Human Pathogens in Environmental Monitoring: Frequency, Risk, and Tools for Smarter Assessment

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1 Abstract

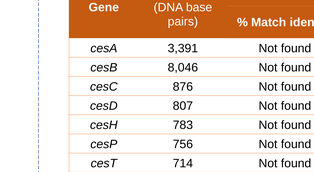
- Objectionable organisms remain a threat to pharmaceutical product safety, and among them, human bacterial pathogens stand out for their potential to cause patient harm.
- Combining a pragmatic definition of pathogenicity with an extensive search strategy, Bartlett *et al.* [1] published a list of nearly 1500 bacterial species known to cause human infections.
- The ***first goal of our study*** was to estimate the coverage and frequency of potential human pathogens recovered during Environmental Monitoring (EM).
  - A cohort of 764,040 EM isolates recovered over a 5-year span from >3,300 global sites identified by Sanger sequencing were used as a representative population for analysis.
  - 56.8% (829 species) of EM isolates recovered were known human pathogens.
- Reliable species level identifications and complementary information such as risk group and physiological attributes provide microbiologists with immediate access to essential information for risk assessments. However, such information does not always provide a comprehensive evaluation for assessing pathogenicity.
- The ***second goal of our study*** was to demonstrate with two case studies how whole genome sequencing can provide deeper insights into the organism of concern when further characterization is required, especially when screened for genes relevant to preservative resistance, cold sensitivity, pathogenicity, antimicrobial and/or disinfectant resistance.
  - Case study 1 (Screening for efflux pump genes that can confer antimicrobial resistance in *S. aureus*).
  - Case study 2 (Screening for genes that can produce the cereulide toxin in *B. cereus*).
- Applying advanced genomic tools during risk assessment can enhance contamination control strategies to support the protection of products and people.

2 Species categorized as human pathogenic bacteria

Based on the publication by Bartlett *et al.* [1]

- Bacterial species were categorized as *established* pathogens if human infections are reported in three or more separate persons. Pathogens are regarded as *putative* if there are fewer than three known cases. In either case, the reported link between the bacterial species and illness is suspected to be causal.
- Building on published information [3-6], IJSEM species lists, and *ad hoc* searches (Google Scholar) a list of 1513 bacterial species was created (as of year 2022).
- Additional curation of pathogen names in 2025 by Charles River Laboratories
  - Species names checked in LPSN [7] and updated as needed
  - Accurate number of human pathogen names reduced from 1513 to **1460**

Top 10 contributing genera to human pathogens [1]



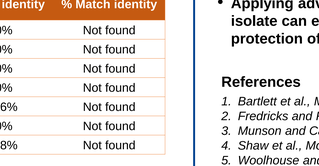
Examples of updated Species names (2021 to 2025)

Original Species name	Corrected Species name	Update reason
Bacteroides ureolyticus	*Campylobacter ureolyticus*	Taxonomic reclassification
Brevibacterium lutescens	*Brevibacterium luteolum*	Incorrect spelling of name
Clostridium bifermentans	*Paraclostridium bifermentans*	Taxonomic reclassification
Corynebacterium nigricans	*Corynebacterium aurimucosum*	Taxonomic reclassification
Legionella bozemanii	*Legionella bozemanae*	Incorrect spelling of name
Mycobacterium massiliense	*Mycobacterium abscessus*	Taxonomic reclassification
Prevotella oulora	*Prevotella oulorum*	Incorrect spelling of name
Pseudomonas pickettii	*Ralstonia pickettii*	Taxonomic reclassification
Streptococcus bovis	*Streptococcus equinus*	Taxonomic reclassification
Legionella bozemanii	*Legionella bozemanae*	Incorrect spelling of name
Mycobacterium massiliense	*Mycobacterium abscessus*	Taxonomic reclassification
Prevotella oulora	*Prevotella oulorum*	Incorrect spelling of name
Pseudomonas pickettii	*Ralstonia pickettii*	Taxonomic reclassification
Streptococcus bovis	*Streptococcus equinus*	Taxonomic reclassification

Discovery of human pathogens over time [1,2]

3 Human pathogenic bacteria recovered in Environmental Monitoring

764,040 Environmental Monitoring (EM) isolates recovered from >3,300 global sites identified at Charles River global testing laboratories



Human pathogenic bacterial species (**1460**) recovered in EM (**829**, 56.8%)

Frequently recovered Genera (top 15)	No. of Species [a]
Corynebacterium	47
Mycobacterium	46
Streptococcus	34
Staphylococcus	29
Clostridium	21
Burkholderia	19
Pseudomonas	19
Acinetobacter	18
Paenibacillus	16
Neisseria	16
Nocardia	16
Achromobacter	13
Actinomyces	13
Bacillus	12
Bacteroides	12

Frequently recovered Species [a] (top 15)	Frequency
Micrococcus luteus	9.2%
Staphylococcus epidermidis	6.6%
Staphylococcus hominis	4.3%
Bacillus cereus	3.7%
Bacillus mycoides	3.7%
Bacillus thuringiensis	3.7%
Staphylococcus capitis	2.7%
Corynebacterium tuberculostearicum	2.7%
Ralstonia pickettii	1.6%
Moraxella osloensis	1.3%
Staphylococcus warneri	1.3%
Staphylococcus haemolyticus	1.1%
Bacillus pumilus	1.1%
Bacillus subtilis	1.0%
Burkholderia cepacia complex	0.95%

[a] List includes Species that are opportunistic pathogens

4 Impact of microbial contaminants and risk assessment

Regulatory requirements for conducting a risk assessment to evaluate potential impact

Impact of microbial contaminants

- Patient harm
- Compromised product quality:
  - degradation of active ingredients
  - altering of properties
  - reduced efficacy or completely ineffective
- Operational state-of-control and cleaning/disinfection
- Product recalls
- Regulatory action
- Financial losses
- Erosion of patient/public trust

Risk assessment toolkit

- Species level identification
  - Tracking and trending
- Hazard/Risk characterization
  - BacDive, Bad Bug Book (FDA), AccuPedia™, etc.
  - Pathogenicity, growth requirements, toxin production, etc.
  - USP <1111> guidance for product: method of application, antimicrobial preservation, intended recipient
- Further characterization via whole genome sequencing
  - Source tracking via strain typing
  - Antimicrobial resistance (AMR) genes
  - Toxin genes

Deeper genomic analysis of isolates of concern is essential, as species-level descriptions alone may not accurately reflect risk or inform appropriate decisions

Whole genome sequencing workflow: A tool for Risk assessment

5 Application of whole genome sequencing for risk assessment (two case studies)

Case Study 1: Screening for genes that can confer antimicrobial resistance (AMR)

- Client evaluation of antimicrobial resistance profile of a *Staphylococcus aureus* isolate.
- Specifically interested in evaluating if the isolate carried the genes *qacA*, *qacB*, *qacC*.
  - These genes code for efflux pumps that confer resistance to various antiseptic agents like **chlorhexidine** and **benzalkonium chloride**, which are used as disinfecting agents or preservatives [8].

Results of screening for genes using a Whole Genome Sequencing workflow

Gene	Query length (DNA base pairs)	% Match identity
qacA	1,932	99.95%
qacB	4,250	Not found
qacC	324	Not found

- The gene *qacA* was present whereas the genes *qacB* and *qacC* were not found.
- The gene *qacA* is carried by a plasmid and could confer resistance through the QacA efflux pump [8].
- **This result assisted the client to make the appropriate choice of a disinfectant.**

Case Study 2: Screening for genes that can produce the cereulide toxin

- Client evaluation of *Bacillus cereus* isolate for cereulide synthetase *(ces)* gene cluster.
- The *ces* gene cluster encodes the enzymatic machinery for the synthesis of cereulide, the toxin responsible for emetic food poisoning [9].
  - Cereulide is a heat-stable toxin acting as a potassium ionophore, which disrupts the mitochondrial membrane potential leading to cell death.

Results of screening for genes using a whole genome sequencing workflow

Gene	Query length (DNA base pairs)	Sample	Positive Control	Negative Control
		% Match identity	% Match identity	% Match identity
cesA	3,391	Not found	100%	Not found
cesB	8,046	Not found	100%	Not found
cesC	876	Not found	100%	Not found
cesD	807	Not found	100%	Not found
cesH	783	Not found	99.66%	Not found
cesP	756	Not found	100%	Not found
cesT	714	Not found	99.58%	Not found

- **All 7 genes of the *ces* gene cluster were not found in the sample (isolate of *Bacillus cereus*), which implies this isolate lacked the capability of producing the cereulide toxin ensuring product safety.**

6 Summary

- 56.8% (829 species) of known human pathogens were recovered from EM of global manufacturing environments over a 5-year span.
- Frequently identified species in EM (examples: *M. luteus, B. cereus, S. epidermidis, R. pickettii, Burkholderia cepacia* complex) have the potential to cause human infection.
- Reliable species level identifications and complementary information such as risk group and physiological attributes provide microbiologists with immediate access to essential information for risk assessments.
- Whole genome sequencing can provide deeper insights into the organism of concern when *source tracking* and/or characterization of *pathogenicity* or *resistance profile* is required.
  - Genes relevant to preservative resistance, cold sensitivity, pathogenicity, or antimicrobial / disinfectant resistance can be rapidly screened.
- **Applying advanced genomic tools during risk assessment for your isolate can enhance contamination control strategies to support the protection of products and people.**

References

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