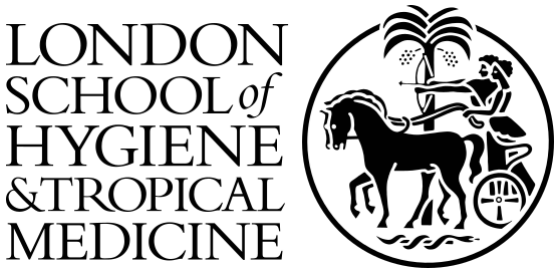

Full title: Managing cardiovascular risk of macrolides: systematic review and meta-analysis

Running title: Managing cardiovascular risk of macrolides

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Abstract (150-250 words, 250 words)

Introduction It was postulated that antibiotics including macrolides could be used for secondary prevention of coronary heart disease but recent studies showed that macrolides increase the cardiovascular risk. We aimed to review the evidence of cardiovascular risk associated with macrolides regarding duration of effect and risk factors; and explore the potential effect of statins for the prevention of cardiovascular events due to macrolide use.

Methods Several electronic databases(PubMed, EMBASE, Cochrane library) were searched to identify eligible studies. Observational studies and randomized controlled trials(RCTs) that investigated the association between macrolides and cardiovascular events in adults aged≥18 years were included. Meta-analysis was conducted to investigate the short and long-term risks of cardiovascular mortality, myocardial infarction, arrhythmia and stroke. Methodological quality was assessed by the Newcastle-Ottawa scale and the Cochrane Collaboration's tool. The body of evidence was evaluated by the Grading of Recommendations Assessment, Development, and Evaluation guidelines.

Results Observational studies were found to have short-term risk of cardiovascular outcomes including cardiovascular mortality, myocardial infarction and arrhythmia associated with macrolides but no risk was found in RCTs. However no association for long-term risk (>3 years) was observed in observational studies or RCTs.

Limitations The included studies reported different units of denominators for absolute risk and used different outcome definitions, which might increase the heterogeneity.

Conclusions More studies are required to investigate the short-term cardiovascular outcomes associated with different types of macrolides. Future studies are warranted to evaluate the effect of statins for preventing excess acute cardiovascular events associated with clarithromycin or other macrolides.

Keypoints

- The short-term risk of cardiovascular outcomes associated with macrolides was found in observational studies but not in randomised controlled trials.
- No long-term cardiovascular risk (>3 years) associated with macrolides was observed.

- There was limited evidence for the use of statins to prevent excess acute cardiovascular events associated with clarithromycin or other macrolides.

1 Introduction

In the past decades, it was postulated that antibiotics could be used for the treatment of *Chlamydia pneumoniae* in order to prevent further cardiovascular events, primarily because *C. pneumoniae* was found to be associated with atherosclerosis and coronary heart disease [1, 2]. Several clinical trials were conducted to investigate this association using the macrolide antibiotics as exposure but most of them used a small sample size [3-9]. Conflicting results were found and there was only limited evidence for a beneficial effect. A meta-analysis including randomized controlled trials (RCTs) found no evidence for antibiotics use to prevent further myocardial infarction [10]. Surprisingly, one of the macrolides, clarithromycin, was shown to have a higher risk of cardiovascular events versus placebo in a RCT (Effect of Clarithromycin on Mortality and Morbidity in Patients with Ischemic Heart Disease trial [CLARICOR trial]) [11]. Recent observational studies also found an elevated risk of cardiovascular events associated with macrolides [12-15] particularly clarithromycin, but the duration of the effects remained unclear [12, 15]. It was proposed that short-term exposure to clarithromycin could activate inflammatory macrophages, which could lead to an accumulation of lipids and vulnerable plaques in long-term, leading to an increased long-term risk of cardiovascular mortality [16, 17]. In addition, the clinical implications of the risk factors for cardiovascular events associated with macrolides have not yet been reported. Current literature suggests that statins may have a potentially protective effect on cardiovascular mortality associated with clarithromycin use [17, 18]. The postulated protective effect of statins was its inhibition of HMG-CoA reductase for reducing cholesterol, which results in reducing plague formation and lowering the risk of plaque rupture [18]. Due to the anti-inflammatory effects of statins, it was also suggested the reduction of C-reactive protein may lower the cardiovascular risk [18] but one study showed no statistically significant cardiovascular risk among clarithromycin users receiving statins [18]. However, the evidence has not yet been systematically evaluated.

This review aims to evaluate the evidence available from those studies that investigated the cardiovascular effects amongst those patients receiving macrolides in terms of duration effect and study designs. We have also summarized the current evidence of risk factors for

cardiovascular risk associated with the macrolide antibiotics and the suggested protective effect of statins in the prevention of cardiovascular events associated with clarithromycin use in the narrative review.**2 Methods**

2.1 Search Strategy and Data Sources

This systematic review was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement [19]. The protocol of this systematic review is available online from the website of the Centre for Safe Medication Practice and Research (http://www.pharma.hku.hk/sweb/CSMPR/). We searched electronic databases as the main information source on 6 May 2016 and updated the search on 3 February 2017. PubMed, EMBASE Classic + EMBASE 1980- via Ovid and the Cochrane library were searched using keywords and/or medical subject headings including 'sudden death', 'cardiovascular diseases', 'cardiac death', 'cardiac mortality', 'heart diseases', 'heart infarct', 'myocardial infarct', 'coronary disease', 'coronary artery disease', 'heart attack', 'out-of-hospital cardiac arrest', 'myocardial ischemia', 'angina', 'angina pectoris', 'arrhythmia', 'ventricular fibrillation', 'brain ischemia', 'stroke', 'clarithromycin', 'azithromycin', 'erythromycin', 'roxithromycin'. There is no specific publication time limit for the search in PubMed and Cochrane Library. For the EMBASE 1980 – via Ovid, the time period of the database for the search was from 1980 to 1 February 2017 in the updated search. Supplementary material 1 (Online Resource 1) shows the search strategy. Potential studies were retrieved after the screening of title and abstract (AYW). A reference list of the retrieved studies was also reviewed for further identification of potential eligible studies (AYW).

2.2 Study Selection

We included published RCTs and observational studies (including cohort, nested case-control, case-control, self-controlled case series, case-crossover or case-time-control studies) that investigated the association between macrolides and cardiovascular events in adults aged ≥18 years old. Studies that did not report any cardiovascular events as an outcome or specify the types of cardiovascular events were excluded. Studies examining drugs other than macrolides or those using other macrolides as comparator only were also excluded. We also excluded conference abstracts, pharmacovigilance signal detection studies, animal studies or studies in languages other than English or Chinese. Two authors determined the eligibility and inclusion of studies in

systematic review and meta-analysis independently (AYW, SA) and the discrepancies were resolved by consensus through discussions.

2.3 Data Collection Process and Quality Assessment

After searching the electronic databases, Endnote X7 was used to store all the citations of identified articles. Data for outcomes were extracted independently by two authors (AYW, SA) and the discrepancies were resolved by consensus through discussions. Data extraction form was compiled to record items in PICO approach such as data source, study period, region, study design, inclusion criteria, exclusion criteria, exposure of interest, follow-up period, outcomes of interest and risk factors for cardiovascular risk associated with macrolides. There were multiple studies using the data from the same source. As they reported results in different follow-up periods in different reports [11, 20, 21], the reported measures of effect were extracted and pooled according to different follow-up periods. For one report of multiple studies which reported estimates from different cohorts of patients, we extracted the data from different cohorts [12, 15, 22, 23]. Each cohort could contribute a proportion of weights in the meta-analysis.

For clinical relevance, the risk of mortality outcomes or composite cardiovascular outcomes was selected as primary outcome. The primary outcome was defined as either cardiac mortality, cardiovascular mortality, sudden death, cardiac arrest, all-cause mortality or composite outcomes including death and/or other cardiovascular events or procedures. We performed sensitivity analysis to exclude studies which merely reported the risk of all-cause mortality or composite outcomes.

Other cardiovascular outcomes such as myocardial infarction, arrhythmia and stroke were selected as secondary outcomes. As the risk of cardiovascular outcomes varied depending on the follow-up period, we separated the analyses into short-term cardiovascular risk (≤30 days) and long-term cardiovascular risk (>30 days). Sensitivity analyses were also conducted to pool the estimate of the studies with same follow-up periods. We also performed stratified analyses in the meta-analysis to examine the risk of cardiovascular outcomes for each type of macrolides if there were at least two studies available for analysis. As some cohort studies defined the comparison group as non-macrolide exposed group, this group of patients could be either those without using any antibiotics or those used antibiotics other than macrolides. We performed additional analysis to remove these studies to test the robustness of the primary result. Factors associated with

cardiovascular risk and the risk of cardiovascular mortality among patients with concomitant use of statins and macrolides were described as the tertiary outcome and were illustrated in the narrative review.

The quality assessment was performed independently (AYW, SA) for each included study. The observational studies were assessed for methodological quality using the Newcastle-Ottawa scale. The risk of bias of the RCTs was assessed using the Cochrane Collaboration's tool. We also assessed the body of evidence according to Grading of Recommendations Assessment, Development, and Evaluation (GRADE) guidelines [24].

2.4 Meta-analysis

Measures of effect including relative risk (RR), hazard ratio (HR), odds ratio (OR) and 95% confidence interval were retrieved and pooled using the generic inverse variance method for observational studies. When the measure of effect was missing, the number of events and total number of subjects for each exposure group were used to estimate the measure of effect. For the RCTs, it was calculated by inputting data into a 2x2 contingency table. When the number of events for each intervention group was not available in the included RCTs, the measure of effect and the total number of patients for each intervention group were identified. They were entered in Review Manager 5.3 to obtain the standard errors for the pooled analysis [25]. The measures of effects were pooled according to different study designs. All summary estimates were calculated using random-effect model in Review Manager 5.3. We assessed the heterogeneity using the I^2 statistic.

3 Results

3.1 Search results and study selection

After the electronic search of three databases, we identified 5905 records of publications. There were 5371 articles for screening after removing the duplicates. We excluded 5075 records as they were animal studies, did not investigate the risk of cardiovascular outcomes associated with macrolides, or conference abstracts. We further screened and evaluated 296 records and subsequently excluded 246 records as they were not observational studies, RCTs, or using other macrolides as comparators. One study was excluded as it was a pharmacovigilance signal generation study without any hypothesis driven process [26]. Another study which was written in languages other than English or Chinese was also excluded [27]. Three additional studies were

identified manually by reviewing the bibliography of the retrieved articles. As a result, we included 41 studies for 49 publications in this systematic review (Fig.1). Among them, thirteen studies investigated the association between the cardiovascular outcomes with at least one macrolide. Eleven studies specifically reported cardiovascular outcomes for azithromycin, Ten studies for clarithromycin, five studies for roxithromycin and two studies for erythromycin. A summary table of the included studies and additional information for the studies included in the meta-analysis are presented in Supplementary material 2 and 3 (Online Resource 2 and 3).

3.2 Methodological quality

For observational cohort studies or self-controlled case series, the exposed and non-exposed cohorts were representative and could demonstrate the risk of incident outcomes in most of the studies. The majority of the studies used rigorous methodologies to adjust confounding. However, many studies did not report the follow-up rate or achieve complete follow-up. In addition, several cohort studies were designed to investigate short-term cardiovascular outcomes, so the follow-up periods were inadequate (Supplementary material 4, Online Resource 4).

For case-control, case-crossover or case-time-control studies, the majority of the studies recruited representative cases and controls. All studies used age and other cardiovascular factors for covariate adjustment in their analyses and properly ascertained the exposure status. However, some studies did not clearly define the controls that they had no history of the outcomes of interest (Supplementary material 5, Online Resource 5).

For RCTs, all studies used randomization method to assign exposure status with blinding of participants and personnel, as well as the outcome assessment in design. However, some studies did not clearly describe the procedures of sequence generation and allocation concealment. In view of these outcome measures, two trials were rated as high risk of incomplete outcome data while another two trials were rated as high risk of selective outcome reporting (Supplementary material 6, Online Resource 6). Supplementary material 7 and 8 (Online Resource 7 and 8) show the GRADE evidence profile and summary of findings respectively.

3.3 Primary outcome

Fig.2 shows the forest plots for short-term primary outcome in different study designs. A higher odds of macrolide exposure was found among cases who had cardiac mortality than that for

controls (OR 1.72; 95% CI 1.09-2.73) during the current use in five case-control or case-time-control studies [23, 28-31]. The current use of macrolides ranged from 7 to 30 days. Only one study reported the risk of outcome in 30 days for clarithromycin [23]. After removing the result for clarithromycin in this study, the risk of cardiac mortality was similarly observed (OR 1.81, 95% CI 1.12-2.93). There were ten cohort studies or self-controlled case series analysis examining the risk of primary outcome associated with macrolides [12-14, 22, 32-37]. Similar to case-control studies, an increased risk was found (RR 1.24; 95% CI 1.04-1.49) with heterogeneity of 90% during the current use (5-14 days). When we excluded those studies that reported all-cause mortality or composite outcomes, the risk found was similar [RR 1.61; 95% CI 1.18-2.20] with a decreased heterogeneity of 73%. For additional analyses, studies defining the comparison group as non-macrolide exposed group only reported the risk of short-term primary outcome. Similar to the primary analysis, an increased risk was still found [RR 1.29; 95% CI 1.07-1.57)]. For RCTs [20, 38-41], all studies reported an all-cause mortality or composite outcomes such as severe recurrent angina, acute myocardial infarction, or cardiac procedures for approximately 30 days follow-up period. The random-effects summary estimate was 0.99 (95% CI 0.74-1.34). We estimated the risk differences ranged from 30.9 to 92.7 per 1 million patients for observational studies using GRADE guideline. The quality of evidence was low and very low for case-control studies and cohort studies respectively. However, the quality of evidence was moderate in RCTs which found no increased risk of short-term primary outcome.

No case-control studies reported the long-term primary outcome but eight cohort studies or self-controlled case series analysis were identified (Fig 3) [15, 22, 33, 37, 42-45]. We could not observe any increased risk of primary outcome associated with macrolides (RR 1.05; 95% CI 0.91-1.22) with heterogeneity of 54%. The follow-up period ranged from 31 days to 5 years. We conducted sensitivity analyses for at least two studies reporting the effect estimates in the same follow-up period (90 days, 1 year, 1-2 year, and 2-3 years). In one year follow-up, two observational studies with three cohorts showed an overall increased risk of 1.41 (95% CI 1.23-1.62) [15, 42]. All other sensitivity analyses showed no increased long-term risk (data not shown). Apart from observational studies, we included 14 RCTs in another analysis [3-9, 21, 40, 46-50] with a RR of 1.03 (95% CI 0.96-1.10). Similarly, we conducted sensitivity analyses for studies which had same follow-up periods (90 days, 180 days, 1 year, 2 years, and ≥3 years). We also excluded studies for reporting all-cause mortality or composite cardiovascular outcomes. All sensitivity analyses

showed no increased risk of long-term primary outcome (data not shown). The quality of evidence was low for cohort studies due to relatively high heterogeneity across studies in the primary analysis but that of RCTs was moderate.

3.4 Myocardial infarction

Fig.4 shows the forest plots for short-term myocardial infarction in different study designs. One case-control study in two publications reported the short-term myocardial infarction and showed no increased risk for exposure to erythromycin 14 days prior to the index date [51, 52]. After pooling the result with a case-crossover analysis which investigated clarithromycin [37], the risk was not significant (OR 1.35; 95% CI 0.57-3.19). Two studies reported the risk of acute myocardial infarction with clarithromycin using both cohort and self-controlled case series study designs [37, 44]. Using results from self-controlled case-series analyses which could eliminate time-invariant confounding, we observed an increased pooled RR of 3.53 (95% CI 2.25-5.54). Although two studies used different follow-up periods (14 days and 30 days), both studies individually showed increased short-term risk of myocardial infarction. In contrast, no increased risk of myocardial infarction, unstable angina or severe recurrent ischemia for clarithromycin and roxithromycin versus placebo could be found for 30 days follow-up periods in three RCTs [3, 20, 38] (RR 0.65; 95% CI 0.24-1.75). Both cohort studies and RCTs had moderate quality of evidence for short-term myocardial infarction and we estimated risk difference of 1787.0 per 1 million patients for cohort studies.

For long-term myocardial infarction (Fig.5), two case-control studies [53, 54] and one case-time-control study [44] did not show an increased long-term risk (OR 0.98; 95% CI 0.89-1.08). A marginal increased risk of myocardial infarction could be observed in cohort studies or self-controlled case series analyses (RR 1.10; 95% CI 1.04-1.17) but the follow-up periods varied considerably from 31 days to 3 years [15, 33, 37, 43, 44, 55]. However, when studies with the same follow-up periods were combined, no increased risk could be observed for most of the follow-up periods (90 days, days 31-90 since prescription start date, days 91-365, 1-2 year, and 2-3 year) (data not shown). Notably, an increased RR of 1.11 (95% CI 1.03-1.19) was observed only for 90 days follow-up due to the relatively high weight (99.0%) of a study which included patients with pneumonia and aged≥65 [33]. A total of 13 RCTs were identified and no increased risk of long-term myocardial infarction and/or unstable angina for macrolides could be observed (RR 0.95;

95% CI 0.87-1.04) versus placebo [3-9, 21, 40, 46, 47, 49, 50]. As the follow-up period varied between RCTs, we separated the analyses for follow-up periods of 90 days, 180 days, 1 year, 2 years and ≥3 years respectively. All analyses showed no significant difference of risk for long-term myocardial infarction versus placebo (data not shown). With the use of GRADE guideline, all study designs showed low quality of evidence that there was no increased or marginal significant long-term risk of myocardial infarction in the primary analysis.

3.5 Arrhythmia

A case-crossover study showed an increased risk of arrhythmia associated with *H. pylori* therapy containing clarithromycin [37]. There were six cohort studies reporting a short-term risk of arrhythmia associated with macrolides [22, 32, 34, 36, 37, 44] and no increased risk was observed (RR 1.36, 95% CI 0.97-1.92) (Fig.6). No RCT was identified in reporting the short-term risk of arrhythmia associated with macrolides. In GRADE, there was only very low quality of evidence for an increased short-term risk of arrhythmia due to serious risk of bias and high heterogeneity in the primary analysis. We estimated the risk difference of 171.7 per 1 million patients for case-crossover study.

One case-control study [56] which separately reported the OR for each type of macrolides and one case-time-control study [44] were identified to report the long-term risk of arrhythmia (Fig.6). Only clarithromycin and erythromycin were shown to have a higher risk of long-term arrhythmia but not with other macrolides in the case-control study [56]. The pooled estimate did not give an increased risk (OR 1.18; 95% CI 0.96-1.44). Six cohort studies or self-controlled case series analysis [15, 22, 33, 37, 44, 56] reported that the pooled estimate for long-term arrhythmia was 1.10 (95% CI 0.99-1.21) with heterogeneity of 69%. However, when results from at least two studies with the same follow-up periods were combined, no increased risk could be found (data not shown). No RCT was identified to report the long-term risk of arrhythmia associated with macrolides. Similarly with short-term arrhythmia, we could only obtain very low quality evidence for no increased long-term risk of arrhythmia in observational studies using GRADE.

3.6 Stroke

For short-term stroke (Fig.7), the overall RR was 1.45 (95% CI 0.92-2.29) in two self-controlled case series analyses [37, 44]. Despite different follow-up periods (14 and 30 days), both studies

showed no significant difference of risk of stroke during risk period versus baseline [37, 44]. Similar result was obtained for two RCTs with follow-up periods of 4 days [38] and 30 days [20] respectively (RR 1.06; 95% CI 0.22-5.02). We found a moderate quality of evidence for two cohort studies and two RCTs and low quality of evidence for one case-crossover study using GRADE.

No increased long-term risk of stroke could be observed in three observational studies [37, 43, 44] (RR 1.07; 95% CI 0.80-1.42) and eight RCTs [4, 6, 9, 11, 46, 48-50] (RR 1.13; 95% CI 0.91-1.41) (Fig.7). All sensitivity analyses that combined the same follow-up periods for cohort studies (90 days, days 31-90 since prescription start date, days 91-365, 1-2 year and 2-3 year) and for RCTs (2 and ≥3 years) showed no long-term risk of stroke associated with macrolides versus placebo (data not shown). We found moderate and low quality of evidence for observational studies and RCTs respectively using GRADE.

3.7 Stratified analyses for each type of macrolides

Due to the limited number of available studies for erythromycin, we did not have sufficient number of studies to perform meta-analyses for all outcomes. We could only observe an increased long-term risk of arrhythmia reported in a study using new antiarrhythmic prescriptions as a proxy of incident arrhythmia [56]. A case-control study showed an increased risk for exposure to macrolides 7 days prior to the date of sudden death [23]. Another cohort study also demonstrated an increased short-term risk of cardiac death associated with erythromycin and the effect was pronounced among patients who concurrently used cytochrome P-450 3A (CYP3A) inhibitors [14] (Supplementary material 9, Online Resource 9).

With clarithromycin, no increased risk of short or long-term primary outcome was observed across study designs. However, the heterogeneity of the pooled estimate was very high among cohort studies (83%). After excluding the study of Chou *et al.* [32] in the analysis, the risk of short-term primary outcome significantly increased (RR 1.40; 95% CI 1.12-1.76) with heterogeneity of 37%. An increased short-term risk of myocardial infarction was observed in observational studies but not in RCTs. No increased long-term risk of myocardial infarction associated with clarithromycin was shown across study designs. We could observe a short-term increased risk of arrhythmia in a case-crossover study (RR 2.49; 95% CI 1.09-5.69) [37] and no increased risk in four cohort studies [22, 32, 37, 44]. For long-term arrhythmia, we observed a marginal increased risk in two case-

control studies (OR 1.19; 95% CI 1.05-1.35) [44, 56]. For stroke, no increased risk in all follow-up periods could be found across study designs. (Supplementary material 10, Online Resource 10)

With azithromycin, we could not observe any significant increased short-term or long-term risk of primary outcome in cohort studies or RCTs. Although the risk did not reach statistical significance, the risk of short-term primary outcome was shown to be doubled in cohort studies and RCTs. No study investigated the short-term risk of myocardial infarction. A cohort study which conducted among patients with high baseline cardiovascular risk showed an increased long-term risk of myocardial infarction in 90 days [33] but five RCTs did not show an overall higher risk ratio of 0.92 (95% 0.79-1.08) versus placebo [4, 7, 8, 46, 47]. A significantly increased short-term risk of arrhythmia was shown in two cohort studies (RR 3.04; 95% CI 1.52-6.07) [32, 34]. No long-term risk of arrhythmia was shown in observational studies. For stroke, no observational study could be identified but no long-term risk was found in three RCTs with an overall risk ratio of 1.07 (95% CI 0.75-1.52) [4, 46, 48] (Supplementary material 11, Online Resource 11).

With roxithromycin, the number of observational studies was inadequate to obtain an overall effect summary for all outcomes. No study investigated the short-term risk of arrhythmia nor short-term risk of stroke associated with roxithromycin. In RCTs, there was no increased risk of primary outcome or myocardial infarction in all follow-up periods. In addition, no increased long-term risk of arrhythmia or stroke could be found (Supplementary material 12, Online Resource 12).

3.8 Narrative review

Supplementary material 2 (Online Resource 2) shows the identified studies which demonstrated subgroup analyses to explore the risk factors for cardiovascular outcomes associated with macrolides. Trac *et al*. performed several subgroup analyses and concluded that the history of chronic kidney disease, congestive heart failure, coronary artery disease or concurrent use of QT wave prolonging drugs did not significantly modify the association between macrolides and ventricular arrhythmia [36]. Regarding erythromycin, one cohort study reported that concurrent use of erythromycin and strong inhibitors of CYP3A such as nitroimidazole antifungal agents, diltiazem, verapamil and troleandomycin was a high risk factor for cardiac death [14]. For azithromycin, Mortensen *et al.* concluded that women had lower risk of 30-day mortality, 90-day mortality and myocardial infarction but the association was not significant due to small number of female patients in veterans administration patient population [33]. Other cohort studies reported

that those patients who received azithromycin had a higher risk of cardiovascular mortality versus non-users among patients who had high cardiovascular baseline risk [13, 35]. However, a cohort study conducted in Taiwan found significant cardiovascular risk with wide confidence intervals among patients without cardiovascular disease and those aged ≤65 years [32]. Two RCTs did not show differences across subgroups according to history of diabetes, hypertension and hypercholesterolemia [8, 46].

Similar to azithromycin, Schembri *et al.* [15] and Wong *et al.* [37] reported that caution should be taken when prescribing clarithromycin for those patients who had a high cardiovascular baseline risk such as history of cardiovascular diseases, hypertensive diseases or diabetes. Patients aged ≥75 years also had higher risk of cardiovascular outcomes associated with clarithromycin versus amoxicillin [37]. In addition, Schembri *et al.* also showed that the longer the duration of clarithromycin administration (more than seven days) then the higher the risk of cardiovascular events [15]. In contrast, Svanstrom *et al.* found no differences across subgroups according to cardiovascular baseline risk but higher risk in women than men for cardiac mortality in current use of clarithromycin versus penicillin V [12].

Regarding the concomitant use of statins and clarithromycin, a subgroup analysis using data from a RCT found that statins has protective prognostic effect of clarithromycin against all-cause and cardiovascular mortality [17, 18]. The concomitant use of statins at entry with clarithromycin showed a non-significant protective effect of cardiovascular mortality (HR 0.68; 95% CI 0.38-1.22) while the risk of cardiovascular mortality confined to those who did not use statins at entry (HR 1.90; 95% CI 1.34-2.67) in 3 years [18]. Similar results were obtained after further adjustment for sex, age, history of myocardial infarction and smoking. Comparing with patients who did not use statins at entry, the risk of all-cause mortality significantly reduced among patients with statins at entry (HR 0.31; 95% CI 0.20-0.49) [17]. In addition, Schembri *et al.* showed no significant differences of cardiovascular events among statins and clarithromycin users versus non-users in both cohorts of patients with community acquired pneumonia and chronic obstructive pulmonary disease given that increased risk could be found in the full cohort [15].

4 Discussion

4.1 Cardiovascular risk

In general, we found a short-term increased risk of cardiovascular mortality, myocardial infarction and arrhythmia associated with the use of macrolides. However, we did not find any long-term cardiovascular risk except for a weak association with myocardial infarction. No short or long-term risk of stroke could be demonstrated in this study. Although we could not observe a short-term cardiovascular risk in RCTs, this may be due to the small sample size of approximately 2000 patients for each study group. For myocardial infarction, we estimated the risk difference was 1.79 events per 1000 patients which may indicate that the included randomized controlled trials were underpowered to detect the short-term risk of myocardial infarction. With large population-based cohorts, observational studies with rigorous study designs and methodologies could detect rare events with robust estimates in short follow-up periods when compared with RCTs. Therefore, observational studies complemented the findings with RCTs for short-term cardiovascular outcomes, especially for short-term myocardial infarction given its moderate quality of evidence in GRADE. Importantly, we estimated 1.79 excess myocardial infarction events in short-term (95% CI 0.88-3.20) per 1000 patients. Further, there was no increased risk of stroke associated with macrolides as supported by the evidence of moderate quality in this study.

In the current literature, there are very few studies for erythromycin and roxithromycin so the class effect of cardiovascular risk for macrolides remains uncertain. In the stratified analysis, we could only demonstrate that erythromycin increased the short-term risk of cardiac death, clarithromycin increased the short-term risk of myocardial infarction, while azithromycin increased the short-term risk of arrhythmia in observational studies. However, one pharmacovigilance study using healthcare data from three European countries found an increased risk of myocardial infarction associated with clarithromycin, azithromycin, roxithromycin and erythromycin individually during the current exposure using several observational study designs [26]. Due to the limited number of studies for macrolides other than clarithromycin, we could not rule out the possibility that macrolides have a class effect upon acute myocardial infarction.

In addition, there were few studies reporting the risk factors for cardiovascular risk associated with macrolides. Most of the studies reported that patients who had high baseline cardiovascular risk had higher risk of cardiovascular outcomes for azithromycin and clarithromycin. Prescribers should be cautious when prescribing azithromycin and clarithromycin to these patients. In view of the cardiovascular protective effect of statins, currently there is only one RCT which was designed

to investigate the long-term risk of cardiovascular mortality and showed that statins can prevent the excess cardiovascular mortality associated with clarithromycin in long-term [18]. No RCT or observational study has been conducted to evaluate the protective effect in short-term. In contrast to the RCT that could find a long-term protective effect of statins for preventing the excess risk of cardiovascular mortality [18], our review could only find a short-term cardiovascular risk associated with clarithromycin. As we could not find a long-term increased cardiovascular risk, we could not evaluate the long-term protective effect of statins suggested by this RCT [18]. In addition, one cohort study reported that no differences of mortality or hospitalization among patients who had overlapping prescriptions of statins and clarithromycin, compared with those who had clarithromycin only after adjustment of age, treatment with other antibiotics, history of diabetes or cardiovascular disease (RR 1.02; 95% CI 0.85-1.22) [57]. Due to the limited evidence, more epidemiological studies are required to evaluate the findings and subsequently perform risk/ benefit assessment before recommendation as co-prescription of statins and clarithromycin could also lead to adverse interaction effects [58].

4.2 Strength and limitation of the review

We updated the systematic review to analyze the risk of cardiovascular events associated with macrolides in terms of different follow-up periods and study designs, as well as summarize the risk factors and the potential cardiovascular protective effect due to the concomitant use of statins and clarithromycin. Similarly, Cheng *et al*. also reported an increased risk of cardiovascular mortality during current use but not former use (365 days) [59]. A study suggested that the risk of mortality could only be observed when the follow-up periods were extended to more than two years [16]. In our review, we demonstrated that there was no increased risk of cardiovascular mortality for more than three years of follow-up. In addition, we pooled data from studies conducted in different countries and thus increased the generalizability of our findings. Moreover, this is also the first systematic review to use GRADE guideline to evaluate the quality of evidence for each cardiovascular outcome associated with macrolides in a better system for further clinical implication.

There are few potential limitations with our systematic review. Firstly, the heterogeneity of short-term increased risk of primary outcome for observational studies is high which resulted in a very low quality of evidence. We attempted to reduce the heterogeneity by performing a sensitivity

analysis to remove studies reporting all-cause mortality only and gave similar result. While randomized controlled trials did not show an increased risk of primary outcome with moderate quality of evidence, these results should be interpreted cautiously due to the limited sample size. Future population-based observational studies should be well-designed to replicate previous studies so as to decrease the heterogeneity across studies for providing higher quality of evidence for the primary outcome. Secondly, several observational studies used different units of denominators for absolute risk (i.e. per prescriptions/ per patients/ per patient years), therefore we could not summarize the total number of patients in exposed and non-exposed groups. Despite the inconsistency of the studies, we estimated the absolute risk of cardiovascular outcomes from large population-based cohort studies and obtained a similar estimate of risk difference of cardiovascular mortality (30.9-92.7 per 1 million patients) reported by Cheng *et al*. (38.2 per 1 million treatment courses) [59]. Thirdly, different RCTs had different definitions of outcomes so it might lead to a certain degree of heterogeneity for our primary outcome. However, the heterogeneity was found to be very low and we also performed several sensitivity analyses to test the robustness of results which gave similar findings. Fourthly, we did not include studies which are in languages other than English and Chinese. However, only one study in Korean which should be eligible for inclusion presented the main findings in an English abstract. It resulted in the same result with our primary analysis (RR 1.03; 95% CI 0.96-1.10). Therefore, the exclusion of non-English/Chinese studies should not affect the interpretation of the results and conclusion of this study.

5 Conclusion

A short-term increased risk of cardiovascular outcome associated with macrolides but no long-term cardiovascular risk could be observed for follow-up periods up to four years. Prescribers should be cautious when prescribing azithromycin and clarithromycin to patients who have high baseline cardiovascular risk such as history of cardiovascular diseases, hypertensive diseases or diabetes. Currently limited observational studies have been conducted to investigate the short-term risk of myocardial infarction associated with macrolides other than clarithromycin. Future studies are required to report subgroup analysis for important risk factors and investigate the potential protective short-term cardiovascular effect from the concomitant use of statins and macrolides.

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Figure captions

Fig. 1 Review flowchart

Fig. 2 The forest plots of the overall risk estimates for short-term primary outcomes

Fig. 3 The forest plots of the overall risk estimates for long-term primary outcomes

Fig. 4 The forest plots of the overall risk estimates for short-term myocardial infarction

Fig. 5 The forest plots of the overall risk estimates for long-term myocardial infarction

Fig. 6 The forest plots of the overall risk estimates for arrhythmia

Fig. 7 The forest plots of the overall risk estimates for stroke

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