

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030747
Article Type:	Protocol
Date Submitted by the Author:	01-Apr-2019
Complete List of Authors:	Ching, Carly; Boston University, Biomedical Engineering Orubu, Ebiowei; Boston University, Institute for Health System Innovation and Policy Wirtz, Veronika; Boston University School of Public Health, Global Health Zaman, Muhammad ; Boston University, Biomedical Engineering
Keywords:	Microbiology < BASIC SCIENCES, INFECTIOUS DISEASES, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT

SCHOLARONE™
Manuscripts

1
2
3 1
4
5
6 2
7
8
9 3
10
11
12 4
13
14
15 5 **Bacterial antibiotic resistance development and mutagenesis following exposure to sub-**
16
17 6 **minimal inhibitory concentrations of fluoroquinolones: a systematic literature review**
18
19
20 7 **protocol**
21
22
23 8•

24
25 9 Carly Ching¹, Ebiowei S.F. Orubu², Veronika J. Wirtz³, Muhammad H. Zaman^{1*}
26
27

28 10 ¹Department of Biomedical Engineering, Boston University, Boston, MA, USA ²Institute for
29 11 Health System Innovation and Policy, Boston University, Boston, MA, USA ³Department of
30 12 Global Health, Boston University School of Public Health, Boston, MA, USA
31
32
33 13

34
35 14• * Muhammad H. Zaman

36
37 15• Department of Biomedical Engineering

38
39 16 Boston University,

40
41 17 44 Cummington Mall

42
43 18 Boston, Massachusetts 02215

44
45 19 617-358-5881

46
47 20 zaman@bu.edu
48

49 21•
50
51 22• Word Count: 1248 (excluding tables)
52
53
54 23
55
56
57
58
59
60

1
2
3 24 **ABSTRACT**
4
5

6
7 25 **Introduction:** Antimicrobial resistance (AMR) is among the most pressing global health
8
9 26 challenges. However, while many drivers are known, the impact of medicine quality on AMR
10
11 27 remains largely elusive. The aim of this review is to systematically evaluate evidence on sub-
12
13 28 inhibitory levels of antibiotic, a major tenant of substandard antibiotics, on AMR, using
14
15 29 fluoroquinolones as a case study.
16
17

18
19 30 **Methods and analysis:** PubMed, EMBASE and Web of Science will be systematically searched
20
21 31 for primary experimental studies related to sub-inhibitory fluoroquinolone treatment and AMR.
22
23 32 A specifically developed non-weighted quality assessment tool will be used. Subgroup analyses
24
25 33 will be performed for different variables and outcomes.
26
27

28
29 34 **Ethics and dissemination:** Ethical approval is not required as no primary data is to be collected.
30
31 35 The completed systematic review will be disseminated through conference meeting presentations
32
33 36 and a peer-reviewed publication.
34
35
36
37

38 37

39
40
41 38

42
43
44
45 39

46
47
48 40

49
50
51 41

52
53
54
55 42

43 STRENGTHS AND LIMITATIONS OF THIS STUDY

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

53 INTRODUCTION

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

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

1
2
3 64 This results in the treatment of bacteria at sub-inhibitory concentrations below their minimal
4
5 65 inhibitory concentration (MIC). Thus, there is not enough API to completely clear the bacterial
6
7
8 66 infection but there may be enough API to provide selective pressure for AMR development. Thus,
9
10 67 medicine quality is a potentially important driver of AMR, however there is currently a lack in
11
12 68 direct evidence to support this hypothesis [2].
13
14

15 69 Here, we seek to fill this important gap by systematically synthesizing experimental evidence on
16
17 70 how sub-inhibitory concentrations of one specific class, fluoroquinolones, impacts AMR. We
18
19 71 further aim to identify gaps in evidence for the relationship between substandard antibiotics usage
20
21 72 and AMR development. Currently, there are only a few broad narrative literature reviews on the
22
23 73 impacts sub-inhibitory concentrations of antibiotics [3–6]. This systematic review aims to merge
24
25 74 experimental evidence with public health implications. Furthermore, to our knowledge, there are
26
27 75 currently few to no systematic reviews of basic microbiological experimental studies. Thus, we
28
29 76 seek to apply Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA)
30
31 77 guidelines to a systematic review on experimental microbiological data in an effort for more
32
33 78 unbiased literature summaries. In summary, the results of this systematic review will contribute to
34
35 79 understanding the potential effects of poor quality medicines on antibiotic resistance acquisition,
36
37 80 identify gaps in evidence, and inform policy-making on the control of substandard medicines.
38
39
40
41
42
43

44 **Systematic review questions**

45
46 82 This review seeks to address the following questions:
47
48

- 49 83 1. Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance
50
51 84 development and mutagenesis?
52
53
54 85 2. What is the potential for substandard drugs to influence antibiotic resistance development?
55
56
57
58
59

86 METHODS

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

90 Patient and Public Involvement

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

92 Eligibility Criteria

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

96 **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) <ul style="list-style-type: none"> • pathogenic • non-pathogenic • clinical • environmental • community-acquired 	Eukaryotes (All) Archaea

	<ul style="list-style-type: none"> lab/domesticated strains 	
Intervention (Treatment)	<p>Treatment with ranges of fluoroquinolone concentrations with levels below the defined minimum inhibitory concentration (MIC), <i>in vitro</i>.</p> <p>All fluoroquinolones will be included.</p>	<p>Treatment at MIC or above MIC, if sub-MIC treatment is not considered.</p> <p>Treatment with sub-MIC fluoroquinolone concentrations in synergy with another class of antibiotic or compound.</p> <p>Treatment with first-generation quinolone antibiotics or other classes of antibiotics.</p> <p>Purely computational models.</p> <p>Studies involving animals.</p>
Comparator	No treatment (same experimental conditions, 0% API)	
Outcomes	<p>Experimental microbiological data related to:</p> <ul style="list-style-type: none"> resistance acquisition (to same or other antibiotic) and mutagenesis rate <p>Examples of data include standard</p>	<p>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</p>

	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

97

98 **Outcomes, Prioritization and Data Extraction**

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

1
2
3 103 resistance acquisition. After quantifying and evaluating the evidence, we aim to assess how
4
5 104 frequently primary scientific papers include public health context (not just clinical). Other
6
7
8 105 variables extracted from each study will include year of publication, bacterial species and number
9
10 106 of strains strain, type of bacterial isolate, drug name and concentration, and study design (duration
11
12 107 of treatment, growth condition, etc.). Study quality and limitations, and gaps in evidence for review
13
14
15 108 questions will also be extracted in addition to reporting on weaknesses in studies and how to
16
17 109 improve experimental design. Data will be extracted to a standardized Excel table.
18
19

20 110 **Search Strategy**

21
22
23 111 The search strategy was based on objectives and a preliminary search of PUBMED to determine
24
25 112 relevant MeSH terms. Using MeSH terms and keyword synonyms along with identified terms for
26
27 113 sub-inhibitory and substandard, a search strategy was designed in PUBMED and translated to Web
28
29
30 114 of Science and Embase to search all fields for articles that fit the inclusion criteria above (Table
31
32 115 1). Complete search terms and details are listed below. Additional records will be identified
33
34 116 through searching the bibliographies of already-identified papers and searching through papers
35
36
37 117 that have cited key studies. Only those papers that also match the above inclusion criteria will be
38
39 118 included. Complete search terms are provided in Appendix 2.
40
41

42 119 Identified terms: *subinhibitory, sub-inhibitory, sub inhibitory, sub-lethal, sublethal, sub lethal,*
43
44 120 *subminimal, sub-minimal, sub minimal, sub-therapeutic, subtherapeutic, sub therapeutic, sub*
45
46 121 *MIC, sub-MIC, low-dose, low dose, substandard, sub-standard, counterfeit, falsified*
47
48
49

50 122 **Study records**

51
52 123 Records will be managed through reference management software Endnote and Mendeley.
53
54
55 124 Additionally, search histories will be saved. Abstract screening and selection of studies will be
56
57
58
59

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

129 **Risk of bias in individual studies**

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

138 **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.	<p>Are there any discrepancies between methods and in-text?</p> <p>Is the methodological section missing any steps or appropriate detail ? (including but not limited to below)</p> <p>Steps/Details:</p> <ul style="list-style-type: none"> -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.

139

140 **Data Synthesis**

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

143 **Meta-bias(es)**

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

150 **Ethics and Dissemination**

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

154 **Authors Contributions**

1
2
3 155 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
4
5 156 protocol.

7
8 157 **Acknowledgements**

9
10
11 158 We would like to acknowledge David Flynn from Boston University Medical Alumni Library for
12
13 159 assistance with search terms.

14
15
16 160 **Funding statement**

17
18
19 161 This work was funded by a fellowship to C.C. by the United States Pharmacopeia.

20
21
22 162 **References:**

- 23
24
25 163 1 World Health Organization. A Study on the Public Health and Socioeconomic Impact of
26
27 Substandard and Falsified medical products. 2017.
28 164
29
30 165 2 Holmes AH, Moore LSP, Steinbakk M, *et al.* Antimicrobials : access and sustainable eff
31
32 ectiveness 2, Understanding the mechanisms and drivers of antimicrobial resistance.
33 166
34 *Lancet* 2016;**387**. doi:10.1016/S0140-6736(15)00473-0
35 167
36
37 168 3 Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat*
38
39 *Rev Microbiol* 2014;**12**:465–78. doi:10.1038/nrmicro3270
40 169
41
42 170 4 Zhanel GG, Kim SO, Davidson RJ, *et al.* Effect of subinhibitory concentrations of
43
44 Ciprofloxacin and gentamicin on the adherence of *Pseudomonas aeruginosa* to Vero cells
45
46 171 and voided uroepithelial cells. *Chemotherapy* 1993;**39**:105–11. doi:10.1159/000239110
47
48 172
49
50 173 5 Blazquez J, Rodriguez-Beltran J, Matic I. Antibiotic-Induced Genetic Variation: How It
51
52 Arises and How It Can Be Prevented. In: Gottesman S, ed. *Annual Review of*
53
54 174 *Microbiology, Vol 72*. 2018. 209–30. doi:10.1146/annurev-micro-090817-062139
55
56 175
57
58
59
60

- 1
2
3 176 6 Broom LJ. The sub-inhibitory theory for antibiotic growth promoters. *Poult Sci*
4
5 177 2007;:3104–8. doi:10.3382/ps/pex114
6
7
8 178 7 Ouzzani M, Hammady H, Fedorowicz Z, *et al.* Rayyan-a web and mobile app for
9
10 179 systematic reviews. *Syst Rev* 2016;5:1–10. doi:10.1186/s13643-016-0384-4
11
12
13 180 8 Hooijmans CR, Rovers MM, Vries RBM De, *et al.* SYRCLE 's risk of bias tool for
14
15 181 animal studies. 2014;:1–9.
16
17
18 182 9 Thomas BH, Ciliska D, Dobbins M, *et al.* A Process for Systematically Reviewing the
19
20 183 Literature : Providing the Research Evidence for Public Health Nursing Interventions.
21
22 184 2004.
23
24
25
26 185
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 ("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*:

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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:	<i>BMJ Open</i>
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

SCHOLARONE™
Manuscripts

1
2
3 1
4
5
6 2
7
8
9 3
10
11
12 4
13
14
15 5 **Bacterial antibiotic resistance development and mutagenesis following exposure to sub-**
16
17 6 **minimal inhibitory concentrations of fluoroquinolones *in vitro*: a systematic literature**
18
19 7 **review protocol**
20
21
22
23 8•

24
25 9 Carly Ching¹, Ebiowei S.F. Orubu², Veronika J. Wirtz³, Muhammad H. Zaman^{1,4*}
26
27

28 10 ¹Department of Biomedical Engineering, Boston University, Boston, MA, USA ²Institute for
29 11 Health System Innovation & Policy, Boston University, Boston, MA, USA ³Department of
30 12 Global Health, Boston University School of Public Health, Boston, MA, USA ⁴Howard Hughes
31 13 Medical Institute, Boston University, Boston, MA USA
32
33

34 14
35
36 15• * Muhammad H. Zaman

37
38 16• Department of Biomedical Engineering

39
40 17 Boston University,

41
42 18 44 Cummington Mall

43
44 19 Boston, Massachusetts 02215

45
46 20 617-358-5881

47
48 21 zaman@bu.edu
49

50 22•
51
52 23• Word Count: 1832 (excluding tables)
53
54
55 24

1
2
3 **25 ABSTRACT**
4
5
6

7 **26 Introduction:** Antibiotic resistance (AR) is among the most pressing global health challenges.
8
9 **27** Fluoroquinolones are a clinically important group of antibiotics that have wide applicability in
10
11 **28** both humans and animals. While many drivers of AR are known, the impact of medicine quality
12
13 **29** on AR remains largely unknown. The aim of this review is to systematically evaluate the evidence
14
15 **30** of the impact of *in vitro* sub-inhibitory antibiotic exposure, a major tenet of substandard antibiotics,
16
17 **31** on the development of AR and mutagenesis, using fluoroquinolones as a case study.
18
19
20

21 **32 Methods and analysis:** EMBASE, Web of Science and PubMed will be systematically searched
22
23 **33** for primary experimental *in vitro* studies, from earliest available dates within each database (1947,
24
25 **34** 1965 and 1966, respectively) through 2018, related to sub-inhibitory fluoroquinolone exposure
26
27 **35** and AR. A specifically developed non-weighted tool will be used to critically assess the evidence.
28
29 **36** Subgroup analyses will be performed for different variables and outcomes.
30
31
32

33
34 **37 Ethics and dissemination:** Ethical approval is not required as no primary data is to be collected.
35
36 **38** The completed systematic review will be disseminated through conference meeting presentations
37
38 **39** and a peer-reviewed publication.
39
40
41

42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

44 STRENGTHS AND LIMITATIONS OF THIS STUDY

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



59 INTRODUCTION

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

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

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

1
2
3 82 To identify important scientific trends and bring awareness to the topic of medicine quality, we
4
5 83 have extracted an underlying scientific question for a systematic review: Does sub-inhibitory
6
7
8 84 fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis?
9

10
11 85 Here, we seek to systematically synthesize and critically appraise experimental evidence on how
12
13 86 sub-inhibitory concentrations of one specific class of antibiotics, fluoroquinolones, impacts AR.
14

15 87 We have chosen fluoroquinolones as they are a commonly used classes of antibiotics, effective
16
17 88 against both Gram-negative and Gram-positive bacteria, in both human and animals. Resistance
18
19 89 emergence against fluoroquinolones has been widely reported for several decades[6–9]. Second to
20
21
22 90 fourth-generation fluoroquinolones stem from the initial non-fluorinated first-generation
23
24 91 quinolone class; these synthetic molecules are technically classified as antimicrobial agents and
25
26
27 92 share a bicyclic quinolone-related core structure, with a fluorine on the sixth or seventh carbon
28
29 93 position. For this review, we will refer to fluoroquinolones as antibiotics [9–11]. In addition to
30
31 94 substandard antibiotic exposure clinically, bacteria are exposed to sub-inhibitory antibiotic
32
33
34 95 concentrations in other situations, such as in the environment from wastewaters or agricultural
35
36 96 soils which can have implications in AR development and transmittance [12].
37
38

39 97 Currently, there are only a few broad narrative literature reviews on the impacts of sub-inhibitory
40
41 98 concentrations of antibiotics [13–16]. To our knowledge, there are currently few to no systematic
42
43
44 99 reviews of basic or fundamental microbiological bench research [17,18]. Thus, we seek to perform
45
46 100 an unbiased systematic literature review using Preferred Reporting Items for Systematic Review
47
48 101 and Meta-Analyses (PRISMA) guidelines on the topic of sub-inhibitory fluoroquinolone exposure
49
50
51 102 and AR development.
52

53 103 The results of this systematic review will contribute to the understanding of the impact of exposure
54
55
56 104 of bacteria to sub-inhibitory levels of fluoroquinolones on antibiotic resistance acquisition.
57
58
59

1
2
3 105 Secondly, we seek to identify gaps in evidence related to medicine quality in an effort to inform
4
5 106 policy-making on the control of substandard medicines. This work can contribute to a rigorous
6
7 107 evidence-base of bench research based on systematic review including critical appraisal of existing
8
9 108 literature instead of narrative review and selective reporting.
10
11
12

13 109 **Systematic review questions**

14
15
16 110 This review seeks to address the following questions:
17
18

- 19 111 1. Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance
20
21 112 development and mutagenesis *in vitro*? (Primary)
- 22
23 113 2. What is the potential for substandard fluoroquinolone drugs to lead to antibiotic resistance
24
25 114 development? (Secondary)

26 27 28 115 **METHODS**

29
30
31 116 Our methodology will conform to the PRISMA reporting standards (Appendix 1, PRISMA-P
32
33 117 Checklist). The protocol does not currently exist elsewhere and is ineligible for hosting on
34
35 118 PROSPERO because it the study participants are not people or animals. The duration of this study
36
37 119 is estimated to be six months.
38
39
40

41 120 **Patient and Public Involvement**

42
43
44 121 It was not appropriate or possible to involve patients or the public in this work.
45
46

47 122 **Eligibility Criteria**

48
49
50 123 To define the search approach and inclusion and exclusion criteria we applied a Population
51
52 124 Intervention Comparator Outcome Study (PICOS) search tool. The criteria is presented in Table.
53
54 125 1.

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

	Include	Exclude
Population	<ul style="list-style-type: none"> Bacteria (All isolates of Gram-negative and Gram-positive species)lab/domesticated strains 	<ul style="list-style-type: none"> Eukaryotes (All) Archaea
Intervention (Exposure)	<ul style="list-style-type: none"> Exposure with ranges of fluoroquinolone (second to fourth-generation) concentrations with levels below the defined MIC*, under controlled <i>in vitro</i> experimental conditions. <p>* defined as the concentration visibly inhibiting growth in the experimental set-up</p>	<ul style="list-style-type: none"> Exposure to first-generation quinolone antibiotics (i.e. nalidixic acid) or other classes of antibiotics Exposure 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	<ul style="list-style-type: none"> No treatment, MIC at 0% API of parental strain 	

<p>Outcomes</p>	<ul style="list-style-type: none"> Quantitative experimental microbiological data related to: <ul style="list-style-type: none"> (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) 	<ul style="list-style-type: none"> 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
<p>Study Design</p>	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> Conference abstracts Review articles (no primary data) Observational Studies

127 MIC=minimum inhibitory concentration, API=active pharmaceutical ingredients,

128 PCR=polymerase chasin reaction

129 **Outcomes, Prioritization and Data Extraction**

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

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

146 **Search Strategy**

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

1
2
3 151 that fit the inclusion criteria above (Table 1). Identified search terms are listed below. Search
4
5 152 strings were designed with a medical librarian. Additional records will be identified through
6
7
8 153 searching the bibliographies of already-identified papers and searching through papers that have
9
10 154 cited key studies. The complete search terms are provided in Appendix 2.

11
12
13 155 Identified terms: *subinhibitory, sub-inhibitory, sub inhibitory, sub-lethal, sublethal, sub lethal,*
14
15 156 *subminimal, sub-minimal, sub minimal, sub-therapeutic, subtherapeutic, sub therapeutic, sub*
16
17 157 *MIC, sub-MIC, low-dose, low dose, substandard, sub-standard, counterfeit, falsified*

18 19 20 21 158 **Study records**

22
23
24 159 Records will be managed through reference management software Endnote and Mendeley.
25
26 160 Additionally, search histories will be saved. Abstract screening and selection of studies will be
27
28 161 performed by two independent reviewers using software Rayyan QCRI [19]. A third researcher
29
30 162 will resolve discrepancies between reviewers selections. The full text of articles from the initial
31
32
33 163 screening will be reviewed for inclusion.

34 35 36 164 **Risk of bias in individual studies**

37
38
39 165 Risk of bias for laboratory microbiology experimentation will be assessed with criteria formulated
40
41 166 by considering and adapting the Systematic Review Centre for Laboratory animal Experimentation
42
43 167 (SYRCLE)'s risk of bias tool for animal studies [20] and the Effective Public Health Practice
44
45 168 Project (EPHPP) quality assessment tool [21]. The criteria is presented in Table 2. Here, we present
46
47
48 169 a non-weighted assessment of individual study quality, including risk of bias. For each of five
49
50 170 domains, studies will be assessed for a series of criteria listed below. For each unmet review criteria
51
52
53 171 within the domain an increased risk of bias point will be assigned. The more points assigned, the
54
55 172 higher the risk of bias associated with the study. There will be no defined cut-off for exclusion of

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

178 **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.	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • 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) <p>Steps/Details:</p>

Domain	Description of domain	Review Criteria
		<ul style="list-style-type: none"> -media used -temperature -time -incubation conditions (static, rolling, shaking, aeration) - reagents used - concentrations used - appropriate control experiments -replication of experiments
<p>Incomplete outcome data (Attrition Bias)</p>	<p>Describe the completeness of outcome data being analyzed, including attrition and exclusions from the analysis.</p>	<ul style="list-style-type: none"> • Is there missing outcome data that was not addressed? • Is the control outcome data mentioned in the paper present?
<p>Selective outcome reporting</p>	<p>Reporting of aim and all outcomes of the study.</p>	<ul style="list-style-type: none"> • Was all data reported for all conditions or just select/statistically significant results?

Domain	Description of domain	Review Criteria
(Reporting bias)		<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • 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.

179

180 Data Synthesis

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

1
2
3 188 study and which may reveal trends. It is clear that more tools need to be developed to move the
4
5 189 field of basic science towards systematic reviews.

6
7
8 190 If meta-analysis is not appropriate, quantitative sub-group analyses and summarization will be
9
10 191 performed. The following protocol, in brief, will be used: Data will be extracted into a standardized
11
12 192 Excel spreadsheet. From here, data will be sorted and grouped for each independent variable, such
13
14 193 as bacterial species, concentration of exposure and antibiotic. The dependent outcome of change
15
16 194 in resistance and mutagenesis will be plotted against these variables. The values of outcomes
17
18 195 (relative change in resistance) will be binned. This will allow us to determine the range and
19
20 196 frequency of magnitudes of resistance changes given different concentrations and different
21
22 197 antibiotics.

23 24 25 26 198 **Meta-bias(es)**

27
28
29 199 Based on data synthesis parameters, the overall quality of the body of evidence will be determined,
30
31 200 if possible. Since we will not be able to make direct clinical recommendations due to the limitations
32
33 201 of our review being focused on *in-vitro* studies we will focus on confidence in our overall summary
34
35 202 of results and trends. For this we will take into account publication bias across studies including
36
37 203 any potential publication biases in authors or types of studies, biases towards certain bacteria,
38
39 204 antibiotics and selective outcomes. Contrastingly, narrative literature reviews of basic science
40
41 205 typically do not critically assess the bias of each study and hence, do not take into account quality
42
43 206 in their summary which is an important limitation of narrative reviews. We will use GRADE
44
45 207 (Grading of Recommendations, Assessment, Development and Evaluations) guidelines on
46
47 208 publication bias to aid us in rating the quality of our evidence [22]. Additionally inconsistencies
48
49 209 in methodology and outcomes will be assessed and taken into account in determining the
50
51 210 confidence of our reported data summary.

1
2
3 **211 Ethics and Dissemination**
4

5
6 212 Ethical approval is not required as no primary data is to be collected. The completed systematic
7
8 213 review will be disseminated through conference meeting presentations and a peer-reviewed
9
10 214 publication.
11
12

13
14 **215 Authors Contributions**
15

16 216 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
17
18 217 protocol.
19
20

21
22 **218 Acknowledgements**
23

24
25 219 We would like to acknowledge David Flynn from Boston University Medical Alumni Library for
26
27 220 assistance with search terms and members of the Zaman lab for their input and feedback.
28
29

30 **221 Funding statement**
31

32
33 222 This work was funded by a fellowship to C.C. by the United States Pharmacopeia.
34
35

36 **223 Competing Interests**
37

38
39 224 The authors declare no competing interests.
40
41

42 **225 References:**
43

- 44
45 226 1 Chatterjee A, Modarai M, Naylor NR, *et al.* Review Quantifying drivers of antibiotic
46
47 227 resistance in humans : a systematic review. *Lancet Infect Dis* 2018;**18**:e368–78.
48
49 228 doi:10.1016/S1473-3099(18)30296-2
50
51
52 229 2 Okeke IN, Klugman KP, Bhutta ZA, *et al.* Antimicrobial resistance in developing
53
54 230 countries. Part II: Strategies for containment. *Lancet Infect Dis* 2005;**5**:568–80.
55
56
57
58
59
60

- 1
2
3 231 doi:10.1016/S1473-3099(05)70217-6
4
5
6 232 3 World Health Organization. A Study on the Public Health and Socieconomic Impact of
7
8 233 Substandard and Falsified medical products. 2017.
9
10
11 234 4 World Health Organization. A Study on the Public Health and Socieconomic Impact of
12
13 235 Substandard and Falsified medical products, Executive Summary. 2017;:1–29.
14
15
16 236 5 Holmes AH, Moore LSP, Steinbakk M, *et al.* Antimicrobials : access and sustainable eff
17
18 237 ectiveness 2, Understanding the mechanisms and drivers of antimicrobial resistance.
19
20 238 *Lancet* 2016;**387**. doi:10.1016/S0140-6736(15)00473-0
21
22
23
24 239 6 Kim ES, Hooper DC. Clinical importance and epidemiology of quinolone resistance.
25
26 240 *Infect Chemother* 2014;**46**:226–38. doi:10.3947/ic.2014.46.4.226
27
28
29 241 7 McEwen SA, Fedorka-Cray PJ. Antimicrobial Use and Resistance in Animals. *Clin Infect*
30
31 242 *Dis* 2002;**34**:Supplement.
32
33
34
35 243 8 Acar JF, Goldstein FW. Trends in Bacterial Resistance to Fluoroquinolones. *Clin Infect*
36
37 244 *Dis* 1999;**24**.
38
39
40 245 9 Dalhoff A. Global Fluoroquinolone Resistance Epidemiology and Implications for Clinical
41
42 246 Use. *Interdiscip Persepctives Infect Dsiseases* 2012;**2012**. doi:10.1155/2012/976273
43
44
45 247 10 Tillotson GS. Quinolones: Structure-activity relationships and future predictions. *J Med*
46
47 248 *Microbiol* 1996;**44**:320–4. doi:10.1099/00222615-44-5-320
48
49
50
51 249 11 Redgrave LS, Sutton SB, Webber MA, *et al.* Fluoroquinolone resistance : mechanisms ,
52
53 250 impact on bacteria , and role in evolutionary success. *Trends Microbiol* 2014;**22**:438–45.
54
55 251 doi:10.1016/j.tim.2014.04.007
56
57
58
59
60

- 1
2
3 252 12 Van Doorslaer X, Dewulf J, Van Langenhove H, *et al.* Fluoroquinolone antibiotics: An
4
5 253 emerging class of environmental micropollutants. *Sci Total Environ* 2014;**500–501**:250–
6
7 254 69. doi:10.1016/j.scitotenv.2014.08.075
8
9
10 255 13 Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat*
11
12 256 *Rev Microbiol* 2014;**12**:465–78. doi:10.1038/nrmicro3270
13
14
15
16 257 14 Zhanel GG, Kim SO, Davidson RJ, *et al.* Effect of subinhibitory concentrations of
17
18 258 Ciprofloxacin and gentamicin on the adherence of *Pseudomonas aeruginosa* to Vero cells
19
20 259 and voided uroepithelial cells. *Chemotherapy* 1993;**39**:105–11. doi:10.1159/000239110
21
22
23 260 15 Blazquez J, Rodriguez-Beltran J, Matic I. Antibiotic-Induced Genetic Variation: How It
24
25 261 Arises and How It Can Be Prevented. In: Gottesman S, ed. *Annual Review of*
26
27 262 *Microbiology, Vol 72*. 2018. 209–30. doi:10.1146/annurev-micro-090817-062139
28
29
30
31 263 16 Broom LJ. The sub-inhibitory theory for antibiotic growth promoters. *Poult Sci*
32
33 264 2007;**3**:104–8. doi:10.3382/ps/pex114
34
35
36 265 17 Hagan ECO, Matalon S, Riesenber LA. Systematic reviews of the literature : a better
37
38 266 way of addressing basic science controversies. 2018;**2017–20**.
39
40 267 doi:10.1152/ajplung.00544.2017
41
42
43
44 268 18 Editorial. Why basic microbiology still matters. *Nat Rev Microbiol* 2011;**9**:480.
45
46 269 doi:10.1038/nrmicro2611
47
48
49 270 19 Ouzzani M, Hammady H, Fedorowicz Z, *et al.* Rayyan-a web and mobile app for
50
51 271 systematic reviews. *Syst Rev* 2016;**5**:1–10. doi:10.1186/s13643-016-0384-4
52
53
54 272 20 Hooijmans CR, Rovers MM, Vries RBM De, *et al.* SYRCLE 's risk of bias tool for
55
56
57
58
59
60

1
2
3 273 animal studies. 2014;:1–9.
4
5

6 274 21 Thomas BH, Ciliska D, Dobbins M, *et al.* A Process for Systematically Reviewing the
7
8 275 Literature : Providing the Research Evidence for Public Health Nursing Interventions.
9
10 276 2004.
11
12

13 277 22 Nolting A, Perleth M, Langer G, *et al.* GRADE guidelines: 5. Rating the quality of
14
15 278 evidence - Publication bias. *Z Evid Fortbild Qual Gesundheitswes* 2012;**106**:670–6.
16
17 279 doi:10.1016/j.zefq.2012.10.015
18
19

20
21 280
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 hosting 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 ("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*:

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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:	<i>BMJ Open</i>
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

SCHOLARONE™
Manuscripts

1
2
3 1
4
5
6 2
7
8
9 3
10
11
12 4
13
14
15 5 **Bacterial antibiotic resistance development and mutagenesis following exposure to sub-**
16
17 6 **minimal inhibitory concentrations of fluoroquinolones *in vitro*: a systematic literature**
18
19 **review protocol**
20 7
21
22
23 8•

24
25 9 Carly Ching¹, Ebiowei S.F. Orubu², Veronika J. Wirtz³, Muhammad H. Zaman^{1,4*}
26
27

28 10 ¹Department of Biomedical Engineering, Boston University, Boston, MA, USA ²Institute for
29 11 Health System Innovation & Policy, Boston University, Boston, MA, USA ³Department of
30 12 Global Health, Boston University School of Public Health, Boston, MA, USA ⁴Howard Hughes
31 13 Medical Institute, Boston University, Boston, MA USA
32
33
34 14

35
36 15• * Muhammad H. Zaman
37

38 16• Department of Biomedical Engineering
39

40 17 Boston University,
41

42 18 44 Cummington Mall
43

44 19 Boston, Massachusetts 02215
45

46 20 617-358-5881
47

48 21 zaman@bu.edu
49

50 22•
51
52 23• Word Count: 1832 (excluding tables)
53
54
55 24
56
57
58
59
60

1
2
3 **25 ABSTRACT**
4
5
6

7 **26 Introduction:** Antibiotic resistance (AR) is among the most pressing global health challenges.
8
9 **27** Fluoroquinolones are a clinically important group of antibiotics that have wide applicability in
10
11 **28** both humans and animals. While many drivers of AR are known, the impact of medicine quality
12
13 **29** on AR remains largely unknown. The aim of this review is to systematically evaluate the evidence
14
15 **30** of the impact of *in vitro* sub-inhibitory antibiotic exposure, a major tenet of substandard antibiotics,
16
17 **31** on the development of AR and mutagenesis, using fluoroquinolones as a case study.
18
19
20

21 **32 Methods and analysis:** EMBASE, Web of Science and PubMed will be systematically searched
22
23 **33** for primary experimental *in vitro* studies, from earliest available dates within each database (1947,
24
25 **34** 1965 and 1966, respectively) through 2018, related to sub-inhibitory fluoroquinolone exposure
26
27 **35** and AR. A specifically developed non-weighted tool will be used to critically assess the evidence.
28
29 **36** Subgroup analyses will be performed for different variables and outcomes.
30
31
32

33
34 **37 Ethics and dissemination:** Ethical approval is not required as no primary data is to be collected.
35
36 **38** The completed systematic review will be disseminated through conference meeting presentations
37
38 **39** and a peer-reviewed publication.
39
40
41

42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

44 STRENGTHS AND LIMITATIONS OF THIS STUDY

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



59 INTRODUCTION

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

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

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

1
2
3 82 To identify important scientific trends and bring awareness to the topic of medicine quality, we
4
5 83 have extracted an underlying scientific question for a systematic review: Does sub-inhibitory
6
7 84 fluoroquinolone exposure increase bacterial antibiotic resistance development and mutagenesis?
8
9

10
11 85 Here, we seek to systematically synthesize and critically appraise experimental evidence on how
12
13 86 sub-inhibitory concentrations of one specific class of antibiotics, fluoroquinolones, impacts AR.
14

15 87 We have chosen fluoroquinolones as they are a commonly used classes of antibiotics, effective
16
17 88 against both Gram-negative and Gram-positive bacteria, in both human and animals. Resistance
18
19 89 emergence against fluoroquinolones has been widely reported for several decades[6–9]. Second to
20
21 90 fourth-generation fluoroquinolones stem from the initial non-fluorinated first-generation
22
23 91 quinolone class; these synthetic molecules are technically classified as antimicrobial agents and
24
25 92 share a bicyclic quinolone-related core structure, with a fluorine on the sixth or seventh carbon
26
27 93 position. For this review, we will refer to fluoroquinolones as antibiotics [9–11]. In addition to
28
29 94 substandard antibiotic exposure clinically, bacteria are exposed to sub-inhibitory antibiotic
30
31 95 concentrations in other situations, such as in the environment from wastewaters or agricultural
32
33 96 soils which can have implications in AR development and transmittance [12].
34
35
36
37
38

39 97 Currently, there are only a few broad narrative literature reviews on the impacts of sub-inhibitory
40
41 98 concentrations of antibiotics [13–16]. To our knowledge, there are currently few systematic
42
43 99 reviews of basic or fundamental microbiological bench research [17–19]. Thus, we seek to perform
44
45 100 an unbiased systematic literature review according to Preferred Reporting Items for Systematic
46
47 101 Review and Meta-Analyses (PRISMA) guidelines on the topic of sub-inhibitory fluoroquinolone
48
49 102 exposure and AR development.
50
51
52

53 103 The results of this systematic review will contribute to the understanding of the impact of exposure
54
55 104 of bacteria to sub-inhibitory levels of fluoroquinolones on antibiotic resistance acquisition.
56
57
58
59
60

1
2
3 105 Secondly, we seek to identify gaps in evidence related to medicine quality in an effort to inform
4
5 106 policy-making on the control of substandard medicines. This work can contribute to a rigorous
6
7 107 evidence-base of bench research based on systematic review including critical appraisal of existing
8
9 108 literature instead of narrative review and selective reporting.
10
11
12

13 109 **Systematic review questions**

14
15
16 110 This review seeks to address the following questions:
17
18

- 19 111 1. Does sub-inhibitory fluoroquinolone exposure increase bacterial antibiotic resistance
20
21 112 development and mutagenesis *in vitro*? (Primary)
- 22
23 113 2. What is the potential for substandard fluoroquinolone drugs to lead to antibiotic resistance
24
25 114 development? (Secondary)

26 27 28 115 **METHODS**

29
30
31 116 Our methodology will conform to the PRISMA reporting standards (Appendix 1, PRISMA-P
32
33 117 Checklist). The protocol does not currently exist elsewhere and is ineligible for hosting on
34
35 118 PROSPERO because it the study participants are not people or animals. The duration of this study
36
37 119 is estimated to be six months.
38
39
40

41 120 **Patient and Public Involvement**

42
43
44 121 It was not appropriate or possible to involve patients or the public in this work.
45
46

47 122 **Eligibility Criteria**

48
49
50 123 To define the search approach and inclusion and exclusion criteria we applied a Population
51
52 124 Intervention Comparator Outcome Study (PICOS) search tool. The criteria is presented in Table.
53
54 125 1.

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

	Include	Exclude
Population	<ul style="list-style-type: none"> Bacteria (All isolates of Gram-negative and Gram-positive species)lab/domesticated strains 	<ul style="list-style-type: none"> Eukaryotes (All) Archaea
Intervention (Exposure)	<ul style="list-style-type: none"> Exposure to ranges of fluoroquinolone (second to fourth-generation) concentrations with levels below the defined MIC*, under controlled <i>in vitro</i> experimental conditions. <p>* 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.</p>	<ul style="list-style-type: none"> Exposure to first-generation quinolone antibiotics (i.e. nalidixic acid) or other classes of antibiotics Exposure 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	<ul style="list-style-type: none"> No treatment, MIC at 0% API of parental strain 	

<p>Outcomes</p>	<ul style="list-style-type: none"> Quantitative experimental microbiological data related to: <ul style="list-style-type: none"> (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) 	<ul style="list-style-type: none"> 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
<p>Study Design</p>	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> Conference abstracts Review articles (no primary data) Observational Studies

127 MIC=minimum inhibitory concentration, API=active pharmaceutical ingredients,

128 PCR=polymerase chasin reaction

129 **Outcomes, Prioritization and Data Extraction**

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

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

146 **Search Strategy**

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

1
2
3 151 that fit the inclusion criteria above (Table 1). Identified search terms are listed below. Search
4
5 152 strings were designed with a medical librarian. Additional records will be identified through
6
7
8 153 searching the bibliographies of already-identified papers and searching through papers that have
9
10 154 cited key studies. The complete search terms are provided in Appendix 2.

11
12
13 155 Identified terms: *subinhibitory, sub-inhibitory, sub inhibitory, sub-lethal, sublethal, sub lethal,*
14
15 156 *subminimal, sub-minimal, sub minimal, sub-therapeutic, subtherapeutic, sub therapeutic, sub*
16
17 157 *MIC, sub-MIC, low-dose, low dose, substandard, sub-standard, counterfeit, falsified*

18 19 20 21 158 **Study records**

22
23
24 159 Records will be managed through reference management software Endnote and Mendeley.
25
26 160 Additionally, search histories will be saved. Abstract screening and selection of studies will be
27
28 161 performed by two independent reviewers using software Rayyan QCRI [20]. A third researcher
29
30 162 will resolve discrepancies between reviewers selections. The full text of articles from the initial
31
32
33 163 screening will be reviewed for inclusion.

34 35 36 164 **Risk of bias in individual studies**

37
38
39 165 Risk of bias for laboratory microbiology experimentation will be assessed with criteria formulated
40
41 166 by considering and adapting the Systematic Review Centre for Laboratory animal Experimentation
42
43 167 (SYRCLE)'s risk of bias tool for animal studies [21] and the Effective Public Health Practice
44
45 168 Project (EPHPP) quality assessment tool [22]. The criteria is presented in Table 2. Here, we present
46
47
48 169 a non-weighted assessment of individual study quality, including risk of bias. For each of five
49
50 170 domains, studies will be assessed for a series of criteria listed below. For each unmet review criteria
51
52 171 within the domain an increased risk of bias point will be assigned. The more points assigned, the
53
54
55 172 higher the risk of bias associated with the study. There will be no defined cut-off for exclusion of

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

178 **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.	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • 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) <p>Steps/Details:</p>

Domain	Description of domain	Review Criteria
		<ul style="list-style-type: none"> -media used -temperature -time -incubation conditions (static, rolling, shaking, aeration) - reagents used - concentrations used - appropriate control experiments -replication of experiments
<p>Incomplete outcome data (Attrition Bias)</p>	<p>Describe the completeness of outcome data being analyzed, including attrition and exclusions from the analysis.</p>	<ul style="list-style-type: none"> • Is there missing outcome data that was not addressed? • Is the control outcome data mentioned in the paper present?
<p>Selective outcome reporting</p>	<p>Reporting of aim and all outcomes of the study.</p>	<ul style="list-style-type: none"> • Was all data reported for all conditions or just select/statistically significant results?

Domain	Description of domain	Review Criteria
(Reporting bias)		<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • 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.

179

180 Data Synthesis

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

1
2
3 188 study and which may reveal trends. It is clear that more tools need to be developed to move the
4
5 189 field of basic science towards systematic reviews.

6
7
8 190 Quantitative sub-group analyses and summarization will be performed. The following protocol, in
9
10 191 brief, will be used: Data will be extracted into a standardized Excel spreadsheet. From here, data
11
12 192 will be sorted and grouped for each independent variable, such as bacterial species, concentration
13
14 193 of exposure and antibiotic. The rationale for subgroups is as follows. First, different bacterial
15
16 194 species often respond differently to stress or have different genetic responses to different stimuli.
17
18 195 For example, the clinically relevant pathogen *Acinetobacter baumannii*, which has a propensity to
19
20 196 gain multi-drug resistances, has a different DNA damage response compared to the conserved
21
22 197 paradigm of *Escherichia coli* [23]. This impacts how these two bacteria respond to stress and such
23
24 198 differences between bacterial species may lead to differences in responses to sub-inhibitory
25
26 199 fluoroquinolone exposure. Concentration of exposure is an important factor, as different
27
28 200 concentrations may present different selective compartments [24]. Similarly, it is of clinical
29
30 201 interest to determine if certain fluoroquinolones impact bacteria differently, given that
31
32 202 fluoroquinolones (with different usage and prescription patterns), display differences in
33
34 203 pharmacodynamics and resistance profiles [25,26]. The dependent outcome of change in resistance
35
36 204 and mutagenesis will be plotted against these factors. The values of outcomes (relative change in
37
38 205 resistance) will also be binned. This will allow us to determine the range and frequency of
39
40 206 magnitudes of resistance changes given different concentrations and different antibiotics.

41 42 43 44 45 46 47 **Meta-bias(es)**

48
49
50 208 Based on data synthesis parameters, the overall quality of the body of evidence will be determined,
51
52 209 if possible. Since we will not be able to make direct clinical recommendations due to the limitations
53
54 210 of our review being focused on *in-vitro* studies, we will focus on confidence in our overall

1
2
3 211 summary of results and trends. For this we will take into account publication bias across studies.
4
5 212 Carroll [27] identified how publication bias may exist in scientific literature and described
6
7
8 213 potential solutions, however these are not best practice. Publication bias could arise from, but is
9
10 214 not limited to, rejection of negative data, researchers not submitting research that present negative
11
12 215 data, publication based on results rather than the quality and rigor of the study design and influence
13
14
15 216 of industry and funding sources. All of these factors can lead to a skewed set of data that does not
16
17 217 fully represent the phenomena being investigated. Narrative literature reviews of basic science
18
19 218 typically do not critically assess the bias of each study and hence, do not take into account quality
20
21 219 in their summary which is an important limitation of narrative reviews. We will use GRADE
22
23
24 220 (Grading of Recommendations, Assessment, Development and Evaluations) guidelines on
25
26 221 publication bias to aid us in rating the quality of our evidence [28]. Specifically, to assess
27
28 222 publication bias we will look at the group of studies to determine how many studies had increased
29
30
31 223 bias for not reporting negative results, or only reporting statistically significant results. This criteria
32
33 224 is already present in our Risk of Bias analysis for individual studies. We will look at the studies
34
35 225 that published negative results and determine if there are differences in the impact factor/prestige
36
37
38 226 of journal that they are published in, and whether data is coming from the same research groups.
39
40 227 We will also look at the final number of studies and time of publication in order to identify if there
41
42 228 is any “lag bias”[28]. Funding sources, specifically frequency of industry funded studies, will be
43
44
45 229 noted. Acknowledging that publication bias is difficult to assess, as suggested by GRADE, we will
46
47 230 then determine if publication bias is “undetected” or “strongly suspected” and rate down a
48
49 231 maximum of one level for suspected bias [28]. Additionally biases in our set of evidence towards
50
51 232 certain bacteria, antibiotics and inconsistencies in methodology and outcomes will be assessed and
52
53
54 233 taken into account in determining the confidence of our reported data summary.
55
56
57
58
59
60

1
2
3 234 **Ethics and Dissemination**
4
5

6 235 Ethical approval is not required as no primary data is to be collected. The completed systematic
7
8 236 review will be disseminated through conference meeting presentations and a peer-reviewed
9
10
11 237 publication.
12

13
14 238 **Authors Contributions**
15

16
17 239 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
18
19 240 protocol.
20
21

22 241 **Acknowledgements**
23

24
25 242 We would like to acknowledge David Flynn from Boston University Medical Alumni Library for
26
27 243 assistance with search terms and members of the Zaman lab for their input and feedback.
28
29

30 244 **Funding statement**
31

32
33 245 This work was funded by a fellowship to C.C. by the United States Pharmacopeia.
34
35

36 246 **Competing Interests**
37

38
39 247 The authors declare no competing interests.
40
41

42 248 **References:**
43

- 44
45 249 1 Chatterjee A, Modarai M, Naylor NR, *et al.* Review Quantifying drivers of antibiotic
46
47 250 resistance in humans : a systematic review. *Lancet Infect Dis* 2018;**18**:e368–78.
48
49 251 doi:10.1016/S1473-3099(18)30296-2
50
51
52 252 2 Okeke IN, Klugman KP, Bhutta ZA, *et al.* Antimicrobial resistance in developing
53
54 253 countries. Part II: Strategies for containment. *Lancet Infect Dis* 2005;**5**:568–80.
55
56
57
58
59
60

- 1
2
3 254 doi:10.1016/S1473-3099(05)70217-6
4
5
6 255 3 World Health Organization. A Study on the Public Health and Socioeconomic Impact of
7
8 256 Substandard and Falsified medical products. 2017.
9
10
11 257 4 World Health Organization. A Study on the Public Health and Socioeconomic Impact of
12
13 258 Substandard and Falsified medical products, Executive Summary. 2017;:1–29.
14
15
16 259 5 Holmes AH, Moore LSP, Steinbakk M, *et al.* Antimicrobials : access and sustainable eff
17
18 260 ectiveness 2, Understanding the mechanisms and drivers of antimicrobial resistance.
19
20 261 *Lancet* 2016;**387**. doi:10.1016/S0140-6736(15)00473-0
21
22
23
24 262 6 Kim ES, Hooper DC. Clinical importance and epidemiology of quinolone resistance.
25
26 263 *Infect Chemother* 2014;**46**:226–38. doi:10.3947/ic.2014.46.4.226
27
28
29 264 7 McEwen SA, Fedorka-Cray PJ. Antimicrobial Use and Resistance in Animals. *Clin Infect*
30
31 265 *Dis* 2002;**34**:Supplement.
32
33
34 266 8 Acar JF, Goldstein FW. Trends in Bacterial Resistance to Fluoroquinolones. *Clin Infect*
35
36 267 *Dis* 1999;**24**.
37
38
39 268 9 Dalhoff A. Global Fluoroquinolone Resistance Epidemiology and Implications for Clinical
40
41 269 Use. *Interdiscip Persepctives Infect Dsiseases* 2012;**2012**. doi:10.1155/2012/976273
42
43
44
45 270 10 Tillotson GS. Quinolones: Structure-activity relationships and future predictions. *J Med*
46
47 271 *Microbiol* 1996;**44**:320–4. doi:10.1099/00222615-44-5-320
48
49
50 272 11 Redgrave LS, Sutton SB, Webber MA, *et al.* Fluoroquinolone resistance : mechanisms ,
51
52 273 impact on bacteria , and role in evolutionary success. *Trends Microbiol* 2014;**22**:438–45.
53
54 274 doi:10.1016/j.tim.2014.04.007
55
56
57
58
59
60

- 1
2
3 275 12 Van Doorslaer X, Dewulf J, Van Langenhove H, *et al.* Fluoroquinolone antibiotics: An
4
5 276 emerging class of environmental micropollutants. *Sci Total Environ* 2014;**500–501**:250–
6
7 277 69. doi:10.1016/j.scitotenv.2014.08.075
8
9
10 278 13 Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. *Nat*
11
12 279 *Rev Microbiol* 2014;**12**:465–78. doi:10.1038/nrmicro3270
13
14
15 280 14 Zhanel GG, Kim SO, Davidson RJ, *et al.* Effect of subinhibitory concentrations of
16
17 281 Ciprofloxacin and gentamicin on the adherence of *Pseudomonas aeruginosa* to Vero cells
18
19 282 and voided uroepithelial cells. *Chemotherapy* 1993;**39**:105–11. doi:10.1159/000239110
20
21 283 15 Blazquez J, Rodriguez-Beltran J, Matic I. Antibiotic-Induced Genetic Variation: How It
22
23 284 Arises and How It Can Be Prevented. In: Gottesman S, ed. *Annual Review of*
24
25 285 *Microbiology, Vol 72*. 2018. 209–30. doi:10.1146/annurev-micro-090817-062139
26
27
28 286 16 Broom LJ. The sub-inhibitory theory for antibiotic growth promoters. *Poult Sci*
29
30 287 2007;**3**:104–8. doi:10.3382/ps/pex114
31
32
33 288 17 Hagan ECO, Matalon S, Riesenber LA. Systematic reviews of the literature : a better
34
35 289 way of addressing basic science controversies. 2018;**2017–20**.
36
37 290 doi:10.1152/ajplung.00544.2017
38
39
40 291 18 Editorial. Why basic microbiology still matters. *Nat Rev Microbiol* 2011;**9**:480.
41
42 292 doi:10.1038/nrmicro2611
43
44
45 293 19 Van Der Putten BCL, Remondini D, Pasquini G, *et al.* Quantifying the contribution of
46
47 294 four resistance mechanisms to ciprofloxacin MIC in *Escherichia coli*: A systematic
48
49 295 review. *J Antimicrob Chemother* 2019;**74**:298–310. doi:10.1093/jac/dky417
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 296 20 Ouzzani M, Hammady H, Fedorowicz Z, *et al.* Rayyan-a web and mobile app for
4
5 297 systematic reviews. *Syst Rev* 2016;**5**:1–10. doi:10.1186/s13643-016-0384-4
6
7
8 298 21 Hooijmans CR, Rovers MM, Vries RBM De, *et al.* SYRCLE 's risk of bias tool for
9
10 299 animal studies. 2014;:1–9.
11
12
13 300 22 Thomas BH, Ciliska D, Dobbins M, *et al.* A Process for Systematically Reviewing the
14
15 301 Literature : Providing the Research Evidence for Public Health Nursing Interventions.
16
17 302 2004.
18
19
20
21 303 23 MacGuire AE, Ching MC, Diamond BH, *et al.* Activation of phenotypic subpopulations in
22
23 304 response to ciprofloxacin treatment in *Acinetobacter baumannii*. *Mol Microbiol*
24
25 305 2014;**92**:138–52. doi:10.1111/mmi.12541
26
27
28
29 306 24 Baquero F. Low-level antibacterial resistance: A gateway to clinical resistance. *Drug*
30
31 307 *Resist Updat* 2001;**4**:93–105. doi:10.1054/drup.2001.0196
32
33
34 308 25 Rolston KVI, Frisbee-Hume S, LeBlanc BM, *et al.* Antimicrobial activity of a novel des-
35
36 309 fluoro (6) quinolone, garenoxacin (BMS-284756), compared to other quinolones, against
37
38 310 clinical isolates from cancer patients. *Diagn Microbiol Infect Dis* 2002;**44**:187–94.
39
40 311 doi:10.1016/S0732-8893(02)00433-9
41
42
43
44 312 26 Boyd LB, Maynard MJ, Morgan-linnell SK, *et al.* Relationships among Ciprofloxacin ,
45
46 313 Gatifloxacin , Levofloxacin , and Norfloxacin MICs for Fluoroquinolone-Resistant
47
48 314 *Escherichia coli* Clinical Isolates □. *Antimicrob Agents Chemother* 2009;**53**:229–34.
49
50 315 doi:10.1128/AAC.00722-08
51
52
53
54 316 27 Carroll HA, Toumpakari Z, Johnson L, *et al.* The perceived feasibility of methods to
55
56
57
58
59
60

1
2
3 317 reduce publication bias. *PLoS One* 2017;**12**:1–19. doi:10.1371/journal.pone.0186472

4
5
6 318 28 Nolting A, Perleth M, Langer G, *et al.* GRADE guidelines: 5. Rating the quality of

7
8 319 evidence - Publication bias. *Z Evid Fortbild Qual Gesundheitswes* 2012;**106**:670–6.

9
10
11 320 doi:10.1016/j.zefq.2012.10.015

12
13
14 321

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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 hosting 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 ("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*:

1
2
3 TS=(quinolone* OR fluoroquinolone* OR ciprofloxacin OR norfloxacin OR ofloxacin OR
4 levofloxacin OR moxifloxacin OR gemifloxacin or enrofloxacin) AND TS=(subinhibitory OR sub-
5 inhibitory OR "sub inhibitory" OR sub-lethal OR sublethal OR "sub lethal" OR subminimal OR
6 sub-minimal OR "sub minimal" OR subminimum OR sub-minimum OR "sub minimum" OR sub-
7 therapeutic OR subtherapeutic OR "sub therapeutic" OR "sub MIC" OR sub-mic OR low-dose OR
8 "low dose" OR substandard OR "sub standard" OR sub-standard OR counterfeit OR falsified)
9 AND TS=(Drug Resistance, Microbial OR Antimicrobial Drug Resistance OR Antimicrobial Drug
10 Resistances OR Antibiotic Resistance OR Microbial Antibiotic Resistance OR Resistance,
11 Antibiotic OR Resistance OR Resistant OR Mutagenesis OR Mutation OR Mutagenicity OR Gene
12 expression OR transcription or transcriptional)
13
14
15

16 Embase

17 Search Term*:

18 (subinhibitory OR 'sub inhibitory' OR 'sub-lethal' OR sublethal OR 'sub
19 lethal' OR subminimal OR 'sub-minimal' OR 'sub minimal' OR subminimum OR 'sub-minimum'
20 OR 'sub minimum' OR 'sub-therapeutic' OR subtherapeutic OR 'sub therapeutic' OR 'sub
21 mic' OR 'sub-mic' OR 'low-dose' OR 'low dose' OR substandard OR 'sub standard' OR 'sub-
22 standard' OR counterfeit OR falsified) AND ('quinolone derived antiinfective agent'/exp
23 OR 'anti infective agents, fluoroquinolone' OR 'anti infective agents, quinolone' OR 'anti-
24 infective agents, fluoroquinolone' OR 'anti-infective agents, quinolone' OR 'antibiotics,
25 quinolones' OR 'chinolone antibiotic' OR 'fluoroquinolone (antibiotic)' OR 'fluoroquinolone
26 antibiotic' OR 'fluoroquinolone antibiotic agent' OR 'fluoroquinolone antiinfective
27 agent' OR 'fluoroquinolone antimicrobial agent' OR 'quinolone derived antiinfective
28 agent' OR 'quinolone (antibiotic)' OR 'quinolone antibiotic' OR 'quinolone
29 antibiotics' OR 'quinolone antiinfective agent') AND ('antibiotic resistance'/exp
30 OR 'antibacterial drug resistance' OR 'antibacterial resistance' OR 'antibiotic
31 resistance' OR 'antimicrobial drug resistance' OR 'antimicrobial resistance' OR 'bacterial drug
32 resistance' OR 'bacterial resistance' OR 'bacterium resistance' OR 'drug resistance,
33 bacterial' OR 'drug resistance, microbial' OR 'microbial drug
34 resistance' OR 'resistance' OR 'resistant' OR 'gene expression'/exp OR 'expression,
35 gene' OR 'gene expression' OR 'genetic expression' OR 'genome expression' OR 'genetic
36 transcription'/exp OR transcription OR transcriptional)
37
38
39
40
41
42
43

44 ***Amendment** – While gene expression is included in the above search terms, gene expression
45 will be excluded from the outcomes.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60