SUPPORTING MATERIAL

Figure S1: Detailed screening flowchart

Screening criteria described for all stages with numbers of passing/excluded records. Examples of excluded records are described at each stage.

Stage one title and abstract relevancy screen

Stage two methods screen

Included papers: 20

Inclusion criteria

Search results (7063)

Primary research

Exclusion questions

Does the paper focus on evaluating STWs – treatment process efficiency or role in selection/emergence of AMR

Collected wastewater sample with human sewage input

Stage one: relevance screen Titles and Abstracts

Reported prevalence of AMR

Does the paper investigate environmental pollution – impact of effluent or overflows on the receiving environment

Performs a comparison of wastewater to another dataset

Inclusion question

Does the paper describe a study which actively attempts to pilot sewage/wastewater surveillance of AMR

Stage two: design screen Full retrieved and methods evaluated (174)

Inclusion question

Does the paper describe a study which actively attempts to pilot sewage/wastewater surveillance of AMR

Yes (12)

Data extraction and risk of bias assessment (44)

Records included: culture-dependent methods Full text reviewed

Records included: culture-independent methods Full text reviewed

Narrative synthesis

Records excluded at stage one screen (6889)

No

Has the paper:

Analysed wastewater samples/data from at least one STW

Compared wastewater to data representing human population (clinical isolates, surveillance data etc)

Yes (32)

Records excluded at data extraction/eligibility with reasons (24)

No

Records excluded at stage two screen (130)

Examples of excluded records at stage one screen
Environmental studies:
- Anthropogenic pollution point source tracking
- Effluent composition, storm events and impact on receiving environment
- Human exposure risk assessment (rivers, beaches etc)

Sewage treatment work evaluations:
- Treatment process comparisons (efficiency) and method development (bioreactors etc)
- Effect of STWs on composition changes between influent and effluent (selection and horizontal gene transfer)

Single source characterisation and lack of human input:
- Reporting of AMR prevalence in wastewater sources without comparison to human prevalence
- Reporting of draft genomes/plasmids isolated from wastewater and phage mining
- Studies investigating wastewater sources such as agriculture, abattoirs and aquaculture

Examples of excluded records at stage two screen and data extraction
Comparison of sewer inputs with no human representing dataset:
- Effect of hospital, other healthcare facilities and urban residential effluent on STW influent composition

Studies which have not sampled/analysed samples from a municipal STW or have very small sample sizes (assessed by data extraction form to have no relevant data to combine):
- One health observational studies with small STW/human component
- Tracking of single isolates from patients to STW

Studies with undefined or vague wastewater metadata

Figure S2: Global density of wastewater sampling of included studies

Count of studies sampling each country plotted by points on country centroids with size reflecting number of studies. World Bank income classifications by study was as follows: high income (n=14), middle income (n=8), low income (n=1).
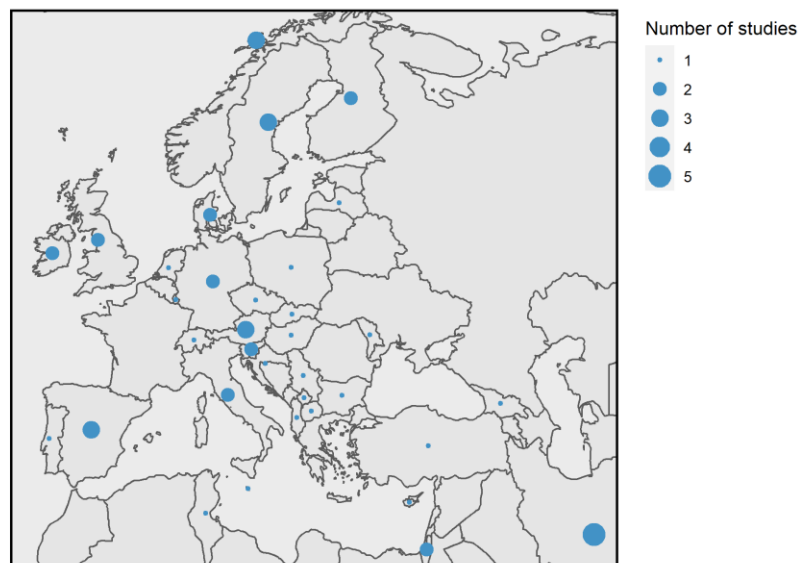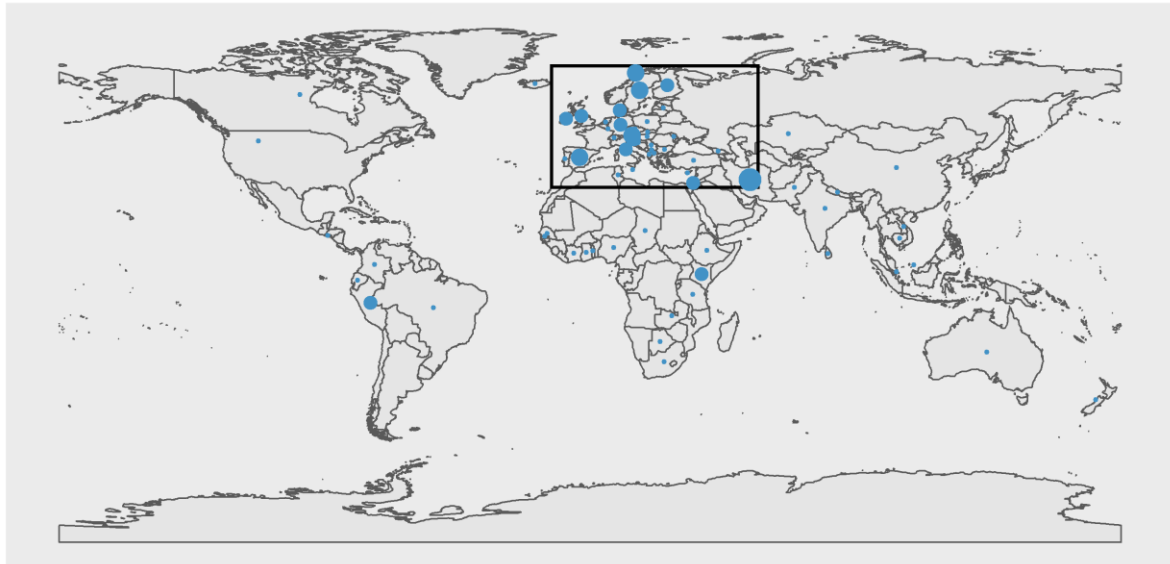
Figure S3: Number of WwTWs sampled and number of wastewater samples collected for each study.

Plotted by study outcome and AMR evaluation approach.
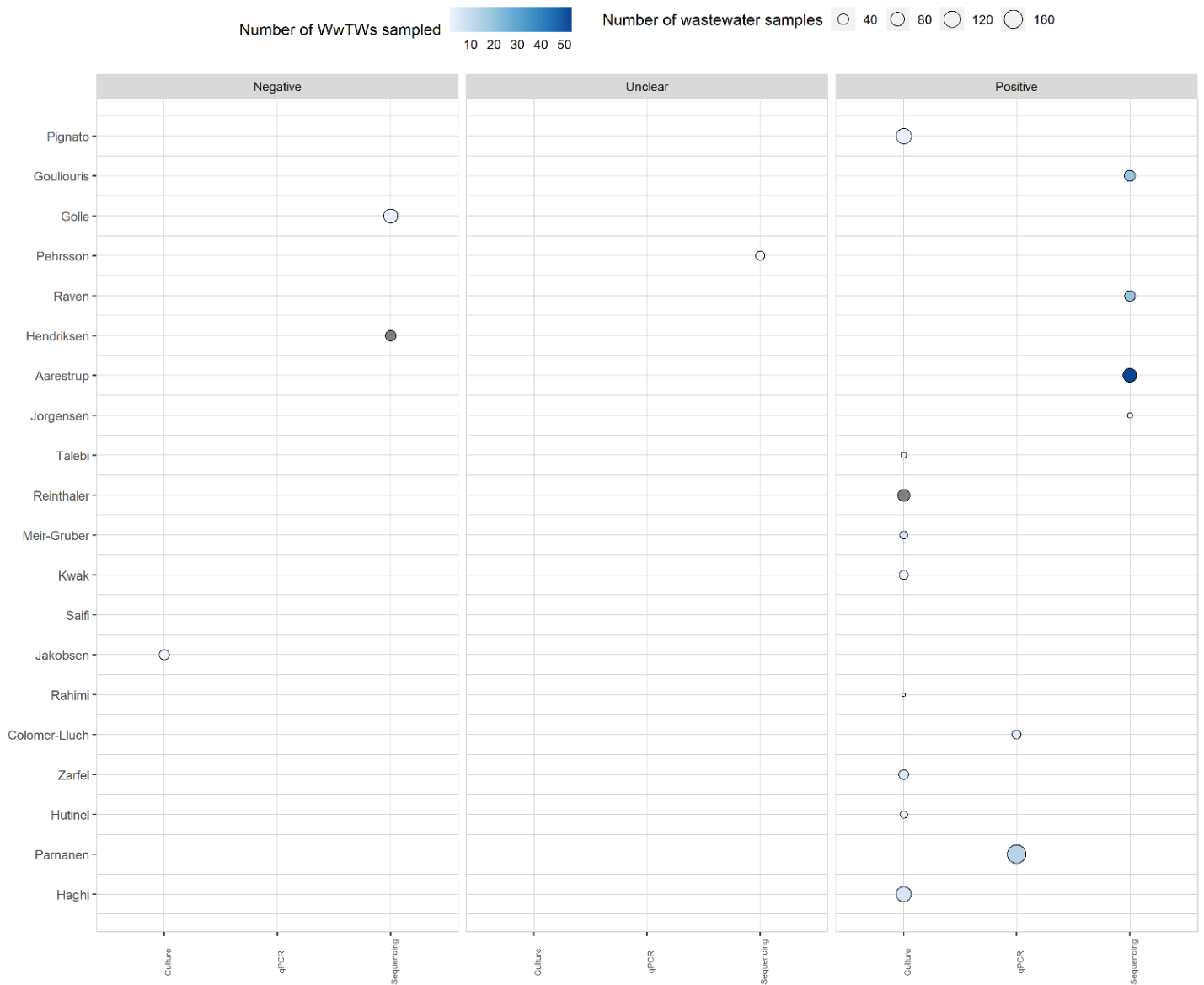
Figure S4: WwTW population equivalent and flow for each study, where reported.

Plotted by study outcome.

Figure S5: Study organism, phenotype and genotype targets.

Plotted by study outcome and AMR evaluation approach.
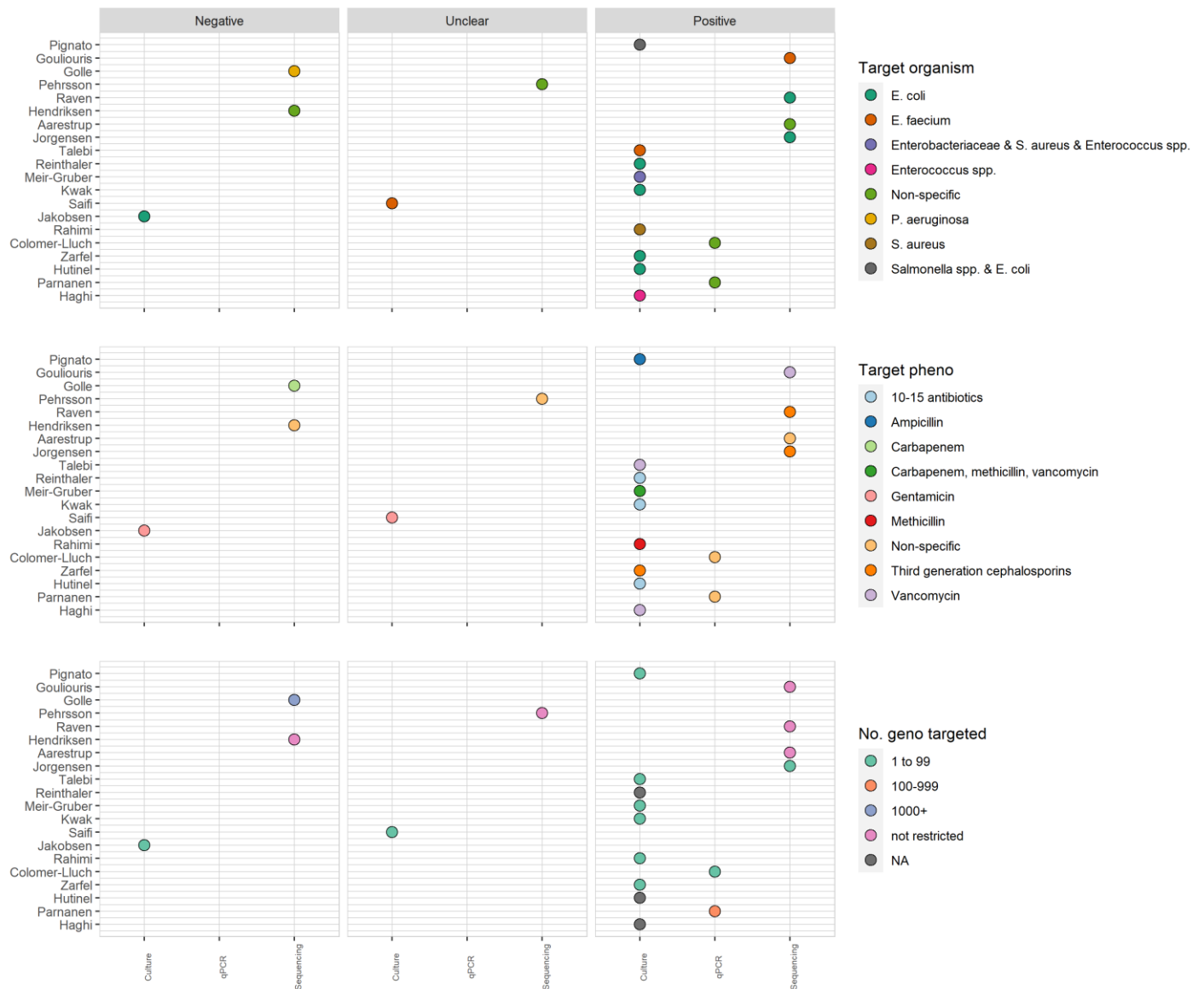
Figure S6: Interactions between study features in relation to outcome, sampling point and AMR evaluation.

Number of studies with a specific study feature (A=wastewater sampling approach (longitudinal vs snapshot), B = longitudinal timeframe, C = sampling method, D = direct vs indirect) plotted against study outcomes, wastewater sampling point and AMR evaluation approach. NAs represent studies without the plotted feature (e.g. snapshot studies for longitudinal timeframe) or did not report feature used. The bar heights represent the number of studies for a given comparison.

Figure S7: Interactions between study features in relation to outcome, sampling point and AMR evaluation.

Number of studies with a specific study feature (A=wastewater-based AMR surveillance as specified study aim - yes/no), B = distinct wastewater inputs, C = longitudinal sampling intervals, D = human comparison dataset description) plotted against study outcomes, wastewater sampling point and AMR evaluation approach. NAs represent studies without the plotted feature or did not report feature plotted (e.g. where no sewer input types were reported). The bar heights represent the number of studies for a given comparison.

Figure S8: Interactions between study features in relation to outcome, sampling point and AMR evaluation.

Number of studies with a specific study feature (A=time to sample processing), B=number of sample freeze-thaws, C=sample filtration, D=sample storage) plotted against study outcomes, wastewater sampling point and AMR evaluation approach. NAs represent studies without the plotted feature or did not report feature plotted (e.g. where no storage details were reported). The bar heights represent the number of studies for a given comparison.