



Molecular detection of extended spectrum beta lactamase producing *E. coli* from diarrheic deer feces

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Abstract

Beta-lactamase-producing *Escherichia coli* pose a public health threat due to their resistance to a wide range of broad-spectrum antibiotics as third-generation cephalosporins. Fifty rectal swabs were obtained from diarrheic Fallow deers, over six months old, of both sexes in Mosul city between September 2024 and December 2025. The samples were sent off immediately to the Lab of Microbiology at the College of Veterinary Medicine under refrigerated and sterile conditions. MacConkey agar plus 2 µg/ml of cefotaxime (MA+) was used to culture all swabs, and methylene blue eosin agar was used to culture suspected colonies. This study indicated that 82% of the isolates were cefotaxime-resistant *E. coli*. The results were confirmed by polymerase chain reaction test and detect some of extended-spectrum beta-lactamase resistance genes. Molecular screening revealed that all isolates subjected to PCR 100% carried the Uid A gene specialized for *E. coli*. also, all isolates gave positive results for the resistance genes (CTX-M and TEM), but negative for the SHV gene. The current study revealed a high rate of isolation of *E. coli* that produced ESBLs with resistance genes (CTX-M, TEM) from the feces of deer with diarrhea, raising ecological and epidemiological concerns, as Fallow deer may serve as reservoirs or vectors of antimicrobial resistance, contributing to its spread in both natural ecosystems and human-associated environments.

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Introduction

Fallow deer (*Dama dama*) farming has recently become widespread in Mosul, both as ornamental animals and for food production, on account of elevation the nutritional value, protein content and depressed cholesterol levels, as well as its distinctive taste (1). Fallow deer breeding requires careful management to maintain their health and ensure a sustainable balance with their environment (2). Antibiotic resistance is constituting a great problem globally. Some bacteria as *Escherichia coli*, are considered as microbial indicators for the development of the prevalence of resistance to antibiotics between human, animals and environment therefor it is use to detect MDR in various animals as cattle, sheep and deer (3) *E. coli* is a commensal normal flora in digestive system of food

producing animal which considered as a reservoir of bacteria that carries the antibiotics resistance genes (4). The randomly employ of antimicrobials in veterinary and human therapy is reflected the important agents in the selection of bacteria that resist to antibiotics may be readily transport between various strains by plasmids (5) in addition to repeatedly use of antibacterial drug in animal management practices with the aims of protection animals from diseases with enhancing the growth rates of animals (6). These practices contribute to increasing in spreading of antibacterial resistance bacteria including the ESBLs producing *E. coli* which constitutes a global challenge to confront this problem (5,7). Therefore, decreasing the use of antibacterial agents is very potent for prevention the spreading of the antibacterial resistance (8). ESBLs are enzymes that degenerate the beta-lactam antibiotics, mainly

include cefotaximase (Ctx-M), sulfhydryl variable (SHV) and Temoneira (TEM) (9). Spreading resist strains of *Escherichia coli* within normal flora in intestinal tract, arise a potential risk to human validity due to the consuming contaminated foods especially meat and milk (10).

Many studies exist about ESBLs producing *E. coli* in food producing animals as chicken, sheep, fish and domestic animals as dogs especially in Mosul city (11-13), but there are no studies are available on wild animals as deer. Therefore, the objective of existing study to isolate the ESBL-resist *E. coli* from fallow deer and detect their ESBL genes.

Materials and methods

Ethical approve

Every sample was taken in accordance with permission granted by an Institutional Animal Care and Use Committee, depending on the authority no UM.VET.2024.113.

Specimens

Fifty rectal swabs were obtained from diarrheic fallow deer of up to 6 months of age and both sexes. The samples collected from Fallow Deer Kingdom in Mosul city from the period of September 2024 to January 2025. The swabs were sent off immediately to the Lab of Microbiology.

Bacterial isolation and identification

The rectal swabs were incubated at 37°C for 24 hours in nutrient broth. A loopful of the broth that incubated were cultured on (MA+) (12). Then incubated at 37°C for 24 h. Suspected isolates were cultured on MA and EMBA for purification, bacterial Identification was performed through morphological and biochemical tests (14).

Extraction

For DNA extraction, all isolates were cultivated on (BHIA) at 37°C overnight. In accordance with the manufactured company (Adprep Genomic bacterial DNA Extraction, Korea), extraction was done for molecular confirmation of species and detection of some virulence genes. The DNA concentration was established using nanodrop (NanoPhotometer® N50/ Germany), and all DNA was stored in -80 °C until used (15).

Determination of bacteria and their virulence genes

For PCR amplification, the mixture 25µl (1 µl from forward and reverse primer, 10 µl of GoTaq® G2 Green Master Mix (Promega, USA), 8 µl of Danase-free water, and extracted DNA at 5 µl) were used to amplification uidA, CTX-M, SHV and TEM genes-specific for detection of *E. coli* and their virulence factor. All primer sequences and amplification cycles were listed in table 1 and 2, amplification was done by using conventional PCR (Sensoquest, Germany).

Table 1: Primers used

Primers		Sequence 5 - 3'	PCR product size	References
Udi A	Forward	CCAAAAGCCAGACAGAGT	623 bp	16
	Reverse	GCACAGCACATCAAAGAG		
CTX-M	Forward	CGCTTTGCGATGTGCAG	550 bp	
	Reverse	ACCGCGATATCGTTGGT		
TEM	Forward	AAACGCTGGTCAAAGTA	720 bp	17
	Reverse	AGCGATCTGTCTAT		
SHV	Forward	ATGCGTTATATTCGCCTGTG	753bp	13
	Reverse	TGCTTTGTTATTCGGCCAA		

Table 2: The amplification programs used for PCR and multiplex PCR

Type of PCR	Initial denaturation	Cycle numbers 35			Extension
		Denaturation	Annealing	Extension	
Udi A	95/3	95/1	57 /0.4	72/1	72/3
CTX-M	95/10	95/1	55 /1	72/1	72/5
TEM	94/5	94/0.3	45 /0.3	72/1	72/5
SHV	94/10	94/1	57/1	72/1	72/5

Results

According to the results of our investigation, the 82% of isolates were ESBL-resist *E. coli* that appear metallic sheen when growing on EMBA as illustrated in figure 1. The

results of molecular confirmation, indicated that all PCR-subjected isolates were given a positive 623 bp amplicon for the uidA gene as shown as figure 2. Also, the molecular identification of CTX-M and TEM genes for *E. coli* confirmed that an amplicon product 550 bp and 720 bp

respectively were obtained as in figures 3 and 4. While all isolate gave negative results for SHV-gene.

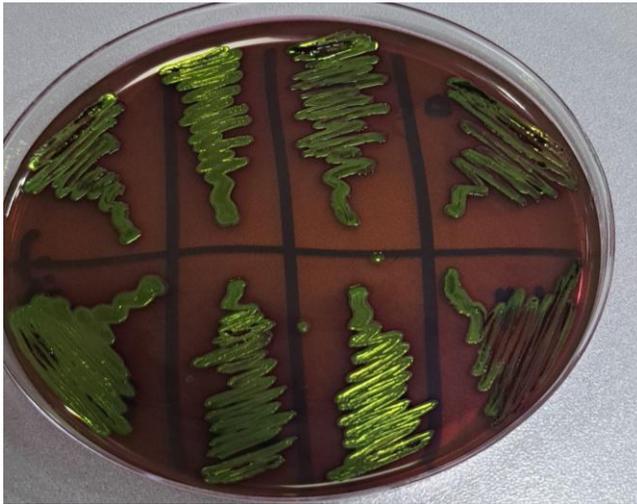


Figure 1: Eosin methylene blue agar shows metallic sheen phenomena of *E. coli*.

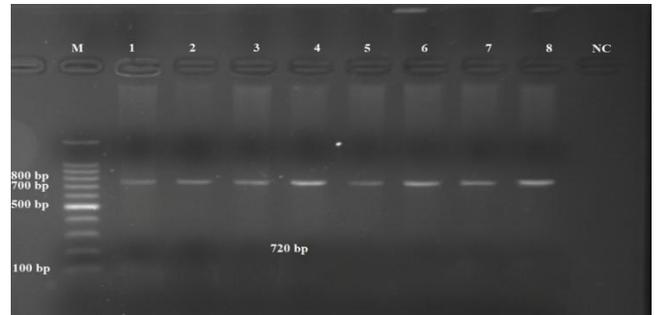


Figure 4: PCR amplicons for TEM genes. M: 100 bp marker, 1-8: *E. coli* isolates, NC: Negative control.

Discussion

β -lactam antibiotics have been used successfully to treat infections induced on by infectious *Escherichia coli*. *E. coli* strains from various pathotypes have evolved to be a critical public validity concern. However, the activity of β -lactams is currently being severely undermined by numerous hydrolytic enzymes, known as β -lactamases, produced by bacteria (18). The current study results detected that the 82% of isolates were resist to cefotaxime when growing on (MA+) medium that belonging to ESBLs which recorded 33% and 54% producing *E. coli* isolated from deer (19,20). The 87.5% of *E. coli*. strains were resisted to ESBL antibiotics (21). Difference in isolation percentage suggests that deer are not being bred hygienically and that *E. coli* is acting opportunistically (18). Additionally, these bacteria have a variety of virulence factors mainly ESBLs enzyme (19). Others factors, including immunity status and nutritional conditions of fallow deer (22), as well as the variation in the geographic distribution (23). According to the molecular detection, the results showed all isolates had the TEM and CTX-M genes, both genes in *E. coli* resist ESBL (24), while only CTX-M genes and others recorded only TEM genes of ESBLs producing *E. coli* in deer (25). Also, current study showed that all isolate were negative to SHV gene. The discrepancy in results is explained by the beta-lactamases of the CTX-M and TEM types are most commonly identified in *E. coli* especially in deer (23,26), in addition to antimicrobials are extensively employed in the farms of animals aimed at disease treatment and prevention, animal growth promotion(27,28),the overuse of antimicrobials leads to spreading of antibacterial resistance bacteria in their surroundings due to west animals (22) also increase the Antibiotic residues in milk and meat. which induces resistance in bacteria present in the environment through mutation (29-31). It thereby provides selective pressure for those antibiotic-resistant bacterial species to survive in the environment where that antibiotic is present (32,33).

The resistance for ESBL antibiotics has become an important public validity concern resulting from their rapid

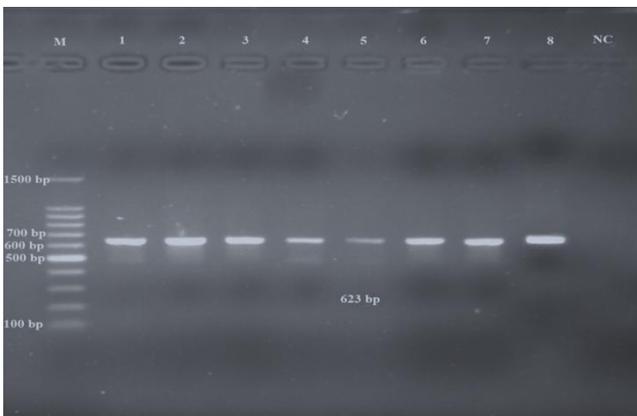


Figure 2: PCR amplicons for uidA genes. M: 100 bp marker, 1-8: *E. coli* isolates, NC: Negative control.

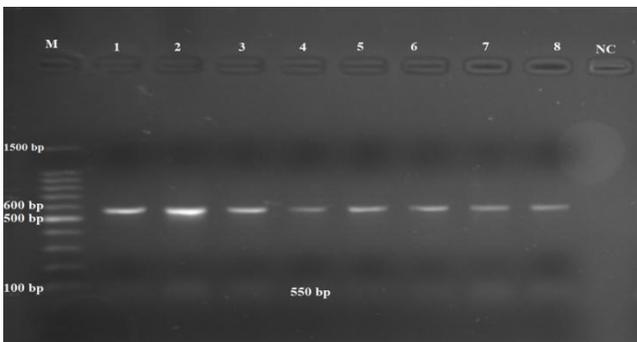


Figure 3: PCR amplicons for CTX-M A genes. M: 100 bp marker, 1-8: *E. coli* isolates, NC: Negative control.

dissemination among commensal and pathogenic *E. coli* (33,34). The existence of ESBL resist *E. coli* in deer suggests possible potential contamination with cross-species transmission, potentially linked to agricultural runoff, human waste, or interaction with livestock (35). The upsurge incidence of strains the beta-lactamase-producing *E. coli*, poses a significant challenge to treatment and highlights the critical requirement for effective antimicrobial stewardship with the establishment of unique therapeutic strategies (36).

Conclusion

In the present investigation, ESBLs -producing *E. coli* that carried the TEM and CTX-M genes were found in Fallow deer. Fallow deer is representing as significant source of distribution of ESBLs producing *E. coli* to other animals, human and environment.

Acknowledgments

None.

Conflict of interest

None.

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الكشف الجزيئي عن الإشريكية القولونية المنتجة لإنزيم البيتا لاكتاميز ممتد الطيف من براز الغزلان المصابة بالإسهال

صبا عبد الرحيم حسين و سمييه ياسين الدباغ

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الموصل، العراق

الخلاصة

تشكل الإشريكية القولونية المنتجة لإنزيمات بيتا لاكتاماز تهديداً للصحة العامة، وذلك لمقاومتها لمجموعة واسعة من المضادات الحيوية ذات الطيف الواسع، كالسيفالوسبورينات من الجيل الثالث. تم الحصول على خمسين مسحة شرجية من غزال الفلو المصاب بالإسهال، بعمر أكثر من ستة أشهر، من كلا الجنسين، في مدينة الموصل، بين أيلول ٢٠٢٤ وكانون الأول ٢٠٢٥. أرسلت العينات على الفور إلى مختبر الأحياء المجهرية بكلية الطب البيطري، تحت ظروف التبريد والتعقيم. استُخدم وسط أجار ماكونكي المُضَاف إليه ٢ ميكروغرام/مل من السيفوتاكسيم MA+ لتنمية جميع المسحات، كما استُخدم أجار الأيوسين ميثيلين الأزرق لزراعة المستعمرات المشتبه بها. أشارت الدراسة الحالية إلى أن ٨٢% من العزلات تنتمي إلى جراثيم الإشريكية القولونية المقاومة للسيفوتاكسيم. تم تأكيد النتائج باستخدام تقنية تفاعل البلمرة المتسلسل والكشف عن بعض جينات مقاومة البيتا لاكتام واسعة الطيف. أظهر الكشف الجزيئي أن جميع العزلات المعرضة لتفاعل البلمرة المتسلسل ١٠٠% تحمل جين Uid A الخاص بالإشريكية القولونية. كما أعطت جميع العزلات نتائج إيجابية لجينات المقاومة CTX-M و TEM، لكنها أعطت نتائج سلبية لجين SHV. كشفت الدراسة الحالية عن ارتفاع معدل عزل الإشريكية القولونية المنتجة للبيتا لاكتاميز والحاملة لجينات المقاومة CTX-M و TEM من براز الغزلان المصابة بالإسهال مما يؤثر مخاوف بيئية ووبائية، حيث يمكن أن يكون غزلان الفلو بمثابة خزانات أو ناقلات لمقاومة مضادات الميكروبات، مما يساهم في انتشارها في كل من النظم البيئية الطبيعية والبيئات المرتبطة بالإنسان.