

EXPERIENCE OF A SURVEILLANCE STRATEGY FOR ANTIMICROBIAL RESISTANCE BASED ON E. COLI ISOLATED FROM MUSSELS GROWN IN THE COASTAL WATERS OF THE VALENCIAN COMMUNITY.

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INTRODUCTION

Combating AMR is a priority for the European Commission. The continuous reinforcement of the surveillance of AMR is essential for the availability of comprehensive and reliable information on the development and spread of resistant bacteria and resistance genes.

The Global Action Plan on antimicrobial resistance recognises that water is an important route for dissemination and promotes the improvement of water management as a key element in tackling AMR from the One Health perspective. However, there are still great uncertainties about the actual role played by the environment in the emergence, spread and persistence of antimicrobial-resistant bacteria.

Bivalve molluscs, including mussels, filter large volumes of water and can concentrate particles and pathogens, which makes them uniquely vulnerable to the bacterial contamination of coastal waters.

In the waters surrounding the port of València, there is a deep-rooted tradition of mussel cultivation, based on a small-scale coastal production using floating rafts.

In line with the EU Action Plan, a harmonised ARM surveillance method is proposed based on the study of E. coli isolated from mussels grown in the coastal waters of the Mediterranean Sea.

METHODOLOGY

The floating rafts are located between 0.591 and 0.113 nautical miles from the coastline and their bathymetry is between -8 and -20 metres.

In 2021, sampling could be carried out in full, as scheduled, but in 2020, because of the Covid-19 pandemic and the restriction measures implemented, the scheduled sampling could not be carried out and a total of 67 samples were taken.

All isolates of indicator E. coli were susceptible to 7 out of 14 of the first harmonised antimicrobial panel in 2020 and 8 of 15 of the updated panel in 2021, including 4 CIAs (WHO,

2019). Moderate resistance levels were detected to four antibiotics, meanwhile high resistance levels to two antibiotics commonly used in veterinary, and to a lesser degree, human medicine (sulfamethoxazole and tetracycline) (JIACRA III) were observed.

Regarding the outcome indicators, extremely high levels of the Primary Indicator, complete susceptibility to the harmonised panel of antimicrobials in *E. coli*, were obtained. As for the secondary indicators, low levels of ESBL/AmpC producing *E. coli* were detected, and moderate levels were observed for the other two (MDR *E. coli* and ciprofloxacin resistant *E. coli*).

RESULTS

The number of resistant and intermediate strains of *E. coli* and *E. faecium* found in rats at both doses of nisin was 1.8-4.4 times higher than in the control. The low dose induced a significantly higher frequency of *E. coli* resistance in the intestines of rats to penicillins, cephalosporins, quinolones and monobactams, and the high dose induced a significantly higher frequency of *E. coli* resistance to penicillins. The GIZ of *E. coli* to azithromycin (13.3 ± 0.43) at the low dose was less than in the control (16.2 ± 1.7), $p=0.037$, and at the high dose (15.3 ± 0.86), $p=0.06$. *E. faecium* resistant to imipenem, vancomycin, erythromycin, tetracycline was found more often among gut isolates in experience groups than in the control (9.1; 27.0; 70.0; 9.1 vs 0; 0; 50 and 0 %, respectively). Moreover, the low dose more often induced resistance to vancomycin, while the high dose induced resistance to erythromycin, linezolid, tetracycline, imipenem. Strains having intermediate resistance to imipenem were identified only among enterococci. The low dose of nisin more strongly promoted the appearance of co-resistance in gut isolates: in 75 % of *E. coli* strains to 2–5 drugs, in 36 % of *E. faecium* to 2–3 drugs. Similar indicators for the aggravated dose were 44 % to 2-3 drugs and 27 % to two drugs, and for the control 20 % to two drugs and 0%.

DISCUSSION

The use of indicators provides a simplified overview of the complex ARM situation. This leads to a loss of information and detail, so the indicators should be interpreted with caution. The detection in the coastal aquatic environment of low prevalence levels of ESBL/AmpC producing *E. coli* and moderate levels of ciprofloxacin resistant *E. coli* may be concerning as regards its relevance to public health.

The proposed surveillance system may be appropriate for AMR monitoring in seafood cultivation. The system is particularly appropriate since it addresses both consumer exposure to resistant bacteria via shellfish and the environmental exposure of shellfish to such bacteria. Additionally, the weighting applied by the PCU allows different biomass to be compared. The aquatic environment acts as a medium for the dissemination of AMR from human and terrestrial livestock faecal waste, so guaranteeing a high microbial quality of the water in wastewater effluents is a priority.

It is necessary to define harmonised environmental AMR surveillance systems in order to evaluate the effect of the Green Pact policy on food producing environments, the consumption of antibiotics and the impacts of climate change.