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Background

Deaths attributable/associated with Antibiotic Resistance (AR)



- Attributable: AR directly caused the death
- Associated: AR was one of the causes of the death

- In 2019, 1.27 million deaths were directly attributable to antibiotic resistance (AR).
- The greatest number of associated deaths was approximately 0.1% of the population in Africa.
- AR is a leading cause of death around the world, with the highest burdens in low-resource settings

Background

How did antibiotic resistant pathogens acquire AR?

- Mutation
- Vertical transmission



- HGT exchanges antibiotic resistance genes (ARGs) faster than vertical transmission and across the different species.
- HGT can occur via conjugation, transformation, and transduction.
- Plasmids can be independently uptaken by bacteria, transferring AR, and replicate independently from the genome, which is fast.
- Plasmids encoded e-ARG is regarded as concerning environmental contaminants.

Introduction

Schematic for transmission paths of e-ARGs



- Humans and livestock consume large amounts of antibiotics while developing AR. The e-ARGs from their waste are transported to wastewater treatment plants (WWTP), which don't completely remove e-ARGs, and discharge them into natural water systems.
- The natural water: a potential drinking water source or recreational water.
- To understand the fate of e-ARGs in natural water system is important in mitigation of AR.

Introduction

Sunlight plays an important role in fate of e-ARGs in natural surface water





Necessity of this research

- There have been different conclusions regarding the importance of ¹O₂, ³DOM^{*}, [•]OH.
- The importance of each pathway was highly dependent on the light spectrum, which makes it hard to derive an overall conclusion.
- In case of ¹O₂ and ³DOM^{*}, even their reactivities with ARG have been less understood.

Research objective and Scope

To advance the understanding of sunlight photolysis mechanisms of e-ARG in aquatic environments



Material & Method



Material & Method

Solution State S

Model	RS system	Reactive Species	Source of RS / Photosensitizer	Competitor (k, M ⁻¹ s ⁻¹)	ref
		¹ O ₂	Rosebengal (RB)	Furfuryl alcohol (FFA) (1.0×10 ⁸)	[5]
¹ O ₂		CO ₃ -	4-Carboxybenzophenone (CBBP)	lsoproturon (IPT) (3.0×10 ⁷)	[6]
CO *	e-ARG	юн	UV _{>290nm} /H ₂ O ₂	Para-chlorobenzoicacid (pCBA) (5.2×10 ⁹)	[7]
			2-Anthraquinone-sulfonate (AQ2S)	Atrazine (ATZ), (1.4×10 ⁹)	[8]
юн	Competitor		4-Carboxybenzophenone (CBBP)	Atrazine (ATZ), (7.2×10 ⁸)	[9]
³ DOM*	³ DOM*	Benzophenone (BP)	2,4,6-Trimethylphenol (TMP) (5.1×10 ⁹)	[10]	
			Methylene blue (MB)	Phenol, (1.7×10 ⁷)	[11]

Material & Method

Sunlight photolysis experiment of e-ARG with model DOM

Light source

Sunlight simulator (> 320 nm) 6.5×10⁻⁵ Ein/L/s by PNA-pyridine actinometry

Why > 320 nm ?

: To focus on indirect pathway, we cut off the UVB region which is the main contributor to direct DNA damage.

Target e-ARG

Linear pUC19 (amp^R) ~ [amp^R]₀=10⁸ copies/mL (30 ppb) Qualitative PCR with 849bp amplicon

DOM list

SRHA II, SRNOM, SRFA II, Effluent [DOC]₀= 6 mg^c/L, Phosphate buffer 10 mM (pH 7)

RS steady state concentration

Reactive Species	probe	K _{RS&probe} [M ⁻¹ s ⁻¹]		
¹ O ₂	Furfuryl alcohol (FFA)	1.0×10 ⁸		
CO ₃ •-	N,N-Dimethylaniline (DMA)	1.85×10 ⁹		
•ОН	Terephthalic Acid (TA)	3.3×10 ⁹		
³ DOM*	2,4,6-Trimethylphenol (TMP)	1.0×10 ⁹		

Second order reaction rate constants between RS & e-ARG

- •OH has the highest reactivity with e-ARG ~ close to theoretical diffusion-controlled reaction rate
- 4 DOM PSs' reactivities with e-ARG ~ a positive relation with their reduction potential (except CBBP).

***CO₃**•- was excluded due to the vulnerability of the probe compound to ³DOM^{*}.

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Contribution of each pathway

(k _{direct})/K _{obs} = Direct	$(k_{obs}-k_{direct}-k_{1O2}-k_{OH})/K_{obs} = {}^{3}DOM^{*}$		
(K _{OH,ARG} × [°OH] _{ss})/K _{obs} = °OH	$(K_{102,ARG} \times [^{1}O_{2}]_{ss})/K_{obs} = {^{1}O_{2}}$		

- No direct pathway (>320 nm): corresponds to k_{direct} prediction
- ➢ ³DOM^{*} was a dominant path in NOM.
- Effluent has slightly higher 'OH path than ³DOM^{*}.
- Effluent showed 2-fold higher ['OH]_{ss} than NOM
- Effluent has a low level of NO₃⁻/DOC ~ photosensitization of DOM is the prevailing 'OH source [12].
- Effluent produces 'OH more efficiently than NOM ~ Effluent has a larger amount of Low Molecular Weight DOM (LC-OCD).
- \succ 1% ~ 4%: little contribution of ¹O₂

Estimated k_{3DOM* & ARG} from the RS model

✤ Comparison of k_{3DOM* & ARG} with literature

 \rightarrow k_{3DOM*,ARG} was calculated using Sunlight photolysis model approach to the literature data value.

Peng et al., 2023, Environmental Science and Technology, 57(18), 7230-7239 Zhang et al., 2022, Chemosphere, 302, 134879

Simulated Photolysis rate & Contribution of each pathway using k_{3DOM* & ARG}

Conclusions

- Second-order reaction rates between 3 RSs (¹O₂, CO₃⁻, ³DOM^{*}) and e-ARG were measured.
- Sunlight photolysis model approach estimated k_{3DOM*&e-ARG} of 2.0(±0.5)×10⁸ M⁻¹s⁻¹, consistent between 4 DOM sources (Effluent, SRHA, SRNOM, SRFA).
- CBBP well predicts the reactivity of ³DOM^{*} of 4 sources of DOM, so it is recommended as model DOM photosensitizers.
- ³DOM^{*} contribution to sunlight photolysis was higher in NOM (71±2%)) than in Effluent (33%), but the reactivity of ³EfOM^{*} can be varied depending on the photochemical properties of EfOM.
- ³DOM^{*} appears to be the most dominant pathway among the indirect pathways and showed contributions comparable to the direct pathway when exposed to a full spectrum.
- The roles of ³DOM^{*} in sunlight photolysis of e-ARG is not negligible and needs more investigation about the key factors of determining the reactivity between ³DOM^{*} and e-ARG.

Thanks for your attention!

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Range of E_T and E^{0*} of ³DOM^{*}

1-4: quinones
5-13: aldehydes and ketones
14-18: coumarins, chromones, and related
19-23: Polycyclic aromatic hydrocarbons (PAHs)
Others

Sunlight spectrum + DNA photoaction spectra+ DOM absorbance $\rightarrow k_{direct}$

RS steady state concentration

- CO_3^{2-}/HCO_3^{-} can be from the CO_2 (g) in the air : At RT, 1 atm, 1×10^{-5} M of CO_2 (g) in water. : $[HCO_3^{-1}] \approx 8 \times 10^{-6}$ M and $[CO_3^{2-}] \approx 4 \times 10^{-9}$ M at pH 7. - Reaction of CO_3^{2-}/HCO_3^{-} with 'OH and/or with ³DOM^{*} $CO^{2-}_3 + \cdot OH \rightarrow OH^- + CO_3 \cdot - k_1 = 3.9 \times 10^8 M^{-1} s^{-1}$ $HCO^-_3 + \cdot OH \rightarrow H_2O + CO_3 \cdot - k_2 = 8.5 \times 10^6 M^{-1} s^{-1}$ $CO^{2-}_3 + ^3DOM^* \rightarrow DOM \cdot - + CO_3 \cdot - k_3 \approx 1.0 \times 10^5 M^{-1} s^{-1}$ $HCO^-_3 + ^3DOM^* \rightarrow DOM \cdot - + H^+ + CO_3 \cdot - k_4 \approx 9.6 \times 10^3 M^{-1} s^{-1}$ - CO_3^{-1} formation rate is $\simeq 3.5 \times 10^{-14}$ M/s. - Predicted $[CO_3^{-1}]_{ss} \simeq 3.5 \times 10^{-16} \simeq 2.2 \times 10^{-17}$ M - Observed $[CO_3^{-1}]_{ss}$ 1,000 times larger than prediction

- **EfOM showed ['OH]**_{ss} below LOD ~ guess to be due to residual MeOH, SPE eluents of EfoM.
- EfOM was excluded from further discussions.
- > CO₃⁻⁻ was larger than expected~ probe compound (DMA) was vulnerable to $^{3}DOM^{*} \rightarrow CO_{3}^{+-}$ excluded from model

Solid Phase Extraction of Effluent Organic Matter (EfOM)

Why EfOM show ['OH]_{ss} below LOD?

 \blacktriangleright We tested **addition of 10ppm NO**₃⁻ to 4 DOM solutions

matrix	NO ₂ ⁻ , mg/L	NO ₃ ⁻ , mg/L	CO ₃ ²⁻ /HCO ₃ ⁻		
Effluent	0.20	11.5	17.82 ppm		
EfOM	0.001	0.35	21.6 ppb		

- [•OH]_{ss} were both below LOD without and with NO₃.
- During the EfOM preparation using SPE, MeOH was used as final eluents, which is commonly used as 'OH quencher. So it's guessed that residual MeOH might have quenched 'OH produced.
- > In all cases, the addition of NO_3^- didn't increase the [**'OH**]_{ss}.
- > NO_3^- was not the major source of **'**OH in effluent.

 \rightarrow EfOM was excluded and Effluent was used in the discussion.

*LOD of [•OH]_{ss} is ~ 4.5×10⁻¹⁸ M

Effect of Nitrate (10 ppm) to the DNA degradation

> Apply each $k_{3DOM^* \& ARG}$ to the model and compare with k_{obs}

- > Overall, model prediction was good (< 30%) with experimentally determined k_{3DOM* & ARG}, except for EfOM.
- Surprisingly, EfOM showed no degradation of ARG with the addition of NO₃⁻, while [³DOM^{*}]_{ss} was 2-fold increased.
- In Chen et al. [14], the counteraction effect of NO₃⁻ to DOM on photo-inactivation of E.coli was reported.
- Addition of NO₃⁻ didn't affect the degradation rate of ARG in case of SRNOM & SRFA. For SRHA, a slight decrease of k_{obs} was observed.

Reaction mechanisms of 4 RSs and e-ARG

Water quality of effluent

Water quality indicator	рН	DOC [ppm]	SS [ppm]	TN [ppm]	TP [ppm]	NO ₂ ⁻ [μM]	NO ₃ ⁻ [μM]
value	7.62	6.2	2.70 (±0.37)	10.36 (±2.82)	0.09 (±0.02)	48.7±0.2	206 ± 0.2

Water treatment trains

DOM characterization

DOM characterization: LC-OCD DOM component analysis

F1: biopolymers; F2: humics; F3: building blocks; F4: LMW humics and acids; F5: LMW neutrals.

DNA purification for SRHA & SRFA

