BMJ Open

Bacterial antibiotic resistance development and mutagenesis following exposure to sub-minimal inhibitory concentrations of fluoroquinolones: a systematic literature review protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030747
Article Type:	Protocol
Date Submitted by the Author:	01-Apr-2019
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Keywords:	Microbiology < BASIC SCIENCES, INFECTIOUS DISEASES, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

Bacterial antibiotic resistance development and mutagenesis following exposure to sub-minimal inhibitory concentrations of fluoroquinolones: a systematic literature review protocol

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Word Count: 1248 (excluding tables)

ABSTRACT

Introduction: Antimicrobial resistance (AMR) is among the most pressing global health challenges. However, while many drivers are known, the impact of medicine quality on AMR remains largely elusive. The aim of this review is to systematically evaluate evidence on sub-inhibitory levels of antibiotic, a major tenant of substandard antibiotics, on AMR, using fluoroquinolones as a case study.

Methods and analysis: PubMed, EMBASE and Web of Science will be systematically searched for primary experimental studies related to sub-inhibitory fluoroquinolone treatment and AMR. A specifically developed non-weighted quality assessment tool will be used. Subgroup analyses will be performed for different variables and outcomes.

Ethics and dissemination: Ethical approval is not required as no primary data is to be collected. The completed systematic review will be disseminated through conference meeting presentations and a peer-reviewed publication.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first systematic review of this body of evidence
- NCBI PubMed, ISI Web of Science and Elsevier Embase will be searched for impact of sub-inhibitory concentrations of fluoroquinolones on antimicrobial resistance without date or language limitation
- Microbiological experimental evidence will be in the context of an important global health issue, medicine quality following Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) protocol standards.
- Even though two researchers will independently review study titles some relevant studies may be missed

INTRODUCTION

Antimicrobial resistance (AMR) is a global health threat. To be able to fully address this problem from clinical and policy standpoints it is paramount to understand all of the drivers of AMR development and spread. An understudied possible driver is the prevalence and use of poor quality medicines, specifically substandard antibiotics. Substandard drugs are defined by the World Health Organization as "*authorized medical products that fail to meet either their quality standards or their specifications, or both*" [1]. The threat of substandard antibiotic usage is highest in low and middle income countries, where the failure rate for antibiotics to meet quality standards has been reported to be greater than 7% [1].

Substandard antibiotics often contain wrong, or inadequate levels of the active pharmaceutical ingredient (API) (below the stated concentration or quality standards) or have poor dissolution.

This results in the treatment of bacteria at sub-inhibitory concentrations below their minimal inhibitory concentration (MIC). Thus, there is not enough API to completely clear the bacterial infection but there may be enough API to provide selective pressure for AMR development. Thus, medicine quality is a potentially important driver of AMR, however there is currently a lack in direct evidence to support this hypothesis [2].

Here, we seek to fill this important gap by systematically synthesizing experimental evidence on how sub-inhibitory concentrations of one specific class, fluoroquinolones, impacts AMR. We further aim to identify gaps in evidence for the relationship between substandard antibiotics usage and AMR development. Currently, there are only a few broad narrative literature reviews on the impacts sub-inhibitory concentrations of antibiotics [3–6]. This systematic review aims to merge experimental evidence with public health implications. Furthermore, to our knowledge, there are currently few to no systematic reviews of basic microbiological experimental studies. Thus, we seek to apply Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines to a systematic review on experimental microbiological data in an effort for more unbiased literature summaries. In summary, the results of this systematic review will contribute to understanding the potential effects of poor quality medicines on antibiotic resistance acquisition, identify gaps in evidence, and inform policy-making on the control of substandard medicines.

Systematic review questions

This review seeks to address the following questions:

1. Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis?
2. What is the potential for substandard drugs to influence antibiotic resistance development?

METHODS

Our methodology will conform to the PRSMA reporting standards (Appendix 1, PRISMA-P Checklist). The protocol does not currently exist elsewhere and is ineligible for hosting on PROSPERO.

Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in this work.

Eligibility Criteria

To define the search approach, inclusion and exclusion criteria and identify the outcomes we applied a Population Intervention Comparator Outcome Study (PICOS) search tool. The criteria is presented in Table. 1.

Table 1. Population Intervention Comparator Outcome Study (PICOS) Design Criteria

	Include	**Exclude**
Population	Bacteria (All Wild and Resistant Isolates of Gram-negative and Gram-positive Species) • pathogenic • non-pathogenic • clinical • environmental • community-acquired	Eukaryotes (All) Archaea

	• lab/domesticated strains	
Intervention (Treatment)	Treatment with ranges of fluoroquinolone concentrations with levels below the defined minimum inhibitory concentration (MIC), *in vitro*. All fluoroquinolones will be included.	Treatment at MIC or above MIC, if sub-MIC treatment is not considered. Treatment with sub-MIC fluoroquinolone concentrations in synergy with another class of antibiotic or compound. Treatment with first-generation quinolone antibiotics or other classes of antibiotics. Purely computational models. Studies involving animals.
Comparator	No treatment (same experimental conditions, 0% API)	
Outcomes	Experimental microbiological data related to: • resistance acquisition (to same or other antibiotic) and mutagenesis rate Examples of data include standard	Outcomes from studies that treat bacteria with sub-lethal levels but do not follow-up with results related to resistance acquisition, mutagenesis or gene expression. Examples of results to exclude include community behavior, such as surface cell adhesion and biofilm formation, virulence (persister formation, toxin/antitoxin systems) and

	microbiological assays, genomic sequencing and transcriptomics. Discussion of substandard of poor quality medicines.	plasmid curing. Studies investigating prophylaxis (long-term low dose treatment).
Study Design	Primary Experimental Studies (All Languages) published from 1966-2018 on NCBI PubMed, from 1965-2018 on ISI Web of Science and from 1947-2018 on Elsevier Embase.	Conference abstracts Foreign language articles Grey literature Review articles (no primary data) Observational Studies

Outcomes, Prioritization and Data Extraction

The primary outcome extracted will be the effect on exposure to sub-inhibitory concentrations of fluoroquinolones on antibiotic resistance acquisition and mutagenesis rates. Secondary outcomes will be whether these papers discuss substandard or poor quality medicines will also be extracted. Our rationale for prioritization is that we first need to determine the link between exposure and

resistance acquisition. After quantifying and evaluating the evidence, we aim to assess how frequently primary scientific papers include public health context (not just clinical). Other variables extracted from each study will include year of publication, bacterial species and number of strains strain, type of bacterial isolate, drug name and concentration, and study design (duration of treatment, growth condition, etc.). Study quality and limitations, and gaps in evidence for review questions will also be extracted in addition to reporting on weaknesses in studies and how to improve experimental design. Data will be extracted to a standardized Excel table.

Search Strategy

The search strategy was based on objectives and a preliminary search of PUBMED to determine relevant MeSH terms. Using MeSH terms and keyword synonyms along with identified terms for sub-inhibitory and substandard, a search strategy was designed in PUBMED and translated to Web of Science and Embase to search all fields for articles that fit the inclusion criteria above (Table 1). Complete search terms and details are listed below. Additional records will be identified through searching the bibliographies of already-identified papers and searching through papers that have cited key studies. Only those papers that also match the above inclusion criteria will be included. Complete search terms are provided in Appendix 2.

Identified terms: *subinhibitory, sub-inhibitory, sub inhibitory, sub-lethal, sublethal, sub lethal, subminimal, sub-minimal, sub minimal, sub-therapeutic, subtherapeutic, sub therapeutic, sub MIC, sub-MIC, low-dose, low dose, substandard, sub-standard, counterfeit, falsified*

Study records

Records will be managed through reference management software Endnote and Mendeley. Additionally, search histories will be saved. Abstract screening and selection of studies will be

performed independently by two independent reviewers using software Rayyan QCRI [7]. A third researcher will resolve discrepancies between reviewers selections. The full text of articles from the initial screening will be reviewed for inclusion. Each paper will be analyzed and key results extracted to a standardized table for comparison.

Risk of bias in individual studies

Risk of Bias for laboratory microbiology experimentation will be assessed with criteria formulated by considering and adapting Systematic Review Centre for Laboratory animal Experimentation (SYRCLE)'s risk of bias tool for animal studies [8] and the Effective Public Health Practice Project (EPHPP) quality assessment tool [9]. The criteria is presented in Table 2. Here, we present a non-weighted assessment of individual study quality, including risk of bias. For each domain, studies will be assessed for a series of criteria listed below. For each unmet criteria an increased risk of bias point will be assigned. The more points assigned, the higher the risk of bias associated with the study.

Table 2. Assessment of risk of bias for laboratory microbiology experimentation

Domain	Description of domain	Review Criteria
Baseline characteristics of bacteria/ Confounders (Selection Bias)	Describe possible genetic or environmental variations to determine whether results for different strains of the same species can be compared. For clinical isolates, genotype is not required.	Were the groups compared individually or were differences discussed in the analysis? Were species and strain details provided?

Domain	Description of domain	Review Criteria
Study Design/Methods	Reproducibility and detail of study design and methods. Description of analysis methods.	Are there any discrepancies between methods and in-text? Is the methodological section missing any steps or appropriate detail ? (including but not limited to below) Steps/Details: -media used -temperature -time -incubation conditions (static, rolling, shaking, aeration) - reagents used - concentrations used - appropriate control experiments

Domain	Description of domain	Review Criteria
		-replication of experiments
Incomplete outcome data (Attrition Bias)	Describe the completeness of outcome data being analyzed, including attrition and exclusions from the analysis.	Is there missing outcome data that was not addressed? Is the control outcome data mentioned in the paper present?
Selective outcome reporting (Reporting bias)	Reporting of aim and all outcomes of the study.	Was all data reported for all conditions or just statistically significant results? Was it clear whether no change results were reported? Was statistical significance noted (if possible)? Is the appropriate comparison to baseline provided?
Other sources of bias	State any important concerns about bias not covered by other domains.	Was the study apparently free of concerns about bias?

Domain	Description of domain	Review Criteria
Global Bias Rating	Summary of all five domains	Calculate total quality points. The more points the higher the risk of bias.

Data Synthesis

Meta-analysis may not be possible based on findings. If possible meta-synthesis will be performed to group sets of results based on bacteria or methodology.

Meta-bias(es)

Depending on data synthesis parameters, the overall quality of the body of evidence will be determined, if possible. We will take into account publication bias across studies. This includes any potential publication biases in authors or types of studies, biases towards certain bacteria, antibiotics and selective outcomes. Additionally inconsistencies in methodology and outcomes will be assessed. This goal will be to determine confidence in reported recommendations or trends.

Ethics and Dissemination

Ethical approval is not required as no primary data is to be collected. The completed systematic review will be disseminated through conference meeting presentations and a peer-reviewed publication.

Authors Contributions

C.C., V.J.W and M.H.Z. initiated work. C.C., E.S.F.O., V.J.W and M.H.Z designed and wrote protocol.

Acknowledgements

We would like to acknowledge David Flynn from Boston University Medical Alumni Library for assistance with search terms.

Funding statement

This work was funded by a fellowship to C.C. by the United States Pharmacopeia.

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Appendix 2: Search Terms

PUBMED

Search Term*:

((quinolone) AND (subinhibitory OR sub-inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR “sub minimum” OR sub-therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified)) AND (Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance, Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene expression OR transcription or transcriptional)

Complete Search Term:

(("quinolones"[MeSH Terms] OR "quinolones"[All Fields] OR "quinolone"[All Fields]) AND (subinhibitory[All Fields] OR sub-inhibitory[All Fields] OR "sub inhibitory"[All Fields] OR sub-lethal[All Fields] OR sublethal[All Fields] OR "sub lethal"[All Fields] OR subminimal[All Fields] OR sub-minimal[All Fields] OR "sub minimal"[All Fields] OR subminimum[All Fields] OR sub-minimum[All Fields] OR "sub minimum"[All Fields] OR sub-therapeutic[All Fields] OR subtherapeutic[All Fields] OR "sub therapeutic"[All Fields] OR "sub MIC"[All Fields] OR sub-mic[All Fields] OR low-dose[All Fields] OR "low dose"[All Fields] OR substandard[All Fields] OR "sub standard"[All Fields] OR sub-standard[All Fields] OR counterfeit[All Fields] OR falsified[All Fields])) AND (("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "drug resistance, microbial"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antimicrobial"[All Fields] AND "drug"[All Fields] AND "resistance"[All Fields]) OR "antimicrobial drug resistance"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antimicrobial"[All Fields] AND "drug"[All Fields] AND "resistances"[All Fields]) OR "antimicrobial drug resistances"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) OR (Microbial[All Fields] AND ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields])) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("resistance"[All Fields] AND "antibiotic"[All Fields]) OR "resistance, antibiotic"[All Fields]) OR Resistance[All Fields] OR Resistant[All Fields] OR ("mutagenesis"[MeSH Terms] OR "mutagenesis"[All Fields]) OR ("mutation"[MeSH Terms] OR "mutation"[All Fields]) OR Mutagenicity[All Fields] OR ("gene expression"[MeSH Terms] OR ("gene"[All Fields] AND "expression"[All Fields]) OR "gene expression"[All Fields]) OR ("transcription, genetic"[MeSH Terms] OR ("transcription"[All Fields] AND "genetic"[All Fields]) OR "genetic transcription"[All Fields] OR "transcription"[All Fields]) OR ("transcription, genetic"[MeSH Terms] OR ("transcription"[All Fields] AND "genetic"[All Fields]) OR "genetic transcription"[All Fields] OR "transcriptional"[All Fields]))

Web of Science

Search Term*:

TS=(quinolone* OR fluoroquinolone* OR ciprofloxacin OR norfloxacin OR ofloxacin OR levofloxacin OR moxifloxacin OR gemifloxacin or enrofloxacin) AND TS=(subinhibitory OR sub-inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR “sub minimum” OR sub-therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified) AND TS=(Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance, Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene expression OR transcription or transcriptional)

Embase

Search Term*:

(subinhibitory OR 'sub inhibitory' OR 'sub-lethal' OR sublethal OR 'sub lethal' OR subminimal OR 'sub-minimal' OR 'sub minimal' OR subminimum OR 'sub-minimum' OR 'sub minimum' OR 'sub-therapeutic' OR subtherapeutic OR 'sub therapeutic' OR 'sub mic' OR 'sub-mic' OR 'low-dose' OR 'low dose' OR substandard OR 'sub standard' OR 'sub-standard' OR counterfeit OR falsified) AND ('quinoline derived antiinfective agent'/exp OR 'anti infective agents, fluoroquinolone' OR 'anti infective agents, quinolone' OR 'anti-infective agents, fluoroquinolone' OR 'anti-infective agents, quinolone' OR 'antibiotics, quinolones' OR 'chinolone antibiotic' OR 'fluoroquinolone (antibiotic)' OR 'fluoroquinolone antibiotic' OR 'fluoroquinolone antibiotic agent' OR 'fluoroquinolone antiinfective agent' OR 'fluoroquinolone antimicrobial agent' OR 'quinoline derived antiinfective agent' OR 'quinolone (antibiotic)' OR 'quinolone antibiotic' OR 'quinolone antibiotics' OR 'quinolone antiinfective agent') AND ('antibiotic resistance'/exp OR 'antibacterial drug resistance' OR 'antibacterial resistance' OR 'antibiotic resistance' OR 'antimicrobial drug resistance' OR 'antimicrobial resistance' OR 'bacterial drug resistance' OR 'bacterial resistance' OR 'bacterium resistance' OR 'drug resistance, bacterial' OR 'drug resistance, microbial' OR 'microbial drug resistance' OR 'resistance' OR 'resistant' OR 'gene expression'/exp OR 'expression, gene' OR 'gene expression' OR 'genetic expression' OR 'genome expression' OR 'genetic transcription'/exp OR transcription OR transcriptional)

***Amendment** – While gene expression is included in the above search terms, gene expression will be excluded from the outcomes.

Appendix 1. PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	(Page No.#)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	5
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	12
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3-4
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	5-7
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	8 and Appendix 1

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	8
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7-8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	5-7
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7-8
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	9-11
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	11-12
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	12
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	12

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

BMJ Open

Bacterial antibiotic resistance development and mutagenesis following exposure to sub-minimal inhibitory concentrations of fluoroquinolones *in vitro*: a systematic literature review protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030747.R1
Article Type:	Protocol
Date Submitted by the Author:	09-Sep-2019
Complete List of Authors:	Ching, Carly; Boston University, Biomedical Engineering Orubu, Ebiowei; Boston University, Institute for Health System Innovation & Policy Wirtz, Veronika; Boston University School of Public Health, Global Health Zaman, Muhammad ; Boston University, Biomedical Engineering
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Genetics and genomics
Keywords:	Microbiology < BASIC SCIENCES, INFECTIOUS DISEASES, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

Bacterial antibiotic resistance development and mutagenesis following exposure to sub-minimal inhibitory concentrations of fluoroquinolones *in vitro*: a systematic literature review protocol

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ABSTRACT

Introduction: Antibiotic resistance (AR) is among the most pressing global health challenges. Fluoroquinolones are a clinically important group of antibiotics that have wide applicability in both humans and animals. While many drivers of AR are known, the impact of medicine quality on AR remains largely unknown. The aim of this review is to systematically evaluate the evidence of the impact of *in vitro* sub-inhibitory antibiotic exposure, a major tenet of substandard antibiotics, on the development of AR and mutagenesis, using fluoroquinolones as a case study.

Methods and analysis: EMBASE, Web of Science and PubMed will be systematically searched for primary experimental *in vitro* studies, from earliest available dates within each database (1947, 1965 and 1966, respectively) through 2018, related to sub-inhibitory fluoroquinolone exposure and AR. A specifically developed non-weighted tool will be used to critically assess the evidence. Subgroup analyses will be performed for different variables and outcomes.

Ethics and dissemination: Ethical approval is not required as no primary data is to be collected. The completed systematic review will be disseminated through conference meeting presentations and a peer-reviewed publication.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study aims to be the first systematic review of this body of evidence.
- NCBI PubMed, ISI Web of Science and Elsevier Embase will be searched for impact of sub-inhibitory concentrations of fluoroquinolones on antimicrobial resistance from earliest available dates within each database through 2018 without language limitation.
- Basic microbiological experimental studies will be reviewed following Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) protocol standards, which is currently not best practice.
- Data will be placed in the context of the important global health issue of medicine quality, with broad implications in mortality, morbidity and antibiotic resistance. The knowledge afforded by the review can provide a foundation for further research studies on substandard antibiotics.
- Review is limited to *in vitro* studies of bacterial monocultures, limiting translation to the clinic.

INTRODUCTION

Antibiotic resistance (AR) is a rapidly growing global health threat. To provide evidence for improved clinical and public health interventions and policies, it is paramount to understand both the social drivers of AR development and the underlying scientific contributors. These drivers include antibiotic usage in the environment and the clinic, as well as access and quality of antibiotics[1,2].

Poor-quality antibiotics, specifically substandard antibiotics, is one possible understudied driver of antibiotic resistance [3]. Substandard drugs are defined by the World Health Organization as "*authorized medical products that fail to meet either their quality standards or their specifications, or both*" [3]. The prevalence, or failure rate, of substandard antibiotics and other anti-infectives in low and middle income countries has been reported to be about 7% [3]. Prevalence estimates are currently limited to low and middle-income countries, with more data needed for high-income countries [4]. Substandard antibiotic products often contain inadequate levels of the active pharmaceutical ingredient (API) (not falling within the stated concentration or quality standards) or have lower than expected/specified bioavailability arising from poor dissolution. This can result in the treatment of bacteria at sub-inhibitory concentrations below their minimal inhibitory concentration (MIC). In this case, there is not enough API to completely clear the bacterial infection but there may be enough API to provide selective pressure for AR development. Thus, medicine quality may be a potentially important driver of AR, however there is currently a lack of direct evidence to support this hypothesis [5].

While systematic reviews of observational studies provide critical evidence for developing clinical interventions and public health policies, there is a lack of a similar systematic approach in reviews of experimental bench research – the science which underlies and explains what occurs clinically.

To identify important scientific trends and bring awareness to the topic of medicine quality, we have extracted an underlying scientific question for a systematic review: Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis?

Here, we seek to systematically synthesize and critically appraise experimental evidence on how sub-inhibitory concentrations of one specific class of antibiotics, fluoroquinolones, impacts AR. We have chosen fluoroquinolones as they are a commonly used classes of antibiotics, effective against both Gram-negative and Gram-positive bacteria, in both human and animals. Resistance emergence against fluoroquinolones has been widely reported for several decades[6–9]. Second to fourth-generation fluoroquinolones stem from the initial non-fluorinated first-generation quinolone class; these synthetic molecules are technically classified as antimicrobial agents and share a bicyclic quinolone-related core structure, with a fluorine on the sixth or seventh carbon position. For this review, we will refer to fluoroquinolones as antibiotics [9–11]. In addition to substandard antibiotic exposure clinically, bacteria are exposed to sub-inhibitory antibiotic concentrations in other situations, such as in the environment from wastewaters or agricultural soils which can have implications in AR development and transmittance [12].

Currently, there are only a few broad narrative literature reviews on the impacts of sub-inhibitory concentrations of antibiotics [13–16]. To our knowledge, there are currently few to no systematic reviews of basic or fundamental microbiological bench research [17,18]. Thus, we seek to perform an unbiased systematic literature review using Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines on the topic of sub-inhibitory fluoroquinolone exposure and AR development.

The results of this systematic review will contribute to the understanding of the impact of exposure of bacteria to sub-inhibitory levels of fluoroquinolones on antibiotic resistance acquisition.

Secondarily, we seek to identify gaps in evidence related to medicine quality in an effort to inform policy-making on the control of substandard medicines. This work can contribute to a rigorous evidence-base of bench research based on systematic review including critical appraisal of existing literature instead of narrative review and selective reporting.

Systematic review questions

This review seeks to address the following questions:

1. Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis *in vitro*? (Primary)
2. What is the potential for substandard fluoroquinolone drugs to lead to antibiotic resistance development? (Secondary)

METHODS

Our methodology will conform to the PRISMA reporting standards (Appendix 1, PRISMA-P Checklist). The protocol does not currently exist elsewhere and is ineligible for hosting on PROSPERO because it the study participants are not people or animals. The duration of this study is estimated to be six months.

Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in this work.

Eligibility Criteria

To define the search approach and inclusion and exclusion criteria we applied a Population Intervention Comparator Outcome Study (PICOS) search tool. The criteria is presented in Table. 1.

Table 1. Population Intervention Comparator Outcome Study (PICOS) Design Criteria

	Include	Exclude
Population	• Bacteria (All isolates of Gram-negative and Gram-positive species)lab/domesticated strains	• Eukaryotes (All) • Archaea
Intervention (Exposure)	• Exposure with ranges of fluoroquinolone (second to fourth-generation) concentrations with levels below the defined MIC*, under controlled *in vitro* experimental conditions. * defined as the concentration visibly inhibiting growth in the experimental set-up	• Exposure to first-generation quinolone antibiotics (i.e. nalidixic acid) or other classes of antibioticsExposure to first-generation quinolone antibiotics, for example nalidixic acid, or other classes of antibiotics. • Exposure to sub-MIC fluoroquinolone concentrations in combination with another class of antibiotic or compound • Purely computational models • Studies involving animals
Comparator	• No treatment, MIC at 0% API of parental strain	

Outcomes	• Quantitative experimental microbiological data related to: (1) resistance acquisition (to same or other antibiotic) and (2) mutagenesis rate • Examples of data include standard microbiological assays (i.e. phenotypical tests, commercially available antibiotic susceptibility tests and molecular and PCR assays for identification of mutations) • Whether any mention of substandard of medicine quality within the paper (yes/no)	• Outcomes from studies that treat bacteria with sub-inhibitory levels but do not follow-up with results related to resistance acquisition or mutagenesis. Examples of results to exclude include community behavior, such as surface cell adhesion and biofilm formation, virulence (persister formation, toxin/antitoxin systems) and plasmid curing
Study Design	• Primary Experimental Studies (All Languages) published from 1966-2018 on NCBI PubMed, from 1965-2018 on ISI Web of Science and from 1947 - 2018 on Elsevier Embase	• Conference abstractsConference abstracts • Review articles (no primary data) • Observational Studies

MIC=minimum inhibitory concentration, API=active pharmaceutical ingredients, PCR=polymerase chasin reaction

Outcomes, Prioritization and Data Extraction

The primary outcome extracted will be the effect of exposure to sub-inhibitory concentrations of fluoroquinolones on (1) antibiotic resistance acquisition (mono-resistance and multi-drug resistance) and (2) mutagenesis. A secondary outcome extracted will be whether these papers discuss substandard or poor quality medicines. Our rationale for prioritization is that we first need to determine the link between exposure and resistance acquisition. After quantifying and evaluating the evidence, we aim to assess how frequently primary scientific papers mention or discuss medicine quality. Other variables extracted from each study will include year of publication, bacterial species and number of strains, type of bacterial isolate (clinically isolated vs reference strain), drug name and concentration, and study design (duration of exposure, growth conditions, etc.). Study quality and limitations after quality assessment, and gaps in evidence for review questions will also be extracted. Data will be extracted to a standardized Excel table. The data will be summarized and standardized as described in the Data Synthesis section.

Each paper will be analyzed and key results extracted to a standardized table for comparison by a single reviewer. For a random sample of 10% of the publications, a second reviewer will extract the data. The results will be compared with the first. If the interrater reliability is moderate or low all data extraction will be done independently by two reviewers.

Search Strategy

The search strategy was based on study objectives and a preliminary search of PUBMED to determine relevant Medical Subject Headings (MeSH) terms. Using MeSH terms and keyword synonyms along with identified terms for sub-inhibitory and substandard, a search strategy was designed in PUBMED and translated to Web of Science and Embase to search all fields for articles

that fit the inclusion criteria above (Table 1). Identified search terms are listed below. Search strings were designed with a medical librarian. Additional records will be identified through searching the bibliographies of already-identified papers and searching through papers that have cited key studies. The complete search terms are provided in Appendix 2.

Identified terms: *subinhibitory, sub-inhibitory, sub inhibitory, sub-lethal, sublethal, sub lethal, subminimal, sub-minimal, sub minimal, sub-therapeutic, subtherapeutic, sub therapeutic, sub MIC, sub-MIC, low-dose, low dose, substandard, sub-standard, counterfeit, falsified*

Study records

Records will be managed through reference management software Endnote and Mendeley. Additionally, search histories will be saved. Abstract screening and selection of studies will be performed by two independent reviewers using software Rayyan QCRI [19]. A third researcher will resolve discrepancies between reviewers selections. The full text of articles from the initial screening will be reviewed for inclusion.

Risk of bias in individual studies

Risk of bias for laboratory microbiology experimentation will be assessed with criteria formulated by considering and adapting the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE)'s risk of bias tool for animal studies [20] and the Effective Public Health Practice Project (EPHPP) quality assessment tool [21]. The criteria is presented in Table 2. Here, we present a non-weighted assessment of individual study quality, including risk of bias. For each of five domains, studies will be assessed for a series of criteria listed below. For each unmet review criteria within the domain an increased risk of bias point will be assigned. The more points assigned, the higher the risk of bias associated with the study. There will be no defined cut-off for exclusion of

papers, in order for the review to be reflective of the evidence base as a whole. This will allow us to determine how strong the body of evidence is as a whole and to perform a qualitative assessment of the most frequent types of gaps in quality to inform recommendations for future studies. Papers will also have to meet a minimum criteria of ability to extract data on methods and results; e.g. appropriate quantitative numerical data on study outcome.

Table 2. Criteria for assessment of the quality of laboratory microbiology experimentation

Domain	Description of domain	Review Criteria
Selection and confounding bias	Describe possible genetic or environmental variations to determine how results for different strains of the same species can be compared. For clinical isolates, genotype is not required.	• Were the groups compared individually or were differences discussed in the analysis? • Were species and strain details provided?
Study Design/Methods	Reproducibility and detail of study design and methods. Description of analysis methods.	• Are there any discrepancies between methods and in-text? • Is the methodological section missing any steps or appropriate detail ? (including but not limited to below) Steps/Details:

Domain	Description of domain	Review Criteria
		-media used -temperature -time -incubation conditions (static, rolling, shaking, aeration) - reagents used - concentrations used - appropriate control experiments -replication of experiments
Incomplete outcome data (Attrition Bias)	Describe the completeness of outcome data being analyzed, including attrition and exclusions from the analysis.	• Is there missing outcome data that was not addressed? • Is the control outcome data mentioned in the paper present?
Selective outcome reporting	Reporting of aim and all outcomes of the study.	• Was all data reported for all conditions or just select/statistically significant results?

Domain	Description of domain	Review Criteria
(Reporting bias)		• Was it clear whether no change results were reported? • Was statistical significance noted (if possible)? • Is the appropriate comparison to baseline provided?
Other sources of bias	State any important concerns about bias not covered by other domains.	• Was the study apparently free of concerns about bias?
Global Bias Rating	Summary of all five domains	Calculate total quality points. The more points the higher the risk of bias.

Data Synthesis

Meta-analysis may not be possible based on findings and will be defined by the limitations of the raw data extracted. It will be dependent on the magnitude of heterogeneity between independent studies and ability to assign an effect-size that would be appropriate. If heterogeneity is too large meta-analyses will not be performed in order to avoid over-interpretation. If we cannot assign a true appropriate control group and true "sample size", meta-analysis will also not be possible. However, despite these potential limitations this is a novel review of experimental evidence that aims to provide a comprehensive synthesis of data that is much more complete than one individual

study and which may reveal trends. It is clear that more tools need to be developed to move the field of basic science towards systematic reviews.

If meta-analysis is not appropriate, quantitative sub-group analyses and summarization will be performed. The following protocol, in brief, will be used: Data will be extracted into a standardized Excel spreadsheet. From here, data will be sorted and grouped for each independent variable, such as bacterial species, concentration of exposure and antibiotic. The dependent outcome of change in resistance and mutagenesis will be plotted against these variables. The values of outcomes (relative change in resistance) will be binned. This will allow us to determine the range and frequency of magnitudes of resistance changes given different concentrations and different antibiotics.

Meta-bias(es)

Based on data synthesis parameters, the overall quality of the body of evidence will be determined, if possible. Since we will not be able to make direct clinical recommendations due to the limitations of our review being focused on *in-vitro* studies we will focus on confidence in our overall summary of results and trends. For this we will take into account publication bias across studies including any potential publication biases in authors or types of studies, biases towards certain bacteria, antibiotics and selective outcomes. Contrastingly, narrative literature reviews of basic science typically do not critically assess the bias of each study and hence, do not take into account quality in their summary which is an important limitation of narrative reviews. We will use GRADE (Grading of Recommendations, Assessment, Development and Evaluations) guidelines on publication bias to aid us in rating the quality of our evidence [22]. Additionally inconsistencies in methodology and outcomes will be assessed and taken into account in determining the confidence of our reported data summary.

Ethics and Dissemination

Ethical approval is not required as no primary data is to be collected. The completed systematic review will be disseminated through conference meeting presentations and a peer-reviewed publication.

Authors Contributions

C.C., V.J.W and M.H.Z. initiated work. C.C., E.S.F.O., V.J.W and M.H.Z designed and wrote protocol.

Acknowledgements

We would like to acknowledge David Flynn from Boston University Medical Alumni Library for assistance with search terms and members of the Zaman lab for their input and feedback.

Funding statement

This work was funded by a fellowship to C.C. by the United States Pharmacopeia.

Competing Interests

The authors declare no competing interests.

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Appendix 1. PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	(Page No.#)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	6 (ineligible for PROSPERO hosing because subjects are not humans or animals)
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	15
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	15
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	4-6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years	7-8

		considered, language, publication status) to be used as criteria for eligibility for the review	
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	9-10 and Appendix 2
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	10
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	10
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8-9
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7-8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8-9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	10-13
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	13-14
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	14
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	14

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Appendix 2: Search Terms

PUBMED

Search Term*:

((quinolone) AND (subinhibitory OR sub-inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR “sub minimum” OR sub-therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified)) AND (Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance, Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene expression OR transcription or transcriptional)

Complete Search Term:

(("quinolones"[MeSH Terms] OR "quinolones"[All Fields] OR "quinolone"[All Fields]) AND (subinhibitory[All Fields] OR sub-inhibitory[All Fields] OR "sub inhibitory"[All Fields] OR sub-lethal[All Fields] OR sublethal[All Fields] OR "sub lethal"[All Fields] OR subminimal[All Fields] OR sub-minimal[All Fields] OR "sub minimal"[All Fields] OR subminimum[All Fields] OR sub-minimum[All Fields] OR "sub minimum"[All Fields] OR sub-therapeutic[All Fields] OR subtherapeutic[All Fields] OR "sub therapeutic"[All Fields] OR "sub MIC"[All Fields] OR sub-mic[All Fields] OR low-dose[All Fields] OR "low dose"[All Fields] OR substandard[All Fields] OR "sub standard"[All Fields] OR sub-standard[All Fields] OR counterfeit[All Fields] OR falsified[All Fields])) AND (("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "drug resistance, microbial"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antimicrobial"[All Fields] AND "drug"[All Fields] AND "resistance"[All Fields]) OR "antimicrobial drug resistance"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antimicrobial"[All Fields] AND "drug"[All Fields] AND "resistances"[All Fields]) OR "antimicrobial drug resistances"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) OR (Microbial[All Fields] AND ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields])) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("resistance"[All Fields] AND "antibiotic"[All Fields]) OR "resistance, antibiotic"[All Fields]) OR Resistance[All Fields] OR Resistant[All Fields] OR ("mutagenesis"[MeSH Terms] OR "mutagenesis"[All Fields]) OR ("mutation"[MeSH Terms] OR "mutation"[All Fields]) OR Mutagenicity[All Fields] OR ("gene expression"[MeSH Terms] OR ("gene"[All Fields] AND "expression"[All Fields]) OR "gene expression"[All Fields]) OR ("transcription, genetic"[MeSH Terms] OR ("transcription"[All Fields] AND "genetic"[All Fields]) OR "genetic transcription"[All Fields] OR "transcription"[All Fields]) OR ("transcription, genetic"[MeSH Terms] OR ("transcription"[All Fields] AND "genetic"[All Fields]) OR "genetic transcription"[All Fields] OR "transcriptional"[All Fields]))

Web of Science

Search Term*:

TS=(quinolone* OR fluoroquinolone* OR ciprofloxacin OR norfloxacin OR ofloxacin OR levofloxacin OR moxifloxacin OR gemifloxacin or enrofloxacin) AND TS=(subinhibitory OR sub-inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR “sub minimum” OR sub-therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified) AND TS=(Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance, Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene expression OR transcription or transcriptional)

Embase

Search Term*:

(subinhibitory OR 'sub inhibitory' OR 'sub-lethal' OR sublethal OR 'sub lethal' OR subminimal OR 'sub-minimal' OR 'sub minimal' OR subminimum OR 'sub-minimum' OR 'sub minimum' OR 'sub-therapeutic' OR subtherapeutic OR 'sub therapeutic' OR 'sub mic' OR 'sub-mic' OR 'low-dose' OR 'low dose' OR substandard OR 'sub standard' OR 'sub-standard' OR counterfeit OR falsified) AND ('quinoline derived antiinfective agent'/exp OR 'anti infective agents, fluoroquinolone' OR 'anti infective agents, quinolone' OR 'anti-infective agents, fluoroquinolone' OR 'anti-infective agents, quinolone' OR 'antibiotics, quinolones' OR 'chinolone antibiotic' OR 'fluoroquinolone (antibiotic)' OR 'fluoroquinolone antibiotic' OR 'fluoroquinolone antibiotic agent' OR 'fluoroquinolone antiinfective agent' OR 'fluoroquinolone antimicrobial agent' OR 'quinoline derived antiinfective agent' OR 'quinolone (antibiotic)' OR 'quinolone antibiotic' OR 'quinolone antibiotics' OR 'quinolone antiinfective agent') AND ('antibiotic resistance'/exp OR 'antibacterial drug resistance' OR 'antibacterial resistance' OR 'antibiotic resistance' OR 'antimicrobial drug resistance' OR 'antimicrobial resistance' OR 'bacterial drug resistance' OR 'bacterial resistance' OR 'bacterium resistance' OR 'drug resistance, bacterial' OR 'drug resistance, microbial' OR 'microbial drug resistance' OR 'resistance' OR 'resistant' OR 'gene expression'/exp OR 'expression, gene' OR 'gene expression' OR 'genetic expression' OR 'genome expression' OR 'genetic transcription'/exp OR transcription OR transcriptional)

***Amendment** – While gene expression is included in the above search terms, gene expression will be excluded from the outcomes.

BMJ Open

Bacterial antibiotic resistance development and mutagenesis following exposure to sub-minimal inhibitory concentrations of fluoroquinolones *in vitro*: a systematic literature review protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-030747.R2
Article Type:	Protocol
Date Submitted by the Author:	07-Oct-2019
Complete List of Authors:	Ching, Carly; Boston University, Biomedical Engineering Orubu, Ebiowei; Boston University, Institute for Health System Innovation & Policy Wirtz, Veronika; Boston University School of Public Health, Global Health Zaman, Muhammad ; Boston University, Biomedical Engineering
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Genetics and genomics, Public health
Keywords:	Microbiology < BASIC SCIENCES, INFECTIOUS DISEASES, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

Bacterial antibiotic resistance development and mutagenesis following exposure to sub-minimal inhibitory concentrations of fluoroquinolones *in vitro*: a systematic literature review protocol

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Word Count: 1832 (excluding tables)

ABSTRACT

Introduction: Antibiotic resistance (AR) is among the most pressing global health challenges. Fluoroquinolones are a clinically important group of antibiotics that have wide applicability in both humans and animals. While many drivers of AR are known, the impact of medicine quality on AR remains largely unknown. The aim of this review is to systematically evaluate the evidence of the impact of *in vitro* sub-inhibitory antibiotic exposure, a major tenet of substandard antibiotics, on the development of AR and mutagenesis, using fluoroquinolones as a case study.

Methods and analysis: EMBASE, Web of Science and PubMed will be systematically searched for primary experimental *in vitro* studies, from earliest available dates within each database (1947, 1965 and 1966, respectively) through 2018, related to sub-inhibitory fluoroquinolone exposure and AR. A specifically developed non-weighted tool will be used to critically assess the evidence. Subgroup analyses will be performed for different variables and outcomes.

Ethics and dissemination: Ethical approval is not required as no primary data is to be collected. The completed systematic review will be disseminated through conference meeting presentations and a peer-reviewed publication.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study aims to be the first systematic review of this body of evidence.
- NCBI PubMed, ISI Web of Science and Elsevier Embase will be searched for impact of sub-inhibitory concentrations of fluoroquinolones on antimicrobial resistance from earliest available dates within each database through 2018 without language limitation.
- Basic microbiological experimental studies will be reviewed following Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) protocol standards, which is currently not best practice.
- Data will be placed in the context of the important global health issue of medicine quality, with broad implications in mortality, morbidity and antibiotic resistance. The knowledge afforded by the review can provide a foundation for further research studies on substandard antibiotics.
- Review is limited to *in vitro* studies of bacterial monocultures, limiting translation to the clinic.

INTRODUCTION

Antibiotic resistance (AR) is a rapidly growing global health threat. To provide evidence for improved clinical and public health interventions and policies, it is paramount to understand both the social drivers of AR development and the underlying scientific mechanisms. These drivers include antibiotic usage in the environment and the clinic, as well as access and quality of antibiotics[1,2].

Poor-quality antibiotics, specifically substandard antibiotics, is one possible understudied driver of antibiotic resistance [3]. Substandard drugs are defined by the World Health Organization as "*authorized medical products that fail to meet either their quality standards or their specifications, or both*" [3]. The prevalence, or failure rate, of substandard antibiotics and other anti-infectives in low and middle income countries has been reported to be about 7% [3]. Prevalence estimates are currently limited to low and middle-income countries, with more data needed for high-income countries [4]. Substandard antibiotic products often contain inadequate levels of the active pharmaceutical ingredient (API) (not falling within the stated concentration or quality standards) or have lower than expected/specified bioavailability arising from poor dissolution. This can result in the treatment of bacteria at sub-inhibitory concentrations below their minimal inhibitory concentration (MIC). In this case, there is not enough API to completely clear the bacterial infection but there may be enough API to provide selective pressure for AR development. Thus, medicine quality may be a potentially important driver of AR, however there is currently a lack of direct evidence to support this hypothesis [5].

While systematic reviews of observational studies provide critical evidence for developing clinical interventions and public health policies, there is a lack of a similar systematic approach in reviews of experimental bench research – the science which underlies and explains what occurs clinically.

To identify important scientific trends and bring awareness to the topic of medicine quality, we have extracted an underlying scientific question for a systematic review: Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis?

Here, we seek to systematically synthesize and critically appraise experimental evidence on how sub-inhibitory concentrations of one specific class of antibiotics, fluoroquinolones, impacts AR. We have chosen fluoroquinolones as they are a commonly used classes of antibiotics, effective against both Gram-negative and Gram-positive bacteria, in both human and animals. Resistance emergence against fluoroquinolones has been widely reported for several decades[6–9]. Second to fourth-generation fluoroquinolones stem from the initial non-fluorinated first-generation quinolone class; these synthetic molecules are technically classified as antimicrobial agents and share a bicyclic quinolone-related core structure, with a fluorine on the sixth or seventh carbon position. For this review, we will refer to fluoroquinolones as antibiotics [9–11]. In addition to substandard antibiotic exposure clinically, bacteria are exposed to sub-inhibitory antibiotic concentrations in other situations, such as in the environment from wastewaters or agricultural soils which can have implications in AR development and transmittance [12].

Currently, there are only a few broad narrative literature reviews on the impacts of sub-inhibitory concentrations of antibiotics [13–16]. To our knowledge, there are currently few systematic reviews of basic or fundamental microbiological bench research [17–19]. Thus, we seek to perform an unbiased systematic literature review according to Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines on the topic of sub-inhibitory fluoroquinolone exposure and AR development.

The results of this systematic review will contribute to the understanding of the impact of exposure of bacteria to sub-inhibitory levels of fluoroquinolones on antibiotic resistance acquisition.

Secondarily, we seek to identify gaps in evidence related to medicine quality in an effort to inform policy-making on the control of substandard medicines. This work can contribute to a rigorous evidence-base of bench research based on systematic review including critical appraisal of existing literature instead of narrative review and selective reporting.

Systematic review questions

This review seeks to address the following questions:

1. Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis *in vitro*? (Primary)
2. What is the potential for substandard fluoroquinolone drugs to lead to antibiotic resistance development? (Secondary)

METHODS

Our methodology will conform to the PRISMA reporting standards (Appendix 1, PRISMA-P Checklist). The protocol does not currently exist elsewhere and is ineligible for hosting on PROSPERO because it the study participants are not people or animals. The duration of this study is estimated to be six months.

Patient and Public Involvement

It was not appropriate or possible to involve patients or the public in this work.

Eligibility Criteria

To define the search approach and inclusion and exclusion criteria we applied a Population Intervention Comparator Outcome Study (PICOS) search tool. The criteria is presented in Table. 1.

Table 1. Population Intervention Comparator Outcome Study (PICOS) Design Criteria

	Include	Exclude
Population	• Bacteria (All isolates of Gram-negative and Gram-positive species)lab/domesticated strains	• Eukaryotes (All) • Archaea
Intervention (Exposure)	• Exposure to ranges of fluoroquinolone (second to fourth-generation) concentrations with levels below the defined MIC*, under controlled *in vitro* experimental conditions. * Defined as the concentration visibly inhibiting growth in the experimental set-up. Methods employed would include broth and agar dilution methods and commercially available MIC test strips.	• Exposure to first-generation quinolone antibiotics (i.e. nalidixic acid) or other classes of antibioticsExposure to first-generation quinolone antibiotics, for example nalidixic acid, or other classes of antibiotics. • Exposure to sub-MIC fluoroquinolone concentrations in combination with another class of antibiotic or compound • Purely computational models • Studies involving animals
Comparator	• No treatment, MIC at 0% API of parental strain	

Outcomes	• Quantitative experimental microbiological data related to: (1) resistance acquisition (to same or other antibiotic) and (2) mutagenesis rate • Examples of data include standard microbiological assays (i.e. phenotypical tests, commercially available antibiotic susceptibility tests and molecular and PCR assays for identification of mutations) • Whether any mention of substandard of medicine quality within the paper (yes/no)	• Outcomes from studies that treat bacteria with sub-inhibitory levels but do not follow-up with results related to resistance acquisition or mutagenesis. Examples of results to exclude include community behavior, such as surface cell adhesion and biofilm formation, virulence (persister formation, toxin/antitoxin systems) and plasmid curing
Study Design	• Primary Experimental Studies (All Languages) published from 1966-2018 on NCBI PubMed, from 1965-2018 on ISI Web of Science and from 1947 - 2018 on Elsevier Embase	• Conference abstractsConference abstracts • Review articles (no primary data) • Observational Studies

MIC=minimum inhibitory concentration, API=active pharmaceutical ingredients, PCR=polymerase chasin reaction

Outcomes, Prioritization and Data Extraction

The primary outcome extracted will be the effect of exposure to sub-inhibitory concentrations of fluoroquinolones on (1) antibiotic resistance acquisition (mono-resistance and multi-drug resistance) and (2) mutagenesis. A secondary outcome extracted will be whether these papers discuss substandard or poor quality medicines. Our rationale for prioritization is that we first need to determine the link between exposure and resistance acquisition. After quantifying and evaluating the evidence, we aim to assess how frequently primary scientific papers mention or discuss medicine quality. Other variables extracted from each study will include year of publication, bacterial species and number of strains, type of bacterial isolate (clinically isolated vs reference strain), drug name and concentration, and study method (duration of exposure, growth conditions, etc.). Study quality and limitations after quality assessment, and gaps in evidence for review questions will also be extracted. Data will be extracted to a standardized Excel table. The data will be summarized and standardized as described in the Data Synthesis section.

Each paper will be analyzed and key results extracted to a standardized table for comparison by a single reviewer. For a random sample of 10% of the publications, a second reviewer will extract the data. The results will be compared with the first. If the interrater reliability is moderate or low all data extraction will be done independently by two reviewers.

Search Strategy

The search strategy was based on study objectives and a preliminary search of PUBMED to determine relevant Medical Subject Headings (MeSH) terms. Using MeSH terms and keyword synonyms along with identified terms for sub-inhibitory and substandard, a search strategy was designed in PUBMED and translated to Web of Science and Embase to search all fields for articles

that fit the inclusion criteria above (Table 1). Identified search terms are listed below. Search strings were designed with a medical librarian. Additional records will be identified through searching the bibliographies of already-identified papers and searching through papers that have cited key studies. The complete search terms are provided in Appendix 2.

Identified terms: *subinhibitory, sub-inhibitory, sub inhibitory, sub-lethal, sublethal, sub lethal, subminimal, sub-minimal, sub minimal, sub-therapeutic, subtherapeutic, sub therapeutic, sub MIC, sub-MIC, low-dose, low dose, substandard, sub-standard, counterfeit, falsified*

Study records

Records will be managed through reference management software Endnote and Mendeley. Additionally, search histories will be saved. Abstract screening and selection of studies will be performed by two independent reviewers using software Rayyan QCRI [20]. A third researcher will resolve discrepancies between reviewers selections. The full text of articles from the initial screening will be reviewed for inclusion.

Risk of bias in individual studies

Risk of bias for laboratory microbiology experimentation will be assessed with criteria formulated by considering and adapting the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE)'s risk of bias tool for animal studies [21] and the Effective Public Health Practice Project (EPHPP) quality assessment tool [22]. The criteria is presented in Table 2. Here, we present a non-weighted assessment of individual study quality, including risk of bias. For each of five domains, studies will be assessed for a series of criteria listed below. For each unmet review criteria within the domain an increased risk of bias point will be assigned. The more points assigned, the higher the risk of bias associated with the study. There will be no defined cut-off for exclusion of

papers, in order for the review to be reflective of the evidence base as a whole. This will allow us to determine how strong the body of evidence is as a whole and to perform a qualitative assessment of the most frequent types of gaps in quality to inform recommendations for future studies. Papers will also have to meet a minimum criteria of ability to extract data on methods and results; e.g. appropriate quantitative numerical data on study outcome.

Table 2. Criteria for assessment of the quality of laboratory microbiology experimentation

Domain	Description of domain	Review Criteria
Selection and confounding bias	Describe possible genetic or environmental variations to determine how results for different strains of the same species can be compared. For clinical isolates, genotype is not required.	• Were the groups compared individually or were differences discussed in the analysis? • Were species and strain details provided?
Study Design/Methods	Reproducibility and detail of study design and methods. Description of analysis methods.	• Are there any discrepancies between methods and in-text? • Is the methodological section missing any steps or appropriate detail ? (including but not limited to below) Steps/Details:

Domain	Description of domain	Review Criteria
		-media used -temperature -time -incubation conditions (static, rolling, shaking, aeration) - reagents used - concentrations used - appropriate control experiments -replication of experiments
Incomplete outcome data (Attrition Bias)	Describe the completeness of outcome data being analyzed, including attrition and exclusions from the analysis.	• Is there missing outcome data that was not addressed? • Is the control outcome data mentioned in the paper present?
Selective outcome reporting	Reporting of aim and all outcomes of the study.	• Was all data reported for all conditions or just select/statistically significant results?

Domain	Description of domain	Review Criteria
(Reporting bias)		• Was it clear whether no change results were reported? • Was statistical significance noted (if possible)? • Is the appropriate comparison to baseline provided?
Other sources of bias	State any important concerns about bias not covered by other domains.	• Was the study apparently free of concerns about bias?
Global Bias Rating	Summary of all five domains	Calculate total quality points. The more points the higher the risk of bias.

Data Synthesis

Meta-analysis may not be possible based on findings and will be defined by the limitations of the raw data extracted. It will be dependent on the magnitude of heterogeneity between independent studies and ability to assign an effect-size that would be appropriate. If heterogeneity is too large meta-analyses will not be performed in order to avoid over-interpretation. If we cannot assign a true appropriate control group and true "sample size", meta-analysis will also not be possible. However, despite these potential limitations this is a novel review of experimental evidence that aims to provide a comprehensive synthesis of data that is much more complete than one individual

study and which may reveal trends. It is clear that more tools need to be developed to move the field of basic science towards systematic reviews.

Quantitative sub-group analyses and summarization will be performed. The following protocol, in brief, will be used: Data will be extracted into a standardized Excel spreadsheet. From here, data will be sorted and grouped for each independent variable, such as bacterial species, concentration of exposure and antibiotic. The rationale for subgroups is as follows. First, different bacterial species often respond differently to stress or have different genetic responses to different stimuli. For example, the clinically relevant pathogen *Acinetobacter baumannii*, which has a propensity to gain multi-drug resistances, has a different DNA damage response compared to the conserved paradigm of *Escherichia coli* [23]. This impacts how these two bacteria respond to stress and such differences between bacterial species may lead to differences in responses to sub-inhibitory fluoroquinolone exposure. Concentration of exposure is an important factor, as different concentrations may present different selective compartments [24]. Similarly, it is of clinical interest to determine if certain fluoroquinolones impact bacteria differently, given that fluoroquinolones (with different usage and prescription patterns), display differences in pharmacodynamics and resistance profiles [25,26]. The dependent outcome of change in resistance and mutagenesis will be plotted against these factors. The values of outcomes (relative change in resistance) will also be binned. This will allow us to determine the range and frequency of magnitudes of resistance changes given different concentrations and different antibiotics.

Meta-bias(es)

Based on data synthesis parameters, the overall quality of the body of evidence will be determined, if possible. Since we will not be able to make direct clinical recommendations due to the limitations of our review being focused on *in-vitro* studies, we will focus on confidence in our overall

summary of results and trends. For this we will take into account publication bias across studies. Carroll [27] identified how publication bias may exist in scientific literature and described potential solutions, however these are not best practice. Publication bias could arise from, but is not limited to, rejection of negative data, researchers not submitting research that present negative data, publication based on results rather than the quality and rigor of the study design and influence of industry and funding sources. All of these factors can lead to a skewed set of data that does not fully represent the phenomena being investigated. Narrative literature reviews of basic science typically do not critically assess the bias of each study and hence, do not take into account quality in their summary which is an important limitation of narrative reviews. We will use GRADE (Grading of Recommendations, Assessment, Development and Evaluations) guidelines on publication bias to aid us in rating the quality of our evidence [28]. Specifically, to assess publication bias we will look at the group of studies to determine how many studies had increased bias for not reporting negative results, or only reporting statistically significant results. This criteria is already present in our Risk of Bias analysis for individual studies. We will look at the studies that published negative results and determine if there are differences in the impact factor/prestige of journal that they are published in, and whether data is coming from the same research groups. We will also look at the final number of studies and time of publication in order to identify if there is any "lag bias"[28]. Funding sources, specifically frequency of industry funded studies, will be noted. Acknowledging that publication bias is difficult to assess, as suggested by GRADE, we will then determine if publication bias is "undetected" or "strongly suspected" and rate down a maximum of one level for suspected bias [28]. Additionally biases in our set of evidence towards certain bacteria, antibiotics and inconsistencies in methodology and outcomes will be assessed and taken into account in determining the confidence of our reported data summary.

Ethics and Dissemination

Ethical approval is not required as no primary data is to be collected. The completed systematic review will be disseminated through conference meeting presentations and a peer-reviewed publication.

Authors Contributions

C.C., V.J.W and M.H.Z. initiated work. C.C., E.S.F.O., V.J.W and M.H.Z designed and wrote protocol.

Acknowledgements

We would like to acknowledge David Flynn from Boston University Medical Alumni Library for assistance with search terms and members of the Zaman lab for their input and feedback.

Funding statement

This work was funded by a fellowship to C.C. by the United States Pharmacopeia.

Competing Interests

The authors declare no competing interests.

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Appendix 1. PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	(Page No.#)
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	6 (ineligible for PROSPERO hosing because subjects are not humans or animals)
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	15
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	15
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	4-6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years	7-8

		considered, language, publication status) to be used as criteria for eligibility for the review	
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	9-10 and Appendix 2
Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	10
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	10
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	8-9
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7-8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8-9
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	10-13
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	13-14
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	14-15
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	14-15

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Appendix 2: Search Terms

PUBMED

Search Term*:

((quinolone) AND (subinhibitory OR sub-inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR “sub minimum” OR sub-therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified)) AND (Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance, Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene expression OR transcription or transcriptional)

Complete Search Term:

(("quinolones"[MeSH Terms] OR "quinolones"[All Fields] OR "quinolone"[All Fields]) AND (subinhibitory[All Fields] OR sub-inhibitory[All Fields] OR "sub inhibitory"[All Fields] OR sub-lethal[All Fields] OR sublethal[All Fields] OR "sub lethal"[All Fields] OR subminimal[All Fields] OR sub-minimal[All Fields] OR "sub minimal"[All Fields] OR subminimum[All Fields] OR sub-minimum[All Fields] OR "sub minimum"[All Fields] OR sub-therapeutic[All Fields] OR subtherapeutic[All Fields] OR "sub therapeutic"[All Fields] OR "sub MIC"[All Fields] OR sub-mic[All Fields] OR low-dose[All Fields] OR "low dose"[All Fields] OR substandard[All Fields] OR "sub standard"[All Fields] OR sub-standard[All Fields] OR counterfeit[All Fields] OR falsified[All Fields])) AND (("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "drug resistance, microbial"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antimicrobial"[All Fields] AND "drug"[All Fields] AND "resistance"[All Fields]) OR "antimicrobial drug resistance"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antimicrobial"[All Fields] AND "drug"[All Fields] AND "resistances"[All Fields]) OR "antimicrobial drug resistances"[All Fields]) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields]) OR (Microbial[All Fields] AND ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("antibiotic"[All Fields] AND "resistance"[All Fields]) OR "antibiotic resistance"[All Fields])) OR ("drug resistance, microbial"[MeSH Terms] OR ("drug"[All Fields] AND "resistance"[All Fields] AND "microbial"[All Fields]) OR "microbial drug resistance"[All Fields] OR ("resistance"[All Fields] AND "antibiotic"[All Fields]) OR "resistance, antibiotic"[All Fields]) OR Resistance[All Fields] OR Resistant[All Fields] OR ("mutagenesis"[MeSH Terms] OR "mutagenesis"[All Fields]) OR ("mutation"[MeSH Terms] OR "mutation"[All Fields]) OR Mutagenicity[All Fields] OR ("gene expression"[MeSH Terms] OR ("gene"[All Fields] AND "expression"[All Fields]) OR "gene expression"[All Fields]) OR ("transcription, genetic"[MeSH Terms] OR ("transcription"[All Fields] AND "genetic"[All Fields]) OR "genetic transcription"[All Fields] OR "transcription"[All Fields]) OR ("transcription, genetic"[MeSH Terms] OR ("transcription"[All Fields] AND "genetic"[All Fields]) OR "genetic transcription"[All Fields] OR "transcriptional"[All Fields]))

Web of Science

Search Term*:

TS=(quinolone* OR fluoroquinolone* OR ciprofloxacin OR norfloxacin OR ofloxacin OR levofloxacin OR moxifloxacin OR gemifloxacin or enrofloxacin) AND TS=(subinhibitory OR sub-inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR “sub minimum” OR sub-therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified) AND TS=(Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance, Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene expression OR transcription or transcriptional)

Embase

Search Term*:

(subinhibitory OR 'sub inhibitory' OR 'sub-lethal' OR sublethal OR 'sub lethal' OR subminimal OR 'sub-minimal' OR 'sub minimal' OR subminimum OR 'sub-minimum' OR 'sub minimum' OR 'sub-therapeutic' OR subtherapeutic OR 'sub therapeutic' OR 'sub mic' OR 'sub-mic' OR 'low-dose' OR 'low dose' OR substandard OR 'sub standard' OR 'sub-standard' OR counterfeit OR falsified) AND ('quinoline derived antiinfective agent'/exp OR 'anti infective agents, fluoroquinolone' OR 'anti infective agents, quinolone' OR 'anti-infective agents, fluoroquinolone' OR 'anti-infective agents, quinolone' OR 'antibiotics, quinolones' OR 'chinolone antibiotic' OR 'fluoroquinolone (antibiotic)' OR 'fluoroquinolone antibiotic' OR 'fluoroquinolone antibiotic agent' OR 'fluoroquinolone antiinfective agent' OR 'fluoroquinolone antimicrobial agent' OR 'quinoline derived antiinfective agent' OR 'quinolone (antibiotic)' OR 'quinolone antibiotic' OR 'quinolone antibiotics' OR 'quinolone antiinfective agent') AND ('antibiotic resistance'/exp OR 'antibacterial drug resistance' OR 'antibacterial resistance' OR 'antibiotic resistance' OR 'antimicrobial drug resistance' OR 'antimicrobial resistance' OR 'bacterial drug resistance' OR 'bacterial resistance' OR 'bacterium resistance' OR 'drug resistance, bacterial' OR 'drug resistance, microbial' OR 'microbial drug resistance' OR 'resistance' OR 'resistant' OR 'gene expression'/exp OR 'expression, gene' OR 'gene expression' OR 'genetic expression' OR 'genome expression' OR 'genetic transcription'/exp OR transcription OR transcriptional)

***Amendment** – While gene expression is included in the above search terms, gene expression will be excluded from the outcomes.