To improve the understanding of the dissemination of antimicrobial resistance and its impact on humans from imported edible shrimp

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Abstract

This UROS funded article investigates antimicrobial resistance (AMR) in bacteria. The project undertaken considers the occurrence of AMR containing bacteria in fresh /frozen shrimp bought from retail outlets and was designed to improve our knowledge of the dissemination of antibiotic resistance genes (ARGs) from the food chain. This work improves our understanding of how AMR in bacteria can impact the health and welfare of the population as modern medicine relies on antibiotics that work to treat many infectious diseases. In the laboratory, samples of shrimp were taken from four UK-wide supermarkets and the countries of origin noted. The samples were processed and plated onto eosin methylene blue (EMB) agar and incubated at 37°C to detect E.coli - the target organism. Of the 14 colonies obtained from Vietnam and Indian sourced shrimp, 6 were identified as E.coli. The 6 E.coli colonies were transferred to nutrient agar containing ampicillin. Antibiotic-resistant E.coli were isolated from all the shrimp samples examined. Plasmid extractions of these isolates were then exposed to multiple temperatures and transfer of ARGs was shown in some of the competent recipient (ampicillin-sensitive strain). DNA analysis of the recipient strain plus plasmid (transformants) detected bands in 50% of the isolates. The conclusion was that ARGs in E.coli plasmids from foodstuffs might survive heat treatment sufficiently intact to transform other possibly commensal strains of E.coli.

Keywords: Antimicrobial resistance, shrimp, *E.coli*, ampicillin, heat treatment

Introduction

Public health is threatened by AMR in bacteria (Thornber *et al.,* 2019). We do not fully understand the spread of AMR but animals, humans, and the environment are all involved (Akter *et al*., 2021). Since many low-income countries have unregulated use of antibiotics and allow untreated waste into local water sources. Food animals imported from these countries such as shrimp increase the risk of AMR dissemination to the UK and worldwide sources (Akter *et al.*, 2021). *E. coli* are of particular interest and targeted in this study as they are widely abundant through excrement cycles in the natural environment globally (Thornber *et al*., 2019). In addition, low levels of antibiotics present in the water appear to drive the emergence

of AMR in *E. coli.* Shrimp containing *E. coli* with AMR may represent a food risk to the consumer unless appropriate heat treatment destroys both the pathogen and ARGs (Verraes *et al.,* 2013). This project will research whether there are antibiotic resistant genes (ARG) present in *E. coli* within shrimp from the far east. Once it is established if ARGs are present the shrimps will be cooked in accordance with government guidelines to investigate whether these ARGs will be destroyed. If the AMR bacteria remain in the shrimps this could increases AMR in humans, this will have detrimental impacts worldwide sources (Akter *et al.*, 2021).

Project Background

This project investigated to what extent antimicrobial resistance occurs in shrimp to enhance knowledge about whether AMR is potentially transferred to humans through our animal food sources. This is an important subject to study to understand AMR dissemination better as part of the 'one health agenda' to consider, animals humans and the environment and their impact on public health threats. We have focussed on *E. coli* which are widely abundant in the environment, populate most mammalian guts and display multi-resistance through a multitude of ARGs. The study aimed to gain an understanding of possible routes ARGs from *E.coli* could be spread from imported shrimp to human intestines. Knowledge of this chain of events may improve our understanding of how resistant bacteria behave in the food chain and what we should do to prevent it. Preliminary studies were aimed at investigating whether cooking (heat treating) the shrimp as recommended by the Food Standards Agency is sufficient to destroy not only *E.coli* but also specific ARGs carried on plasmids. Although the carriage of AMR bacteria in food is well documented there is little evidence that heat treated ARGs can transfer intact to other organisms. Antimicrobial resistance was first presented to me as a major issue in first term at university, the scary realisation that medicine used to treat many illness, such as tonsillitis, which I viewed as a minor illness could soon not be treated. If antibiotics can no longer cure patients, then significantly more people will suffer and die. Mainstream media outlets barely shed light on AMR and its catastrophic impacts it could have on public health in the future. Therefore, I developed an interest into how ARGs pathways work and how they affect humans, in order to highlight where we go wrong and how we could prevent the dissemination AMR.

Review of literature

Since emergence and dissemination of AMR caused by the use and misuse of antibiotics is a 'One Health' problem that affects humans, animals and the environment, all sectors should work together to reduce the negative impacts on the population (Thornber *et al.,* 2019). Changes that can be achieved locally often lead to international consequences and since food supply chains are usually international national governments and organisations in producer countries should coordinate and develop 'action plans' to reduce the risk that the shrimp industry is disseminating AMR globally through distribution (Thornber *et al.,* 2019). Thornber *et al.* have formulated potential risk mitigation strategies for the shrimp industry which could be

used to reduce AMR transmission and produce food in a more sustainable way for the planet (Thornber *et al.,* 2019). Several authors have described the pathways that antimicrobial-resistant zoonotic pathogens can become present in the food (Founou et al., 2016; James *et al.,* 2021; Verraes *et al.,* 2013). The risk is that without intervention ARGs from bacteria in shrimp (and other food) will be capable of transfer to the microbiome of the human gut and prevent adequate treatment by antibiotics when infections occur (Akter *et al.,* 2021). It has been clear that a raft of mechanisms exists (vectors such as plasmids, bacteriophages etc) to transport ARGs in microorganisms such as *E. coli* and subsequently rapid wide-ranging transfer between bacteria is the rule in the laboratory (Akter *et al.,* 2021). A recent review showed that recommended heat treatment such as cooking reliably kill bacteria in food products and found it unlikely that ARGs could survive this heat treatment but whether this intervention is sufficient to prevent transmission of ARGs into commensal or pathogenic bacteria in the human gut in practical terms is not proven (James *et al.,* 2021).

Methodology

Retail shrimps were obtained from 4 different national supermarkets, who sourced from Vietnam (Supermarkets A, B, C) or from India (Supermarket D). The shrimps were dissected out and tissue measured into aliquots 0.5g, 1g, 5g, 10g and 20g, and 10ml of sterile water added to each. The suspensions were macerated in a stomacher for 35 seconds at 260rpm. 100ul of this suspension was aseptically spread plated onto EMB agar, and the plates incubated overnight at 37°C. Resulting colonies underwent tests (Gram staining, oxidase, and catalase tests) to confirm their identity - Gram-negative, oxidase negative and catalase-positive aligning with the characteristics of *E. coli* (Figure 1).

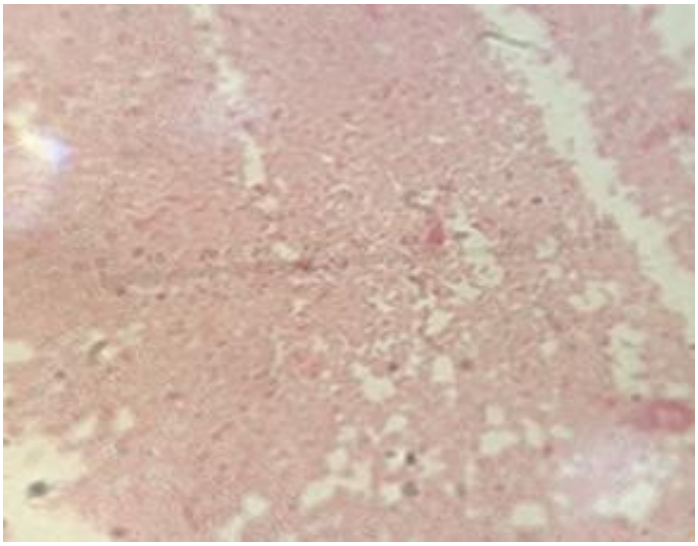

Figure 1 Gram-stained colony from Supermarket B with the characteristics of *E. coli.*

Putative *E. coli* colonies were streak plated onto nutrient agar containing ampicillin at 20% concentration and incubated overnight at 37°C. Colonies that had grown on the antibiotic-containing agar were sub-cultured into the nutrient broth and incubated at 37°C overnight. Ampicillin-resistant cultures were subjected to an extraction technique using a standard lab methodology and their DNA concentrations were measured on a nanodrop estimator. Each concentration (over 50% DNA), along with a control sample of *E.coli* ampicillin sensitive recipient DH5α, were then heated to 3 different temperatures - 63°C, 72°C and 90°C in a heat block - all for 2 minutes. Subsequent measurements were taken by nanodrop to observe whether the effect of heat directly reduces the concentration of DNA.

Attempts to transfer ampicillin-resistant ARG from each isolated and heat-treated plasmid to *E.coli* ampicillin-sensitive recipient (DH5α) were undertaken using standard lab procedures from previously prepared competent cells.

Results

Colonies grew on 14 out of the 40 samples tested with EMB agar and showed distinctive iridescent colonies. Supermarket C samples did not produce any *E. coli* colonies whereas the 4 plates of shrimp at different concentrations from supermarket D are shown (Figure 2).

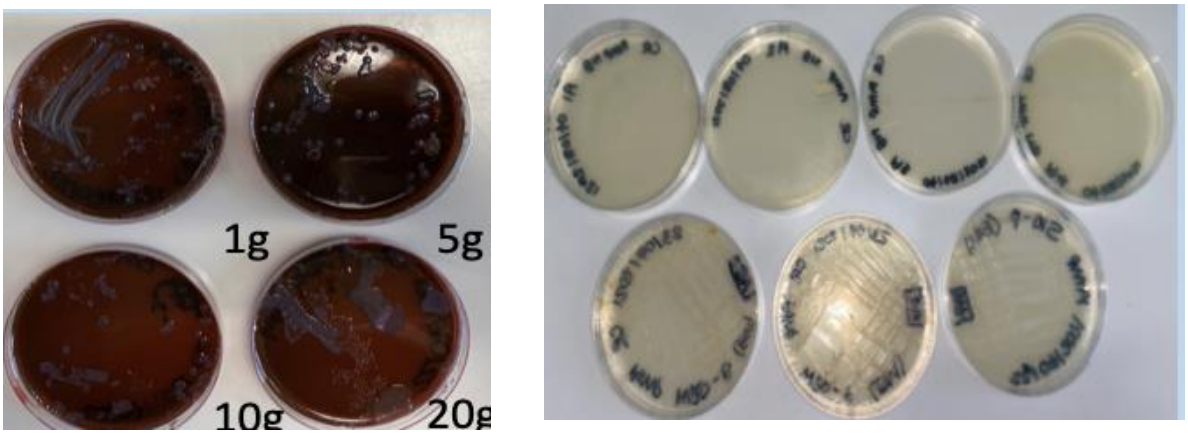

Figure 2 EMB agar with 1g, 5g, 10g and 20g of shrimp from supermarket D and nutrient agar with 20% ampicillin from 6 samples from Supermarkets A, B and D showing growth plus DH5α.

Bacteria from these 14 colonies were Gram stained, catalase and oxidase tested and 6 were identified as *E.coli* obtained from supermarkets A, B (Vietnam) and D (India). The resulting six isolates were then streak plated onto nutrient agar with ampicillin added. All samples identified as *E. coli* from the shrimp sourced showed resistance against ampicillin. Preliminary experiments showed no reduction in DNA from

plasmid preparations following heat treatments. Following the transfer experiments of plasmid preparation from isolates into antibiotic-sensitive recipient *E.coli* (DH5α) only 9 out of 18 isolates contained a plasmid. The heat treatments (63°C, 72°C or 90°C for 2 minutes) made no impact on the plasmid transfers. The heat treatments did not appear to disrupt the plasmids, as transfer occurred with all 3 isolates from the three heat treatments tested.

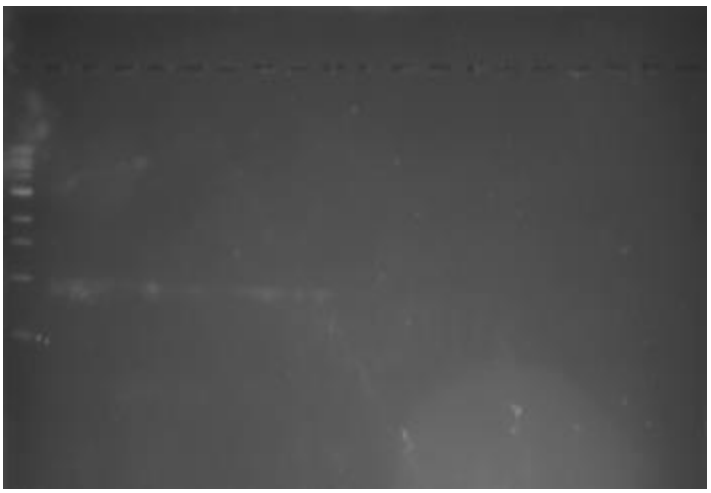

Figure 3 Faint bands for nine isolates

Faint bands for nine isolates alongside a 1kb ladder are also shown (Figure 3). The plasmids all had a molecular size of approximately 700kb. These nine isolates are plasmids originating from DH5α, supermarket B (Vietnam) and D (India).

The nine isolates that contained plasmids still only showed light bands, this means that there are very small amounts of the plasmids in the isolates. These results are insignificant into showing whether these plasmids are affected by heat as the isolates found ranged from being from all the temperature conditions. However, this project does show that antibiotic resistant E.coli were present in three out of four of the shrimp samples. This raises the question, would cooking these shrimps remove the ARGs, decreasing the dissemination of AMR, this would require further research to be carried out.

UROS Experience

This opportunity to gain first-hand experience in conducting laboratory research has been an invaluable experience, especially due to the support of my supervisor who is exceptionally knowledgeable in microbiology. He shared his in-depth knowledge of the subject, which helped me push through, and meet the challenges I otherwise may not have. This scheme has given me a deeper understanding of how I can conduct and present my final year project research, and it has enabled me to improve my academic writing and research skills. My passion for research has increased throughout this

experience, and this project has helped me realise which master's course I would like to apply for in the future.

However, several challenges arose while conducting my research in the laboratory. It took almost five weeks for me to find the best method to isolate *E. coli* from shrimp from three out of the four supermarkets. This is due to the agars being insufficiently toxic to other bacteria. Seven types of agar were tested, including 6 of them being specialised to grow *E. coli*. Additionally, my first plasmid extraction was unsuccessful as they produced <10 % DNA concentrations. Finally, my first gel electrophoresis did not work very well, and I am unsure as to the reason. I believe these issues further developed my understanding of protocols, allowed me more time to enhance my practical skills in the laboratory, moreover, taught me how to troubleshoot.

Conclusion

Shrimps from three out of four supermarkets tested were found to grow colonies of *E.coli*. These colonies were confirmed to be ampicillin-resistant on nutrient agar with supplemented ampicillin by virtue of ARGs. The question is how significant the resistance from food is and whether it is important for AMR dissemination, potentially resulting in modern medicine being ineffective to treat illnesses. We wanted to investigate whether heat treating (cooking) shrimps reduces the risk of dissemination of *E.coli* or the associated ARGs and therefore the ability to transfer to other bacteria in the human gut microbiome. Little work has been reported on the ability of ARGs (in plasmids) to survive cooking or whether heat disruption of the ARG's DNA leads to inability to transfer to recipient bacteria. Our results were unfortunately not conclusive although with sufficient time we could determine in a statistically robust way that ARGs could be transferred to competent cells through transformation following some levels of heat treatment. If so, this means that the ARGs could potentially be spread into commensal and pathogens in the human digestive tract increasing human resistance to antibiotics.

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