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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Cor	firmed
\boxtimes		The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
\boxtimes		An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
\boxtimes		A description of all covariates tested
	\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
\boxtimes		A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
	\boxtimes	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
\boxtimes		Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)
		Our web collection on <u>statistics for biologists</u> may be useful.

Software and code

Policy information about availability of computer code

Data collectionThe seugence data was retrieved using MG-RAST API and getSeqENA script (https://github.com/B-UMMI/getSeqENA).Data analysisBowtie2 v. 2.3.0, DIAMOND v. 0.9.14. R v. 3.3.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data was downloaded from public repositories and is publicly available.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Kelogical, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Public metagenomic data was used to assess the relationship between fecal pollution and antibiotic resistance gene abundance in human impacted environments such as downstream from a waste water treatment plant.
Research sample	n/a
Sampling strategy	n/a
Data collection	Data was downloaded from public repositories (SRA/ENA/MG-RAST)
Timing and spatial scale	n/a
Data exclusions	n/a
Reproducibility	n/a
Randomization	n/a
Blinding	n/a
Did the study involve field	d work? Yes XNo

Reporting for specific materials, systems and methods

Materials	&	experimental	S١	/stems
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Methods

n/a	Involved in the study
\boxtimes	Unique biological materials
\ge	Antibodies
\ge	Eukaryotic cell lines
\ge	Palaeontology
\boxtimes	Animals and other organisms
\boxtimes	Human research participants

n/a	Involved in the study
\boxtimes	ChIP-seq
\ge	Flow cytometry

	non of comeny
\boxtimes	MRI-based neuroimaging