

## THE ROLE OF ESBLs HARBOURING PLASMIDS ON ANTIMICROBIAL RESISTANCE AMONG *ESCHERICHIA COLI* FROM HUMANS AND DOGS

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### ABSTRACT

Rising antimicrobial resistance is considered one of the major health concerns faced today. *Escherichia coli* has become one of the real causes of this crisis. In this study, we examined how *E. coli* from humans and dogs were resistant to treatment, paying special attention to the presence of certain extended-spectrum beta-lactamase (ESBL) genes carried on plasmids. To investigate the risk of bacterial resistance in the community, fecal samples were collected from humans (69) and dogs (67). *E. coli* was isolated using conventional methods, and its identity was confirmed by molecular technique (PCR). Antimicrobial ability against some beta-lactam antibiotics was detected, in addition to the presence of plasmid harbouring *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes. The findings showed that 73.68% of *E. coli* isolates from dogs and 91.83% from humans produced ESBLs, making them resistant to several antibiotics. Generally, 83.07% of the isolated *E. coli* showed this worrying resistance. Furthermore, the study highlighted the role of plasmid-mediated genes (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>). Approximately 30.95% of dog isolates and 35.56% of human isolates carried *bla*<sub>TEM</sub>, while *bla*<sub>SHV</sub> appeared in about 14.28% and 17.78% of dog and human isolates, respectively. In some cases, bacteria carried both genes, suggesting the potential for these resistance traits to spread rapidly. These findings suggested that antimicrobial resistance is a two-way path between humans and animals. The bacterial resistance transfer between humans and animals, particularly through direct contact, is a continuing risk. The study demonstrated a clear fact about antibiotic resistance that bridges human and veterinary medicine so control and management can slow the spread of these dangerous microorganisms and protect the effectiveness of antibiotics for future generations.

**Keywords:** ESBL, *Escherichia coli*, Plasmid, *bla*<sub>TEM</sub>, and *bla*<sub>SHV</sub>

### INTRODUCTION

The bacterial antimicrobial resistance has become a big health issue and one of the

most significant worldwide human threats. It has effectively contributed to complicating infectious disease treatment, leading to bad clinical results, longer hospitalization periods, and much higher healthcare costs (Salam *et al.*, 2023). Among several bacterial resistance mechanisms, the production of extended-spectrum beta-lactamases (ESBLs) has

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obtained significant attention regarding its ability to damage broad-spectrum beta-lactam antibiotics, including penicillins and cephalosporins (Castanheira *et al.*, 2021). *Escherichia vast* has been found to be one of the most common bacteria harbouring ESBL genes. ESBL-producing *E. coli* is associated with a huge range of infections, including digestive tract infections and urinary tract infections, in addition to more severe bloodstream infections. Remarkably, they pose a significant treatment challenge, as these strains are usually resistant to multiple antibiotic classes (Husna *et al.*, 2023).

The spreading of ESBL-producing bacteria is not limited to human populations only. Animals, especially those associated with humans, serve as significant reservoirs for these bacteria. Particularly, companion pet animals, such as dogs, become common carriers of ESBL-producing *E. coli* (Tseng *et al.*, 2023). The presence of these bacterial types in animals raises a specific concern, which suggests the possibility of cross-species transmission. Complex infections could occur when resistant *E. coli* strains pass from animals to humans through direct contact (Biswas *et al.*, 2024). This potential zoonotic transmission emphasizes the importance of monitoring the bacterial resistance in human and animal populations to get a better understanding and clear vision for the issue.

A significant factor of ESBL distribution among bacterial isolates is the presence of effective genes on plasmids. Plasmids are non-chromosomal mobile genetic elements (MGEs) that can transfer genes between diverse bacterial strains and species (Tokuda and Shintani 2024). Many antimicrobial genes, such as *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>, which encode ESBL enzyme production, are usually carried on plasmids, making them highly mobile and contributing to widespread resistance (Sivaraman *et al.*, 2021). Even though both of them share functional similarities, they differ in origin, genetic variability, and

prevalence. The *bla*<sub>TEM</sub> gene is more common globally. Both genes contribute to  $\beta$ -lactam resistance, particularly in ESBL-producing bacteria (Ejaz *et al.*, 2021). ESBLs are grouped into four classes (A, B, C, and D) of enzymes. The cefotaximase (CTX-M), the temoneira (TEM), and the sulfhydryl variable (SHV) are class A ESBLs (Shahid *et al.*, 2011). In addition to enhancing *E. coli* resistance against beta-lactam antibiotics, these plasmid-borne genes frequently confer bacterial resistance to other antibiotic classes, forming multidrug-resistant isolates. This mobility highlighted the importance of investigating plasmid-mediated antimicrobial resistance, as it facilitates horizontal resistance gene transmission, accelerating the antimicrobial resistance (AMR) crisis (Nasrollahian *et al.*, 2024).

During this study, the main aim was to assess the prevalence of ESBL-producing *E. coli* in human and dog fecal samples, concentrating on the plasmid role in harbouring *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes. By investigating the genetic determinants of ESBL resistance, the current findings offer a better understanding of the plasmid-borne resistance mediated in the spread of AMR. Our results contribute to previous studies on the role of animals in the transmission of antimicrobial-resistant pathogens, and highlight the requirement for a One Health approach to tackling and controlling AMR.

## Methods:

### Ethical approval:

The current study was ethically approved by the Veterinary Medicine Ethical Approval Committee, College of Veterinary Medicine, University of Basrah, Basrah, Iraq. The ethical approval certificate number is 72/37/2025.

### Sample collection:

A total of 136 fresh fecal samples were collected in sterile containers during the period from August 2023 to January 2024. Samples were included: 67 human and 69 dog fecal samples. They were either

processed immediately or stored at 4°C for less than 24 hours. Approximately 200 ng of each sample was homogenized in phosphate buffer saline, and then the prepared samples were used for bacterial culturing (Gemmell *et al.*, 2024).

#### Isolation and identification of *E. coli*:

*E. coli* was detected using the direct plating method described by (Bartoloni, *et al.*, 2006). Recently collected fecal samples were plated directly onto MacConkey's agar and Eosin Methylene Blue agar (EMB) using a plain cotton swab. Furthermore, the identification of *E. coli* isolates was performed using the Vitek®2 system (Vitek®2 GN ID Card, Product number 21341, bioMérieux, USA). For preservation, the isolates are routinely stored in a brain-heart infusion (BHI) medium supplemented with 15% glycerol at -20°C.

#### Genomic DNA extraction:

Genomic DNA was extracted from the isolated *E. coli* using a gDNA extraction kit (Wizard® Genomic DNA Purification Kit, Promega, USA). All the extraction steps

were applied as recommended by the manufacturer's protocol.

#### Detection of the *E. coli* housekeeping gene (*malB* promoter gene):

Molecular bacterial identification of the isolated *E. coli* was conducted by applying the polymerase chain reaction (PCR) technique using a GoTaq® PCR master mix (Promega, USA). A particular region of the *E. coli malB* promoter gene, approximately 585 bp, was amplified using a species-specific pair of primers (Table 1). The following amplification program was applied: 95°C for 4 minutes followed by 33 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. Finally, an extra 5 minutes at 72°C was applied. The resulting amplicons were loaded on 1% agarose gel electrophoresis alongside a DNA ladder (either the 100 bp DNA ladder Bioneer, South Korea, or the 100 bp DNA ladder, Promega, USA). All the loaded samples were exposed to 70 volts for approximately 45 minutes. The amplified regions (amplicons) were detected by adding DNA safe dye (red safe dye, amb, Canada) to the agarose gel and visualized under a UV transilluminator.

**Table 1:** Primers used during the current study.

primers	Sequence	length	Size (bp)	References
ECO-2 F	5'- GACCTCGGTTTAGTTCACAGA-3'	21	585	(Wang <i>et al.</i> , 1996)
ECO-2 R	5'- CACACGCTGACGCTGACCA-3'	19		
<i>bla</i> <sub>TEM</sub> -F	5'-CATTTCCTGTGCGCCCTTATTC -3'	22	800	(Dallenne <i>et al.</i> , 2010)
<i>bla</i> <sub>TEM</sub> -R	5'-CGTTCATCCATAGTTGCCTGAC-3'	22		
<i>bla</i> <sub>SHV</sub> -F	5'-AGCCGCTTGAGCAAATTAAAC-3'	21	713	
<i>bla</i> <sub>SHV</sub> -R	5'- ATCCCGCAGATAAATCACCAC -3'	21		

#### Detection of Extended Spectrum $\beta$ -lactamase (ESBL) activity, Double disk approximation method (DAM)

The double disk approximation method (DAM) was performed according to CLSI 2020 and Coelho *et al.* (2022). An Amoxicillin-Clavulanate 20µg/10µg disk was placed in the center of a Muller Hinton agar plate, followed by three discs of third-generation cephalosporin: ceftriaxone µg, ceftazidime µg, and cefotaxime 30 µg; and one disc of the monobactam antibiotic

(aztreonam 30 µg) around the central disc. The plates were incubated at 37°C for 24 hours. Positive isolates were identified regarding the inhibition zone appearance around the antibiotic disks or between two discs on the agar surface.

#### Plasmid DNA extraction:

Plasmid DNA (pDNA) was extracted from *E. coli* isolates using the Wizard® Plasmid DNA Purification Kit (Promega, USA) commercial kit. After overnight growth in

nutrient broth (Difco, UK), *E. coli* was harvested and processed as described by the manufacturer's instructions protocol.

### Detection of some extended-spectrum $\beta$ -lactamase (ESBL) genes.

The PCR technique was used to detect the *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes in the plasmid DNA of the isolated *E. coli*. A unique sequence of each gene was amplified using a pair of gene-specific primers (Table 1). A single-plex PCR reaction was applied to detect *bla*<sub>TEM</sub> (800 bp) and *bla*<sub>SHV</sub> (713 bp) genes. The amplified genes were detected and visualized, as described previously in PCR amplicon detection. The genes of interest were amplified using the GoTaq® master mix (Promega, USA) and the following thermocycler conditions: initial denaturation at 95°C, then 30 cycles of 94°C for 30 seconds, 50°C (*bla*<sub>TEM</sub>)/56°C (*bla*<sub>SHV</sub>) for 30 seconds, and 72°C for 1 minute. Finally, the PCR mixtures were exposed for an

additional 10 minutes at 72°C (Dallenne *et al.*, 2010).

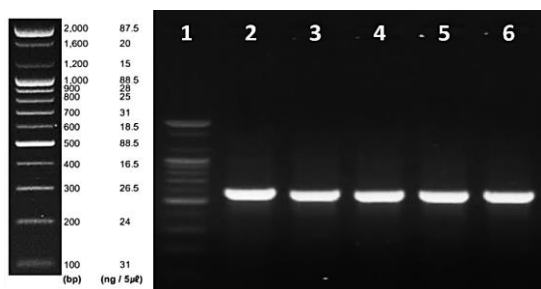
## RESULTS

### *Escherichia coli* identification:

The primary results of the current study reported that out of 136 faeces samples (from dogs and humans), 122 tested positives for suspected *E. coli* growth regarding their cultural growth characteristics on MacConkey and EMB agar. These 122 faeces samples were obtained from 64 dogs and 58 humans (Table 2). The suspected *E. coli* isolates were identified using the Vitek®2 system test. Furthermore, 106 *E. coli* isolates (57 from dogs and 49 from humans) were genetically confirmed by partially amplifying the *malB* promoter gene using an *E. coli* species-specific pair of primers (Figure 1, Table 2).

**Table 2:** Distribution and identification of *E. coli* isolates in dog and human faeces samples.

Sample type	No.	growth on MacConkey agar & EMB		Vitek®2 system test for n=114 suspected <i>E. coli</i> isolate		<i>malB</i> using a species-specific for n=106	
		Suspected <i>E. coli</i>	Other Gram-ve	<i>E. coli</i> isolates	another Gram-ve	<i>E. coli</i>	Negative results
dog feces	69	64 (96.96%)	18	59 (92.18%)	5 (7.82%)	57 (96.61%)	2 (3.39%)
human feces	67	58 (93.54%)	24	55 (94.82%)	3 (5.18%)	49 (89.09%)	6 (10.91%)
Total	136	122 (89.70%)	42	114 (93.44%)	8 (6.56%)	106 (92.98%)	8 (7.02%)



**Figure 1:** An agarose gel electrophoresis image displays the positive result of the *malB* promoter gene detection. This result is represented by only one clear band at approximately 585 bp for each sample. Lane 1: DNA ladder 100 bp (Bioneer, South Korea); lanes 2-6: single amplicon approximately 585 bp.

### Extended spectrum $\beta$ -lactamase-producing *E. coli* detection:

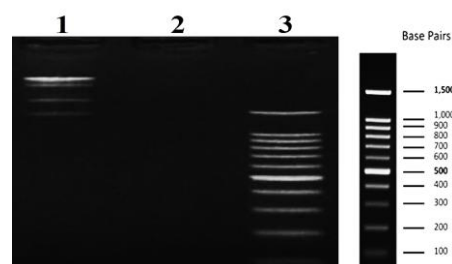
The current study found that 42 (73.68%) *E. coli* isolates from dogs and 45 (91.83%) from humans were positive for extended-spectrum  $\beta$ -lactamases (ESBLs) activity using the double disk approximation method (DAM) (Figure 2). On the other hand, 15 (26.32%) and 4 (8.17%) of *E. coli* isolates from dogs and humans showed negative results for producing ESBLs, respectively (Table 3).

**Table 3:** Distribution and detection of *E. coli* isolates producing extended-spectrum  $\beta$ -lactamases (ESBLs) in dog and human fecal samples depending on the double disk approximation method.

Extended-spectrum $\beta$ -lactamases (ESBLs)	<i>E. coli</i> isolates from dogs faeces (n=57)	<i>E. coli</i> isolates from human faeces (n=49)	Total 106
Positive (ESBLs)	42 (73.68%)	45 (91.83%)	87 (82.07%)
Negative (ESBLs)	15 (26.32%)	4 (8.17%)	19 (17.92%)
Total	57	49	106

**Figure 2:** The double disk approximation method for the isolated *E. coli*. The image shows the zones of inhibition around the tested antibiotics (amoxicillin-clavulanate 20  $\mu$ g/10  $\mu$ g disc—in the center of a Muller Hinton agar plate—ceftriaxone 30  $\mu$ g, ceftazidime  $\mu$ g, cefotaxime  $\mu$ g, and aztreonam 30  $\mu$ g) as a positive result for the ESBL test.**Molecular (plasmids and genes) findings:**

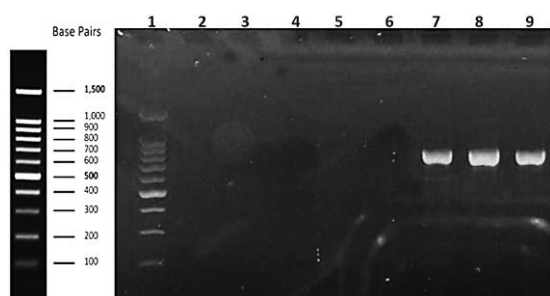
Plasmid DNA was isolated successfully from all *E. coli* producing extended-spectrum  $\beta$ -lactamase isolates. However, the plasmid bands varied on agarose gel electrophoresis. The majority of isolated samples showed more than one band. The maximum number of bands was five, whereas one was the lowest (Figure 3).

**Figure 3:** An agarose gel electrophoresis image shows the isolated plasmids from different *Escherichia coli* isolates. A variable number of plasmids were detected in lanes 1 and 2. Lane 3: DNA ladder 100 bp (Promega, USA)

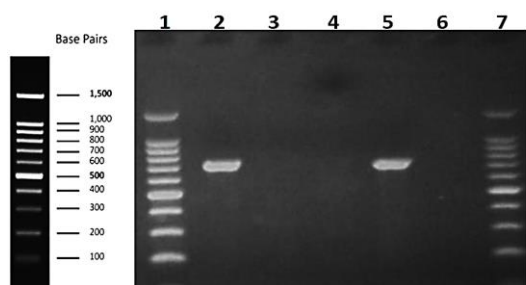
The ESBL gene (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>) amplification results showed that out of 42 *E. coli* isolates from dogs, only 12 (30.95%) isolates harboured *bla*<sub>TEM</sub>, whereas 6 (14.28%) isolates had positive results for *bla*<sub>SHV</sub> (Figures 4 and 5). Remarkably, 4 isolates were found to carry both genes (Table 4). On the other hand, 16 (35.56%) of *E. coli* isolates from humans had positive results for *bla*<sub>TEM</sub>. In contrast, 8 (17.78%) isolates showed positive results for *bla*<sub>SHV</sub>. Moreover, both genes were found together in 6 isolates.

**Table 4:** Detection of some extended-spectrum  $\beta$ -lactamases genes (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>) in plasmid DNA

Source of isolates	ESBLs on plasmid DNA			
	<i>bla</i> <sub>TEM</sub> positive	<i>bla</i> <sub>SHV</sub> positive	<i>bla</i> <sub>TEM</sub> and <i>bla</i> <sub>SHV</sub> positive	<i>bla</i> <sub>TEM</sub> and <i>bla</i> <sub>SHV</sub> negative
<i>E. coli</i> from dogs (n=42)	12 (30.95%)	6 (14.28%)	4	28 (66.67%)
<i>E. coli</i> from humans (n=45)	16 (35.56%)	8 (17.78%)	6	27 (60%)
Total	28 (32.18%)	14 (16.09%)	10	55 (63.21%)



**Figure 4:** An agarose gel image displays the positive result of the *bla*<sub>TEM</sub> gene detection, which is represented by one clear band at approximately 800 bp for each sample. Lane 1: DNA ladder 100 bp (Promega, USA); lane 2: negative control; lanes 3-6: show no band as negative results; lanes 7-9: single amplicon approximately at 800 bp.



**Figure 5:** An agarose gel image displays the positive result of the *bla*<sub>SHV</sub> gene detection, which is represented by one clear band at approximately 713 bp for each sample. Lane 1 and 7: DNA ladder 100 bp (Promega, USA); lane 6: negative control; lanes 2 and 5: single amplicon approximately at 713 bp; lanes 3 and 4 show no band as negative results.

## DISCUSSION

Antimicrobial resistance has recently become one of the most important public health issues these days. A microorganism with an extended-spectrum beta-lactamase (ESBL) gene, like *Escherichia coli*, poses a particularly concerning threat. This enzyme enables the bacterial cells to resist a varied range of beta-lactam antibiotics. Which are frequently used to cure human and animal bacterial infections. The current study was conducted to determine the *E. coli* (isolated from human and dog fecal samples) resistance ability against ESBL antibiotics.

Furthermore, it investigates the role of plasmid mediation in some ESBL genes (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>). The obtained findings highlight important worrying trends in the distribution of antimicrobial resistance and propose that humans and animals contribute to this issue.

During the primary screening of *E. coli* from fecal samples, they were cultured on two culture media (MacConkey agar and EMB), which helps to recover a high number of bacterial isolates efficiently. Approximately 96.96% and 93.54% of dog and human samples showed growth on these selective media, respectively. Then, the growing bacteria confirmed with the Vitek®2 system that 92.18% of dog isolates and 94.82% of human isolates were correctly recognized as *E. coli*. Furthermore, 96.61% of dogs and 89.09% of human isolates were molecularly identified using a species-specific pair of primers, indicating that our isolation methods were highly successful. This high accuracy is reassuring and supports the reliability of our findings. This is in line with several previously conducted studies in humans and dogs (Almousawi and Al-hejjaj, 2024; Massella *et al.*, 2021), which detected 85% and 92.59% *E. coli* from dogs and human fecal samples, respectively.

## Prevalence of ESBL Activity

The overall rate of ESBL producers in our study was high, with 73.68% of dog isolates and 91.83% of human isolates testing positive for ESBL activity. This shows a high prevalence combined (82.08%) across all the tested samples, which brings it into line with similar discoveries worldwide. For instance, in research conducted in Thailand, a similar high prevalence (96.5%) of ESBL-producing *E. coli* was found in both hospital patients (Chaisaeng, *et al.*, 2024). Whereas a lower rate (16.56%) was reported in *E. coli* samples from dogs in Africa (Salgado-Caxito *et al.*, 2021). The higher prevalence in human isolates could be related to the fact that human medicine

often contains more aggressive antibiotics, which were widely used (Muteeb *et al.*, 2023), leading to increased selective pressure and allowing for the existence of resistant bacteria. Remarkably, dogs also harboured a high number of ESBL-producing *E. coli* isolates (75.43%). This finding suggests that animals, especially those closely connected to humans, might serve as significant reservoirs of this type of bacteria. These results support the evidence that antimicrobial resistance is not restricted to humans only; however, it can extend to some animals as well. Direct contact with some animals can return bacterial resistance to the human population (Zhang *et al.*, 2024).

#### Detection of some ESBL genes (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>)

Plasmids, which are extrachromosomal DNA, play a fundamental role in the spread of bacterial antimicrobial resistance, as they can mediate several resistance genes and transfer them between different bacterial isolates and strains (Wang *et al.*, 2024). Remarkably, all the isolated *E. coli* has harboured a range of plasmids. The highest number was 5, while one plasmid was the lowest. The current study focused on the presence of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes, as significant ESBL genes are frequently located on plasmids. The results showed that 30.95% and 35.56% of *E. coli* isolated from dogs and humans, respectively, carried *bla*<sub>TEM</sub>. While 14.28% of dog isolates and 17.78% of human isolates carried *bla*<sub>SHV</sub>. Moreover, it has been found that four dog isolates and six human isolates had *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes together, demonstrating the co-carriage of these types of genes in some *E. coli* isolates. Among the investigated genes, the *bla*<sub>TEM</sub> gene was the most frequently detected. This is consistent with several studies demonstrating that it is the dominant ESBL gene in *E. coli* strains, as it was reported in 100% and 81% of urinary tract infection and thalassaemia patients' *E. coli* isolates, respectively (Mohammed *et al.*, 2024; Pishtiwan and Khadija, 2019). This gene spread broadly

and can be transferred among many bacterial species and isolates, making it a public health concern. Whereas, the *bla*<sub>SHV</sub> gene was found in lower isolate numbers in (17.78%) humans and (14.28%) dogs. This agrees with previous studies demonstrating that this gene is more frequently associated with hospital-acquired bacterial infections (Elsayed *et al.*, 2024) and less frequently detected (25%) in animal samples, such as chicken ceca, eggs, and fish, in comparison to (81.25%) *bla*<sub>TEM</sub> (Ariyanti *et al.*, 2024). Although *bla*<sub>SHV</sub> is less present in dog isolates, its prevalence on plasmids in humans and dog isolates raises the probability of cross-species transmission of these gene types. There are no significant differences in the prevalence of these genes between animal and human *E. coli* isolates, which supports the theory that antimicrobial resistance is not restricted to one host. Remarkably, the ESBL-producing *E. coli* prevalence was higher than the presence of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes on their plasmid. This phenomenon could be related to the role of other ESBL family gene members (Mohammed *et al.*, 2024). The presence of these ESBL genes on plasmids indicates the possibility of horizontal gene transfer. The ability of resistant bacterial isolates to transmit their resistance genes (traits) to sensitive bacterial strains aids in the dissemination of resistance among various bacterial isolates (Husna *et al.*, 2023).

#### The Role of Plasmids in Resistance

One of the significant findings of the current study is the recognition of ESBL genes on plasmids, which is a principally concerning matter. Plasmids are non-chromosomal mobile genetic elements (MGEs) that can easily transfer genes, including antimicrobial resistance, among bacterial cells either from the same species or from different ones (Partridge *et al.*, 2018). This plasmid motility is the crucial reason why resistance (genes) can spread quickly, even between human and animal bacterial isolates. The presence of ESBL genes (*bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>) on plasmids in



human and dog *E. coli* isolates highlights the role of plasmid-borne resistance in the distribution of antimicrobial resistance. Plasmid-mediated resistance had another hard impact by making the bacterial treatment more problematic, since these genes usually carry resistance against several antibiotic classes, forming multidrug-resistant (MDR) bacterial strains (Gauba & Rahman 2023). Plasmid-borne resistance ability certainly increases concerns about the current antimicrobial efficiency of stewardship policies. As plasmids can transfer these genes among several bacterial strains, managing and controlling resistance in one population, such as humans, might not be enough if the same genes resistance are circulating in animals as well (Solanki and Das 2024). Therefore, a One Health approach, which addresses antimicrobial resistance in the human and veterinary world, is fundamental for controlling the spread of resistant bacteria.

## CONCLUSION

The current study findings emphasize the importance of managing and controlling the spread of antimicrobial resistance among humans and animals. The high presence and frequency of ESBL-producing *E. coli* in both populations proposes that serious efforts are required to curb antimicrobial resistance, and they must involve both the human and veterinary worlds. It is essential to highlight how plasmids help spread resistance genes and to manage antibiotic use in both humans and animals to stop resistance from spreading further. Furthermore, monitoring the prevalence of ESBL-producing bacteria in humans and animals would help track and control resistance trends.

## REFERENCES

- Almousawi N.F. and Al-Hejjaj M.Y. (2024): A New Blend of Phenotypic and Genotypic Application as a Zoonosis*
- Escherichia coli Transmission Detector (2024). Iran J War Public Health. 16 (3): 279-287 URL: <http://ijwph.ir/article-1-1398-en.html>*
- Ariyanti, T., Suhaemi, S., Mulyati, S., Sukatma, S., Sumirah, S., Noor, S. M., Rachmawati, F., Widiyanti, P. M., Sukmawinata, E., Andriani, A., Kusumaningtyas, E., and Khairullah, A. R. (2025): Dissemination and phenotypic characterization of ESBL-producing Escherichia coli in Indonesia. Open veterinary journal, 15(3), 1340–1348. <https://doi.org/10.5455/OVJ.2025.v15.i3.25>*
- Bartoloni, A., Benedetti, M., Pallecchi, L., Larsson, M., Mantella, A., Strohmeyer, M., Bartalesi, F., Fernandez, C., Guzman, E., Vallejos, Y., Villagran, A. L., Guerra, H., Gotuzzo, E., Paradisi, F., Falkenberg, T., Rossolini, G.M. and Kronvall, G. (2006): Evaluation of a rapid screening method for detection of antimicrobial resistance in the commensal microbiota of the gut. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(2), 119–125. <https://doi.org/10.1016/j.trstmh.2005.06.027>*
- Biswas, S., Bal, M., Pati, S., Rna, R., Dixit, S. and Ranjit, M. (2024): Antibiotic resistance in toxigenic *E. coli*: a severe threat to global health. Discov Med 1, 72. <https://doi.org/10.1007/s44337-024-00102-x>*
- Castanheira, M., Simner, P. J. and Bradford, P.A. (2021): Extended-spectrum  $\beta$ -lactamases: an update on their characteristics, epidemiology and detection. JAC-antimicrobial resistance, 3(3), dlab092. <https://doi.org/10.1093/jacamr/dlab092>*
- Chaisaeng, S., Chopjitt, P., Kasemsiri, P., Putthanachote, N., Boueroy, P., Takeuchi, D., Akeda, Y., Hamada, S. and Kerdsin, A. (2024): High prevalence of ESBL-producing *E. coli* phylogroup B2 clinical isolates in northeastern Thailand. BMC*



- Microbiol 24, 425. <https://doi.org/10.1186/s12866-024-03582-0>
- Clinical and Laboratory Standards Institute.* (2020): Performance standards for antimicrobial susceptibility testing, 30th Edition. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
- Coelho, N.T.A., Silva, R.S.d., Delmondes, G.M., Lima, W.G., Jensen, C.E.d.M. and Paiva, M.C.d.* (2022): Occurrence of extended-spectrum betalactamase (esbl) and carbapenemases among ampicillin-resistant enterobacteriales recovered from a municipal raw sewage in minas gerais, brazil. *Revista Colombiana De Ciencias Químico-Farmacéuticas*, 50(3). <https://doi.org/10.15446/rcciquifa.v50n3.100228>
- Dallenne, C., Da Costa, A., Decré, D., Favier, C. and Arlet, G.* (2010): Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *The Journal of antimicrobial chemotherapy*, 65(3), 490–495. <https://doi.org/10.1093/jac/dkp498>
- Ejaz, H., Younas, S., Abosalif, K.O.A., Junaid, K., Alzahrani, B., Alsrhani, A., Abdalla, A.E., Ullah, M.I., Qamar, M. U. and Hamam, S.S.M.* (2021): Molecular analysis of blaSHV, blaTEM, and blaCTX-M in extended-spectrum  $\beta$ -lactamase producing Enterobacteriaceae recovered from fecal specimens of animals. *PloS one*, 16(1), e0245126. <https://doi.org/10.1371/journal.pone.0245126>
- Elsayed, A.G.A., Badr, D.F., El Kheir, N.Y.A., Zaki, M.E., Mossad, A.E.M. and Mahmoud, E.M.F.* (2024): Prevalence of extended-spectrum beta-lactamase and molecular detection of blaTEM, blaSHV, and blaCTX-M genotypes among gram-negative Bacilli isolates from hospital acquired infections in pediatrics, one institutional study. *Ital J Pediatr* 50, 31. <https://doi.org/10.1186/s13052-024-01599-9>
- Gemmell, M.R., Jayawardana, T., Koentgen, S., Brooks, E., Kennedy, N., Berry, S., Lees, C. and Hold, J.* (2024): Optimised human stool sample collection for multi-omic microbiota analysis. *Sci Rep* 14, 16816. <https://doi.org/10.1038/s41598-024-67499-4>
- Gaub, A. and Rahman, K.M.* (2023): Evaluation of Antibiotic Resistance Mechanisms in Gram-Negative Bacteria. *Antibiotics*, 12(11), 1590. <https://doi.org/10.3390/antibiotics12111590>
- Husna, A., Rahman, M.M., Badruzzaman, A.T.M., Sikder, M.H., Islam, M.R., Rahman, M.T., Alam, J. and Ashour, H.M.* (2023): Extended-Spectrum  $\beta$ -Lactamases (ESBL): Challenges and Opportunities. *Biomedicines*, 11(11), 2937. <https://doi.org/10.3390/biomedicines11112937>
- Massella, E., Giacometti, F., Bonilauri, P., Reid, C.J., Djordjevic, S.P., Meriadi, G., Bacci, C., Fiorentini, L., Massi, P., Bardasi, L., Rubini, S., Savini, F., Serraino, A. and Piva, S.* (2021): Antimicrobial Resistance Profile and ExPEC Virulence Potential in Commensal Escherichia coli of Multiple Sources. *Antibiotics*, 10(4), 351. <https://doi.org/10.3390/antibiotics10040351>
- Mohammed, A.J., Al-Amara, S.S.M. and Al-Hejjaj, M.Y.* (2024): Molecular characterization of blaTEM and blaCTX-M ESBLs genes producing Escherichia coli isolates from urinary tract infections (UTIs) in Al-Basrah province, Iraq. *South Eastern European Journal of Public Health*, 389–396. <https://doi.org/10.70135/seejph.vi.1146>
- Muteeb, G., Rehman, M.T., Shahwan, M. and Aatif, M.* (2023): Origin of Antibiotics and Antibiotic Resistance,

- and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals* (Basel, Switzerland), 16(11), 1615. <https://doi.org/10.3390/ph16111615>
- Nasrollahian, S., Graham, J.P. and Halaji, M. (2024): A review of the mechanisms that confer antibiotic resistance in pathotypes of *E. coli*. *Frontiers in cellular and infection microbiology*, 14, 1387497. <https://doi.org/10.3389/fcimb.2024.1387497>
- Partridge, S.R., Kwong, S.M., Firth, N. and Jensen, S.O. (2018): Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clinical microbiology reviews*, 31(4), e00088-17. <https://doi.org/10.1128/CMR.00088-17>
- Pishtiwan, A.H. and Khadija, K.M. (2019): Prevalence of bla<sub>TEM</sub>, bla<sub>SHV</sub>, and bla<sub>CTX-M</sub> Genes among ESBL-Producing *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Thalassemia Patients in Erbil, Iraq. *Mediterranean journal of hematology and infectious diseases*, 11(1), e2019041. <https://doi.org/10.4084/MJHID.2019.041>
- Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A. and Alqumber, M.A.A. (2023): Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare* (Basel, Switzerland), 11(13), 1946. <https://doi.org/10.3390/healthcare11131946>
- Salgado-Caxito, M., Benavides, J.A., Adell, A.D., Paes, A.C. and Moreno-Switt, A.I. (2021): Global prevalence and molecular characterization of extended-spectrum  $\beta$ -lactamase producing-*Escherichia coli* in dogs and cats – A scoping review and meta-analysis, *One Health*, 12, 100236. <https://doi.org/10.1016/j.onehlt.2021.100236>
- Shahid, M., Singh, A., Sobia, F., Rashid, M., Malik, A., Shukla, I. and Khan, M. (2011): bla<sub>CTX-M</sub>, bla<sub>TEM</sub>, and bla<sub>SHV</sub> in Enterobacteriaceae from North-Indian tertiary hospital: High occurrence of combination genes. *Asian Pac J. Trop. Med.* 4, 101–105. doi: 10.1016/S1995-7645(11)60046-1
- Sivaraman, G.K., Rajan, V., Vijayan, A., Elangovan, R., Prendiville, A. and Bachmann, T.T. (2021): Antibiotic Resistance Profiles and Molecular Characteristics of Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated From Shrimp Aquaculture Farms in Kerala, India. *Front. Microbiol.* 12:622891. doi: 10.3389/fmicb.2021.622891
- Solanki, S. and Das, K. (2024): Antimicrobial Resistance: Molecular Drivers and Underlying Mechanisms. *J. Med. Surg. Public Health* 2024, 3, 100122. <https://doi.org/10.1016/j.glmedi.2024.100122>
- Tokuda, M. and Shintani, M. (2024): Microbial evolution through horizontal gene transfer by mobile genetic elements. *Microbial biotechnology*, 17(1), e14408. <https://doi.org/10.1111/1751-7915.14408>
- Tseng, C.H., Liu, C.W. and Liu, P.Y. (2023): Extended-Spectrum  $\beta$ -Lactamases (ESBL) Producing Bacteria in Animals. *Antibiotics* (Basel, Switzerland), 12(4), 661. <https://doi.org/10.3390/antibiotics12040661>
- Wang, B., Farhan, M.H.R., Yuan, L., Sui, Y., Chu, J., Yang, X., Li, Y., Huang, L. and Cheng, G. (2024): Transfer dynamics of antimicrobial resistance among gram-negative bacteria. *The Science of the total environment*, 954, 176347. <https://doi.org/10.1016/j.scitotenv.2024.176347>
- Wang, R.F., Cao, W.W. and Cerniglia, C.E. (1996): PCR detection and

quantitation of predominant anaerobic bacteria in human and animal fecal samples. Applied and environmental microbiology, 62(4), 1242–1247.

<https://doi.org/10.1128/aem.62.4.1242-1247.1996>

Zhang, T., Nickerson, R., Zhang, W., Peng, X., Shang, Y., Zhou, Y., Luo, Q., Wen,

G. and Cheng, Z. (2024): The impacts of animal agriculture on One Health-Bacterial zoonosis, antimicrobial resistance, and beyond. One health (Amsterdam, Netherlands), 18, 100748.

<https://doi.org/10.1016/j.onehlt.2024.100748>

## دور البلازميدات الحاملة لإنزيمات بيتا لاكتيميز ذو الطيف الواسع في مقاومة مضادات الميكروبات بين الإشريكية القولونية المعزولة من الانسان والكلاب

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تعتبر مقاومة مضادات الميكروبات المتزايدة واحدة من أكبر المخاوف الصحية التي نواجهها اليوم. أصبحت الإشريكية القولونية واحدة من الأسباب الحقيقية لهذه الأزمة. خلال هذا البحث، تم إلقاء نظرة فاحصة على مقاومة الإشريكية القولونية المعزولة من الانسان والكلاب للعلاج، مع التركيز على وجود وانتشار بعض إنزيمات بيتا لاكتيميز ذات الطيف الواسع. للتحقيق في خطر مقاومة البكتيريا في المجتمع، تم جمع عينات البراز من البشر (٦٩) والكلاب (٦٧). تم عزل الإشريكية القولونية باستخدام الطرق التقليدية وتم تأكيد هويتها من خلال تقنية تفاعل البلمرة المتسلسل. تم الكشف عن قدرة البكتيريا المعزولة ضد بعض المضادات الحيوية من نوع بيتا لاكتام، بالإضافة إلى الكشف عن وجود بلازميد طبيعية تحمل جينات *bla*<sup>SHV</sup> و *bla*<sup>TEM</sup>. أظهرت النتائج أن ٧٣,٦٨٪ من عزلات الإشريكية القولونية من الكلاب و ٩١,٨٣٪ من البشر أنتجت إنزيمات بيتا لاكتاماز ذات الطيف الواسع، مما يجعلها مقاومة للعديد من المضادات الحيوية. وبشكل عام، أظهرت ٨٣,٠٧٪ من عزلات الإشريكية القولونية هذه المقاومة. وعلاوة على ذلك، سلطت الدراسة الضوء على دور الجينات (*bla*<sup>SHV</sup> و *bla*<sup>TEM</sup>) التي تنتقل عبر البلازميد. حيث حملت حوالي ٣٠,٩٥٪ من عزلات الكلاب و ٣٥,٥٦٪ من عزلات البشر جين *bla*<sup>TEM</sup>، بينما ظهر جين *bla*<sup>SHV</sup> في حوالي ١٤,٢٨٪ و ١٧,٧٨٪ من عزلات الكلاب والبشر على التوالي. وفي بعض الحالات، حملت البكتيريا كلا الجينين، مما يشير إلى إمكانية انتشار هذه السمات المقاومة بسرعة. وأشارت النتائج إلى أن مقاومة مضادات الميكروبات هي مسار ذو اتجاهين بين البشر والحيوانات. وبشكل انتقالي مقاومة البكتيريا بين البشر والحيوانات، وخاصة من خلال الاتصال المباشر، خطرًا مستمرًا. وأظهرت الدراسة حقيقة واضحة حول مقاومة المضادات الحيوية، والتي تربط بين الطب البشري والبيطري، وبالتالي فإن السيطرة وإدارة استخدام المضادات الحيوية يمكن أن تؤدي إلى إبطاء انتشار هذه الكائنات الحية الدقيقة الخطيرة وحماية فعالية المضادات الحيوية للأجيال القادمة.