Use of azithromycin and risk of ventricular arrhythmia

Association of azithromycin and ventricular arrhythmia: a European multi-database, population-based study

Gianluca Trifirò MD, PhD[1,2*], Maria de Ridder PhD[1], Janet Sultana MSc[1,3], Alessandro Oteri PhD[1], Peter Rijnbeek PhD[1], Serena Pecchioli MD, PhD[4], Giampiero Mazzaglia MD PhD[4], Irene Bezemer PhD[5], Edeltraut Garbe MD PhD[6], Tania Schink PhD[6], Elisabetta Poluzzi MD PhD[7], Trine Frøslev PhD[8],Mariam Molokhia PhD[9], Igor Diemberger PhD[10] and Miriam CJM Sturkenboom PhD[1]

[1]Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, Netherlands; [2]Department of Biomedical and Dental Sciences and Morpho-functional Imaging, University of Messina, Italy; [3]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; [4]Health Search, Italian College of General Practitioners, Florence, Italy; [5]PHARMO Institute for Drug Outcomes Research, Utrecht, Netherlands; [6]Leibniz Institute for Epidemiology and Prevention Research - BIPS GmbH, Germany; [7]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [8]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark; [9]Department of Primary Care and Public Health Sciences, King's College, London, United Kingdom; [10]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy

***Corresponding author:** GianlucaTrifirò, Department of Biomedical and Dental Sciences and Morpho-functional Imaging, Policlinico Universitario G. Martino, University of Messina, 1, Via Consolare Valeria, 98125, Messina, Italy; Email: trifirog@unime.it; Tel. + 39 0902213264; Fax: + 39 0902213300

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Conflicts of interest

EP, ID, MdR, AO, SP, IB, GT, GM, TF, PRand JS declare no support from any organisation. TS is working in departments that occasionally perform studies funded by pharmaceutical industries (Bayer, Celgene, GlaxoSmithKline, Mundipharma, Novartis, Purdue Pharma, Sanofi-Aventis, Sanofi Pasteur MSD, and STADA). MS is heading a research unit that holds unconditional research contracts with some pharmaceutical companies (Eli Lilly, Pfizer, AstraZeneca Novartis, Boehringer, GSK, Servier), none related to this study. MM reports grants from Pfizer, Astra-Zeneca and International Serious Adverse Reactions Events Consortium,outside the submitted work. EG has in the past run a department that occasionally performed studies for pharmaceutical industries. These companies include Bayer, Celgene, GSK, Mundipharma, Novartis, Sanofi, Sanofi Pasteur MSD, and STADA. EG has been a consultant to Bayer, Nycomed, Teva, GSK, Schwabe and Novartis.

Abstract

Background: There are conflicting findings from observational studies investigating the association of azithromycin and potentially arrhythmogenic cardiovascular death, of which only one study was conducted in Europe. This study was aimed at studying the association between ventricular arrhythmia and azithromycin.

Methods: A case-control study was nested in a cohort of incident antibiotic users using seven population-based healthcare databases from Denmark, Germany, Italy, Netherlands, and United Kingdom using data from 1996-2010.Cases were matched to up to 100 controls on age, sex and database. Recency of use and type of antibiotic (azithromycin was the exposure of interest) at the index date (occurrence of ventricular arrhythmia) were identified. The odds ratio (OR) of ventricular arrhythmia occurrence with current azithromycin use relative to current amoxicillin use or non-use (>365 days treatment discontinuation) of any antibiotic was estimated using conditional logistic regression, while adjusting for confounders.

Results: There were 4,040,688 new antibiotic users, of whom 13,536 (0.33%)developed ventricular arrhythmia. Among ventricular arrhythmia cases, 30 were current azithromycin users. In the pooled data analyses across databases, compared to non-use of antibiotics, azithromycin use was associated with higher ventricular arrhythmia risk (OR_{Adj}: 1.97; 95% CI: 1.35-2.86). This increase disappeared using current amoxicillin use as comparator (OR_{Adj}: 0.90; 95%CI: 0.48-1.71). Database-specific estimates and meta-analysis of single database estimates confirmed results from the pooled data analysis.

Interpretation: Azithromycin is not associated with ventricular arrhythmia compared to amoxicillin, while confounding by indication may explain the observed increased VA risk of azithromycin use versus non-use of antibiotic.

Key words: azithromycin, case-control study, ventricular arrhythmia, databases

Introduction

Azithromycin is a widely-prescribed broad spectrum macrolide mainly used inrespiratory and urinary tract bacterial infections. Concerns were raised recently regarding its arrhythmogenic potential, which is already known to exist for first marketed macrolide erythromycin.[2-7]There are several case reports describing QT prolongation,[8-10]*torsades de pointes*[11-13]or polymorphic ventricular tachycardia [14] following azithromycin use. Several contrasting observational studies assessed the association of azithromycin use and cardiovascular death.[15-22] Since the known arrhythmogenic potential of azithromycin is related to QT interval prolongation, *torsades de pointes* and ventricular arrhythmia[19,20] the above-mentioned studies are limited by the broad category of cardiovascular death they use as an outcome, which likely only partially captures azithromycin-associated cardiac arrhythmia risk. To date, only one observational study investigated specifically the association of ventricular arrhythmia and azithromycin use.[23] In light of limited and contrasting findings on this drug-adverse event association, we investigated the risk of ventricular arrhythmia associated to azithromycin using a network of 7 healthcare databases from 5 European countries participating in the ARITMO project.[24]

Methods

Design and setting

A case-control study was nested in acohort of antibiotic users, using datafrom the ARITMO network. These comprised seven healthcare databases from five European countries, covering a population of approximately 28 million subjectsin the period running from January 1st 1997 to December 31st 2010. The databases used were Health Search Cegedim-Strategic Data-Longitudinal-Patient Database (Italian nationwide), Integrated Primary Care Information database (Dutch nationwide), The Health Improvement Network (United Kingdom nationwide), PHARMO database network (Dutch nationwide), AARHUS (Denmark, North and Central Denmark), German Pharmacoepidemiological Research Database (Germany) and Emilia-Romagna Database (Italy, Emilia-Romagna region) (**eTable1**). Harmonized data extraction, quality assurance and analyses when combining multiple healthcare databases for drug safety studies has been described elsewhere.[25] The databases in the ARITMO network have been previously used for pharmacoepidemiological studies[26,41] and trends in antibiotic use across databases have already been investigated.[42] This study is registered in the ENCePP registry of studies held at the European Medicines Agency (http://www.encepp.eu/encepp/viewResource.htm?id=4669).

Cohort

The cohort consisted of new antibiotic users (no antibiotic in prior year). Cohort entry occurred on the date of the first recorded antibiotic drug prescription in patients with ≥1 year of database history. Patients >85 years were excluded to databases to restrict to patients with more reliable data records as previously done[43]. Persons with malignant cancer were excluded as they may be hospitalized for long periods thus precluding full capture of medical history. Cohort members were followed from the first antibiotic prescription until the earliest of the following: a) end of study, b) occurrence of ventricular arrhythmia; c) transfer out of database; d) malignant cancer; e) 85th birthday or f) death.

Cases and controls

The primary outcome of this study was ventricular arrhythmia (case definition and coding algorithms reported in **eFigure1** and **eTable2**). Outcome validation was conducted in every database for a random sample of 200 cases through independent manual medical record review by two experts per database who were blinded to drug exposure. In the Integrated Primary Care Information database, manual validation of all automatically detected cases was performed due to extensive use of unstructured free-text patient notes. A positive predictive value $\geq$90% of final coding algorithm was achieved.[44] Each case was matched with up to 100 controls using incidence density sampling on year of birth (±1 year), sex, calendar time and database.

Exposure definition

Data on antibiotics (Anatomical Therapeutic Chemical classification codes J01*) were obtained from the electronic drug prescription/dispensing databases. The exposure period was calculated dividing the total number of units per prescription by the prescribed daily number of units (or defined daily dose, if prescribed dosage was unavailable). Exposure to antibiotics was categorized as: a) current: exposure period covered the index date or ended <7 days before the index date (to account for lack of compliance or late registration of outcome, i.e. to reduce spurious attribution of outcome onset to the exposure period); b) recent: exposure period ends between 7 and <90 days before the index date (to account for complications of severe infections and delayed antibiotic effects); c) past: exposure period ends between 90 and <365 days before the index date (both antibiotic exposure and infection are likely to have no effect at all on occurrence of the outcome); d) non-use: no exposure within 365 days before the index date.

Covariates

We considered the following risk factors of ventricular arrhythmia as covariates: a) age and sex; b) cardiovascular disease; c) metabolic diseases; d) other diseases related to increased risk of ventricular arrhythmia (e.g. atrial fibrillation/flutter); e) prior use of anti-arrhythmia drug and concomitant use of medication inducing hypokalemia or QT prolonging drugs (i.e. drugs with established torsadogenic liability based on the CredibleMeds list).[45] Covariates and identification algorithms are listed in **AppendiceseTables3-6**using the harmonization process that has been described before.[46]

Statistical analysis

Data were extracted locally and transformed into a simple common data model according to a process we have described before.[47] Using the custom-built JAVA-based softwareJerboa data were transformed locally from the common input files into fully anonymized datasets.[25]

Unadjusted and adjusted odds ratios (OR and OR_{adj} respectively) with 95% confidence intervals (CIs) were calculated using conditional logistic regression analysis for current, recent and past use of azithromycin using current use of

amoxicillin as a primary comparator. ORs were also calculated for current, recent and past use of azithromycin, amoxicillin and other antibiotics using non-use of antibiotics (J01*) as a secondary comparator.

Confounders included in the final models were selected through a stepwise approach. Well-known risk factors for ventricular arrhythmia were included *a priori* as confounders in the final multivariate model (**eTable 3**). Other potential confounders were included if they had a prevalence of >5% among the controls and were associated with the outcome in the univariate model. All analyses were conducted in every database and a meta-analysis of single database risk estimates was carried out; only databases with more than 3 cases currently using azithromycin were included. Heterogeneity across databases was estimated using Cochran's Q (Q statistic). The I^2 statistic was then used to express the percentage variation across the databases due to heterogeneity, with values >75% considered to denote a high heterogeneity. A conservative approach was taken for the two-stage pooling, favoring a random effects model.[48]

The 7-day carry-over period that was used to account for lack of compliance or late registration of VA was excluded in a sensitivity analysis. An additional sensitivity analysis was carried out in which all persons with an acute myocardial infarction recorded within 15 days prior to the index date were excluded in order to explore the possible confounding effect due to recent history of acute myocardial infarction.

Ethical approval

This study was not conducted with direct patient involvement and all data was analysed retrospectively; prior ethical approval for the study was obtained in line with national regulations.

Results

In the source population of 28,760,406 subjects, 4,040,688 (14%) incident antibiotics users free of cancer and aged <85 years were identified during the study period. Within the study cohort, 12,947 (0.04%) cases of ventricular arrhythmia and 1,240,783 (4.31%) matched controls were identified (**eFigure2**). Mean age of cases and controls was 63 years and two-thirds of patients were male (**Table 1**).

Of the ventricular arrhythmia cases, 30 were current azithromycin users, matched to 1,344 controls. When comparing the occurrence of ventricular arrhythmia in current use of azithromycin versus current use of amoxicillin, no significant increase in risk was found (OR_{Adj}: 0.94; 95% CI: 0.50-1.77) (**Table 2**). Similarly, there was a decreased risk of ventricular arrhythmia in recent and past users of azithromycin as compared to current amoxicillin users (OR_{Adj}: 0.58 (95% CI: 0.38-0.87) and 0.52 (95% CI: 0.37-0.73) (**Table 2**).

Compared to non-use of any antibiotic, current use of azithromycin was associated with a significant increased risk of ventricular arrhythmia, which was decreased substantially but remained significant upon adjustment for potential confoundersin the one stage pooling (OR_{Unadj}: 2.83; 95% CI: 1.97-4.08; OR_{Adj}: 1.97; 95% CI: 1.35-2.86) (**Table 3**). There was no increased risk of ventricular arrhythmia in recent and past users of azithromycin compared to non-use of any antibiotic (OR_{Adj}: 1.12 (95% CI: 0.92-1.37) and 1.10 (95% CI: 0.95-1.28) respectively). Current use of other antibiotics was also associated with a higher risk of ventricular arrhythmia (OR_{Adj}: 1.83; 95% CI: 1.71-1.97) compared

to non-use of antibiotics, while this risk was lower for recent users (OR_{Adj}: 1.32; 95% CI: 1.25-1.39) and past users (OR_{Adj}: 1.11; 95% CI: 1.06-1.16). The meta-analysis included Aarhus database, the Emilia-Romagna Database, the German Pharmacoepidemiological Research Database and PHARMO database only since the other databases had less than 3 ventricular arrhythmia cases who were current users of azithromycin. Heterogeneity among databases was low and non-significant (Q statistic: 3.39; p-value 0.3; I^2: 11%). The meta-analysis of the comparison of current users of azithromycin with current amoxicillin usersshowed no increased risk of ventricular arrhythmia (OR: 1.01; 95% CI: 0.38-2.64) (**Figure 1**). However, the meta-analysis estimate showed an increased risk of ventricular arrhythmia during current use of azithromycin as compared to non-use of any antibiotic (OR: 2.44; 95% CI: 1.61-3.69) (**Figure 2**).

Main findings remained consistent when excluding the 7 day carry-over period: compared to non-use, as both azithromycin and amoxicillin current use were associated with a similar increased risk of ventricular arrhythmia (OR_{Adj}: 1.9 (95%CI: 1.1-3.1) and OR_{Adj}: 1.9 (95%CI: 2.5-2.3) respectively). After excluding patients with a diagnosis of acute myocardial infarction within 15 days prior to the index date, both azithromycin and amoxicillin had a higher risk of ventricular arrhythmia compared to non-use of antibiotics (OR_{Adj}: 1.7; 95%CI: 1.2-2.5 and OR_{Adj}: 1.9; 95%CI: 1.7-2.2 respectively).

Interpretation

Main findings

This study shows that the risk of ventricular arrhythmia with current azithromycin use was higher compared to non-use of any antibiotic. However, confounding by indication played a major role in this association as suggested by the VA risk disappearance when amoxicillin use was employed as comparator. This result was consistent across separate databases as well as in the one or two stage pooling. Confounding by indication in context of the comparison between azithromycin use and non-use of any antibiotic refers to the increased baseline risk of ventricular arrhythmia associated with the *indication* of the antibiotic use, i.e. the infection, rather than the exposure itself, i.e. azithromycin. This comparison between amoxicillin and azithromycin is more likely to be a true reflection of the risk of ventricular arrhythmia.The rationale behind using amoxicillin as a control is therefore two-fold: 1) it is not expected to have an increased risk of ventricular arrhythmia; 2) it would avoid introducing confounding by indication as both amoxicillin and azithromycin have a similar indication of use and spectrum of action. Amoxicillin has been used as a comparator in similar studies previously.[15,18]

Using the upper limit of the confidence interval and the crude incidence rate of ventricular arrhythmia in the general population in all 7 databases, we would expect at most 8.07 excess cases of ventricular arrhythmiaduring use of azithromycin use per 100,000 person years compared to non-use of any antibiotic.

Comparison to other studies

There are conflicting results from large epidemiological studies reporting the risk of cardiovascular death and more rarely, cardiac arrhythmia. A United States-based cohort study reported a 2.5- and 2.9-fold increased risk of cardiovascular death compared to amoxicillin and non-use of antibiotics use respectively in Medicare beneficiaries.[15] Following publication of this study, the Food and Drug Association issued a safety warning about azithromycin-related cardiovascular death.[16]Asubsequent Danish study showed that the increased risk of cardiovascular death with azithromycin use compared to non-use of antibiotics (rate ratio (RR): 2.85;(95%CI: 1.13-7.24)) disappeared when penicillin V use was used as comparator (RR: 0.93; (95%CI: 0.56-1.55)).[17] More recently, another cohort study using United States Veterans' Affairs data reported an almost two-fold increased risk of serious arrhythmia with azithromycin compared to current amoxicillin use.[18] In 2014, another US study reported that among older patients hospitalized with pneumonia, azithromycin use was associated with a small increased risk of myocardial infarction for azithromycin users compared to non-azithromycin antibiotic users, while no effect on cardiac arrhythmias was observed.[21]The findings in outpatients from the United States[15,18,21] contrast with our findings and those of Svanstrom et al.[17]A possible explanation is that Medicaid beneficiaries and retired veterans have a more marked presence of multiple confounders such as older age, lower socioeconomic status, increased number of co-morbidities, obesity, current smoking status and/or disability, which may render them more vulnerable to the cardiovascular adverse effects of azithromycin than other populations.

Our findings are also in agreement with a cohort study carried out in a Canadian elderly population (set in Ontario), which found no increased risk of ventricular arrhythmia with macrolide use compared to non-macrolide antibiotic use.[22] This study is based on the assumption that a potential increased risk of ventricular arrhythmia is a class effect, potentially masking the risk of ventricular arrhythmia associated with individual agents. This was illustrated by Chou et al., who conducted the only published cohort study investigating the association between ventricular arrhythmia and azithromycin use, set in the Taiwan National Health Insurance Database.[23] Electrophysiological evidence supports our findings and suggests that azithromycin lacks arrhythmogenic potential at therapeutic doses.[48] Fever itself may be a risk factor for arrhythmia, and therefore confounding by indication is pronounced when antibiotics are used in patients with high fever.[50]

Strengths and limitations

A main strength of this study is the size of the network since ventricular arrhythmiais a rare outcome, as well as the common methodology used. All of the databases except Emilia-Romagna Database and Aarhus database are representative of the countries from which data is drawn. In addition, exposure to individual antibiotics was identified in such a way that only users of a single antibiotic were included in the analyses. Results from the one-stage pooling were confirmed in the meta-analysis of individual database risk estimates.Taken together, these factors significantly increase the reliability of study findings herein. Although it may be considered a limitation that three of the databases were excluded from the two-stage pooling due to the low number of cases, it should be noted that all seven databases were included in the one-stage pooling. The present study had some other limitations. Exposure misclassification is possible if patients do not fill their prescriptions or take their medications. However, since risk estimates were consistent across databases using either drug dispensing or prescriptions as source for exposure assessment these biases are likely to be negligible. In addition, removal of the 7 day carry-over period to take into account delay in the intake of antibiotic did not change the results.[26] The exclusion of cancer patients and those aged over 85 may also be considered a limitation,

although the latter approach has been used previously[43]. In addition, findings from the present paper may not be directly extrapolated to the hospital setting, both because the health status of patients as well as the nature of antibiotic use is likely to be very different than in the community setting.

Conclusion

This ARITMO study showed that azithromycin is not associated with ventricular arrhythmia when compared to use of amoxicillin in various European countries. The increase in risk when using non-use of antibiotics as comparator suggests significant confounding by indication.

References

1. Mosholder AD, Mathew J, Alexander JJ, et al. Cardiovascular risks with azithromycin and other antibacterial drugs. *N Engl J Med* 2013;368(18):1665-1668.
2. Vogt AW, ZolloRA.Long Q-T syndrome associated with oral erythromycin used in preoperative bowel preparation.*AnesthAnalg* 1997;85(5):1011-1013.
3. Tschida SJ, Guay DR, Straka RJ, et al.QTc-interval prolongation associated with slow intravenous erythromycin lactobionate infusions in critically ill patients: a prospective evaluation and review of theliterature.*Pharmacotherapy* 1996;16(4):663-674.
4. De Ponti F, Poluzzi E, Montanaro N.QT-interval prolongation by non-cardiac drugs: lessons to be learned from recent experience.*Eur J ClinPharmacol* 2000;56(1):1-18.
5. Drici MD, Knollmann BC, Wang WX, et al.Cardiac actions of erythromycin: influence of female sex.*JAMA* 1998;280(20):1774-1776.
6. Shaffer D, Singer S, Korvick J, et al. Concomitant risk factors in reports of torsades de pointes associated with macrolide use: review of the United States Food and Drug Administration Adverse Event Reporting System.*Clin Infect Dis* 2002;35(2):197-200.
7. KohTW.Risk of torsades de pointes from oral erythromycin with concomitant carbimazole (methimazole) administration.*PacingClinElectrophysiol* 2001;24(10):1575-1576.
8. Matsunaga N, Oki Y, Prigollini A.A case of QT-interval prolongation precipitated by azithromycin.*N Z Med J* 2003;116(1185):U666.
9. Samarendra P, Kumari S, Evans SJ, etal.QT prolongation associated with azithromycin/amiodarone combination.*PacingClinElectrophysiol* 2001;24(10):1572-1574.
10. Russo V, Puzio G, SiniscalchiN.Azithromycin-induced QT prolongation in elderly patient.*Acta Biomed* 2006;77(1):30-32.
11. Arellano-Rodrigo E, García A, Mont L, et al.Torsade de pointes and cardiorespiratory arrest induced by azithromycin in a patient with congenital long QT syndrome[Article in Spanish]. *Med Clin (Barc)* 2001;117(3):118-119.

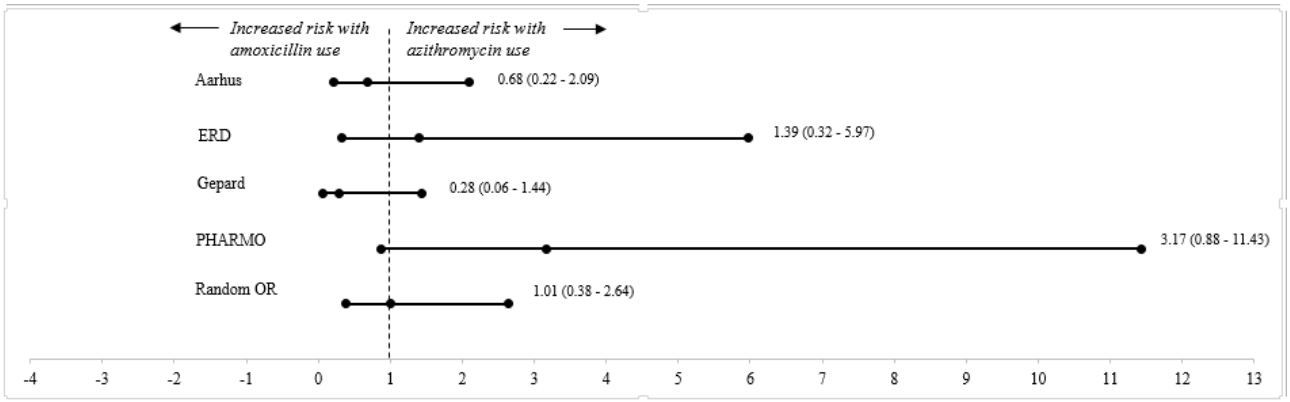
12. Kezerashvili A, Khattak H, Barsky A, et al.Azithromycin as a cause of QT-interval prolongation and torsade de pointes in the absence of other known precipitating factors.*JInterv Card Electrophysiol* 2007;18(3):243-246.
13. Huang BH, Wu CH, Hsia CP, et al.Azithromycin-induced torsade de pointes.*PacingClinElectrophysiol* 2007;30(12):1579-1582.
14. Kim MH, Berkowitz C, TrohmanRG.Polymorphic ventricular tachycardia with a normal QT interval following azithromycin.*PacingClinElectrophysiol* 2005;28(11):1221-1222.
15. Ray WA, Murray KT, Hall K, et al.Azithromycin and the risk of cardiovascular death.*NEngl J Med* 2012;366(20):1881-90.
16. Food and Drug Association (FDA). FDA Statement regarding azithromycin (Zithromax) and the risk of cardiovascular death. Maryland: FDA, 2012.(Accessed June 24, 2015 at http://www.fda.gov/Drugs/DrugSafety/ucm304372.htm)
17. Svanström H, Pasternak B, HviidA.Use of azithromycin and death from cardiovascular causes.*NEngl J Med* 2013;368(18):1704-1712.
18. Rao GA, Mann JR, Shoaibi A, et al. Azithromycin and levofloxacin use and increased risk of cardiac arrhythmia and death. *Ann Fam Med* 2014;12(2):121-127.
19. Hinkle LE Jr, ThalerHT.Clinical classification of cardiac deaths.*Circulation* 1982;65(3):457-464.
20. Marcus FI, Cobb LA, Edwards JE, et al.Mechanism of death and prevalence of myocardial ischemic symptoms in the terminal event after acute myocardial infarction.*Am J Cardiol* 1988;61(1):8-15.
21. Mortensen EM, Halm EA, Pugh MJ, et al. Association of azithromycin with mortality and cardiovascular events among older patients hospitalized with pneumonia.*JAMA* 2014;311(21):2199-2208.
22. Trac MH, McArthur E, Jandoc R, et al. Macrolide antibiotics and the risk of ventricular arrhythmia in older adults. *CMAJ* 2016 Feb 22. pii: cmaj.150901. [Epub ahead of print]
23. Chou HW, Wang JL, Chang CH, et al. Risks of cardiac arrhythmia and mortality among patients using new-generation macrolides, fluoroquinolones, and β-lactam/β-lactamase inhibitors: a Taiwanese nationwide study. *Clin Infect Dis* 2015;60(4):566-577.
24. Molokhia M, De Ponti F, Behr ER, et al. Academic output from EU-funded health research projects.*Lancet* 2012;380(9857):1903-1094.
25. Trifirò G, Coloma PM, Rijnbeek PR, et al.Combining multiple healthcare databases for postmarketing drug and vaccine safety surveillance: why and how?*J Intern Med* 2014;275(6):551-561.
26. Mor A, Frøslev T, Thomsen RW, et al.Antibiotic use varies substantially among adults: a cross-national study from five European Countries in the ARITMO project. *Infection* 2015. [Epub ahead of print]
27. Coloma PM, Schuemie MJ, Trifirò G, et al. Combining electronic healthcare databases in Europe to allow for large-scale drug safety monitoring: the EU-ADR Project.*Pharmacoepidemiol Drug Saf* 2011;20(1):1-11
28. Laforest L, Licaj I, Devouassoux G, et al. Prescribed therapy for asthma: therapeutic ratios and outcomes. *BMC FamPract* 2015;14(16):49.
29. James GD, Petersen I, Nazareth I, et al. Use of long-term antibiotic treatment in COPD patients in the UK: a retrospective cohort study. *Prim Care Respir J* 2013;22(3):271-7.
30. Ben Ghezala I1, Arendt JF, Erichsen R, et al. Positive predictive value of the diagnosis coding for vitamin B12 deficiency anemia in the Danish National Patient Register. *ClinEpidemiol* 2012;4:333-8.

31. Galatti L, Sessa A, Mazzaglia G, et al. Antibiotic prescribing for acute and recurrent cystitis in primary care: a 4 year descriptive study. *J AntimicrobChemother* 2006;57(3):551-6.
32. Mazzaglia G, Caputi AP, Rossi A, et al. Exploring patient- and doctor-related variables associated with antibiotic prescribing for respiratory infections in primary care. *Eur J ClinPharmacol* 2003; 59: 651-7.
33. Poluzzi E, Piccinni C, Sangiorgi E, et al. Trend in SSRI-SNRI antidepressants prescription over a 6-year period and predictors of poor adherence. *Eur J ClinPharmacol* 2013;69(12):2095-101.
34. Ohlmeier C, Langner I, Hillebrand K, et al. Mortality in the German Pharmacoepidemiological Research Database (GePaRD) compared to national data in Germany: results from a validation study. *BMC Public Health* 2015;15:570.
35. Kollhorst B, Abrahamowicz M, Pigeot I. The proportion of all previous patients was a potential instrument for patients' actual prescriptions of nonsteroidal anti-inflammatory drugs. *J ClinEpidemiol* 2016;69:96-106.
36. Valkhoff VE, Coloma PM, Masclee GM, et al. Validation study in four health-care databases: upper gastrointestinal bleeding misclassification affects precision but not magnitude of drug-related upper gastrointestinal bleeding risk. *J ClinEpidemiol* 2014;67(8):921-31.
37. Cheung K, Aarts N, Noordam R, et al. Antidepressant use and the risk of suicide: a population-based cohort study. *J Affect Disord* 2015;174:479-84.
38. Coloma PM, de Ridder M, Bezemer I, et al. Risk of cardiac valvulopathy with use of bisphosphonates: a population-based, multi-country case-control study. *OsteoporosInt* 2015. [Epub ahead of print]
39. Overbeek J, van Wijngaarden R, Diels J, et al. Relationship between Glycaemic Burden and Micro- and Macrovascular Complications in Patients with Type 2 Diabetes Mellitus: A real-world study in the Pharmo Database Network. *Value Health* 2015;18(7):A597.
40. Bezemer ID, Verhamme KM, Gini R, et al. Use of oral contraceptives in three European countries: a population-based multi-database study. *Eur J ContraceptReprod Health Care* 2016;21(1):81-7.
41. Mor A, Frøslev T, Thomsen RW, et al. Antibiotic use varies substantially among adults: a cross- national study from five European Countries in the ARITMO project.*Infection* 2015;43(4):453-472.
42. Diemberger I, Oteri A, Rijnbeek P, et al. Epidemiology of Ventricular Arrhythmias in five European Countries – Abstract submitted to World Congress in Cardiac Electrophysiology and Cardiac Techniques, June 18-21, 2014 - Nice France. Europace. 2014;16:2.
43. García Rodríguez LA, Cea Soriano L, Hill C, Johansson S. Increased risk of stroke after discontinuation of acetylsalicylic acid: a UK primary care study. *Neurology*. 2011 Feb 22;76(8):740-6.
44. Avillach P, Coloma PM, Gini R, et al.Harmonization process for the identification of medical events in eight European healthcare databases: the experience from the EU-ADR project. *J Am Med Inform Assoc* 2013;20(1):184-92.
45. CredibleMeds. CredibleMeds webpage. (Accessed January 15, 2014 at https://crediblemeds.org/)
46. Coloma PM, Schuemie MJ, Trifirò G, et al. Combining electronic healthcare databases in Europe to allow for large-scale drug safety monitoring: the EU-ADR Project. *Pharmacoepidemiol Drug Saf* 2011;20(1):1-11.
47. Higgins JPT, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. *J R Stat SocSer A Stat Soc* 2009;172(1):137–159.
48. Ohara H, Nakamura Y, Watanabe Y, et al. Azithromycin Can Prolong QT Interval and Suppress Ventricular Contraction, but Will Not Induce Torsade de Pointes. *Cardiovasc Toxicol* 2015;15(3):232-240.

49. Nakajima T, Kaneko Y, Kurabayashi M. Unveiling specific triggers and precipitating factors for fatal cardiac events in inherited arrhythmia syndromes. *Circ J* 2015;79(6):1185-1192.
50. Pasquié JL, Sanders P, Hocini M, et al. Fever as a precipitant of idiopathic ventricular fibrillation in patients with normal hearts. *J Cardiovasc Electrophysiol* 2004;15(11):1271-1276.

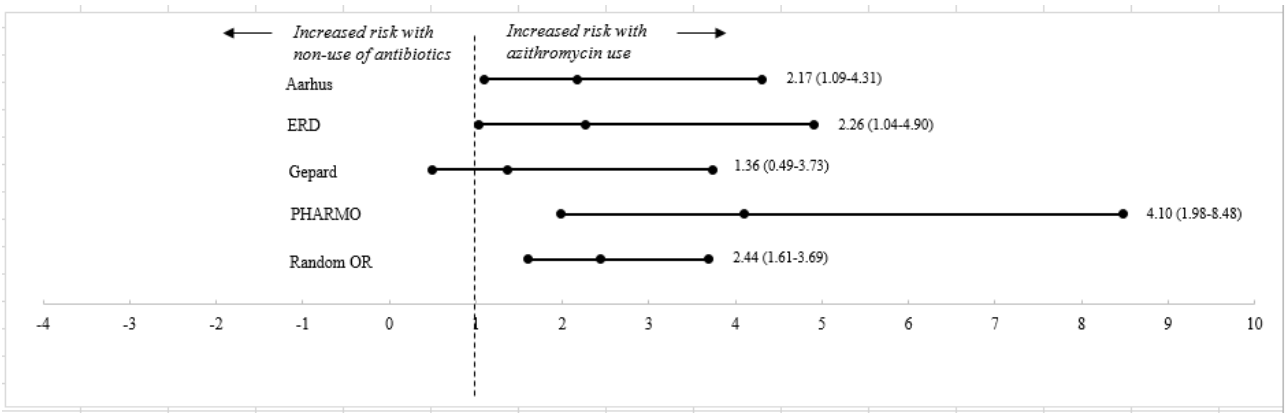
Figures

Figure 1: Meta-analysis of database-specific risk of ventricular arrhythmia for current use of azithromycin vs. current use of amoxicillin using a random effects model (two-stage pooling). Cochran's Q: 0.08; p-value: 0.1; I^2: 50.68. Databases with less than 3 exposed cases were not included in the model.



Database	Description	X	Y
Aarhus	ES	0.68	5
	Lower CI	0.22	5
	Upper CI	2.09	5
ERD	ES	1.39	4
	Lower CI	0.32	4
	Upper CI	5.97	4
Gepard	ES	0.28	3
	Lower CI	0.06	3
	Upper CI	1.44	3
PHARMO	ES	3.17	2
	Lower CI	0.88	2
	Upper CI	11.43	2
Random OR	ES	1.01	1
	Lower CI	0.38	1
	Upper CI	2.64	1

Figure 2: Meta-analysis of database-specific risk estimates for current use of azithromycin vs. non-use of any antibiotic and ventricular arrhythmia using a random effects model (two-stage pooling. Cochran's Q: 3.4; p-value 0.3; I^2:11.4. Databases with less than 3 exposed cases were not included in the model.



Study	Description	X	Y
Aarhus	ES	2.17	5
	Lower CI	1.09	5
	Upper CI	4.31	5
ERD	ES	2.26	4
	Lower CI	1.04	4
	Upper CI	4.90	4
Gepard	ES	1.36	3
	Lower CI	0.49	3
	Upper CI	3.73	3
PHARMO	ES	4.10	2
	Lower CI	1.98	2
	Upper CI	8.48	2
Random OR	ES	2.44	1
	Lower CI	1.61	1
	Upper CI	3.69	1

Table 1. Demographic and clinical characteristics of ventricular arrhythmia cases and controls in an inception cohort of new users of antibiotics across all the databases (one-stage pooling).

Covariates	Cases N= 12,874 (%)	Controls N=1,240,431 (%)	p-value
Mean age ± SD (years)	63.5 ± 15.3	63.6 ± 15.4	Matching factor
Age groups			
< 60	4,210 (32.7)	405,621 (32.7)	
60-79	7,106 (55.1)	678,516 (54.7)	
≥80	1,558 (12.1)	156,294 (12.6)	
Sex			Matching factor
Males	8,561 (66.5)	824,887 (66.5)	
Females	4,313 (33.5)	415,544 (33.5)	
Well- known risk factors for VA			
Atrial fibrillation/flutter	2,332 (18.1)	61,844 (5.0)	<0.001
Cardiomyopathies	1,097 (8.0)	10,887 (0.9)	<0.001
Cerebrovascular events	1,539 (11.9)	81,444 (6.6)	<0.001
Coronary heart disease	5,919 (46.0)	229,811 (18.5)	<0.001
Electrolyte imbalance	1,100 (8.5)	57,663 (4.6)	<0.001
Heart failure	2,913 (22.6)	54,114 (4.4)	<0.001
Hypertension	10,254 (79.6)	730,389 (58.9)	<0.001
Peripheral arterial disease	626 (4.9)	26,970 (2.2)	<0.001
Prior use of anti-arrhythmic drugs	1,009 (7.8)	13,865 (1.1)	<0.001
Concomitant use* of medications inducing hypokalemia	4,661 (36.2)	211,284 (17.0)	<0.001
Concomitant use* of QT prolonging drugs**	2,089 (16.2)	75,177 (6.1)	<0.001
Potential risk factors for ventricular arrhythmia			
Alcohol abuse	343 (2.6)	12,157 (1.0)	<0.001
Chronic liver disease	683 (5.3)	43,325 (3.5)	<0.001
Chronic respiratory disease	5,310 (41.2)	390,364 (31.5)	<0.001
Conduction disorders	1,146 (8.9)	27,911 (2.2)	0.001
Congenital heart disease	151 (1.2)	2,968 (0.2)	<0.001
Diabetes mellitus	2,395 (18.6)	140,208 (11.3)	<0.001
Hyperthyroidism	300 (2.3)	16,195 (1.3)	<0.001
Hypothyroidism	1033 (8.0)	68,233 (5.5)	<0.001
Acute and chronic renal failure	802 (6.2)	23,324 (1.9)	<0.001
Lipid metabolism disorders	5906 (45.9)	351,428 (28.3)	<0.001
Obesity	1,223 (9.5)	71,835 (5.8)	<0.001
Other cardiac arrhythmias***	1,394 (10.8)	34,920 (2.8)	<0.001
Cardiac valve disorders	1,012 (7.9)	28,970 (2.3)	<0.001

Legend- SD: standard deviation.
* Within three months prior to index date;
** Use of drugs with established TdP liability, based on CredibleMeds list;
***Except for atrial fibrillation/flutter, conduction disorders, QT prolongation, ventricular arrhythmia and sudden cardiac death;
Smoking information was partly available only in two databases and for this reason it was not included in the final multivariate models.

Table 2. Risk of ventricular arrhythmia associated with current, recent and past use of azithromycin compared to current use of amoxicillin (one-stage pooling).

	Cases N (%)	**Controls N (%)**	**Unadjusted OR* (95% CI)**	**Adjusted OR** (95% CI)**
Current use of amoxicillin	165 (34.45)	370 (20.02)	**Reference**	**Reference**
Azithromycin				
Current use	30 (6.26)	88 (4.76)	1.05 (0.63-1.74)	0.94 (0.50-1.77)
Recent use	107 (22.34)	483 (26.14)	0.60 (0.44-0.83)	0.58 (0.38-0.87)
Past use	177 (36.95)	907 (49.08)	0.49 (0.36-0.66)	0.52 (0.37-0.73)

Abbreviations: CI- Confidence Interval; OR- odds ratios; **Legend**: Current use: exposure period covered the index date or ended <7 days before the index date; recent use: exposure period ended between 7 and <90 days before the index date; past use: exposure period ended between 90 and <365 days before the index date.

* The crude ORs are estimated for matched case-control pairs and cannot be calculated directly from the table above.

**Adjusted for risk factors of ventricular arrhythmia: atrial flutter/fibrillation, cardiomyopathy, coronary heart disease, cerebrovascular disorders, chronic obstructive lung disease, electrolytic imbalance, heart failure, hypertension, diabetes mellitus, lipid disorders, peripheral arterial disease, hypothyroidism, prior use of antiarrhythmic drugs and concomitant use of QT prolonging drugs and drugs which cause hypokalemia.

Current users of both azithromycin with other antibiotics were not included in the analysis.

Table 3. Risk of ventricular arrhythmia associated with exposure to azithromycin and other antibiotics by recency of use versus non-use of any antibiotic (one-stage pooling).

	Cases N (%)	**Controls N (%)**	**Unadjusted OR* (95% CI)**	**Adjusted OR** (95% CI)**
Non-use of any antibiotic	5,060 (39.1)	601,049 (48.4)	**Reference**	**Reference**
Azithromycin				
Current use	30 (0.2)	1,344 (0.1)	2.83 (1.97-4.08)	1.97 (1.35-2.86)
Recent use	109 (0.8)	8,315 (0.7)	1.65 (1.36-2.00)	1.12 (0.92-1.37)
Past use	187 (1.4)	18,000 (1.5)	1.28 (1.10-1.48)	1.10 (0.95-1.28)
Any other antibiotic				
Current use	1,026 (8.0)	52,543 (4.2)	2.43 (2.27-2.60)	1.83 (1.71-1.97)
Recent use	2,617 (20.3)	193,154 (15.6)	1.69 (1.61-1.77)	1.32 (1.25-1.39)
Past use	3,845 (29.9)	366,026 (29.5)	1.29 (1.23-1.34)	1.11 (1.06-1.16)

Abbreviations: OR: odds ratio; CI: Confidence Interval; **Legend**: Current use: exposure period covered the index date or ended <7 days before the index date; recent use: exposure period ended between 7 and <90 days before the index date; past use: exposure period ended between 90 and <365 days before the index date.

* The crude ORs are estimated for matched case-control pairs and cannot be calculated directly from the table above.

**Adjusted for atrial flutter/fibrillation, cardiomyopathy, coronary heart disease, cerebrovascular disorders, chronic obstructive lung disease, electrolytic imbalance, heart failure, hypertension, diabetes mellitus, lipid disorders, peripheral arterial disease, hypothyroidism, prior use of antiarrhythmic drugs and concomitant use of QT prolonging drugs and drugs which cause hypokalemia.

Current users of both azithromycin with other antibiotics are not included in the analysis.