84 persons from Taiwan found proton pump inhibitors were an independent risk factor for development of *C. difficile* infections (OR 3.2, p=0.014)[27], while a second study found of those with *C. difficile* colonization, use of proton pump inhibitors was more common in those who subsequently developed infection.[28]

While it has been postulated that the acid suppressions mechanism of PPIs may play a role by allowing *C. difficile* spores to survive the gastric environment, this may not be the mechanism as *C. difficile* spores can survive acid gastric contents.[29] Acid levels, though, may affect

toxin expression. As we previously noted, one in-vitro study found that expression of toxin genes and their regulators were different in the presence of PPIs in some *C. difficile* ribotypes.[9] Another in vitro study also examining the effect of PPIs on expression of colonocyte genes found PPIs decreased their expression.[30] The consequence of decreased expression includes loss of maintenance of cell junction, and reduced production of proteins known to protect the intestinal epithelium, one of which is known to protect against *C. difficile* induced intestinal damage. An effect on bile acid metabolism and transport was also observed.[30] All of these effects have the potential to increase susceptibility to or worsen *C. difficile* infection.

Given one of the possible mechanisms by which PPIs may exert their influence on *C. difficile* infection is toxin expression and that toxin expression differs by ribotype,[9] our results, highlight the importance of obtaining risk estimates, where possible, from local data as data from other countries may not be directly applicable. Our results show slightly higher risk estimates in the Asian countries, however, we did not have access to clinical records and so could not ascertain any information on whether this could be related to the strain of *C. difficile* being treated. Another possible reason for higher risk estimates in the Asian countries, and Japan in particular, is because of inter-ethnic differences in the prevalence of CYP2C19 polymorphisms. There is a higher prevalence of the poor metaboliser phenotype of CYP2C19 in the Japanese (18-22.5%) and the Koreans (12.6%) compared to Caucasian populations (2.5-3.5%). The poor metaboliser phenotype results in raised and sustained plasma levels of PPIs, thus potentially increasing infection risk.[31]

Strengths of our research include the access to national data sets for Australia, Canada, Korea, and Taiwan. Our research used a common method and a common analytic approach which ensures results are comparative with regards to method and data variables used. This is

the first study of this association using a within person design, which has the advantage of inherently adjusting for measured and unmeasured confounders that are stable over time.[10] A prior study utilising SSA demonstrated that patient characteristics are often similar in the causal and non-causal groups and therefore are unlikely to affect the sequence order providing further support that the method effectively adjusts for stable confounders.[10] Given the association between *C. difficile* infections and PPIs has been previously described,[1, 2, 3, 4, 5] there is potential for prescribers who are aware of the association to minimise prescribing PPIs in people with *C. difficile* infections. However, our temporal analysis shows an increase in oral vancomycin initiation in the non-causal groups, in the weeks immediately preceding PPI initiation, suggesting this potential confounding effect was not present to any great extent in our study (Figure 2). In addition the sequence symmetry method only includes persons who got both medicines, thus, eliminating the bias that could occur if those with no PPI prescription after *C. difficile* treatment were included. Additionally, our sensitivity analysis, using metronidazole as the outcome indicator, provides further evidence supporting the result.

Limitations of our study include lack of diagnostic information at the outpatient level in the majority of countries, and hence the reliance on oral vancomycin as the proxy indicator for *C. difficile* infections. Oral vancomycin, however, has been used as a marker of *C. difficile* infection in previous research[17] and is the sole reimbursed indication for oral vancomycin in Australia[32], Canada and Korea[33]. In Japan it is indicated for 1) "infectious enteritis (including pseudomembranous colitis)" caused by vancomycin-sensitive MRSA colitis and Clostridium difficile, and 2) "sterilization of gastrointestinal tract at bone-marrow transplantation". In the other countries it may be used for either *C. difficile* or staphylococcal enterocolitis. A limitation of the method was the inability to include data from Hong Kong and the inclusion of only limited data from Taiwan. In both countries the injectable

formulation of vancomycin is used orally as a treatment for *C. difficile* infections and these dispensings could not be distinguished from vancomycin given in the injectable form for other reasons. It is possible that injectable vancomycin was also given orally in countries outside Taiwan and Hong Kong, however, we were unable to ascertain the extent of this practice. The implications of this means we may have missed some *C. difficile* infections, however, if this bias does exist, it is likely to have been systematic and not influenced one group more than the other. A further limitation is that oral vancomycin is second line therapy in many countries, with metronidazole being first line therapy for *C. difficile* infections; including in Australia, Japan and Canada. We did not use metronidazole as our primary indicator of *C. difficile* infections because metronidazole is also indicated for other conditions and thus may have biased the results. This limitation means that we did not include cases of *C. difficile* treated by metronidazole only in our primary analysis and so will not have captured all events. In addition, there are differences in the extent of capture of *C. difficile* infections across data sets. The Korean data set, which is a whole of country data set that includes hospital in-patient data, had the greatest number of *C. difficile* events. The only other data set to include hospital infections was the Japan Hamamatsu data set. All other data sets represent community treated infections. While the community treated infections may have been less serious infections, our analysis shows the risk estimates were similar across all data sets, suggesting an effect due to proton pump inhibitor use and not to differences in data capture or health systems. The variation in the data sets available limited our ability to assess differences in risk between countries. The risk of protopathic bias, where PPIs may be used in the prodromal phase of the disease, is unlikely as indigestion type symptoms are not usually associated with *C. difficile* infections. Further the temporal analysis shows that while the counts of persons receiving oral vancomycin is highest in the first few weeks post-initiation, the effect remains elevated over time compared to those receiving oral vancomycin

prior to PPI initiation. Finally, we cannot exclude the possibility that some of the differences observed across countries may be due to differences in health care practice.

5. Conclusion

Our study confirms the association between PPI initiation and *C. difficile* infections across countries in the Asia-Pacific region. Further research to confirm reasons underpinning any risk difference in the Asian region is required. C. difficile infections are of growing concern across the world, particularly as resistance to treatments increases.[8, 34] Use of proton pump inhibitors is a modifiable risk factor for clostridium difficile infections and clinicians need to take this into consideration when treating at-risk patients.

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Authorship statement

EER, NP developed the study protocol, were responsible for analysis and interpretation of the data, and manuscript preparation. TN was responsible for analysis and interpretation of the data, and manuscript preparation. JG, TTW, conceived the project, developed the study protocol, were responsible for analysis and interpretation of the Canadian data and manuscript preparation. KKCM, EWC, ICKW were responsible for analysis and interpretation of the Hong Kong data, and manuscript preparation. NKC, JL, JYS, BJP, were responsible for analysis and interpretation of the Korea data, and manuscript preparation. KK, MK, TK, were responsible for analysis and interpretation of the Japanese Hamamatsu data, and manuscript preparation. KK, NO, TS were responsible for analysis and interpretation of the Japan Medical Data Center data, and manuscript preparation. ECCL, YHKY were responsible for analysis and interpretation of the Taiwan data, and manuscript preparation. All authors approved the final version of the manuscript.

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Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Table 1: Data sources for the study

Data source	Data type	Population covered	Years of coverage	Comments
DUSC secretariat, Australian Government Department of Human Services	National pharmacy claims data	23 million persons	2003-2013	Excludes inpatient use in public hospitals
Australian Government Department of Veterans' Affairs	Pharmacy claims data for Australian veterans and their dependents	300,000	2001-2012	Excludes inpatient use in public hospitals. Predominantly older population (median age 80 years)
Canadian Institute for Health Information (CIHI)'s National Prescription Drug Utilization Information System (NPDUIS) Database	National Pharmacy Claims from public drug programs	3.5 million	2001-2012	Data from 7 Canadian provinces included in this study. Includes population aged 65 years and over who are eligible for coverage by provincial public drug programs. Includes only drugs dispensed in a community-based setting.
Hong Kong Clinical Data Analysis and Reporting System	National electronic healthcare record of public hospitals and their ambulatory clinics.	7 million	2008-2012	Oral vancomycin not reimbursed. Injectable formulation given orally.
Japan Medical Data Center database	Private health insurance data set for workers	1,000,000	2008-2013	
Japan Hamamatsu Medical University Database	Hospital data set	200,000	1996-2014	Includes all inpatient and outpatient dispensing
Korea Health Insurance Review and	National Health Insurance data	50 million	2009-2013	Includes inpatient use

Assessment Service database (HIRA DB)	set			
Taiwan National Health Insurance Research Database	National Health insurance data set	23 million	2001-2012	3 million person random sample data set utilised. Oral vancomycin not used after 2005. Injectable formulation given orally across whole time frame.

Table 2: Sequence symmetry results for association between incident proton pump inhibitor (class) supply and incident oral vancomycin supply.

	Study Population n	Oral vancomycin dispensed after PPIN (Causal)	Oral vancomycin dispensed before PPIN (Non-causal)	Crude Sequence Ratio (causal/non-causal)	Null Effect Sequence ratio	Adjusted* sequence ratio (Crude SR/Null Effect SR) (95% confidence intervals)
Korea	53820	37113	16707	2.22	1.03	2.15 (2.11-2.19)
Australia	351	257	94	2.734	1.10	2.48 (1.90-3.12)
Australia DVA	77	49	28	1.75	0.99	1.76 (0.97-2.71)
Japan Hamamatsu	171	128	43	2.98	0.93	3.21 (2.12-4.55)
Japan Medical Data Center	139	116	23	5.04	0.93	5.40 (2.73-8.75)
Canada	388	270	118	2.29	1.58	1.45 (1.16-1.79)
Taiwan[#]	11	8	3	2.67	0.99	2.70 (-1.60-8.91)

Low numbers for Taiwan as a result of preferential use of injectable vancomycin

* Adjusted for changing temporal trends in medicine use.

Table 3: Sequence symmetry results for association between incident proton pump inhibitor (individual agent) supply and incident oral vancomycin supply.

	Study Population	Number of people with oral vancomycin dispensed after PPI	Number of people with oral vancomycin dispensed before PPI	Adjusted sequence ratio (95%confidence intervals)
Omeprazole				
Australia	113	82	31	2.41 (1.45, 3.58)
Korea	14878	9512	5366	1.67 (1.61, 1.72)
Pantoprazole				
Australia	299	209	90	2.08 (1.57, 2.65)
Korea	32070	20219	11851	1.71 (1.67, 1.75)
Lansoprazole				
Australia	18	16	2	7.66 (1.76, 33.31*)
Korea	25917	15731	10186	1.53 (1.50,1.57)
Rabeprazole				
Australia	104	70	34	1.84 (1.10, 2.69)
Korea	23021	15474	7547	2.07 (2.02, 2.13)
Esomeprazole				
Australia	284	192	92	1.90 (1.44, 2.41)
Korea	20261	12000	8261	1.67 (1.62, 1.71)

* Due to small sample size, CI calculated using approximate interval estimation.[35]

Figure 1: Pooled estimate for association between incident proton pump inhibitor supply and incident oral vancomycin supply.

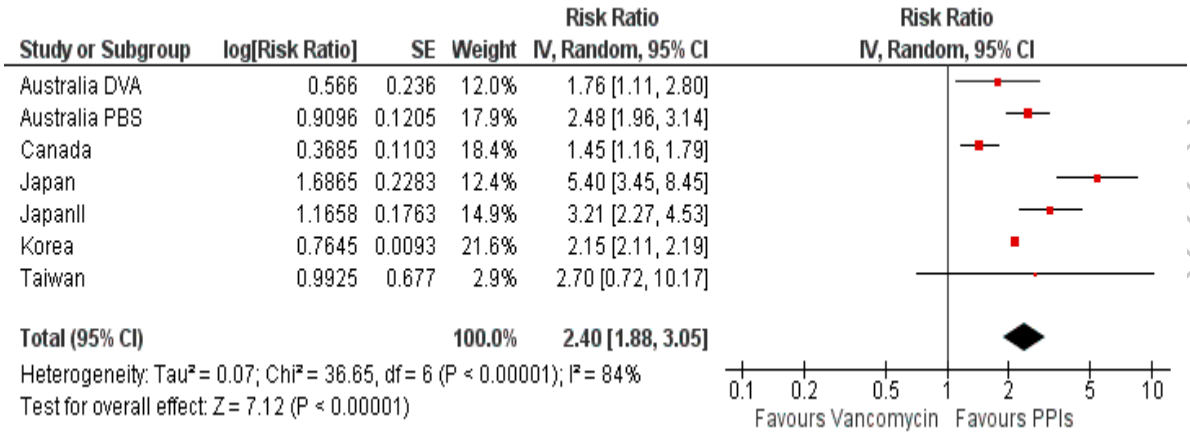
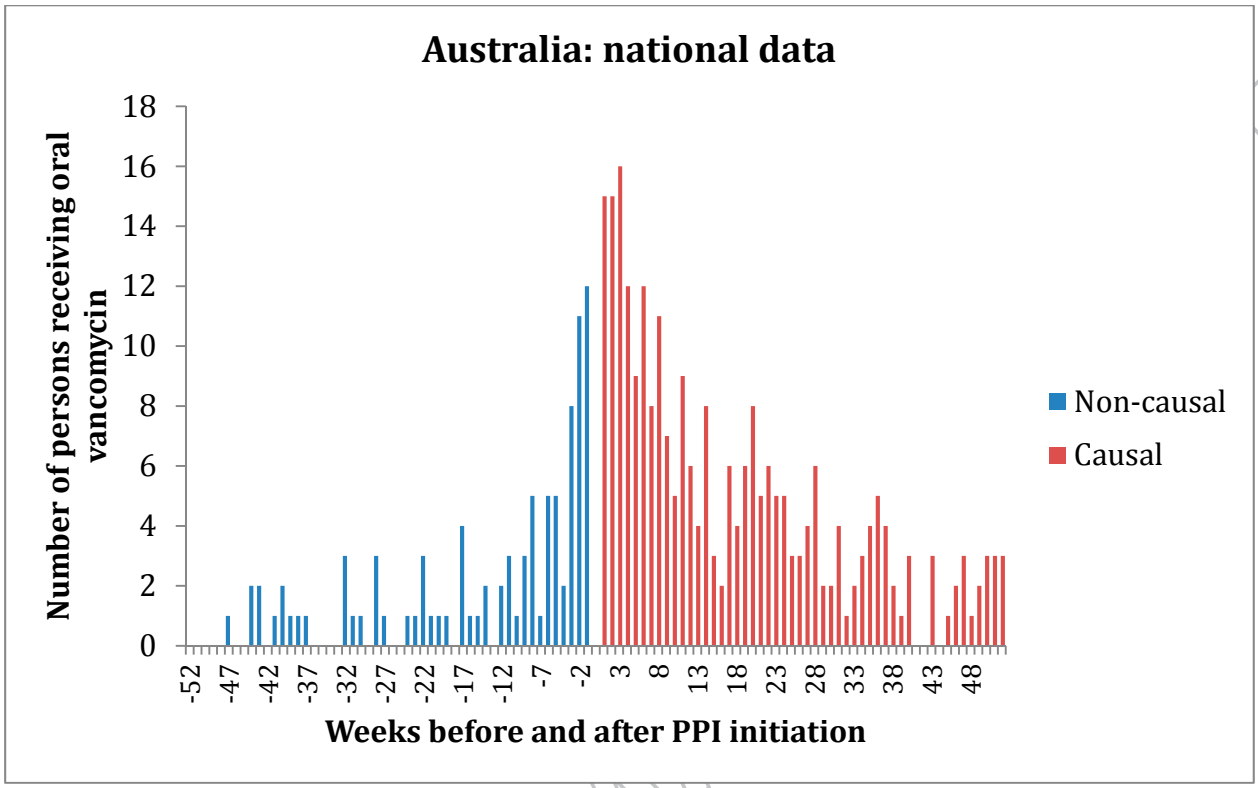


Figure 2: Temporal relationship of incident PPI supply and incident vancomycin use



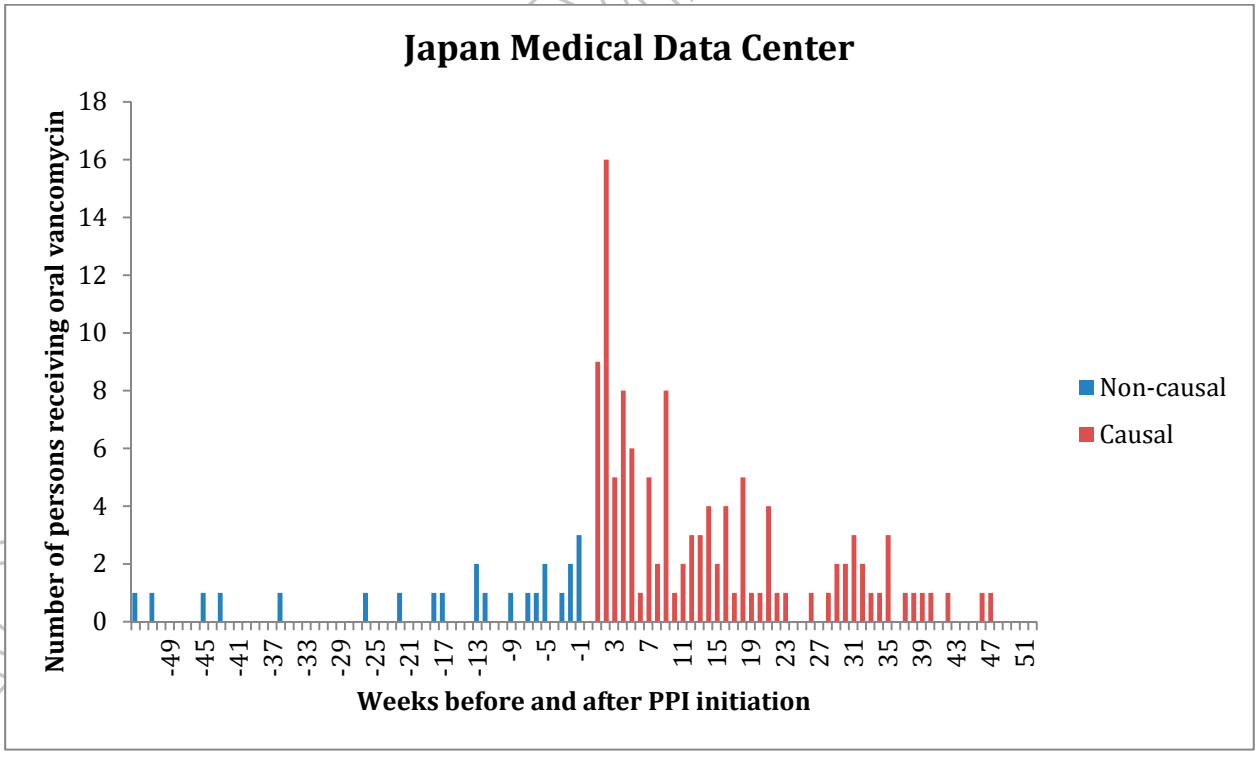


Figure 3: Pooled estimate for association between incident proton pump inhibitor supply and incident metronidazole supply.