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Plasmid-Encoded Beta-Lactamase Production in *Escherichia coli* Isolated from River and Aquaculture Fish in Kirkuk, Iraq

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ABSTRACT

This study aimed to investigate the presence of plasmid-encoded beta-lactamase enzymes in *Escherichia coli* isolated from fish sourced from rivers and private aquaculture ponds in Kirkuk, Iraq. Fifty samples (25 from each source) were collected and analyzed using morphological, biochemical, and API 20E identification methods. Beta-lactamase production was found in 12% of river isolates and 24% of pond isolates. Plasmid extraction and curing were performed using alkaline lysis and SDS treatment. The findings demonstrated that most isolates lost their beta-lactamase activity after plasmid curing, suggesting that the resistance genes were plasmid-borne. This research highlights the potential health risks associated with antibiotic resistance in aquaculture environments.

INTRODUCTION

Escherichia coli belongs to the Enterobacteriaceae family. It is a Gram-negative, rod-shaped, often motile bacterium that can grow under both aerobic and anaerobic (facultative anaerobic) conditions. It ferments sugars such as lactose, rhamnose, and sorbitol, and prefers temperatures between 36– 37°C for optimal growth **(Murray *et al.*, 2021)**. It is indole-positive, catalase-positive, methyl red-positive, oxidase-negative, Voges–Proskauer-negative, and citrate-negative **(Sastry & Bhat, 2019)**.

It naturally inhabits the intestinal tract of humans and animals as part of the normal flora but can become opportunistic, causing diseases such as sepsis, meningitis, diarrhea, bacteremia, and urinary tract infections—being responsible for 90% of UTIs globally. It can also persist in environmental reservoirs, contaminating water, food, and soil **(Nascimento *et al.*, 2021; Obaid & Jasim, 2023a)**.

E. coli possesses numerous virulence factors, including endotoxins, exotoxins, cytotoxins, siderophores, hemolysin, colicin, and surface structures such as flagella,

capsules, and lipopolysaccharides. These structures carry various antigens (Flagellar "F," Capsular "K," and Somatic "O") that contribute to its pathogenicity **(Dmurena *et al.*, 2021)**.

It is characterized by multidrug resistance (MDR) **(Cornelissen & Hobbs, 2020; Obaid & Jasim, 2024b)**, posing a growing global health threat **(Masoud *et al.*, 2021)**. This resistance arises from mechanisms such as extended-spectrum beta-lactamase (ESβL) production, quinolone and aminoglycoside resistance, target site modification, membrane permeability changes, and efflux pumps **(Nji *et al.*, 2021)**, making *E. coli* clinically challenging **(Prmohoammad *et al.*, 2019)**. Despite the development of new antibiotics in recent years, their misuse and overuse have resulted in resistant strains emerging globally **(Sultan *et al.*, 2018)**.

Plasmids are widespread among bacterial species. While not essential for survival, they carry vital traits such as resistance and toxin production. Plasmid curing experiments help determine the genetic basis of such traits **(Laxminarayan *et al.*, 2020)**.

Given the importance and widespread presence of *E. coli* in animals and its association with various diseases, this study aimed to explore its virulence factors and investigate whether their genes are plasmid-encoded or chromosomal.

MATERIALS AND METHODS

Samples collection

Fifty fish samples were collected from the 1st of February to the 1st of March 2022. All relevant information was recorded in survey forms. The samples were taken to the Microbiology Laboratory, College of Health and Medical Technology, Northern Technical University, Kirkuk. Fish were dissected, and intestinal samples were cultured on MacConkey agar and incubated at 37°C for 24 hours.

Bacterial identification

Isolates were identified based on morphology, microscopy, and biochemical tests (indole, methyl red, oxidase, catalase, citrate, motility) (Fig. 1) and confirmed using the API 20E system (Fig. 2).

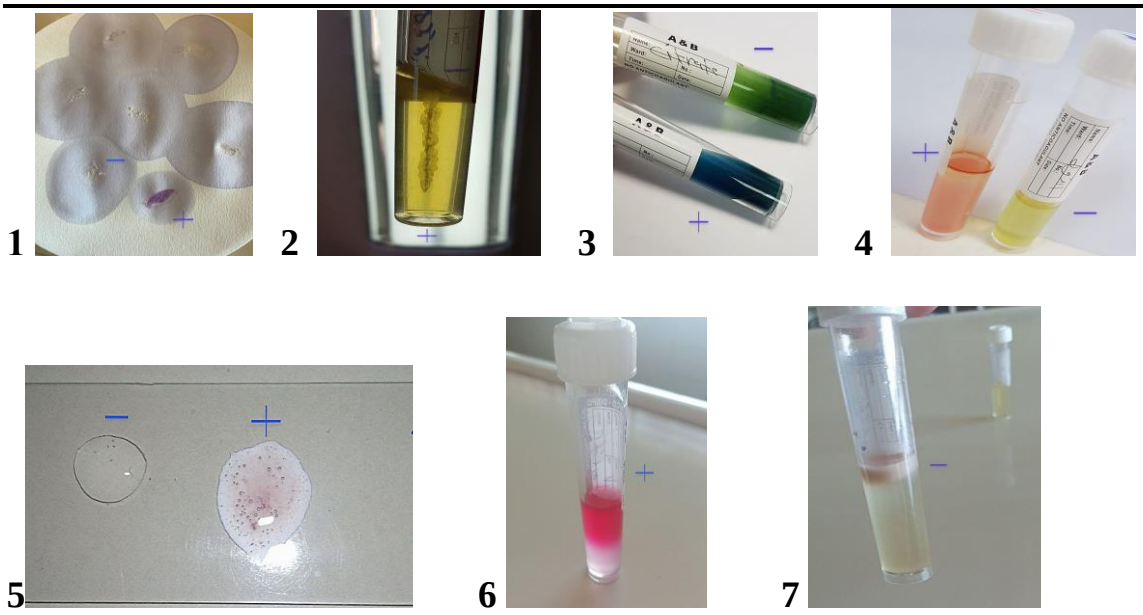

Fig. 1. biochemical tests:

1. Oxidase Test
2. Motility Test
3. Citrate Test
4. Indole Test
5. Catalase Test
6. Methyl Red Test
7. Voges-Proskauer Test

Detection of ESβL production

The disk approximation method was used, following **Rozwandowicz *et al.* (2018)**. An Augmentin disk (amoxicillin/clavulanic acid) was placed in the center, with Ceftazidime, Cefotaxime, and Piperacillin disks placed 3cm away. Enhanced inhibition zones indicated ESβL production.

Plasmid extraction

Plasmids were extracted using the alkaline lysis method with the Accuprep Kit (Bioneer, South Korea).

Plasmid curing

Plasmid curing was performed using sodium dodecyl sulfate (SDS) according to **Garrec *et al.* (2011)**.

ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
+	-	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	+

Fig. 2. *E. coli* bacteria cultured on an API 20E strip

RESULTS AND DISCUSSION

Fifty intestinal samples (25 each from rivers and ponds) yielded *E. coli* in 90% of cases: 10 from river fish and 12 from pond fish.

Plasmid content screening

In this study, the plasmid content of the selected isolates with the highest resistance to various antibiotics was investigated using the alkaline lysis method, as provided by the manufacturer. This method allows for the extraction of plasmids up to 10,000 base pairs in size. The results of agarose gel electrophoresis of the isolates from which plasmids were extracted showed that 5 out of 6 isolates (83%) contained plasmids, while one isolate from river fish did not contain a plasmid (17%), as shown in Fig. (3).

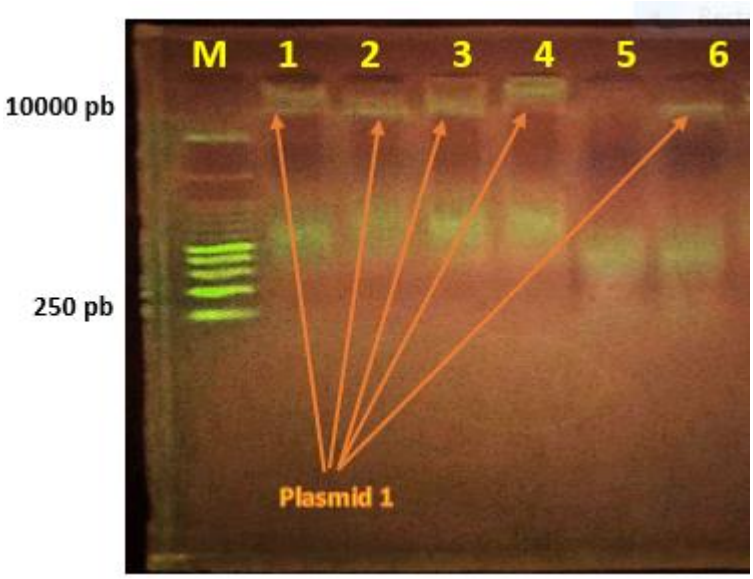

Fig. 3. The plasmid bands electrophoresed on agarose gel (1, 2, and 3 are isolates from pond fish, while 4, 5, and 6 are isolates from river fish)

This is consistent with previous studies showing that *E. coli* commonly harbors at least one plasmid of varying molecular size.

It was also found that there is no clear relationship between the genes encoding virulence factors and the size of plasmids. This confirms that virulence factors may be located on plasmids of different molecular weights. Plasmid-borne traits are considered more significant than those carried on the chromosome due to their higher ability to transfer and rapidly spread among various bacterial species. The importance of plasmids lies in their ability to encode traits such as resistance to multiple antibiotics. These traits can be transferred from pathogenic bacteria to non-pathogenic ones through conjugation, leading to serious health problems for the host.

This study also found that isolates from river fish possess fewer virulence factors compared to those from pond fish. This is likely because the natural river environment is generally better than that of fish-farming ponds, which often contain high levels of antibiotics, treatments, and vaccines. Additionally, the quality and type of water have a direct impact on the presence of virulence factors.

In this study, the bacterial isolates were found to produce and secrete multiple virulence factors to resist various antibiotics. Sodium dodecyl sulfate (SDS) was used for plasmid curing at concentrations of 6000, 5000, 3000, 1000, 500, and 100µg/ mL on six selected multidrug-resistant isolates. The study showed that the minimum inhibitory concentration (MIC) of SDS was 5000µg/ mL, which is relatively consistent with the

findings reported by **Vogwill and MacLean (2015)**. After the curing process, the presence of virulence factors in the bacteria changed. Initially, all isolates showed β-lactamase enzyme production at a rate of 100% in both sources. However, after plasmid curing, the presence of this virulence factor was reduced to 66.6% in river water isolates, while it remained at 100% in pond water isolates.

These results align with those in **Lopatkin *et al.* (2017)**, **San Millan (2018)** and **Osuntokun *et al.* (2019)**, who achieved similar curing effects. Most of the isolates were found to contain β-lactamase enzymes even after the plasmid curing process, a finding confirmed by **Bharathan *et al.* (2019)**. They emphasized the clinical significance of *E. coli* as it poses a serious concern to human health due to its multidrug resistance. These plasmids are considered a top priority for researchers, as they represent a severe and potentially deadly threat to public health **(Wangkheimayum *et al.*, 2022)**.

The presence of virulence factors in bacteria is attributed to the existence of specific genes located either on plasmids or on the chromosome, which confer the ability to express these virulence traits **(Sun *et al.*, 2019; Rodríguez *et al.*, 2020)**. The mechanism of action of SDS lies in its ability to disrupt part of the bacterial cell surface structures, thereby leading to the destruction or removal of plasmids, which are typically located near the bacterial cell surface **(Ibekwe *et al.*, 2011; Sharef *et al.*, 2025)**.

CONCLUSION

This study demonstrated a higher prevalence of plasmid-borne β-lactamase enzymes in *E. coli* isolates from aquaculture ponds compared to those from river fish in Kirkuk. The use of sodium dodecyl sulfate (SDS) in plasmid curing revealed that most resistance traits were plasmid-encoded, posing a significant public health risk due to the potential for horizontal gene transfer. These findings highlight the need for continuous environmental monitoring and the implementation of responsible antibiotic use practices in aquaculture to help mitigate the spread of antimicrobial resistance.

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