# SUPPLEMENTAL INFORMATION

## Metaphylogenetic analysis of global sewage reveals that bacterial strains associated with human disease show less degree of geographic clustering

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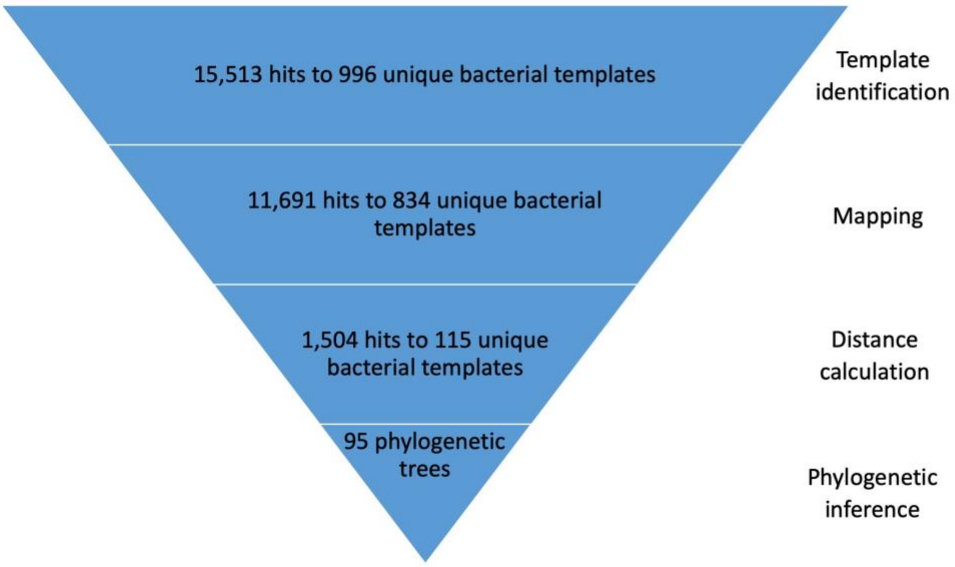
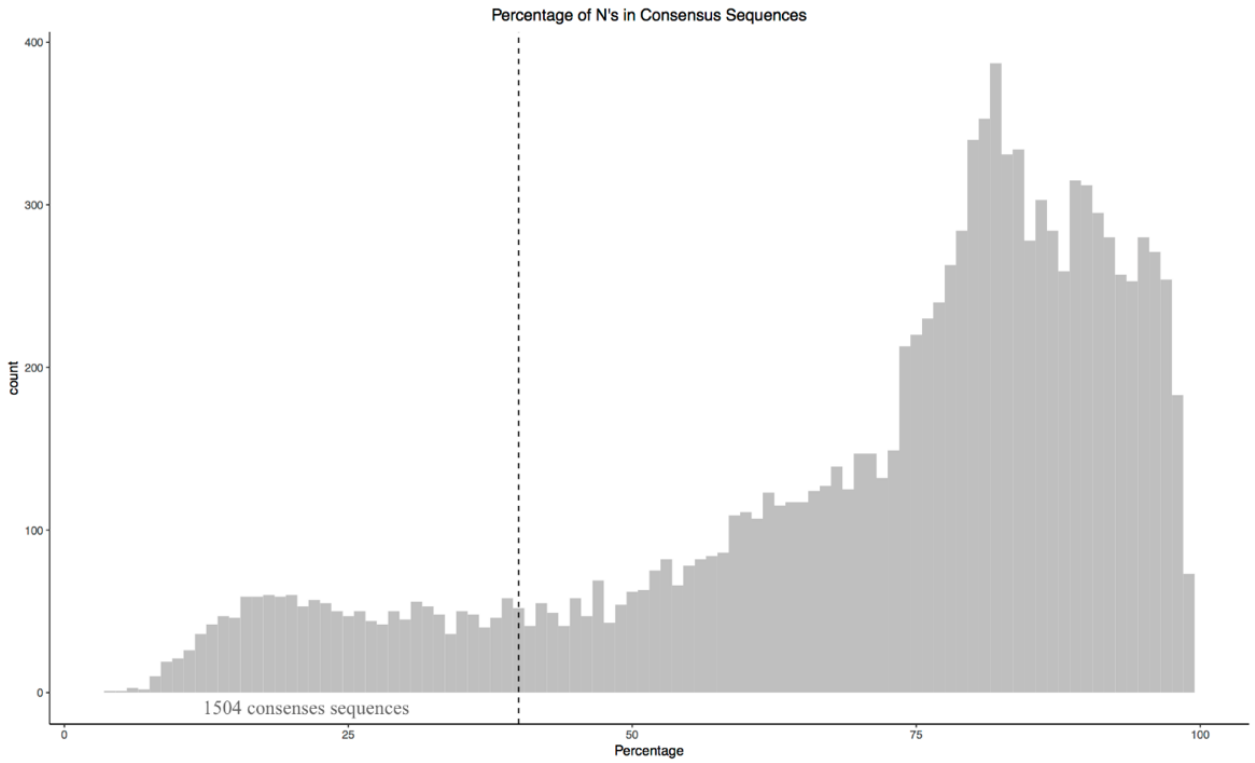
# SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure S1: Quantitative overview after major steps of the pipeline**. Overview of the number of bacterial templates and unique bacterial templates left, after each of the four major steps in the pipeline.

**Supplementary Figure S2: Unknown bases in the consensus sequences**. A barchart of percentage of unknown bases in the consensus sequences. X-axis illustrates percentage of unknown bases and Y-axis shows number of consensus sequence counts. The dotted line shows the cut-off at 40% which we ended up using.




Supplementary Figure S1




Supplementary Figure S2

# SUPPLEMENTAL TABLES

**Supplementary Table S1:** Country and regional information on each of the 80 samples from the Global Sewage project. Only 79 samples were used in the final results, the sample from Hungary was removed due to insufficient reads.

| Samplefile ID | Sample_country_region | Sample ID | Country | Region | ISO_alpha_3 |
|---|---|---|---|---|---|
| 17.fsa | 17_Albania_Europe | 17 | Albania | Europe | ALB |
| 18.fsa | 18_Australia_Oceania | 18 | Australia | Oceania | AUS |
| 18a.fsa | 18a_Australia_Oceania | 18a | Australia | Oceania | AUS |
| 70.fsa | 70_Austria_Europe | 70 | Austria | Europe | AUT |
| 19.fsa | 19_Botswana_Africa | 19 | Botswana | Africa | BWA |
| 53.fsa | 53_Brasil_SouthAmerica | 53 | Brasil | South America | BRA |
| 53a.fsa | 53a_Brasil_SouthAmerica | 53a | Brasil | South America | BRA |
| 66.fsa | 66_Bulgaria_Europe | 66 | Bulgaria | Europe | BGR |
| 21.fsa | 21_Cambodia_Asia | 21 | Cambodia | Asia | KHM |
| 22.fsa | 22_Canada_NorthAmerica | 22 | Canada | North America | CAN |
| 22a.fsa | 22a_Canada_NorthAmerica | 22a | Canada | North America | CAN |
| 22b.fsa | 22b_Canada_NorthAmerica | 22b | Canada | North America | CAN |
| 22c.fsa | 22c_Canada_NorthAmerica | 22c | Canada | North America | CAN |
| 1.fsa | 1_Chad_Africa | 1 | Chad | Africa | TCD |
| 64.fsa | 64_China_Asia | 64 | China | Asia | CHN |
| 2.fsa | 2_Colombia_SouthAmerica | 2 | Colombia | South America | COL |
| 13.fsa | 13_CoteD'ivoire_Africa | 13 | Cote D'ivoire | Africa | CIV |
| 68.fsa | 68_Croatia_Europe | 68 | Croatia | Europe | HRV |
| 23.fsa | 23_CzechRepublic_Europe | 23 | Czech Republic | Europe | CZE |
| RA.fsa | 71RA_Denmark_Europe | 71RA | Denmark | Europe | DNK |
| RD.fsa | 71RD_Denmark_Europe | 71RD | Denmark | Europe | DNK |
| RL.fsa | 71RL_Denmark_Europe | 71RL | Denmark | Europe | DNK |
| 14.fsa | 14_Ecuador_SouthAmerica | 14 | Ecuador | South America | ECU |
| 14a.fsa | 14a_Ecuador_SouthAmerica | 14a | Ecuador | South America | ECU |
| 24.fsa | 24_Ethiopia_Africa | 24 | Ethiopia | Africa | ETH |
| 25.fsa | 25_Finland_Europe | 25 | Finland | Europe | FIN |
| 10.fsa | 10_Gambia_Africa | 10 | Gambia | Africa | GMB |
| 59.fsa | 59_Georgia_Asia | 59 | Georgia | Asia | GEO |
| 27.fsa | 27_Germany_Europe | 27 | Germany | Europe | DEU |
| 4.fsa | 4_Ghana_Africa | 4 | Ghana | Africa | GHA |
| 61.fsa | 61_Hungary_Europe | 61 | Hungary | Europe | HUN |
| 28.fsa | 28_Iceland_Europe | 28 | Iceland | Europe | ISL |
| 11.fsa | 11_India_Asia | 11 | India | Asia | IND |
| 12.fsa | 12_Iran_MiddleEast | 12 | Iran | Middle East | IRN |

| | | | | | |
|---|---|---|---|---|---|
| 69.fsa | 69_Ireland_Europe | 69 | Ireland | Europe | IRL |
| 29.fsa | 29_Israel_MiddleEast | 29 | Israel | Middle East | ISR |
| 30.fsa | 30_Italy_Europe | 30 | Italy | Europe | ITA |
| 6.fsa | 6_Kazakhstan_Asia | 6 | Kazakhstan | Asia | KAZ |
| 72.fsa | 72_Kenya_Africa | 72 | Kenya | Africa | KEN |
| 31.fsa | 31_Latvia_Europe | 31 | Latvia | Europe | LVA |
| 32.fsa | 32_Luxembourg_Europe | 32 | Luxembourg | Europe | LUX |
| 54.fsa | 54_Malaysia_Asia | 54 | Malaysia | Asia | MYS |
| 63.fsa | 63_Malta_Europe | 63 | Malta | Europe | MLT |
| 33.fsa | 33_Nepal_Asia | 33 | Nepal | Asia | NPL |
| 43.fsa | 43_Netherlands_Europe | 43 | Netherlands | Europe | NLD |
| 56.fsa | 56_NewZealand_Oceania | 56 | New Zealand | Oceania | NZL |
| 50.fsa | 50_Nigeria_Africa | 50 | Nigeria | Africa | NGA |
| 34.fsa | 34_Norway_Europe | 34 | Norway | Europe | NOR |
| 7.fsa | 7_Pakistan_Asia | 7 | Pakistan | Asia | PAK |
| 35.fsa | 35_Peru_SouthAmerica | 35 | Peru | South America | PER |
| 36.fsa | 36_Poland_Europe | 36 | Poland | Europe | POL |
| 62.fsa | 62_RepublicOfMacedonia_Europe | 62 | Republic Of Macedonia | Europe | MKD |
| 65.fsa | 65_RepublicOfMoldova_Europe | 65 | Republic Of Moldova | Europe | MDA |
| 8.fsa | 8_Senegal_Africa | 8 | Senegal | Africa | SEN |
| 37.fsa | 37_Serbia_Europe | 37 | Serbia | Europe | SRB |
| 52.fsa | 52_Singapore_Asia | 52 | Singapore | Asia | SGP |
| 9.fsa | 9_SlovakRepublic_Europe | 9 | Slovak Republic | Europe | SVK |
| 38.fsa | 38_Slovenia_Europe | 38 | Slovenia | Europe | SVN |
| 39.fsa | 39_SouthAfrica_Africa | 39 | South Africa | Africa | ZAF |
| 75.fsa | 75_Spain_Europe | 75 | Spain | Europe | ESP |
| 40.fsa | 40_SriLanka_Asia | 40 | Sri Lanka | Asia | LKA |
| 41.fsa | 41_Sweden_Europe | 41 | Sweden | Europe | SWE |
| 41a.fsa | 41a_Sweden_Europe | 41a | Sweden | Europe | SWE |
| 67.fsa | 67_Switzerland_Europe | 67 | Switzerland | Europe | CHE |
| 15.fsa | 15_Tanzania_Africa | 15 | Tanzania | Africa | TZA |
| 44.fsa | 44_Togo_Africa | 44 | Togo | Africa | TGO |
| 46.fsa | 46_Turkey_Europe | 46 | Turkey | Europe | TUR |
| 74.fsa | 74_USA_NorthAmerica | 74 | USA | North America | USA |
| 74a.fsa | 74a_USA_NorthAmerica | 74a | USA | North America | USA |
| 74b.fsa | 74b_USA_NorthAmerica | 74b | USA | North America | USA |
| 74c.fsa | 74c_USA_NorthAmerica | 74c | USA | North America | USA |
| 74d.fsa | 74d_USA_NorthAmerica | 74d | USA | North America | USA |
| 74e.fsa | 74e_USA_NorthAmerica | 74e | USA | North America | USA |
| 74f.fsa | 74f_USA_NorthAmerica | 74f | USA | North America | USA |
| 74g.fsa | 74g_USA_NorthAmerica | 74g | USA | North America | USA |

| | | | | | |
|---|---|---|---|---|---|
| 74h.fsa | 74h_USA_NorthAmerica | 74h | USA | North America | USA |
| 74i.fsa | 74i_USA_NorthAmerica | 74i | USA | North America | USA |
| 48.fsa | 48_Vietnam_Asia | 48 | Vietnam | Asia | VNM |
| 49.fsa | 49_Zambia_Africa | 49 | Zambia | Africa | ZMB |
| 49b.fsa | 49b_Zambia_Africa | 49b | Zambia | Africa | ZMB |

**Supplementary Table S2:** Taxonomic rank and classification at phylum, order, class, genus, species, and template level of the bacterial database

| Taxonomic rank | Count |
|---|---|
| Phylum | 34 |
| Order | 64 |
| Class | 138 |
| Family | 301 |
| Genus | 843 |
| Species | 2,319 |
| Unique bacterial templates | 3,721 |

**Supplementary Table S3:** Number of each taxonomic rank and classification at phylum, order, class, genus, species, and template level of the bacterial templates identified in the 79 sewage samples.

| Taxonomic rank | After step B | After step D | Phylogenetic trees |
|---|---|---|---|
| Phylum | 15 | 6 | 6 |
| Class | 25 | 10 | 10 |
| Order | 61 | 15 | 13 |
| Family | 120 | 19 | 18 |
| Genus | 279 | 31 | 27 |
| Species | 653 | 73 | 62 |
| Unique bacterial templates | 834 | 115 | 95 |

**Supplementary Table S4:** p-values for tests of organism group differences in clustering. EID2p organism classifications. P-values < 0.05 are white, while those above are colored grey.

| | World Bank Regions | World Bank Income Levels | WHO Regions | WHO Health Impact |
|---|---|---|---|---|
| Commensal ↔ Environmental | 0.79 | 0.94 | 0.91 | 0.029 |
| Commensal ↔ Pathogen | 0.014 | 0.77 | 0.0039 | 0.022 |
| Environmental ↔ Pathogen | 0.039 | 0.75 | 0.039 | 0.94 |

**Supplementary Table S5:** p-values for tests of organism group differences in clustering. 5CC organism classification. P-values < 0.05 are white, while those above are colored grey.

| | World Bank Regions | World Bank Income Levels | WHO Regions | WHO Health Impact |
|---|---|---|---|---|
| Commensal ↔ COP | 0.077 | 0.29 | 0.042 | 0.00078 |
| Commensal ↔ Environmental | 0.27 | 0.79 | 0.86 | 0.10 |
| Commensal ↔ Opportunistic pathogen | 0.026 | 0.54 | 0.028 | 0.66 |
| Commensal ↔ Pathogen | 0.12 | 0.17 | 0.074 | 0.23 |
| COP ↔ Environmental | 0.048 | 0.23 | 0.32 | 0.034 |
| COP ↔ Opportunistic pathogen | 0.33 | 0.067 | 0.48 | 0.0034 |
| COP ↔ Pathogen | 0.39 | 0.038 | 0.24 | 0.50 |
| Environmental ↔ Opportunistic pathogen | 0.019 | 0.80 | 0.14 | 0.31 |
| Environmental ↔ Pathogen | 0.21 | 0.24 | 0.18 | 0.39 |
| Opportunistic pathogen ↔ Pathogen | 0.58 | 0.30 | 0.69 | 0.28 |

**Supplementary Table S6:** Number of countries sampled for each of the region groupings used. The regions listed in the same row are those with the highest amount of countries in common.

| World Bank Regions | Countries sampled | WHO Regions | Countries sampled |
|---|---|---|---|
| Sub-Saharan Africa | 13 | Africa | 13 |
| Latin America & Caribbean | 4 | Americas | 6 |
| North America | 2 | | |
| Middle East & North Africa | 3 | Eastern Mediterranean | 2 |
| Europe & Central Asia | 28 | Europe | 30 |
| South Asia | 4 | South-East Asia | 3 |

| | | | |
|---|---|---|---|
| East Asia & Pacific | 7 | Western Pacific | 7 |

**Supplementary Table S7:** Regional differences in country groupings between WB-R and WHO-R

| **ISO alpha 3** | **Country name** | **World Bank Regions** | **WHO Regions** |
|---|---|---|---|
| PAK | Pakistan | South Asia | Eastern Mediterranean |
| ISR | Israel | Middle East & North Africa | Europe |
| MLT | Malta | Middle East & North Africa | Europe |
| COL | Colombia | Latin America & Caribbean | Americas |
| ECU | Ecuador | Latin America & Caribbean | Americas |
| PER | Peru | Latin America & Caribbean | Americas |
| BRA | Brazil | Latin America & Caribbean | Americas |
| CAN | Canada | North America | Americas |
| USA | United States of America | North America | Americas |

# SUPPLEMENTAL METHODS

## Classifications of bacterial templates

Here follows the step by step description of how the two classifications schemes EID2 plus (EID2p) and Five class classification (5CC) was made. EID2p is made by step 1-3 and is mostly built on the EID2 database [1], but with an additional step (step 3) hence the name EID2 plus. The 5CC is made by adding step 4 to the EID2p scheme

### Step 1 – Annotate for human interaction

The used database: EID2 (enhanced infectious diseases database) [1]: https://eid2.liverpool.ac.uk/
Terms and conditions: https://eid2.liverpool.ac.uk/Home/TermsAndConditions

EID2 categorized the information as carrier and cargo.
Carrier correspond to a vector or a host.
Cargo correspond to a potential pathogen, parasite, commensal etc.

Furthermore, all organisms are categorized into taxa: bacteria, invertebrates, mammal, plant, primates, rodents, vertebrates, and virus.

The database was scraped for information about interactions where the cargo taxa were bacteria, and carrier taxa were every possible taxon.

The scrape output was then separated according to carrier taxa, where information had to be about carriers at rank 'species' and cargo at either rank 'no rank' or 'species'. This resulted in files containing carrier information about bacteria, invertebrates, mammal, plant primates, rodents, and vertebrates.

Lastly the information about homo sapiens interactions with bacteria were extracted from the file containing information about primate interactions with bacteria.

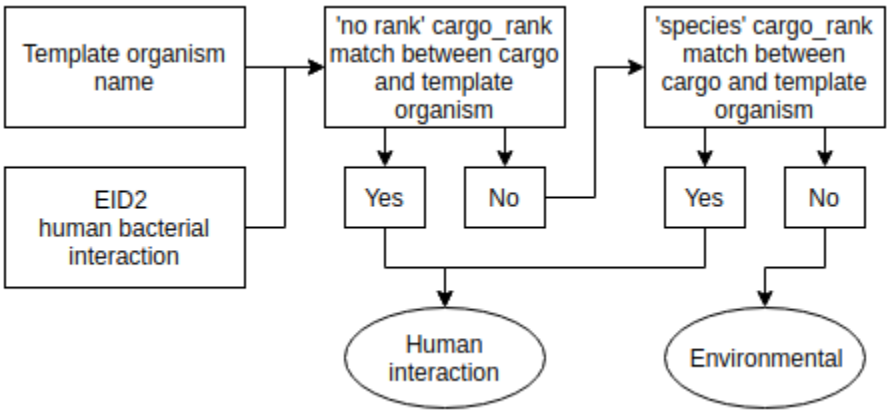
### Step 2 – Lookup of intersection between the human interaction list and the template list

Next the EID2 homo sapiens information was used to assign each template organism to either 'human interaction' or 'environmental'.

Firstly, a match between the template organism name and cargo at rank 'no rank' was searched for. If a match is found the template is classified as 'human interaction'. If no match was found at 'no rank' level, a match at species level is searched for. If a match was found the organism is classified as 'human interaction', else it was classified as 'environmental'.

When a match was identified, the name from the EID2 was noted down. If no match was found, NA was noted.

The conditions for which classification was assigned can be seen in the flow diagram below.




Information can be found in the columns:
- EID2.interaction.step2
Listing the classification either 'human interaction' or 'environmental'
- EID2.closest.match.interactions.step2
Listing the closest match between the template organism name and the EID2 database of bacterial cargos in humans.

### Step 3 – Lookup for pathogenesis in the list from the article: Risk factors for human disease emergence (2001), Taylor, L. H. et *al*. [2]

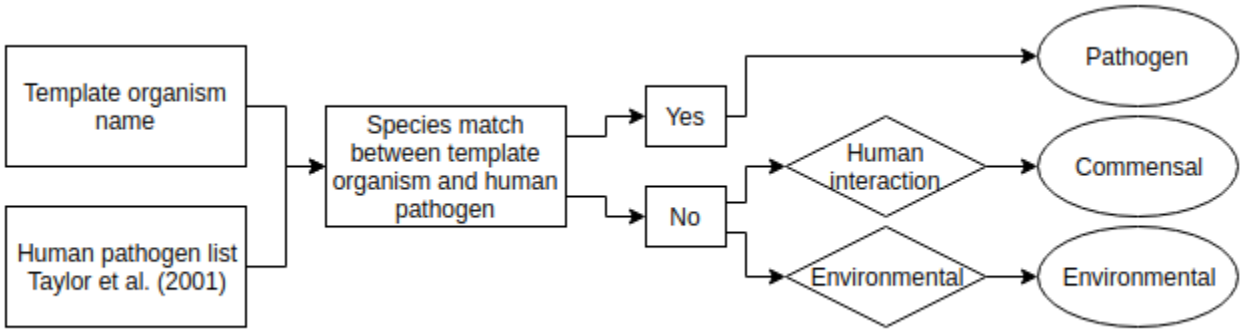
Search for a match between the list of pathogens from Taylor et *al*. and the template organism name. This can only be done at species level since Taylor et *al*. only include information about pathogenic bacteria at species level.

The template organisms were further classified as commensal, pathogen, or environmental.

If the template has a match to the Taylor list, it is classified as 'pathogen' regardless of its classification in step 2. If the template does not have a match in the Taylor list it will be classified as 'commensal' if it was classified as 'human interaction' in step 2. If it was classified as 'environmental' in step 2, it will keep its 'environmental' classification.

Note that if Taylor et *al*. has listed a genus (e.g. *Lactobacillus sp*. and *Megaspaera sp*.) as pathogenic, this will not be accepted as an evidence to classify the relevant templates as 'pathogen'.

The flow diagram below gives an overview of the decision flow.




Information can be found in the columns:
- classification.pathogen.Taylor.step3
List classification of either 'pathogen', 'commensal', or 'environmental'

- classification.pathogen.zoonotic.Taylor.step3
From Taylor et al. 2001, list if pathogen is defined as zoonotic

**Step 4 – Five class classification (5CC). Hand curated classification, made by web search for pathogenesis grouping as well as confirming commensal and environmental status.**

In this step pathogens are grouped according to the definition made by Price L.B et *al.* in Colonizing opportunistic pathogens (COPs): The beast in all of us (2017) [3]

**In the paper, pathogens are divided into three groups:**
Colonizing opportunistic pathogens (COPs)
Simple opportunistic pathogens (SOPs)
Frank pathogens

**Definitions listed by the paper:**
Colonizing opportunistic pathogens (COPs) are microbes that asymptomatically colonize the human body and, when the conditions are right, can cause infections.

The broad category of opportunistic pathogens can be divided into two distinct subgroups: the COPs and the noncolonizing, simple opportunistic pathogens (SOP).

The defining feature of all opportunistic pathogens is their capacity to cause disease when they are introduced into a susceptible body site or when hosts are immunologically compromised.

Whereas SOPs, such as *Vibrio vulnificus*, *Mycobacterium marinum*, and *Legionella pneumophila*, are only present in environmental reservoirs, COPs can also take up long-term residence in/on the human body as part of the "normal" human microbiome.

Frank pathogens, can cause acute, chronic, or latent infections that can be symptomatic or asymptomatic.

The detection of frank pathogens is often associated with a diseased status, whether active or latent, and identifying cases of active or recent infections is usually enough to trace transmission routes.

**Groupings used in our study:**
- Commensal
- COP
- Environmental
- Opportunistic pathogen
- Pathogen

Pathogens = frank pathogens
Opportunistic pathogens = noncolonizing/simple opportunistic pathogens

**Search protocol**
First look for the templates' names, and investigate what the specific paper for the sequencing of the organism (if it exists) mention about the organism.
Afterward, search if there are any other articles mentioning the same organism.

Hereafter, search for descriptions of the species itself, to investigate what tendencies the species has.

Search also for the keywords pathogen, commensal, human, environment in relation to the organism.

<u>Commensal definition:</u>
If an article search gives results mentioning human interaction. For example, used in food production of yogurt, fermentation procedures *etc*.
If found in human derived samples, and there are no results when searching for the organism with pathogen as an additional search term.

<u>COPs definition:</u>
Can cause disease in immunocompromised people. Mentioned as pathogen.
Can colonize the microbiome without causing disease, in some instances the bacteria are ubiquitous to the environment, meaning it can be present in soil, domestic animals as well as human microbiomes, plants etc.

<u>Environmental definition:</u>
If an article search shows no evidence of human interaction, or optimal growth conditions are far removed from those of the human body, e.g. a psychrophilic bacterium with optimal growth temperature below that of the human body.

<u>Opportunistic pathogen definition:</u>
Can cause disease in immunocompromised people.
Does not colonize the human microbiome, normally act as environmental bacteria except when causing infections.

<u>Pathogen defintions:</u>
Obligate/frank pathogen, cause infection when in contact with human, both immunocompetent as well as immunocompromised people.
There should be no evidence of the bacteria colonizing the human microbiome, it normally acts as environmental when not infecting humans.

Columns
- Web.search.step4
Listing classification; commensal, COP, environmental, opportunistic pathogen, pathogen
- Search.notes.step4
Notes taken while searching for information regarding each organism
- Ref1.step4
Link to paper with information regarding the organism
- Ref2.step4
Additional link to paper with information regarding the organism
- Ref3.step4
Additional link to paper with information regarding the organism

## References


1. Wardeh, M., Risley, C., McIntyre, M. K., Setzkorn, C. & Baylis, M. Database of host-pathogen and related species interactions, and their global distribution. *Sci. Data* **2,** 150049 (2015).
2. Taylor, L. H., Latham, S. M. & Woolhouse, M. E. Risk factors for human disease emergence. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **356,** 983–9 (2001).
3. Price, L. B., Hungate, B. A., Koch, B. J., Davis, G. S. & Liu, C. M. Colonizing opportunistic pathogens (COPs): The beasts in all of us. *PLOS Pathog.* **13,** e1006369 (2017).