CATÓLICA
FACULTY
OF BIOTECHNOLOGY
PORTO

Towards Antibiotic Resistance Genes (ARGs)

New module on Antibiotic Resistant Bacteria/Antibiotic Resistance Genes and update of DCTs on waste water, sewage sludge, soil and terrestrial environment

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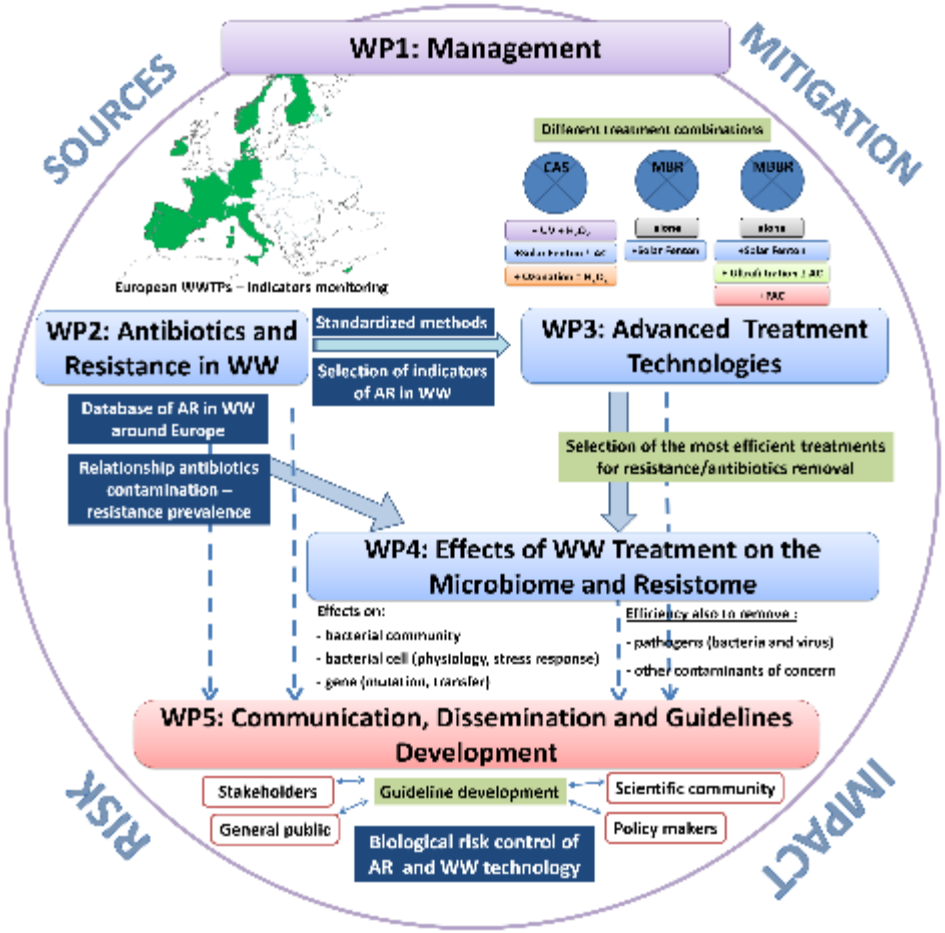
StARE (Stopping Antibiotic Resistance Evolution)



WaterJPI/0001/2013

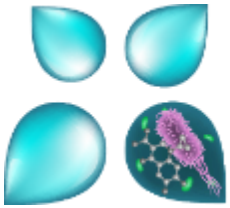
https://stareeurope.wordpress.com/

Other projects

mpare

DTU **Global Sewage Surveillance Project**

HEARD PIRE



ANSWER

ANtibioticS and mobile resistance elements in WastEwater Reuse applications: risks and innovative solutions European Commission Horizon 2020 - MSCA-ITN-2015-ETN: Marie Sklodowska-Curie Innovative Training Networks (ITN-ETN)

Antibiotic resistance contaminants

- Antibiotic residues
- Antibiotics Transformation Products

Chemical compounds (as possible stressor for resistance)

- Antibiotic Resistant Bacteria (ARB)
- Antibiotic Resistance Genes (ARG)

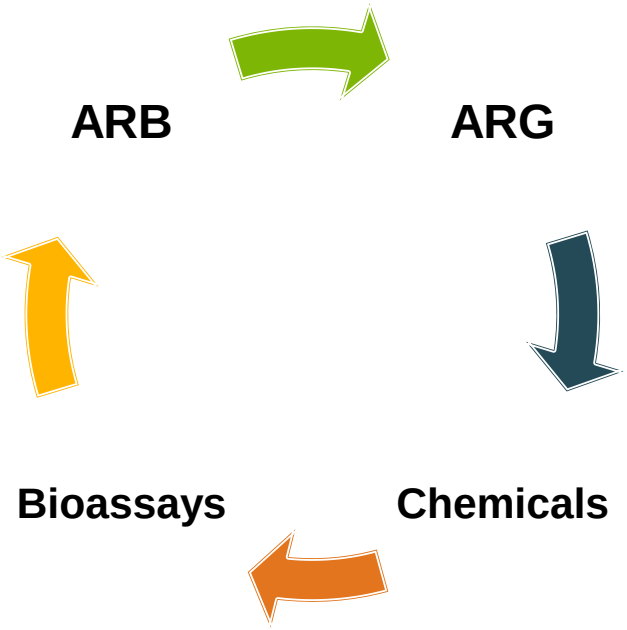
Emerging (biological) contaminants

ARB and ARGs - databases

- ARG databases (e.g. CARD, ARDB, SARG)
- **Innovation: quantitative data / correlation with other variables (geography, quality, etc…)**
- Inclusion in the EMPODAT - preparation of specialized DCTs

EMPODAT – a possible good solution!

- Previous knowledge
- Possible links with complementary information



Wastewater - vulnerable environmental matrices

- Wastewater
- Surface water
- Groundwater
- Sewage sludge
- Soil
- Crops/wildlife

https://www.melbournewater.com.au/

Data Collection Templates (DCTs)

- Data source (define information about the data provider, laboratory and references)
- Analysis (describe the sampling station, the measured values and relevant metadata)
- Analytical method (define the analytical methods used for the determinands)

DCTs for ARB

- Suggested changes on *Data source*

Bacteriological group/Determinand		**OBLIGATORY FIELD. Drop-down list: MRSA (Methicillin resistant Staphylococcus aureus); VRE (Vancomycin resistant Enterococci); Gram-negative carbapenem resistant; Gram-negative cephalosporin resistant; other**
	Other	**Please indicate the other bacteriological group**

Data can only be submitted to the database after accepted for publication

Important to guarantee the confidentiality of the samples suppliers, if they asked for!

DCTs for ARB

- Suggested changes on *Analysis*

Bacteriological group/Determinand			OBLIGATORY FIELD. Drop-down list: MRSA (Methicillin resistant Staphylococcus aureus); VRE (Vancomycin resistant Enterococci); Gram-negative carbapenem resistant; Gram-negative cephalosporin resistant; other
	Other		Please indicate the other bacteriological group
DETERMINAND / MEASURAND	Individual compound		**OBLIGATORY FIELD.** Select *Determinand* from the drop-down list. After selecting *Determinand* information on *Class*, *Subclass*, *CAS#* will be filled out automatically.
	Class		This field will be filled in automatically.
	Subclass		This field will be filled in automatically.
	CAS No.		This field will be filled in automatically.
AR Phenotype	**Multiple compounds**		Drop-down list with antimicrobials (possibility of multiple selection)
AR genotype	**Multiple genes**		Drop-down list with antimicrobials (possibility of multiple selection)
INDIVIDUAL CONCENTRATION	Concentration		**~~OBLIGATORY FIELD.~~** Select type of *Concentration* from the drop-down list.
	Abundance	(CFUs/mL)	Fill in the abundance of the bacteria in the sample (CFU's/mL). Only positive numbers should be inserted (0 is not accepted).
	Unit		Unit in which the measurement (determination) is expressed. This field will be filled in automatically after selection of *Matrix* and *Individual compound* (see above).
	Sampling date	Day	**~~OBLIGATORY FIELD~~.** Fill in *Day, Month, Year* of sampling. Drop-down list available.
		Month	
		Year	
		Hour	Fill in *Hour and Minute* of sampling. Drop-down list available.
		Minute	

DCTs for ARB

- Suggested changes on *Analytical method*

QA / QC INFORMATION ABOUT phenotype DATA	**Limit of Detection (LoD)**	[CFU/ml]	**OBLIGATORY FIELD.** Fill in the value of *Limit of Detection (LoD)*. Only positive numbers should be inserted (0 is not accepted).
	Limit of Quantification (LoQ)	[CFU/ml]	~~**OBLIGATORY FIELD**~~**.** Fill in the value of *Limit of Quantification (LoQ)*. Only positive numbers should be inserted (0 is not accepted).
	Uncertainty at LoQ	[%]	Fill in the value of *Uncertainty at LoQ* - number only. **Please, do not type "%" sign.** Only numbers between 0 and 100 should be used.
		Coverage factor	Select *Coverage factor* from the drop-down list.
	Bacteria isolation method		**OBLIGATORY FIELD.** Select ~~*Sample preparation*~~ *Bacteria isolation method*. When choosing "Other" specify the information in the next field.
		Other	
	Phenotype determination method		**OBLIGATORY FIELD.** Select ~~*Analytical*~~ *Phenotype determination method* from the drop-down list. When choosing "Other" specify the information in the next field.
		Other	
	Interpretation criteria		**OBLIGATORY FIELD. Drop-down list (e.g. CLSI; EUCAST; SFM; other)**
		Other	

Bacteria isolation method:
- Culture on non-selective culture medium
- Culture on selective culture medium
- Under selective pressure
- other

Phenotype determination method:
- Disk diffusion
- Microdilution
- Etest or similar
- VITEK
- other

DCTs for ARG

- Suggested changes on *Data source*

Genetic determinant		Gene name	OBLIGATORY FIELD. Drop-down list with an intensive list of genes (e.g. blaVIM-7)
		Gene description	OBLIGATORY FIELD. Drop-down list with an intensive list of genes (e.g. metallo-beta-lactamase VIM-7)
		Gene family	Drop-down list with an intensive list of genes (e.g. lactamases)
		Associated phenotype	Indicate the resistance phenotype associated to the gene (e.g. ESBL)
		Monogenic phenotype?	Drop-down list (yes; no)
		Multi-drug resistance phenotype	Indicate if it is a multidrug resistance phenotype (e.g. efflux pumps)
		Genetic marker?	Drop-down list (yes; no)
		If yes, detail	Indicate if it is a plasmid replicon type, integron related, etc…
		Common bacterial host	Indicate the common bacterial host (species, genus or family)

DCTs for ARG

- Suggested changes on *Analysis*

Sample preparation	**Type of sample**		OBLIGATORY FIELD. Drop-down list (composite, grab, microcosm)
		Details	Give details of how the sample was collected
	Volume of sample used for DNA extraction	(mL)	Indicate the volume of sample used for the DNA extraction
	Method used for DNA extraction		Indicate if the DNA extraction was performed with a kit (indicate which kit) or other method (indicate a reference for the method)
	Targeted analysis		Drop-down list (conventional PCR, qPCR, FISH, RT-PCR (gene expression), qPCR (gene abundance e.g. qPCR chip), other)
		other	Indicated if other targeted analysis was used
	Non-targeted analysis		Drop-down list (metagenomics, metatranscriptomics, functional metagenomics, other)
		other	Indicate if other non-targeted analysis were used
	Analysis of pooled DNA extracts		Drop-down list (yes; no)
		details	If yes, give details of how the pool was done
	DNA concentration	(ng/µL)	Indicate the concentration of the DNA in which the quantification of the gene was done
Genetic determinant		Details	Indicate the method used for the quantification. Drop-down list (Qubit, Nanodrop, other)
		Gene name	OBLIGATORY FIELD. Drop-down list with an intensive list of genes (e.g. blaVIM-7)
		Gene description	OBLIGATORY FIELD. Drop-down list with an intensive list of genes (e.g. metallo-beta-lactamase VIM-7)
		Gene family	Drop-down list with an intensive list of genes (e.g. lactamases)
		Associated phenotype	Indicate the resistance phenotype associated to the gene (e.g. ESBL)
		Monogenic phenotype?	Drop-down list (yes; no)
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		If yes, detail	Indicate if it is a plasmid replicon type, integron related, etc…
		Common bacterial host	Indicate the common bacterial host (species, genus or family)

DCTs for ARG

- Suggested changes on *Analysis*

INDIVIDUAL CONCENTRATION	**Concentration/ Abundance**	[gene copy number/mL of sample]	Indicate the abundance of the gene per mL of sample
		[gene copy number/mL of sample]	Indicate the abundance of the gene per ng of DNA
		[number of reads/total number of sequence reads]	Indicate the abundance of the gene
	Prevalence	[gene copy number/16S rRNA gene copy number]	If determined, indicate the gene prevalence
	Unit		Unit in which the measurement (determination) is expressed. This field will be filled in automatically after selection of *Matrix* and *Individual compound* (see above).
	Sampling date	Day	**OBLIGATORY FIELD.** Fill in *Day, Month, Year* of sampling. Drop-down list available.
		Month	
		Year	
		Hour	Fill in *Hour and Minute* of sampling. Drop-down list available.
		Minute	

DCTs for ARG

- Suggested changes on *Analytical method*

QA / QC INFORMATION ABOUT genes DATA	**Limit of Detection (LoD)**	[number of copies]	~~**OBLIGATORY FIELD.**~~ Fill in the value of *Limit of Detection (LoD)*. Only positive numbers should be inserted (0 is not accepted).
	Limit of Quantification (LoQ)	[number of copies]	~~**OBLIGATORY FIELD.**~~ Fill in the value of *Limit of Quantification (LoQ)*. Only positive numbers should be inserted (0 is not accepted).
	Uncertainty of the quantification	[%]	Fill in the value of *Uncertainty at LoQ* - number only. **Please, do not type "%" sign.** Only numbers between 0 and 100 should be used.
		Coverage factor	Select *Coverage factor* from the drop-down list.
	Efficiency		If quantitative methods were used indicate the efficiency
	Sequencing read depth		If the gene was detected with metagenomic approaches, indicate the number of reads analysed
	Sample preparation method		**OBLIGATORY FIELD.** Select *Sample preparation method*. When choosing "Other" specify the information in the next field.
	Analytical method		**OBLIGATORY FIELD.** Select *Analytical method* from the drop-down list (Conventional PCR; Real-time PCR; Illumina Myseq; Whole genome sequencing; LAMP-PCR; Other) .When choosing "Other" specify the information in the next field.
		Other	

Minimum quality-related information

- **ARB**

Difficult to define QA/QC parameters due to the high variety of methods that may be used

- **ARG**

When quantitative methods are used (e.g. qPCR) the quality may be evaluated based on:

- LOD; LOQ and uncertainty (%)
- Efficiency
- Melting curve

Rule: Submission to EMPODAT only after publication in peer reviewed journals or curated databases

Acknowledgements

WaterJPI/0001/2013

European Commission Horizon 2020 - MSCA-ITN-2015-ETN:
Marie Sklodowska-Curie Innovative Training Networks (ITN-ETN)