TO IMPROVE THE UNDERSTANDING OF THE DISSEMINATION OF ANTIMICROBIAL RESISTANCE AND ITS IMPACT ON HUMANS FROM IMPORTED EDIBLE SHRIMP

INTRODUCTION

Public health is threatened by antibiotic resistance in bacteria (Thornber et al., 2019). There is an increased need for further understanding the spread of antimicrobial resistance (AMR) between animals, humans, and the environment, as AMR can compromise the safety of our food (Akter, Chowdhury and Mina, 2021). Many low-income countries have unregulated antibiotic use and allow untreated waste to local water sources (Akter, Chowdhury and Mina, 2021). Therefore, these countries are increasing the risk of AMR dissemination through animals in these waters, such as shrimp. Escherichia coli (E. coli) bacteria are of particular interest in this study as they are widely abundant through excrement in the natural environment. This can lead them to be exposed to antibiotics and disseminate AMR rapidly through the environment, causing a threat to human health (Thornber et al., 2019).

OBJECTIVES

The first objective is to improve our understanding of possible routes of transfer and spread of AMR bacteria from imported shrimp. The second objective is to gain a better understanding of how resistant bacteria behave in the food chain. In order to meet these objectives, in vitro data will be collected on the prevalence of AMR E. coli from country labelled shrimp imports from UK supermarkets. Furthermore, tests will be carried out to check whether heating the imported shrimp to 72 °C for 2 minutes will break down the antibiotic resistance gene, as the government recommends these cooking instructions (Cooking your food, 2021).

METHODOLOGY

Shrimp were obtained from 3 different supermarkets, three from Vietnam (Supermarket A, B, C) and the other from India (Supermarket D). The shrimps were measured into 0.5g, 1g, 5g, 10g and 20g and 10ml of water was added to each before being put into a stomacher for 35 seconds at 260rpm. 100ul of this water was spread plated onto eosin methylene blue agar and left in an incubator overnight at 37°C.

The colonies underwent tests such as gram staining, oxidase, and catalase. Those which came back gram-negative, oxidase negative and catalase-positive align with the characteristics of E. coli (Figure 1); supermarket C samples did not have any E. coli. Then the E. coli samples are streak plated onto nutrient agar containing ampicillin at 20% concentration and placed overnight in the 37°C incubator. All samples identified as E. coli showed antibiotic resistance against ampicillin.

Colonies from the antibiotic agar were placed into the nutrient broth and left in the 37°C incubator. Then plasmids were extracted, and their DNA concentrations were measured on a nanodrop. Each concentration of over 50% DNA, along with a control sample of DH5α, were then put into a heat block and heated to 3 different temperatures - 63°C, 72°C and 90°C all for 2 minutes. Then these were tested on the nanodrop for the DNA concentrations again to observe whether heat breaks down the antimicrobial resistance genes.

The plasmids were prepared for polymerase chain reaction (PCR) and put into the thermal cycler for 95°C for 30 seconds, 53.5°C for 60 seconds, 72°C for 15 seconds and repeated 30 cycles. These samples were then used for gel electrophoresis.

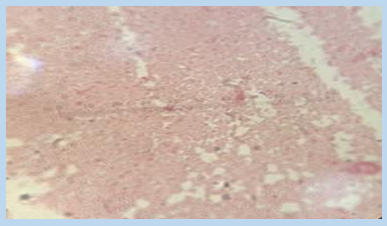
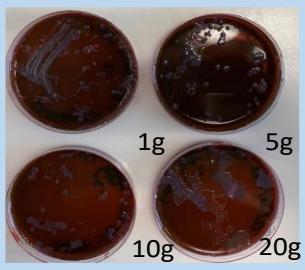


Figure 1. Gram-stained colony from supermarket B with the characteristics of Escherichia coli.

RESULTS

Colonies grew from 14 out of the 40 on eosin methylene blue agar, 4 plates from supermarket D shown in Figure 2. (A). These were gram stained and underwent catalase and oxidase tests, and these tests established seven samples of E. coli. These seven samples were then streak plated onto the nutrient agar with ampicillin added, Figure 2. (B) shows all seven plates with bacterial growths.

(A)



B)

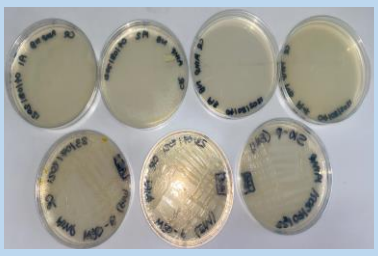


Figure 2. (A) Eosin methylene blue agar with 1g, 5g, 10g and 20g of shrimp from supermarket D. (B) Nutrient agar with 20% ampicillin from 6 samples from Supermarkets A, B and D and control from DH5α.

The gel electrophoresis investigates the genomes behind antibiotic resistance. However, only 9 out of the 18 isolates contained a plasmid. Figure 3. shows these nine isolates alongside the 1kb ladder. The plasmids all had a genomic size of approximately 700kb. These nine isolates which are shown are plasmids originating from DH5α, supermarket B and D.

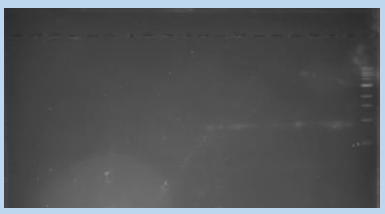


Figure 3. 1kb DNA ladder; B, Identification of Plasmid DNA through gel electrophoresis.

CONCLUSIONS

Three out of four of the supermarkets' shrimps were found to have colonies that had the same qualities as E. coli. These colonies all grew on the nutrient agar containing the antibiotics. Therefore, the E. coli must have genes that are ampicillin resistant to grow on these plates. This resistance is significant as it could lead to AMR dissemination, potentially resulting in modern medicine being ineffective to treat illnesses. It is therefore crucial to know whether cooking the shrimps correctly reduces the risk of dissemination. However, the gel electrophoresis results for the plasmids showed no difference in gene size between the temperature groups. This means that the temperature most likely has not broken down the gene responsible for AMR. It can be argued therefore that further research would be required to determine whether the AMR gene could be transferred to competent cells through a transformation. If so, this means that the AMR gene could potentially be spread in the human gut.

REFERENCES

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