Prevalence and characterization of plasmid-mediated quinolone resistance in various aquatic sources

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Introduction

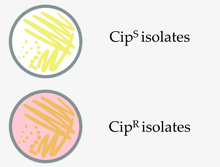
- Ciprofloxacin is a fully synthetic antibiotic developed in 1987 and remains among the most widely used antibiotics worldwide
- Excessive usage has resulted in small quinolone concentrations found in aquatic sources[1]
- Environments with subinhibitory antibiotic concentrations and high microbial diversity and nutrient loads (e.g. wastewater) foster the development of antibiotic resistance genes[2]
- *qnr* genes conferring quinolone resistance were first found in 1998 and have since been observed on a global scale

Methodology

Sample Collection:

Collect samples from 5 aquatic sources

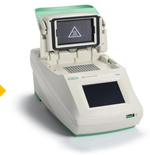
Spread plate samples onto media



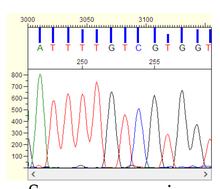
Streak to obtain pure cultures

Genera Identification and *qnr* Gene Detection:

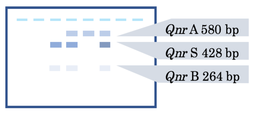
Whole genomic DNA extraction



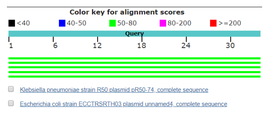
PCR amplification for 16S rRNA gene and *qnr* genes



Sanger sequencing



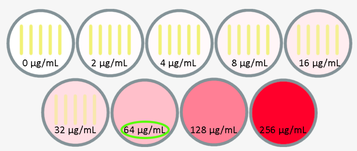
Visualize *qnr* presence by gel electrophoresis



Match sequences to species on BLAST database

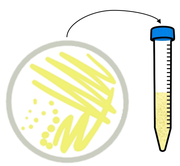
Minimum Inhibitory Concentration Testing:

Streak isolate onto media of incrementally increasing antibiotic concentration

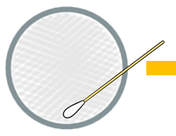


Observe lowest concentration with complete inhibition after 24 hours

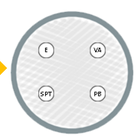
Antibiotic Susceptibility Testing:



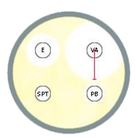
Resuspend isolate to uniform turbidity



Swab liquid evenly across R2A media



Insert antibiotic test disks



Record radius of inhibition after 24 hours

Purpose

Goal: To profile patterns of *qnr* gene prevalence in aquatic environments

Objectives:
- Determine the extent of ciprofloxacin resistance present in wastewater communities
- Determine co-resistance patterns with other antibiotics
- Profile genera diversity and its connection with ciprofloxacin resistance
- Quantify the potency of *qnr* genes on the extent of ciprofloxacin resistance

Results

- The proportion of ciprofloxacin-resistant bacteria across all locations ranged from 0.12 – 1.62% of the culturable population

Table 1. Differences in multiple antibiotic resistance in isolates resistant or sensitive to ciprofloxacin

Population	ARI Score	Percentage of bacteria resistant to x or more antibiotics											
		0	1	2	3	4	5	6	7	8	9	10	11	12
Cip^S bacteria	0.26	15	85	69	56	46	30	17	7	2	0	0	0	0
Cip^R bacteria	0.60	0	100	100	98	86	76	64	52	37	31	27	26	21

Bacteria with ciprofloxacin resistance had a greater tendency to be resistant to other antibiotics tested.

Table 2. Diversity of species among the sampled ciprofloxacin-sensitive and resistant populations

	Cip^S	Cip^R
Isolate total (N)	104	80
# of genera	40	20
Shannon Diversity index (H)	3.26	2.63
Genera richness (S)	3.69	3.00
Sorenson Co-efficient (SC)	0.3	

The ciprofloxacin-resistant population has moderately lower diversity, as measured by Shannon-Weaver Diversity Index and genera richness, compared to the ciprofloxacin-sensitive population. The overlap in genera is relatively low, as indicated by SC = 0.3.

Table 3. Minimum inhibitory concentrations of ciprofloxacin for isolates with and without *qnr* genes

Qnr Genes	Sample Size	Ciprofloxacin MIC (µg/mL)			
		≤ 32	64	128	≥ 256
Present	n = 34	5.88%	26.47%	29.41%	38.24%
Absent	n = 56	17.86%	23.21%	23.21%	35.71%
Total	n = 90	13.33%	24.44%	25.56%	36.67%

No significant differences in ciprofloxacin's minimum inhibitory concentrations of *qnr*+ and *qnr*- isolates were observed.

Conclusions

- Ciprofloxacin resistance genes were observed in all aquatic sources sampled
- Ciprofloxacin resistant bacteria had a greater tendency for co-resistance to other antibiotics, suggesting that a single mobile genetic element may harbor multiple ARGs
- Ciprofloxacin resistance is restricted to a relatively distinct and less diverse subset of the bacterial community
- Minimum inhibitory concentration is not significantly enhanced in bacteria with *qnr* genes, indicating that a multitude of ciprofloxacin resistance genes may be relevant

Acknowledgements

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Prospects of CRISPR/Cas cloning vectors to combat antibiotic resistance gene dissemination

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Introduction

- CRISPR (Clustered Regularly Interspaced Small Palindromic Repeats) and Cas (CRISPR-associated) proteins comprise an adaptive immune system in prokaryotes
- CRISPR-Cas systems digest invading nucleic acids and incorporate a ~30 bp snippet ("spacer") of the invader into the host genome
- Spacers are transcribed into CRISPR RNAs (crRNAs) that recognize the same nucleic acid in the event of a repeat invasion and guide nucleases to the target for elimination

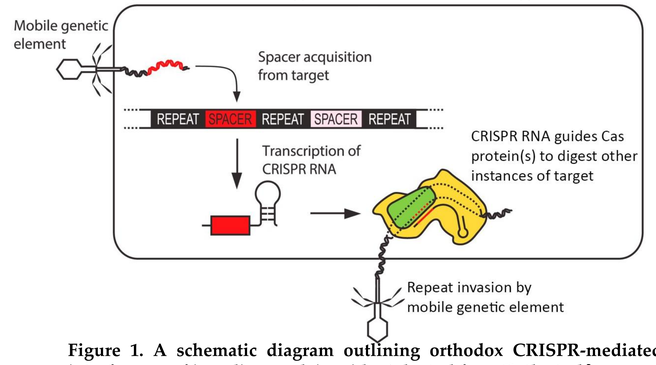
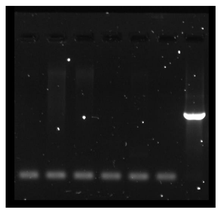


Figure 1. A schematic diagram outlining orthodox CRISPR-mediated interference of invading nucleic acids. Adapted from Rath *et al.*[3]

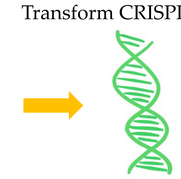
- We hypothesize that a plasmid vector, when transformed into a host cell, could exogenously express a CRISPR/Cas system and prevent the host's participation in conjugative processes

Proposed Methodology

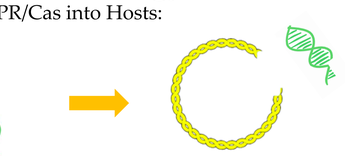
Transform CRISPR/Cas into Hosts:



Screen bacteria for CRISPR/Cas system

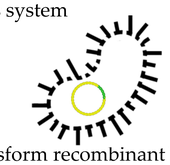


PCR to obtain sufficient CRISPR/Cas DNA



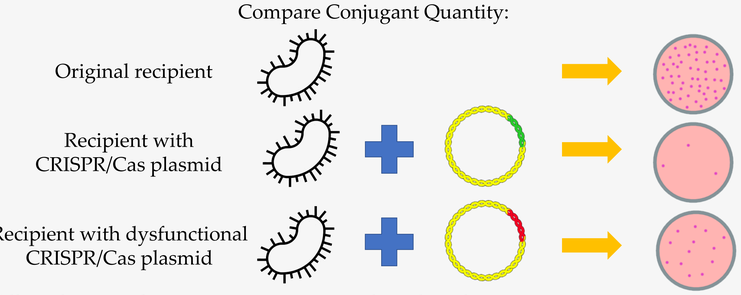
Digest DNA and vector plasmid with restriction enzymes

Ligate to create recombinant DNA



Transform recombinant DNA into host cell (heat shock, electroporation)

Compare Conjugant Quantity:



Allow donor and recipient to conjugate in liquid media

Spread plate and count CFUs

References

1. Batt, A. L., Bruce, I. B. & Aga, D. S. (2006). Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. *Environ Pollut, 142*, 295–302.
2. Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P&T, 40*, 277–83.
3. Rath, D., Amlinger, L., Rath, A. & Lundgren, M. (2015). The CRISPR-Cas immune system: Biology, mechanisms and applications. *Biochimie, 117*, 119-128.