Shellfish as bioindicators to assess antimicrobial resistance

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This PATH-SAFE pilot research study sought to develop a suite of testing approaches to assess the presence and identity of antimicrobials and resistant microorganisms in bivalve shellfish. The filter feeding ability of bivalve shellfish means they may provide a sentinel species to report on water quality in respect of antimicrobial resistance while minimising costs through using samples already collected for other purposes.

INTRODUCTION

Antimicrobial resistance represents a major concern for humans, animals, plants and the wider environment. The government's 5-year National Plan outlines how the UK will address the AMR challenge and includes specific reference to the need for better understanding of the spread, transmission and risk of AMR in the environment. Filter-feeding shellfish present a potentially useful sentinel bioindicator model to help address this current gap in environmental AMR surveillance. Bivalve species are capable of greatly concentrating microbial contamination from the environment and are already examined in Europe for bacterial faecal indicator organisms (*Escherichia coli, E. coli*) as part of existing monitoring programmes.

METHODS

Shellfish waters are currently monitored for a range of substances and the microbiological quality of shellfish flesh. For this work we used some of the bivalve shellfish samples obtained during routine official control sampling and further tested for AMR.

Shellfish were obtained from five sites in July 2022 to January 2023 and were tested using chemical (Figure 1), molecular (AMR gene and metagenomic analysis, Figure 2) and microbiological approaches (*E. coli* isolation and susceptibility testing, Table 1). We chose shellfish from a range of "impacted" and "reference" study sites in England.

RATIONALE

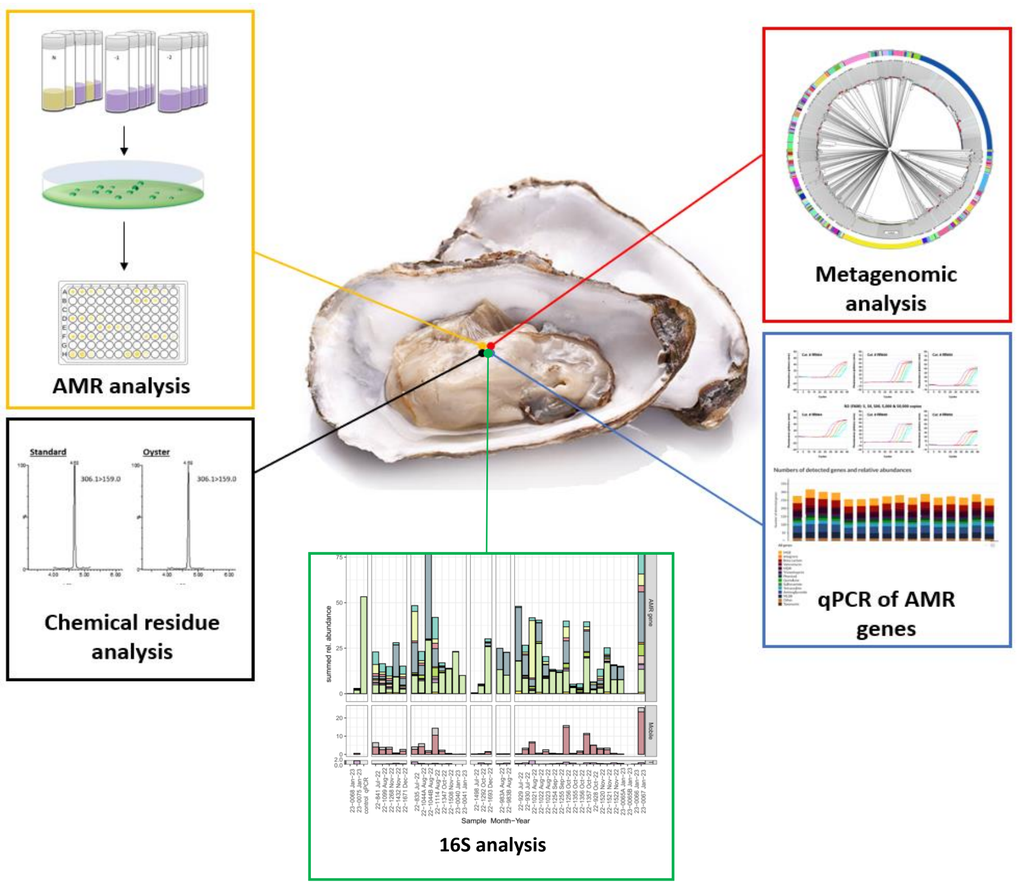

Figure 1. One sample = many analyses (OSMA). Here, a shellfish sample, could be simultaneously analysed for the presence of AMR of bacteria and associated genes, and various molecular and chemical targets, ultimately to assess the use of shellfish samples as sentinels of environmental health[1,2] Because samples are already obtained for existing monitoring purposes, this may be a cost effect approach for wider environmental surveillance efforts.

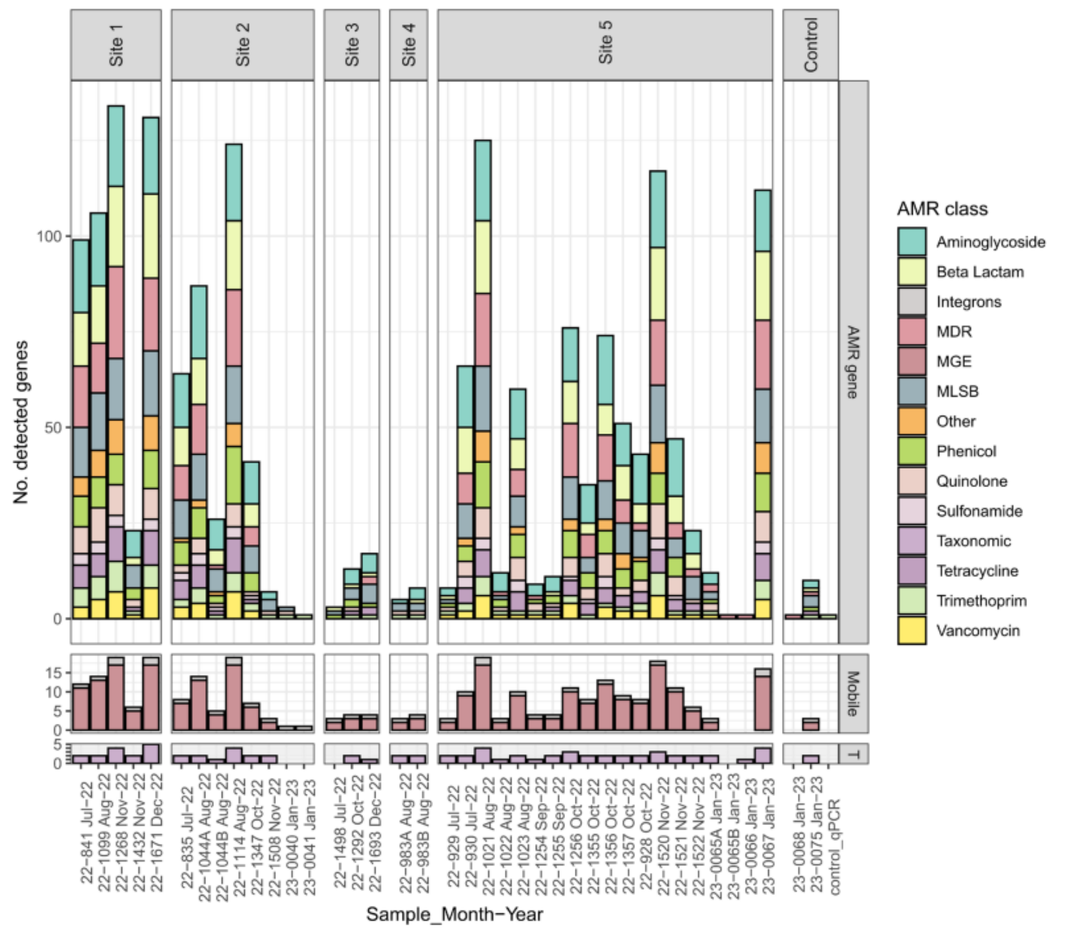

Figure 3. Numbers of AMR genes detected in each sample from different sites between July 2022 and January 2023 using SmartChip array.

Select antimicrobial capabilities of multi-drug resistant *E. coli* strains isolated from shellfish samples				
Cefas ID of sample	**Site of isolation of tested strain**	**Month and year**	**Number of resistances**	**Resistance profile**
22-1671A	Site 1	Dec 2022	4	Amp, Smx, Tet, Tmp
22-1671B	Site 1	Dec 2022	4	Amp, Smx, Tet, Tmp
22-1021	Site 5	August 2022	11	Amp, Fot, Cf, Taz,, Chl, Cip, Col, Mer, Nal, Smx and Tet
22-1355B	Site 5	October 2022	9	Ami, Fot, Taz, Cip, Col, Gen, Mer, Nal and Smx

Table 1. Isolation of highly-AMR *E. coli* strains from this study.

RESULTS

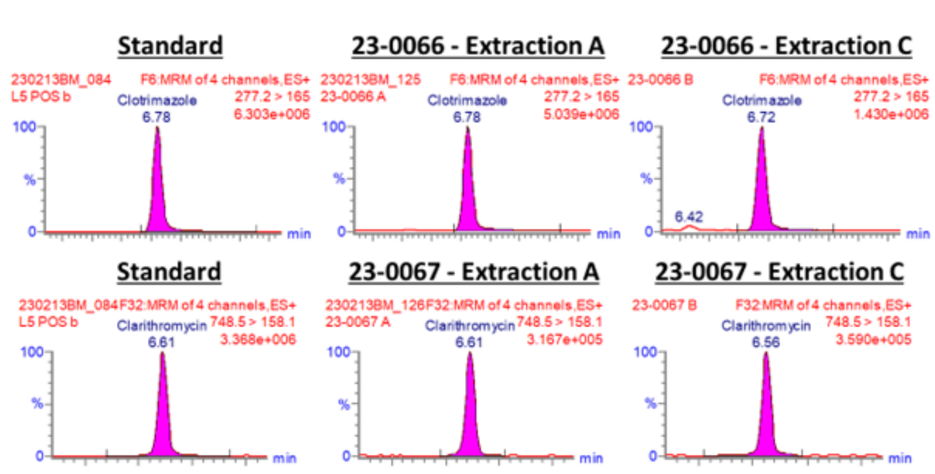

Figure 2. We successfully developed extraction and testing methods to identify various antibiotics in shellfish. Analysis of the shellfish samples identified the presence of the antibiotic's clarithromycin and trimethoprim, as well as the antifungal clotrimazole. These compounds appeared to show a trend to be at higher levels in samples from contaminated sites – potentially associated with human impact.

CONCLUSIONS

- We successfully developed a range of different testing approaches, **using shellfish as the focus testing matrix.** Many of the methods trailed as part of this project have **never been successfully carried out before**.

- All highly resistant isolates identified from this pilot study were isolated from potentially "impacted" sites. We were able to test of the same matrices for various **pharmaceutical residues** (e.g. antibiotics) using **targeted** and **untargeted chemical analysis** approaches. The antibiotics **clarithromycin** and **trimethoprim**, as well as the antifungal **clotrimazole** were identified. Successful detection of **AMR genes** and associated mobile genetic elements using a combination of metagenomic and SmartChip arrays, **with higher overall number of AMR genes found in "impacted" sites compared to cleaner reference sites**. Metagenomic approaches developed need optimising as the coverage for **bacterial DNA was low** (data not shown).

- Overall, the pilot study demonstrated that shellfish may be a **highly useful testing matrix** to assess AMR in the environment. The final shellfish AMR report published with EA on 23rd November 2023[3].

REFERENCES

[1] Hill *et al.* (2024). Realising a global One Health surveillance and response strategy. *In review.*

[2] https://www.gov.uk/government/publications/antimicrobial-resistance-surveillance-strategies-within-wild-flora-and-fauna-of-england

[3] Shellfish as bioindicators for coastal antimicrobial resistance. Cefas/EA report