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Plasmidic content of certain virulence factors of Escherichia coli bacteria isolated from different organisms in Kirkuk City

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***Abstract*---**The study included isolation and diagnosis of Escherichia coli from clinical and animal sources (humans, cows, sheep, birds, chickens, fish, and gecko). It collected 275 samples distributed to 125 clinical samples taken from urine and 150 samples distributed to other organisms with 25 samples per organism. The isolates used in the study were diagnosed on the appearance, microscopy, and chemical tests, as well as confirmatory testing of isolations through the use of the Api 20 E tape, where 41 isolations of E. coli bacteria were obtained from the clinical source and 89 isolation from other organisms. Some fertility factors of bacterial isolates were studied, all bacterial isolates were found to be Capsule productive, while all were non-Hemolysin enzymes, and Extended Spectrum β-lactamase enzymes were 41% (53/130), and isolates' ability to form biofilm was investigated where isolates were producers 60% (77/130). Three isolates were selected for each source to study their plasmid content, Electrically phased isolators on agarose gels have shown a different variety in their plasmid content. The results of the curing of plasmid DNA and the use of sodium dodecyl sulfate(SDS) showed that most of the insulation produced by the virulence factor was based on plasmid because the insulation lost its production of virulence factors after curing.

***Keywords*---**escherichia coli, virulent factors, plasmid curing, plasmid isolation, bacterial isolates.

Introduction

The Escherichia coli is a family member of the Enterobacteriaceae intestinal and gram-negative, the stick-shaped, moving by flagella or non-moving, with optional aerobic or anaerobic living, fermented by many sugars, including lactose and Ramnose and SSorbitol. PPerfecttemperature (36^{o} - 37^{o}) For its growth (Murray and Rosenthal,2021), it is a product of Indole and a positive for red methyl, catalase and negative test for Vogase Proskauer Oxidase test and non-consuming Citrate (Sastry and Bhat, 2019). Normal Flora lives in the human and animal intestine and therefore has the ability to infect the body with an opportunist in the Opportunistic manner when it has an opportunity, causing many diseases including diarrhea Diarrhea, Spesis, meningitis, Bacteremia in addition to that caused the causes of pathways Urinary Tract Infections where it is responsible for 90% of urinary tract infection and diseases around the world, as it is found in different environments outside the body of living organisms, causing water, food and soil pollution (Nascimento *et al.*,2021).

E.coli bacteria have been characterized by the possession of many virulent factors that have increased the diseases of these bacteria, the most important of which are their possession of the toxic necrosis agent Cytotoxin necrotizing the production of endotoxin internal and external exotoxin, siderophores iron carriers, and enzymes such as Hemolysin and Bacteriocin it contains surface compositions such as Flagella, Capsule, and Polysular lipopolysaccharides (LPS), which give it antigen qualities through the production of capsular Antigen(k) and Somatic antigen(o) and the Flagellar antigen(H), also possess pili and fimbriae, which help them stick inside the host tissues and Thus it gives them the susceptibility to biofilm production and increases the seriousness of these bacteria(Denamur *et al.*,2021).

E.coli bacteria have been distinguished from having a multi-resistant recipe for different antibiotics Multi drug Resistance (MDR) (Cornelissen and Hbbs,2021), which has become one of the most important growing problems and poses a major threat to global health and over time (Masoud *et al.*,2021), this problem is caused by its high resistance to various antibiotics as a result of its possession of enzymes responsible for resistance qualities such as Extended Spectrum β - lactamase (ESβLS), which accords resistance to anti β - lactam, as well as possessing enzymes that accord resistance to Quinolones and aminoglycosides, and in addition having other mechanisms that give them resistance, such as a hange in the target location, change in cell membrane permeability and possession of pumps efflux (Nji *et al.*,2021), All these mechanisms increase the seriousness of these bacteria, especially in clinical terms (Pormohammad *et al.*,2019).

In recent years, many antibiotics have been developed and have proven to be highly efficient in treating many dangerous diseases, but poor use, intensive and unstudied has led to the emergence of bacterial strains with a wide range of resistance to known antibiotics (Sultan *et al.*,2018). Plasmids are highly prevalent in many different bacterial genes and these plasmids are usually unnecessary to their lives but give them some important qualities such as antibiotic resistance and the production of different toxins so often curing plasmids neutralization

processes are used to remove these qualities and know the relationship of plasmids in carrying genes that are responsible for this quality axminarayan *et al.*, 2020). Different materials with the ability to curing plasmid, such as orange acarine sodium dodecyl sulfate (SDS), and ethidium, as well as some medical and plant extracts, are used in curing processes (Murray and Rosenthal,2021).

Materials and Methods

Sample Collection

275 samples of different organisms were collected for the period from 2021-12-1 to 2022-4-1 with each sample's information recorded in the questionnaire form, These samples included:

- *Human Samples*
  125 samples of people infected with urinary tract infection of different ages and sex were collected from the reviewers of the following hospitals (Kirkuk General Hospital - Dakuk General Hospital - Kirkuk Children's Hospital) After consulting the competent physician and referring the patient to the laboratory, he took the information of each sample and fixed it in her questionnaire form. The samples were collected with the first droplets neglected to be contaminated with normal flora and the middle drops were taken, placed in sterile plastic cans and transported to the laboratory ,and planted on the McConkey agar media.
- *Animal samples*
  150 stool samples were collected from the intestines distributed by reality (25 samples) of cows, sheep, chickens, birds, fish, and geckos from different places in Kirkuk, province poop samples of cows, sheep, chickens, and birds were collected and placed in tubes containing a nutrient Swap with media for the non-damage of the sample and the death of bacterial isolates, The samples were transported to the laboratory and planted in the McConkey aga. The fish and the gecko were anatomy in the laboratory and took a sample of the intestine. samples collected were planted on the McConkey agar and cuddled for 24 hours at a temperature of 37^{o}.

Diagnostic

Bacterial isolates were diagnosed through a phenomenal and microscopic examination as well as chemical tests that included (indole, methyl red, catalase, oxidase, citrate, and movement), and the diagnosis was confirmed using the diagnosis kit Api 20 E.

Detection of virulence factors

- *Detecion the production of Extended Spectrum β-Lactamases (ESβLS)*
  The Disk Approximation method was used to detect the dispersed bacteria of these enzymes according to (Garrec *et al.*, 2011), placing Augmentin (Amoxicillin/Clavulanic acid) in the middle of the petri dish and arranging other discs (Cefotaxime, Cefotaxime, piperacillin) on the ends and 3 cm from the disk Central . Dishes are petri dish and inhibition areas between the

central disc and one or more of the disks used are observed to indicate a positive result and the production of these enzymes by the bacterial isolates used.

- Hemolysin production test
  The production of hemolysin was detected by bacterial isolates through the transplantation of isolates on the middle of the blood agar, incubating the dishes with an incubator temperature of 37° and for 24 hours, then observing the bacteria's ability to produce hemolysin enzyme depending on the type of decomposition (Karam *et al.*,2018).
- *Biofilm Formation*
  The bacteria's ability to form the biofilm in the Congo red method was revealed by the transfer of a pure, developing single colony on the MacConkey agar to 5 ml container test tube of a selfie brine and compared with McFarland Standard and then I planted on the of the Congo red agar and incubator the dishes at 37° for 24 hours, The result is positive when the colonies appear black, while the colour remains pink as an indication of the negative result (Mathur *et al.*, 2006).
- *Capsule Formation*
  A drop of Indian ink dye was placed on a glass slice after which a pure colony of bacteria was transported and well mixed with the dye drop and the drop of oil was placed and examined with a microscope using the oil lens, as the presence of a lit area around the bacterial cells indicates the presence of the capsule (Murray and Rosenthal,2021).

Plasmid Extraction

The plasmid was isolated by Accuprep Kit by the Alkaline lysis method according to the Korean-origin manufacturer Bioneer.

Plasmid curing

A plasmid curing test for *E.coli* bacteria was conducted according to (Zaman *et al.*,2010) through the use of SDS (Sodium dodec sulfat) as a plasmid curing.

Results and Discussions

This study collected 125 clinical samples taken from median urine and urinary tract infections, 150 samples taken from animal feces and 25 specimens each. (cows, sheep, chicken, birds, fish, gecko) The *E.coli* bacteria ratio was found to be isolation 41 (33%) ،18 (72%) ،15 (60%) ،20 (80%) ،17 (78%), 11 (44%), 8 (32%) for humans, cows, sheep, chicken, birds, fish and geckos, respectively.

Plasmid Content Investigation

In this study, the plasmidic content of elected isolates and the most resistant to various antibiotics was investigated in a (Alkaline lysis method) and equipped by its manufacturer, this method can obtain plasmids in the size of 3500-3650 base pairs, showing the results of the electrical relay of the isolators from which the plasmid was isolated on the gel acarose containment (12) Isolation of origin (21) Isolation of plasmid at 59% as shown in the photographs 1.

The images showed (1) that isolation No. (5) contains two plasmid packages, the first package with a size of 1900bp and the second 1800 bp, and isolators (4,9,10,11,12,13,14,17,19) with one plasmid package with a size of 1800 bp, and isolators No. (1,2,3,7, 8, 15, 16, 18, 20, 21, 22) There is no plasmid package as shown in table (1).

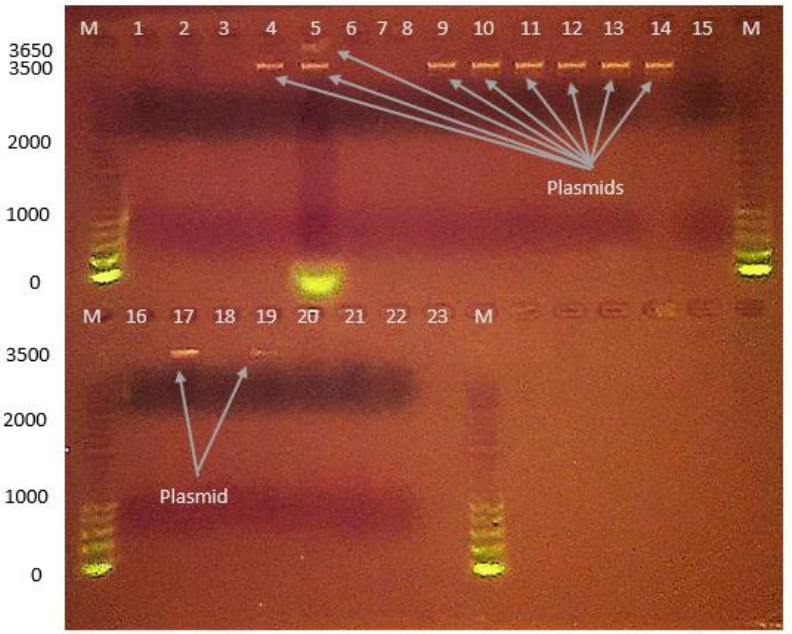


Figure 1. Showing electrically phased plasmidic beams on agarose gel

Table 1
Shows the number of plasmidic content found in bacterial isolates

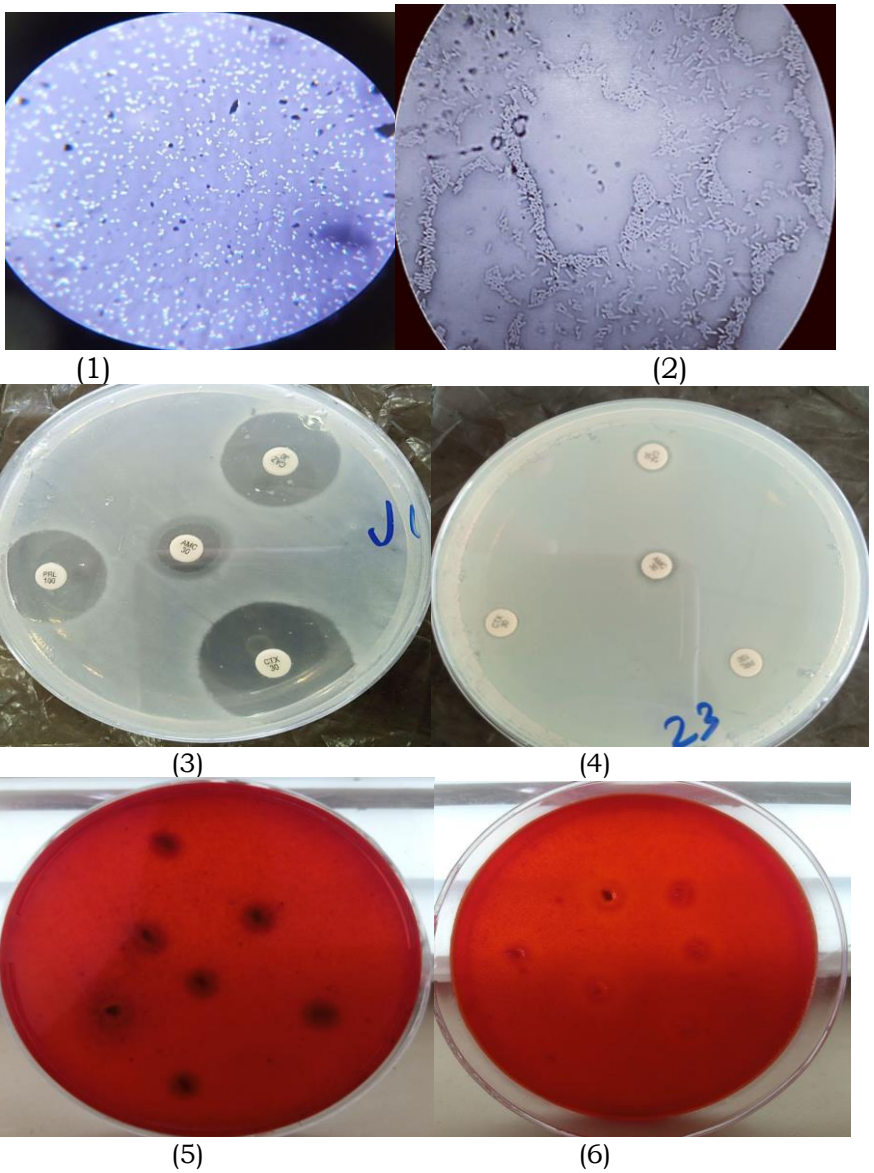
Heading	Heading	Heading
	1	4
Human	1	9
	None	15
	None	16
Cows	1	10
	None	18
	None	1
Sheep	1	11
	None	7
	2	5
Birds	None	8
	1	12
	None	21
Chicken	1	13
	1	14
	None	22
Fish	1	17

	None	2
	None	3
Gecko	1	19
	None	20

These results are consistent with several findings of previous studies that showed *E.coli* isolated containment of at least one plasmidic content , including a large molecular weight, including a ,small molecular weight and a variety of (Li *et al.*,2019; Sabencac *et al.*,2021; Aworh *et al.*,2021) (Zhang *et al.*,2022). It was also found that there was no clear correlation between the genes encoding the virulence factor and the size of the plasmids and thus that the presence of the virulence factor may be on plasmids with different molecular weights (Vogwill and Maclean, 2015). The plasmid-borne characteristic is more important than chromosome-borne in its ability to transmit and rapidly spread between different types of bacteria (Lopatkin et al.,2017), the importance of plasmids lies in their encryption of qualities such as resistance to a number of antibiotics. These qualities have the potential to move from pathogenic bacteria to not pathogenic bacteria by cojugation with serious pathogenic problems of the host (San, 2018).

Plasmid Curing

In this study, the ability of bacterial isolates to form and produce numerous virulence factors to resist different antibSodium is shown. SDS (Sodium dodecyl sulfate) has been used to curing plasmid and concentrations (6000-5000-3000-1000-500-100) µg/ml and for elected isolators with multiple antibiotic resistance and reality (21) isolation. The study showed that the lower stabilized concentration of SDS was (5000) µg/ml and these were relatively consistent with what the researcher referred to as (Osuntokun *et al.*,2019). After the neutralization process, the incidence of virulence factors in the bacteria changed, with clinical isolations containing (capsule, biofilm, lactamese) in the rate of (100%, 100%, 67%) respectively, but after the curing procedure the ratio of virulence factors was shown to be in the following ratios (33%, 67%, 67%). As for cows isolates, the proportion of necessity factors (100%, 33%, 67%) after the curing process became the ratios (100%, 33%, 0%) . Sheep isolates were virulence factors (100%, 67%, 67%), but the ratios changed completely after the curing process, becoming (33%, 0%, 0%). Also, bird isolates also changed the proportions of the presence of factors but less than other isolates where they were before curing (100%, 100%, 67%) and after curing (100%, 33%, 33%). For chicken insulators, two isolates remained preserving the presence of the capsule, and the three isolates lost the biofilm , where the ratios before curing (100%, 100%, 33%) and after curing became (33%, 0%, 33%).Similarly, fish isolates also lost two biofilm enzymes and maintained other factors where they were pre-curing (100%, 100%, 0%). Gecko isolations maintained and did not lose fertility after curing , as shown in the following ratios (100%, 100. 67%) and after curing (100%, 100%, 67%).



(1) (2)

(3) (4)

(5) (6)

Figure 1. Images showing the factors of fertility before and after curing Plasmid, (1,2) Capsule Test, (3,4) β -lactamase Enzymes Test, (5,6) Biofilm Test

Table 2
Shows the number of plasmids content and virulence factors before and after curing

source of isolation	No	Plasmid content	Before the curing	After the curing
Human	4	1	Capsule, biofilm, β-lactamase	β -lactamase
	9	1	Capsule, biofilm,	Capsule

			β-lactamase	
	15	None	Capsule, biofilm	Capsule, biofilm
Cows	16	None	Capsule, biofilm	Capsule, biofilm
	10	1	Capsule, B-lactamase	Capsule
	18	None	Capsule, *β*-lactamase	Capsule
Sheep	1	None	Capsule, biofilm, *β*-lactamase	Capsule
	11	1	Capsule, *β*-lactamase	None
	7	None	Capsule, biofilm	Capsule
Birds	5	2	Capsule, biofilm, *β*-lactamase	Capsule, *β*-lactamase
	8	None	Capsule, biofilm	Capsule, biofilm
	12	1	Capsule, biofilm, *β*-lactamase	Capsule
Chicken	21	None	Capsule, biofilm, *β*-lactamase	Capsule, *β*-lactamase
	13	1	Capsule, biofilm	None
	14	1	Capsule, biofilm	Capsule
Fish	22	None	Capsule, biofilm	Capsule, biofilm
	17	1	Capsule, biofilm	Capsule
	2	None	Capsule, biofilm	Capsule
Gecko	3	None	Capsule, biofilm, *β*-lactamase	Capsule, biofilm, *β*-lactamase
	19	1	Capsule, biofilm, *β*-lactamase	Capsule, biofilm, *β*-lactamase
	20	None	Capsule, biofilm	Capsule, biofilm

According to the plasmid curing process, the genes encoding the secretion and composition of the capsule are genes that are not essentially plasmid-borne and may be present on the chromosome. This explains why isolates contain the capsule after curing the plasmid. This is confirmed by the researcher (Rodriguez ,2020) in his study. As for the biofilm, most isolates maintained the biofilm after the process of curing the plasmid and this is what the researcher (He,2021) when pointed that the genes encoding the formation of the biofilm are portable on the plasmid in *Escherichia coli* bacteria. Likewise, *β*-lactamese enzymes were also mostly isolates that did not contain *β*-lactamese enzymes after the plasmid curing process, which the researcher (Juraschek,2022) confirmed in his study and explained the importance of E. coli bacteria clinically because they are a concern for human health for their multiple drug resistance, and that these plasmids are a top priority for researchers because they pose a dangerous and deadly threat of public health (Juraschek,2022). The bacteria's containment of virulence factors is due to the presence of a particular transported gene on plasmid or chromosome and this gene gives the quality of containing virulence factors (Sun *et al.*,2019). The mechanism of action of SDS is in the material's ability to analyze part of the

surface structures of bacterial cells and create holes and thus destroy or remove plasmids that are near the surface of bacterial cells (Woodford *et al.*,2018).

Conclusion

This study concludes that bacteria was found in various sources of organisms, which are considered to have a serious source of diseases, including urinary tract diseases. The bacteria possessed virulent factors agents, which increased their disease and resistance to antibiotics, as confirmed in the study virulence factors are portable on plasmid, which increases the risk of this bacteria due to the ability of plasmid to quickly transmit to other bacteria and increase its pathology.

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Conflict of interest

There are no conflicting interests in publishing or funding this research.

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