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Supplementary File 1

PRISMA 2020 Checklist—items to include when reporting a systematic review

Antibiofilm and Quorum-sensing Agents Against Pseudomonas aeruginosa and Staphylococcus aureus: a Systematic Review of Preclinical Studies, 2022-2026

Ma'aitah, S., AlAmairyin, B. (2026). Journal of Basic and Applied Research in Biomedicine, 12(1): 33-50

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title	1	Identify the report as a systematic review.	Title, page 33	"…a systematic review of preclinical studies, 2022-2026"
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract, page 33	Structured abstract (Objective, Methods, Results, Conclusion). Methods: "PubMed and Scopus were searched without a date limit (3,349 records). Eligible in vitro and in vivo studies were published from 2022 onward, tested an exogenous agent and named either organism in the title." Results: "We included 252 studies, led by natural products (115). A QS assay in the target pathogen was present in 140 studies (56%)… No meta-analysis was done, and title-only organism matching may have missed studies."
Introduction				
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction, page 33	"Its quality is uneven. Many papers claim QS inhibition from indirect readouts such as pyocyanin or swarming, concentrations at or above the minimum inhibitory concentration (MIC) confound antibiofilm effects with killing, and docking scores are used as if they were evidence of activity… Earlier reviews tend to cover one agent class or one pathogen, and few separate studies that measured QS directly from those that inferred it (Shariati et al., 2024)."
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction, page 33	"Our aims were to (1) describe the agents, models and outcomes, (2) grade each study by how directly it tested QS, and (3) appraise risk of bias in the in vitro and animal evidence."
Methods				
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Section 2.2, page 33; Section 2.4, page 34	"We included preclinical in vitro and in vivo studies published in English from 2022 onward that tested an exogenous antibiofilm or anti-quorum-sensing agent… and that named Pseudomonas aeruginosa or Staphylococcus aureus/MRSA in the title. … We excluded human trials, narrative and systematic reviews, editorials and correction notices, retracted articles, studies of native genes or regulators without an exogenous agent (knockout-only), and purely in silico studies." Grouping: each study was assigned one primary agent class by a fixed order of rules (Section 2.4) and one QS evidence tier (Section 2.5).
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Sections 2.1 and 2.3, pages 33–34; Table S1	"The databases were last searched in August 2026." "PubMed and Scopus were searched (full strategies in Supplementary Table S1)." Cochrane CENTRAL was searched at first; its 2 records were not eligible and it was removed from the review. Full-text retrieval used PubMed Central, Unpaywall, Zotero and institutional access.

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Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Section 2.3, page 34; Table S1	"The PubMed query combined antibiofilm terms, quorum-sensing terms and biofilm terms and excluded clinical trials, randomised controlled trials and records indexed to humans. The Scopus query used the same three concept groups in title, abstract and keywords and excluded clinical-trial terms and the Medicine subject area." "The searches had no date limit." Verbatim queries, filters and record counts are in Supplementary Table S1.
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Section 2.3, page 34	"Duplicates were removed across databases before loading (892 records) and within the screening log by DOI, exact-title and fuzzy-title matching (1 record). Records were screened by title and abstract, and the full texts of the remaining records were assessed against the criteria." The number of screeners per record is not stated in the manuscript.
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Section 2.4, page 34; Section 2.7, page 34; AI declaration, page 40	"Extraction was done by the author, with the aid of AI tool (see Use of generative AI), and was verified against the full text by two reviewers." "Two reviewers independently checked the extraction, the tiers and the ratings against the full texts, and disagreements were settled by discussion." Study investigators were not contacted. AI use is declared in the Declaration of Generative AI section (page 40).
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Sections 2.4 and 2.5, page 34	Extracted: "the country, study type, agent and class, target pathogen and strain, model system, concentrations, exposure time, biofilm and QS measures and results, in vivo outcomes, cytotoxicity testing and funding." The main outcome was the QS evidence tier, defined by "what the authors themselves measured" (Direct, Surrogate reporter, Indirect, In silico only, Not assessed).
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Section 2.4, page 34	Other variables are listed in Section 2.4 above (country, study type, agent, strain, model, concentrations, exposure time, cytotoxicity, funding). "Where a paper contradicted itself, we used the value in the Results or the figure and recorded the discrepancy in the workbook."
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Section 2.6, page 34; Table S3	In vitro studies: a nine-item checklist built for this review ("not a published or validated instrument"), scored yes/no/unclear (rubric in Table S3). Vertebrate efficacy studies: SYRCLE's risk-of-bias tool (Hooijmans et al., 2014). Ratings came from a rule-based first pass (automation), after which "the two reviewers checked all ratings, including these, against the full texts." Invertebrate-only and toxicity-only studies were not rated.
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Section 2.7, page 34	Not applicable. "Because outcomes were reported in different units (percent, fold change, log reduction) and with different designs, no meta-analysis was done; results are summarised descriptively as counts of studies."
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item 5)).	Sections 2.4 and 2.5, page 34	All 252 included studies were summarised descriptively. Studies were grouped by primary agent class (order of rules in Section 2.4), by target organism and by QS evidence tier (Section 2.5).
	13b	Describe any methods used to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Section 2.7, page 34	Not applicable; no summary statistics were pooled or converted. "Docking scores were not compared across studies because the scoring programs differ."

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	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Sections 3.2–3.5, pages 35–39; Tables 1–3; Figures 1–4; Table S2	Tables 1–3 (QS tier by organism, QS tier by agent class, animal studies), Figure 2 (classes), Figure 3 (keyword co-occurrence map; construction rules in the legend), Figure 4 (item-level risk of bias) and Supplementary Table S2 (one row per study).
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Section 2.7, page 34	"…no meta-analysis was done; results are summarised descriptively as counts of studies." Results are described by class because "agents from different classes were not assumed to be comparable at a given concentration."
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Sections 2.4–2.5, page 34	Not applicable (no meta-analysis). The QS evidence tier was tabulated by target organism and by agent class (Tables 1–2).
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Section 2.7, page 34; Section 3.6, page 39	"A sensitivity analysis repeated the main summaries for studies with Direct QS evidence." A second sensitivity analysis considered the 18 abstract-only records that were later excluded (Section 3.6).
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Section 4.5, page 40	Not done. Search coverage (PubMed and Scopus only, English only, organism named in the title) is discussed as a limitation in Section 4.5.
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Sections 2.5–2.6, page 34	No formal certainty grading (such as GRADE) was applied. QS evidence tiers (Section 2.5) and risk-of-bias ratings (Section 2.6) were used to describe the strength of the evidence.
Results				
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Section 3.1, page 34; Figure 1, page 35	"The searches identified 3,349 records. After 893 duplicates were removed, 2,456 records were screened and 520 were excluded on title and abstract. Of 1,936 records assessed for eligibility, 1,684 were excluded… In total, 252 studies were included (Figure 1)."
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Section 3.1, page 34; Section 4.5, page 40; Supplementary Materials, page 40	Reasons for the 1,684 exclusions are given with counts: 774 target organism not in the title, 740 published before 2022, 145 full text not obtained, 18 different species, 7 other reasons. The list of the 18 abstract-only records excluded after extraction is provided in the Supplementary Materials. Individual excluded records are not cited in the text.
Study characteristics	17	Cite each included study and present its characteristics.	Sections 3.2–3.3.1, pages 35–36; Table 3, page 37; Table S2	All 252 included studies are listed in Supplementary Table S2 (agent, class, organism, model, concentrations, results, QS tier, in vivo model). Agent classes are described in Section 3.3.1 with citations; animal studies are in Table 3.
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Section 3.5, pages 38–39; Figure 4, page 39; Table S3	"Of the 252 in vitro appraisals, 143 were rated low, 93 moderate and 16 high risk of bias." "Of the 46 animal infection or efficacy studies, 31 included a vertebrate model; 30 were rated with SYRCLE, all with some concerns." Study-level ratings are in the extraction workbook (QA - In Vitro, QA - In Vivo (SYRCLE)).

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Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table S2 (Biofilm result, QS result)	Not applicable as effect estimates. Biofilm and QS results for each study are summarised in text form in Supplementary Table S2; no effect estimates were pooled (Section 2.7).
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Sections 3.2–3.5, pages 35–39	Characteristics: Section 3.2 (country, year, model, organism, class). Risk of bias: Section 3.5 (in vitro and SYRCLE ratings).
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Section 3.3, page 35; Tables 1–2	No meta-analysis. Descriptive counts: "A Direct QS assay… was present in 140 of 252 studies (56%)." "Direct evidence was more common for P. aeruginosa (58%) than for S. aureus/MRSA (54%) and for studies on both species (35%)."
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Section 3.3, page 35; Table 2	Not applicable (no meta-analysis). QS evidence tier by agent class: "Direct evidence was highest for repurposed drugs (13 of 18) and lowest for multi-class combinations (6 of 15)."
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Section 3.6, page 39	"Among the 140 studies with Direct QS evidence, natural products accounted for 63 (45%, against 46% of all 252 studies) and P. aeruginosa for 95 (68%, against 65%)." "Had these 18 been kept, the Direct share would have been 54% (145 of 270) instead of 56%."
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Section 4.5, page 40	Not assessed (see item 14).
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Sections 3.3 and 3.5, pages 35–39	Not graded (see item 15). Strength of evidence is described through the QS evidence tiers and the risk-of-bias ratings.
Discussion				
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Section 4.1, page 39	"This review mapped 252 preclinical studies from 2022 to 2026… Earlier reviews have covered natural compounds against P. aeruginosa (Shariati et al., 2024) and the P. aeruginosa QS systems as drug targets (Soukarieh et al., 2018); the present review adds S. aureus/MRSA and separates studies by what was actually measured."
	23b	Discuss any limitations of the evidence included in the review.	Sections 4.2–4.4, pages 39–40	Surrogate and indirect QS readouts, assays at or above the MIC, growth-delay artefacts (Section 4.2), docking scores (Section 4.3), and sparse animal and cytotoxicity data (Section 4.4).
	23c	Discuss any limitations of the review processes used.	Section 4.5, page 40	"Eligibility required the organism to be named in the title…" "Only PubMed and Scopus were searched… Language was limited to English." 145 records could not be retrieved in full text; 15 invertebrate studies were not rated with SYRCLE; no meta-analysis was done.
	23d	Discuss implications of the results for practice, policy, and future research.	Section 4.6, page 40; Section 5, page 40	"Future studies should test QS in the target pathogen with a specific reporter or gene-expression assay at sub-MIC doses… Reviews and funders should treat docking-only and phenotype-only claims as preliminary."
Other information				

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Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Section 2.2, page 33	"Because the protocol was not registered, no criteria were fixed in advance in a public record."
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Section 2.2, page 33	No registered or published protocol (see item 24a).
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Sections 2.2 and 2.5, pages 33–34	"The restriction to studies published from 2022 onward and naming either organism in the title was decided after the initial search and is therefore a post hoc criterion." "The Surrogate reporter tier was added after the first extraction round; 27 studies first coded Direct were moved into it…"
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Funding, page 40	"This research received no external funding."
Competing interests	26	Declare any competing interests of review authors.	Conflict of Interest, pages 33 and 40	"The authors declare no conflict of interest related to the research. Editorial disclosure: Prof. Dr. Haitham Qaralleh, Editor-in-Chief of this journal, has a supervisory relationship with the authors. He was not involved in the editorial handling of this manuscript, the selection of reviewers, or any decision on it. Peer review and the editorial decision were managed independently by another member of the editorial board."
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Data Availability and Supplementary Materials, page 40	"No new primary data were generated. All extracted data, the search strategies (Table S1) and the list of included studies (Table S2) are provided as Supplementary Materials." The extraction workbook (Screening Log, Data Extraction, QA - In Vitro, QA - In Vivo (SYRCLE), PRISMA Tracker, Synthesis, Sensitivity by verification, Change Log) is also supplied.

*Page numbers refer to the pages of this article in J. Basic Appl. Res. Biomed. 12(1): 33-50.

Note: From Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/strobe