

# **EMBRACE-WATERS** statement: **Recommendations for reporting of** studies on antimicrobial resistance in wastewater and related aquatic environments



Nasreen Hassoun-Kheir<sup>1</sup>, Yoav Stabholz<sup>1</sup>, Jan-Ulrich Kreft<sup>2</sup>, Roberto de la Cruz<sup>2</sup>, Arnaud Dechesne<sup>3</sup>, Barth F Smets<sup>3</sup>, Jesús L Romalde<sup>4</sup>, Alberto Lema<sup>4</sup>, Sabela Balboa<sup>4</sup>, Carlos García-Riestra<sup>5</sup>, Eva Torres-Sangiao<sup>5</sup>, Ami Neuberger<sup>1</sup>, Graham David<sup>6</sup>, Marcos Quintela-Baluja<sup>6</sup>, Dov J Stekel<sup>7</sup>, Jay Graham<sup>8</sup>, Amy Pruden<sup>9</sup>, Joseph Nesme<sup>10</sup>, Søren Johannes Sørensen<sup>10</sup>, Rupert Lloyd Hough<sup>11</sup>, Michal Paul<sup>1</sup>, Cansu Uluseker<sup>2</sup>



DARWIN

1-Rambam Health Care Campus, Israel, 2-University of Birmingham, UK, 3- Technical University of Denmark, 4-Universidadede Santiago de Compostela, Spain, 5-University Hospital Complex of Santiago (CHUS), Spain, 6-Newcastle University, UK, 7-University of Nottingham, UK, 8-University of California, USA, 9- Virginia Tech, USA, 10-University of Copenhagen, Denmark, 11-The James Hutton Institute, UK

# **Rationale and Objectives**

We attempted a meta-analysis of studies comparing AMR in hospital versus community wastewater (1). To our frustration, this was not possible because different studies reported AMR prevalence on a distinct basis (see Example). We also found a high risk of bias in several elements of sampling, comparability and analysis categories in most studies (1). And there are other shortcomings.

# Why Do We Need Standards of Reporting?



Current observations are piecemeal and need to be brought together in order to

Generate reliable and accurate large-scale trends Enable meta-analysis and direct comparisons

Therefore, we decided to include many experts to produce reporting standards to enable meta-analyses and other value-adding integration of studies in the future (2).

- Improve the quality of scientific papers
- Enhance the communication among different experts

### **Example:** Studies report AMR on a distinct basis

Vancomycin resistant Enterococci (VRE) in hospital (H) and community (C) untreated (u) or treated (t) wastewater (1).

Study	Sam H	ples C	ARB units	Hospital (H)	Community (C)
Blanch, 2003	59	207	Log CFU/100 ml VRE	334.59 (u)	2146.15 (u)
Caplin, 2008	26	48	Log CFU/100 ml VRE	1.3 x 10^5 (u)	5.7 x 10^5 (u)
Hamiwe, 2019	4	44			
lversen, 2002	14	67	VRE + samples/all samples (%)	5/14 (36) (u)	21/35 (60) (u)
Kotzamanidis, 2009	16	42	VRE + samples/all samples (%)	14/16 (87) (u)	20/30 (67) (u)
Meir-Gruber, 2017	Not Reported		VRE + sampling location (%)	18/54 (33) (u)	6/55 (11) (u)
Narciso-da- Rocha, 2014	7	42		H >>	C (u)

# **Result: Reporting recommendations**

EMBRACE-WATERS checklist – recommendations for reporting on AMR in wastewater and related aquatic environments (2).

Торіс		Checklist item		
Title	1	Describe the environmental compartment and antimicrobial resistance studied		
Abstract	2	Provide a structured summary including implications of key findings		
Background	3	Describe the scientific background and the rationale of the study		
		Methods		
Planned location	4	Report on predefined sampling locations		
Sample types	5	Describe sample types in each location		
Technique	6	Describe the sampling techniques		
Equipment	7	Describe the type of equipment used for sampling		
Sample volume	8	Report the volume of the samples from all locations for each analysis		
Sample processing	9	Report sample processing by sample type and on-site preservation methods		
Source characterization	10	If sampling water from a point source (agricultural water, raw sewage inlet, WWTP effluent, etc.) or downstream the point source, report the exact source and its characteristics; In the absence of a point source, report characteristics of the watershed		
Microbiological methods	11a	Describe the microbiological methods used to detect bacteria		
	11b	Report how antibiotic resistance was assessed		
Analysis plan	12	Describe the data analysis or analytical pipeline planned for comparison. Report on use of statistical tests		
Sample size	13	Calculate the number of samples required to address the research question (statistical power calculation)		
Results				
Locations	14	Report and describe actual sampling locations		
	15a	Report the season, dates and frequency of sampling		
Dates and weather	15b	Provide description of weather conditions in the period leading up to the sampling, precipitation and any other external factors		
Water quality indicators and metadata	16	Report general water quality conditions and any other meta-data		
Results	17	Report results per location, including negative results		

Novais, 2005	14	12	VRE + samples/all samples (%)	11/14 (78.6) (u)	0/12 (0) (u)
Schwartz, 2003	6	10	% VRE	25% (u)	12.5 (t)
Silva, 2005	2	4	% Intermediate VRE/all isolates (%)	3/26 (11.54) (u)	9/37 (24.32) (u)

# **Method:** Consensus building through a Delphi process



The expert panel was expanded and questionnaire rounds were used to solicit feedback

Units of analysis	18	If possible, report outcomes as concentration units (and normalized concentration) and provide confidence intervals for all results	
Post hoc analysis	19	Describe statistical analysis performed if different than planned report on additional post-hoc analyses if done	
Discussion			
Interpretation	20	Discuss the study findings in context of existing evidence	
Limitations	21	Address the study limitations	

## **Conclusions and Future Outlook**

- EMBRACE-WATERS reporting standards facilitate comparability and integration of studies that would otherwise remain disjoint
- Huge potential for added value if widely adopted
- Standards can and should evolve

#### **References**

- 1 Hassoun-Kheir N, Stabholtz Y, Kreft J-U, de la Cruz R, Romalde JL, Nesme J, Sørensen SJ, Smets BF, Graham D, Paul M (2020). Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. Science of The Total Environment 743: 140804
- 2 Hassoun-Kheir N, Stabholz Y, Kreft J-U, de la Cruz R, Dechesne A, Smets BF, Romalde JL, Lema A, Balboa S, García-Riestra C, Torres-Sangiao E, Neuberger A, Graham D, Quintela-Baluja M, Stekel DJ, Graham J, Pruden A Nesme J, Sørensen SJ, Hough R, Paul M (2021). EMBRACE-WATERS statement: Recommendations for reporting of studies on antimicrobial resistance in wastewater and related aquatic environments. One Health 13: 100339

