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Infection-associated biofilms and statins: protocol for systematic review

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ABSTRACT

Introduction Owing to their propensity for being associated with infections, biofilms have become a focus in infectious disease research. There is evidence suggesting that statins, which are commonly used for prevention of cardiovascular disease, may prevent biofilm-associated infections, but this association has not been well-understood.

Methods and analysis This systematic review protocol will include six database searches from their inception to 20 August 2020. A medical librarian will conduct the searches in PubMed, EMBASE, Web of Science, CINAHL, LILACS and CENTRAL, without any limits. Bibliographies of selected articles, previously published reviews and high-yield journals that publish on statins and/or biofilms will be searched to identify additional articles. The screening and data extraction will be conducted by two independent reviewers using DistillerSR. All included papers will also be evaluated for quality using Cochrane Risk of Bias Assessment tool, and we will examine for publication bias. If there are two or more studies with quantitative estimates that can be combined, we will conduct a meta-analysis after assessing for heterogeneity. We will report all findings according to the Preferred Reporting Items for Systematic reviews and Analyses-P framework.

Ethics and dissemination There are conflicting results on the effect of statins on biofilm-associated infections. The rise of antibiotic resistance in medical settings warrants a deeper understanding of this association, especially if statins can be used as a novel antibiotic. The findings of this review will assess the association between statin use and biofilm-associated infection to inform future medical practice. No formal ethical review is required for this protocol. All findings will be published in a peer-reviewed journal.

PROSPERO registration number CRD42020193985.

Strengths and limitations of this study

- This review will access >3 databases, multiple other sites, and will have no language limits.
- We will be assessing the quality of studies.
- If data are available, we will conduct meta-analysis.
- There is potential for heterogeneity in included articles.
- Reproducibility of the study based on ability to pay for access to the databases like EMBASE and Web of Science, and shift in the PubMed platform from Legacy PubMed to the New PubMed might be limited.

BACKGROUND

Biofilms are an 'assemblage of surface-associated microbial cells enclosed in an extracellular polymeric substance matrix'.[1] Although they are sometimes beneficial to cellular processes in both plants and animals, biofilms also promote chronic infection by *Staphylococcus aureus* and other bacteria.[2] Developing frequently at the intersection between surface and aqueous media, they also pose significant risks for patients with implanted medical devices.[1] In addition, a growing number of studies demonstrate that biofilms are a significant source of antibiotic-resistance, particularly in nosocomial settings.[1 2] With a shrinking number of tools available to address common bacterial infections, they have therefore become a major focus of research on infectious disease.[3 4]

Statins are commonly used for primary prevention of cardiovascular disease.[5–8] As a class, they have high safety and tolerability with few side effects when used for lowering lipids in patients with elevated cholesterol.[5] Although all statins act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase to reduce cholesterol synthesis, they can be lipophilic or hydrophilic depending on their tissue selectivity.[2 6] Studies suggest that while both classes have similar lipid-lowering outcomes, they vary in their anti-inflammatory, anticancer and antimicrobial effects.[5] Simvastatin, for instance, has been shown to have activity against clinical isolates and biofilms of methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus*—suggesting it might have a role in treatment of *S. aureus* infections.[5] Atorvastatin, another lipophilic statin has also been shown to abrogate biofilm formation in periodontal disease.[7 8] Fewer antimicrobial and antibiofilm effects have been documented in hydrophilic statins.[9 10] Whether either class of drug is efficacious eradicating or preventing

biofilms remains controversial; however, as research shows mixed results for statin treatment of biofilm-forming bacterial infections.[10]

The mechanisms by which statins inhibit bacterial growth is unclear. There is some evidence that they modulate toll-like receptors, host sentinels of innate and adaptive immunity and operate through anti-inflammatory pathways shown to reduce the formation of biofilms.[11 12] Another possible mechanism is statin effects on metalloproteases (matrix metalloproteases (MMPs)) shown to prolong inflammatory response and fuel biofilm formation.[13] Studies have shown that specific statins inhibit the expression of MMP-1, MMP-2, MMP-8 and MMP-9 both in vivo and in vitro.[14 15] Finally, the cholesterol-lowering effects of statins may extend to bacterial pathogens as cholesterol is a critical component of bacteria membrane integrity.[16] Some biofilm-producing organisms like *Salmonella* also bind and form biofilms on cholesterol particles. Therefore, reducing levels of circulating lipids may limit the potential for biofilm formation.[17]

Research on the control and prevention of biofilms is currently being pursued in variety of fields including wound treatment, periodontal infections, vaginal conditions like bacterial vaginosis and vaginal candidiasis, nosocomial infections, medical device implantation and antimicrobial resistance.[18] Statins may offer new hope for the treatment and the prevention of one of bacteria's most recalcitrant features—but there currently remains controversy around their usefulness. As a consequence, there is a compelling need for systematic review of the literature and determination of whether a meta-analysis of statin effects on biofilm is warranted. Consequently, this review will examine the literature on statins and their effects on biofilm formation and prevention.

METHODS

This systematic review and meta-analysis will be conducted according to the Cochrane Handbook for Systematic Reviews of Interventions (V.6.1) and the findings will be reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[19 20]

Patient and public involvement

Patients and the public were not involved in setting the research question or the outcome measures.

Purpose of the protocol

To conduct a detailed synthesis of the literature examining the effects of statins on biofilm-associated infections.

Eligibility criteria

Types of studies to be included

Observational and experimental study designs will be included (cohort, case–control, cross-sectional and clinical studies). We will not include case reports. There will be no limitations with regards to the number of participants in the studies.

Participants/population

Participants may be of any age, with and without evidence of an infection associated with biofilm formation.

1. Inclusion: Individuals who were treated with any statins and had a biofilm-associated infection.
2. Exclusion: Animal studies, lab studies and in vitro studies.

Intervention(s), exposure(s)

Studies comparing any antibiotic regimen plus statins (intervention group) as compared with the same antibiotic regimen without statins (control group). Studies in which statins were offered by any route of administration (orally or intravenously), and with any dose, provided the same route and the same dose were used in both experimental and control groups.

Comparator(s)/control

We will include studies with a placebo replacing the statin, as well as studies of antibiotic regimens alone (without placebo).

Types of outcome measures

Successful reduction of the infection associated with biofilm formation shown from measures of effect such as standard mean difference and HR.

Eligibility criteria

Inclusion criteria

1. Research findings published in peer-reviewed journals.
2. The iterature published since September 1987.

Exclusion criteria

1. Animal studies.
2. Dissertation/theses.
3. Studies without reported estimates.
4. Laboratory in vitro studies.

Information sources and search strategy

Primary search databases

As the first statin was approved for use in September 1987, only paper published between that date and August 2020, will be included. However, no other restrictions on language or status of publication when searching the electronic databases or other resources will be included. A literature search will be conducted to identify all published and unpublished studies. The following electronic databases will be searched for identifying potential studies: MEDLINE, EMBASE, Cochrane Central Register for Clinical Trials, Web of Science, CINAHL and LILACS. We will include all articles published in these databases beginning in September 1987. Search strategies will be translated for each database. We will include controlled vocabulary (eg, MeSH and Emtree) and keyword search terms to identify studies. An example of the search strategy for PubMed is listed below. The full set of search terms for each database will be included at the time of publication of the systematic review. We will scan the reference lists of eligible studies and relevant review articles to identify any

potentially eligible studies to be included into the review. The searches will be conducted by a medical librarian (DJMC) who has the necessary training and skills in conducting database searches. There will be no language limits, and translations will be requested for articles published in languages other than English.

Secondary hand-search of databases

We will also include the following databases, including pharmaceutical company databases, to be searched for additional studies on the association between statins and biofilm:

1. Health Management Information Consortium database.
2. National Technical Information Service database.
3. OpenGrey.
4. AstraZeneca Clinical Trials.
5. Bristol-Myers Squibb Clinical Trial Registry.
6. Merck Clinical Trial Registry.
7. Pfizer Clinical Trial Registry.
8. ClinicalTrials.gov.
9. Current Controlled Trials metaRegister of Controlled Trials.
10. Eli Lilly and Company Clinical Trial Registry.
11. EU Clinical Trials Register.
12. International Clinical Trials Registry Platform Search Portal.
13. International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) Clinical Trials Portal.
14. Roche Clinical Trials Results Database.

Within the above databases, studies will be selected depending on their use of statins for infection-related treatments.

We will conduct manual searches of the table of contents for the following journals published between September1987 and up to August 2020:

1. *New England Journal of Medicine*.
2. *Antimicrobial Agents and Chemotherapy*.
3. *Cell, Host and Microbe*.
4. *Medical Mycology*.
5. *JAMA Cardiology*.
6. *Medical Mycology*.
7. *JAMA Internal Medicine*.

Search terms for PubMed

Infection OR "infections" [MeSH]
AND
biofilm OR biofilms OR bacterial adhesions OR bacterial adhesin OR biofouling OR plaque OR Microcosm OR microcosms OR multi-species OR defined-multispecies OR multispecies OR microbial consortia OR microbial consortium OR "biofilms" [mesh] OR "bacterial adhesion" [mesh] OR "Adhesins, Bacterial" [Mesh] OR "dental plaque" [mesh] OR "Microbial Consortia" [Mesh]
AND
statin OR statins OR hydroxymethylglutaryl-coa reductase inhibitors OR HMG-CoA OR HMGCoA OR "Hydroxymethylglutaryl-Coenzyme A" OR atorvastatin OR Lipitor OR Torvast OR Fluvastatin OR Lescol OR Pravachol OR Lipostat OR Selektine OR Rosuvastatin OR Crestor OR Simvastatin OR Zocor OR Lipex OR Pravastatin OR Lovastatin OR Mevacor OR Altocor OR Altoprev OR Pitavastatin OR Livalo OR Pitava OR cerivastatin OR "hydroxymethylglutaryl-coa reductase inhibitors"[MeSH] OR "Lovastatin" [Mesh] OR "Pravastatin" [Mesh] OR "Simvastatin" [Mesh].

Study records

Screening of articles to be selected into the review

Titles and abstracts will be identified and retrieved from the included databases using the search strategy. Hand-searches or high-yield journals, grey literature and pharmaceutical company databases will also be conducted to identify additional papers pertaining to statins and their use to prevent or treat infection. Hand-searches will also be conducted according to our inclusion and exclusion criteria. Once all database and hand-searches have been completed, references will be deduplicated in EndNote and exported to DistillerSR for review. This will be done by an experienced librarian familiar with database searches.

DistillerSR is a systematic review software that streamlines screening and data extraction for reviewers. Within this software, the review team will be assigned to specific tasks in the screening, full-text review and data extraction processes. Each title and abstract will be screened independently by two review authors to identify studies that will be included in the review based on previously stated inclusion criteria. Studies that meet the criteria for inclusion after title and abstract review will undergo full-text review by two study authors. Discrepancies will be resolved by a third member of the review team. Data will be extracted from studies that are included after full-text review using standardised extraction forms that are automated, but also customisable, in DistillerSR. Data extracted within DistillerSR will be exported for further analysis.

Data

Data management and extraction

A standardised form created in DistillerSR will be used to extract data from the included studies. The data we will collect from each study include study setting, study population, participant demographics, description of the intervention including statin(s) used, dosage, route of administration and duration. We will also describe primary and secondary outcomes, frequency of outcome measurements, attrition rates, longitudinal studies outcomes and times of measurement. At all of these stages, if there is any discordance between two review authors, we will have a third author serve as a tiebreaker.

If statistical measures of effect are presented in two or more papers, we will conduct subgroup analyses for the kinds of infections, populations, study design, age (paediatric vs adult) groups and types of statins. Sensitivity

analysis will be performed by study design (experimental and observational studies) and by quality of the studies.

Risk of bias and quality assessment

We will use the Cochrane Bias Assessment Tool to determine the risk of bias in the studies.[21] This will be assessed independently by two reviewers. We will use the Newcastle-Ottawa Quality Assessment Scale to assess the risk of bias of cohort and case–control studies.[22]

Data synthesis

If there are measures that can be synthesised appropriately, we will summarise our findings using quantitative methods. However, if quantitative synthesis is inappropriate, we will describe the study findings using narrative analysis. A spreadsheet will be created to abstract data on all variables, and to determine concordant and discordant conclusions about articles included in the review. Data will be summarised in tables. We will extract both the unadjusted and adjusted measures of effect from the selected studies.

If there are statistical measures of effect presented in two or more papers, we will assess the heterogeneity of the studies. A meta-analysis will be conducted if heterogeneity is not a major concern. Heterogeneity will be determined using the χ^2 and I^2 tests. We will pool data with a random effects model when there are sufficient studies to summarise. If heterogeneity is identified (I^2 >40%) and there are sufficient number of studies included in the review, we will examine for reasons for heterogeneity. Subgroup analyses and sensitivity analyses will be considered if they are necessary.

We will assess for publication bias by use of funnel plots when the number of studies reported is >9 studies. All analyses will be conducted using STATA statistical software. All tests will be two-tailed, and p<0.05 will be considered statistically significant.

We will use the Grading of Recommendations Assessment, Development and Evaluation methodology to rate the certainty of evidence as high, moderate, low or very low.[23] Register of Controlled Trials begin as high certainty evidence but can be rated down because of risk of bias, imprecision, inconsistency, indirectness and publication bias. If the limitation of the evidence is considered serious, the evidence is downgraded by one level; if the limitation of the evidence is considered serious, the evidence is downgraded by two levels. Observational studies begin as low-quality evidence but can be rated upwards for a large magnitude of effect, a dose–response gradient or the presence of plausible confounders or other biases that increase confidence in the estimated effect.

ETHICS AND DISSEMINATION

There are conflicting results on the effect of statins on biofilm-associated infections. The rise of antibiotic resistance in medical settings warrants a deeper understanding of this association, especially if statins can be used as a novel antibiotic. The findings of this review will assess the association between statin use and biofilm-associated infection to inform future medical practice. No formal ethical review is required for this protocol. All findings will be published in a peer-reviewed journal.

Contributors PM and KK: developed research question. KK: responsible for writing the background of the protocol. DJMC: developed the search strategy presented in the protocol. DYV and MH: responsible for writing the protocol under the supervision of PM and DJMC. AD and PM: worked with quantitative analysis.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

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