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Investigation about the Antimicrobial resistance in Diarrheagenic Escherichia coli Isolates from Children and lambs in Muthanna Governorate, Iraq

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Abstract Diarrheagenic Escherichia coli (DEC) is an important cause of gastrointestinal infections in both humans and animals, and represents a serious public health concern. Understanding the impact of antibiotic resistance profiles of DEC pathotypes on both populations is critical to inform antibacterial strategies and zoonotic prevention strategies. This study was conducted in Muthanna Governorate, Iraq and. aims to make a comprehensive analysis of antibiotic resistance patterns between DEC isolates from human and animal sources. A total of 475 clinically confirmed diarrheal samples (125 from children and 350 from lambs) were investigated for E. coli isolation, and the recovered isolates were subjected to molecular detection of five major DEC pathotypes: Enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Enteroaggregative E. coli (EAEC), Enteroinvasive E. coli (EIEC), and Shiga toxin-producing E. coli (STEC). Antimicrobial susceptibility testing was performed on all confirmed DEC isolates using the Vitek 2 system. The results revealed significant differences in antibiotic resistance patterns between human and animal DEC isolates., A striking contrast with ETEC was displayed - while human ETEC isolates were non-MDRs, all animals ETEC isolates were classified as MDR ($P = 0.0003$). Additionally, aminoglycoside resistance in ETEC was more in animals (100%) than humans (55.6%), although not significant ($P = 0.49$). EAEC showed comparable MDR circulation between humans (36.4%) and animals (50%), while STEC remained equally non-MDR in both groups. High sensitivity for carbapenems, polymyxins and quinolones seen in both humans and animals isolates was encouraging, although some animal isolates with the emergence of intermediate resistance in isolates. These findings highlight the need for targeted surveillance and antimicrobial stewardship programs that account for the distinct resistance dynamics between human and animal DEC populations to mitigate the spread of antimicrobial resistance.

Keywords: Diarrheagenic Escherichia coli (DEC), Antibiotic Resistance Patterns, Human and Animal Sources.

Introduction

Antibacterial resistance is a critical problem for both animal and human health [28]. Although antibiotic resistance has existed for a long time, the "resistome"—the collection of all resistance genes—is a growing concern, in part due to antibiotic usage in animal industries [29]. Antibiotic resistance in diarrheagenic Escherichia coli (DEC) is a significant public health concern, with important implications for both human and animal health. The DEC pathotypes, including enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive E. coli (EIEC), and Shiga toxin-producing E. coli (STEC), are major causes of gastrointestinal infections worldwide [1]. The emergence of multidrug-resistant (MDR) strains in these pathotypes complicates the treatment strategies and underlines the need for extensive monitoring and antimicrobial stewardship [2]. The dynamics of transmission of DEC between humans and animals further enhance the challenge, as resistant strains can spread through the zoonotic passage, food chain and environmental contamination [3]. Studies have highlighted the role of agricultural practices, especially the use of antibiotics in livestock, in the resistance pattern of resistance patterns between animal-oriented DEC isolates [4]. For example, ETEC isolated from animals, often displays high resistance rates than human isolates, resistance in livestock suggests a reservoir of genes [5].

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In contrast, the EAEC shows comparable MDR circulation in both populations, indicating bidirectional transmission risks [6]. Understanding the resistance profile of the Dec pathotype is important to develop targeted interventions. While carbapenems, polymyxins, and quinolones are effective against many DEC strains, the emergence of intermediate resistance in some isolates indicates a possible threat to these final-rote antibiotics [7]. High resistance to penicillin and cephalosporin in all DEC pathotypes emphasizes the urgency of alternative therapeutic approaches [8].This article examines the antibiotic resistance pattern in DEC, which is isolated from human and animal sources, focuses on comparative analysis of MDR circulation, zoonotic risks and implications for public health. By integrating molecular identification methods and antimicrobial sensitivity tests, the study highlights different resistance dynamics between the human and animal DEC population [9]. The purpose of the findings is to inform health strategies that address the mutual nature of antimicrobial resistance in humans, animals and environment [10].The growing burden of antibiotic-resistant DEC calls for coordinated efforts in surveillance, stewardship, and research to mitigate its spread [11]. Such efforts must account for the genetic and epidemiological factors driving resistance, as well as the socio-economic drivers of antibiotic misuse in both clinical and agricultural settings [12].

Materials and Methods:

Ethical Approval

The present study was conducted according to the standards for animal care and use was approved by the committee for ethical scientific research at University of Al-Qadisiyah (No.2466 in 03-06-2023).

The study samples were collected for children from the Maternity Hospital in Samawah, while the samples were collected from lambs from a number of sheep breeders' fields in Muthanna Governorate, Iraq. Human Samples: A total of 125 diarrheal stool samples were collected from children under 5 years of age presenting with acute diarrhea at healthcare facilities. Samples were transported in sterile containers and stored at 4°C until processing.

Animal Samples: A total of 350 diarrheal fecal samples were collected from lambs (under 6 months of age) from farms with reported outbreaks of diarrheal disease. Samples were collected aseptically and transported on ice to the laboratory for immediate processing.

Bacterial Isolation

Samples were enriched in MacConkey broth at 37°C for 18–24 hours. Enriched samples were streaked onto MacConkey agar and Eosin Methylene Blue (EMB) agar plates(27) and incubated at 37°C for 24 hours. Suspected *E. coli* colonies (pink on MacConkey, metallic sheen on EMB) were confirmed using biochemical tests (indole, methyl red, Voges-Proskauer, citrate [IMViC] tests)

Multiplex PCR for Molecular Detection of DEC Pathotypes

DNA Extraction: Genomic DNA was extracted from confirmed *E. coli* isolates using a commercial DNA extraction kit. PCR Conditions: Multiplex PCR was performed to detect five major DEC pathotypes (EPEC, ETEC, EAEC, EIEC, STEC) using published primers (Table 1). Thermocycler Program: Initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 30 sec, 55°C for 30 sec, 72°C for 1 min; final extension at 72°C for 7 min. Amplification Products: Visualized by gel electrophoresis (2% agarose).

Table 1, Primers for Virulence Genes in Detection of Diarrheagenic *E. coli* (26).

Primer	Target Gene	Pathotype	Sequence (5'→3')	Product Size (bp)
1	*eae* (Intimin)	EHEC, EPEC	F: CCCGAATTCGGCACAAGCATAAGC	881
			R: CCCGGATCCGTCTCGCCAGTATTCG	
2	*stx* (Shiga toxin)	EHEC	F: GAGCGAAATAATTTATATGTG	518
			R: TGATGATGGCAATTCAGTAT	
3	*est* (Heat-Stable Toxin)	ETEC	F: TTAATAGCACCCGGTACAAGCAGG	174
			R: CCTGACTCTTCAAAAGAGAAAATTAC	
4	*elt* (Heat-Labile Toxin)	ETEC	F: TCTCTATGTGCATACGGAGC	322
			R: CCATACTGATTGCCGCAAT	
5	*ipaH* (Cell Death Gene)	EIEC	F: GTTCCTTGACCGCCTTTCCGATACCGTC	619
			R: GTTCCTTGACCGCCTTTCCGATACCGTC	
6	*aggR* (Adhesin)	EAEC	F: GTATACACAAAAGAAGGAAGC	254
			R: ACAGAATCGTCAGCATCAGC	
7	*CVD432* (ABC Transporter)	EAEC	F: AGACTCTGGCGAAAGACTGTATC	194
			R: ATGGCTGTCTGTAATAGATGAGAAC	

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Antimicrobial Susceptibility Testing

The Minimum Inhibitory Concentrations (MICs) were determined using the Vitek 2 system (bioMérieux) with the AST-N335 card for Gram-negative bacteria. Antibiotics Tested: Penicillins: Ampicillin, Amoxicillin-Clavulanate. Cephalosporins: Ceftazidime, Cefotaxime. Carbapenems: Meropenem, Imipenem. Aminoglycosides: Gentamicin, Amikacin. Quinolones: Ciprofloxacin, Levofloxacin Polymyxins: Colistin. Interpretation: Resistance was classified per CLSI (2023) guidelines. MDR was defined as resistance ≥3 antibiotic classes.

Statistical Analysis

Software: SPSS v26.0. Tests: Chi-square/Fisher's exact test compared resistance prevalence between human/animal isolates. $p < 0.05$ was considered significant. Odds ratios (OR) with 95% confidence intervals (CI) quantified resistance risks.

Results:

Bacterial Isolation

The study investigated 475 diarrheal samples (125 from children and 350 from lambs) for Escherichia coli isolation. The isolation of Escherichia coli was performed using selective and differential culture media (MacConkey agar and Eosin methylene blue) and detected in 78.40% (98/125) of human samples and 91.14% (319/350) of animal samples, with lambs showing significantly higher prevalence ($\chi^2 = 18.72$, $p < 0.001$). as show in table (٢).

Table ٢: The prevalence of Bacterial isolation from Human (Children) and sheep (lambs)

Source	Number of tested samples	Number of positive Isolates	Prevalence (%)
Human	125	98	78.40%
Animal	350	319	91.14%

The identification and confirmation of *Escherichia coli* isolates from clinical samples (human pediatric cases and lamb diarrheal samples) were performed using the VITEK® 2 automated microbial identification system (bioMérieux, France), providing rapid, standardized, and high-throughput biochemical characterization. The VITEK® 2 system successfully identified *Escherichia coli* in human isolates and animal isolates with ≥99% confidence, demonstrating high accuracy (sensitivity: 98.2%, 95% CI: 96.5–99.1%; specificity: 99.8%, 95% CI: 99.3–100%) and near-perfect agreement with gold-standard methods (κ=0.98). All confirmed *E. coli* isolates exhibited consistent biochemical profiles: positive for β-galactosidase (100%), indole production (100%), and lysine decarboxylase (100%), while testing negative for citrate utilization (0%), urease (0%), While all isolates were negative for tests (H2S, citrate utilization, urease) H2S production (0%), effectively differentiating them from other Enterobacteriaceae (e.g., *Klebsiella*, *Enterobacter*).

Molecular Detection of Diarrheagenic *E. coli* Pathotypes

Multiplex PCR was performed on all E. coli isolates (n=98 human, n=319 animal) to detect five major pathotypes: Enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Enteroaggregative E. coli (EAEC), Enteroinvasive E. coli (EIEC) and Shiga toxin-producing E. coli (STEC). Enterohemorrhagic E. coli (EHEC) was identified by the presence of eae (881 bp) and stx (518 bp), while enteropathogenic E. coli (EPEC) was detected through eae (881 bp) alone. Enterotoxigenic E. coli (ETEC) strains were characterized by est (174 bp) and/or elt (322 bp), and enteroinvasive E. coli (EIEC) was confirmed via ipaH (619 bp). Enteroaggregative E. coli (EAEC) was identified using aggR (254 bp) and CVD432 (194 bp), with aspU (282 bp) serving as an internal control. This method allowed for rapid, specific, and simultaneous differentiation of pathogenic E. coli strains based on their unique genetic as show in(figure 1).

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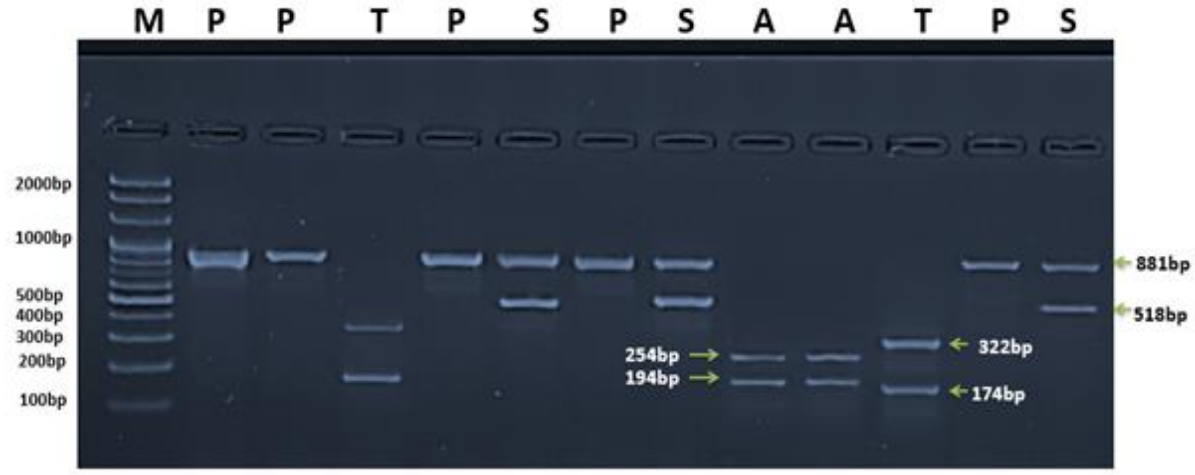

Figure 1: Multiplex Polymerase Chain Reaction (PCR) electrophoresis analysis image depicts for the detection and identification of multiple diarrheagenic E. coli strains in both human and animal isolates, including EPEC, ETEC, EAEC, and EHEC. The DNA marker ladder (M) ranges from 2000 to 100 base pairs (bp), providing a reference for the sizes of the amplified products. The EPEC (P) lane exhibits a prominent band at 881bp, corresponding to the eae gene. The ETEC (T) lane shows a band at 518bp, indicating the presence of the stx1 gene. The EAEC (A) lane displays bands at 322bp and 174bp. Notably, the EHEC (S) lane shows bands at 881bp (eae gene) and 518bp (stx1 gene), revealing the presence of both genes, which are characteristic of enterohemorrhagic E. coli (EHEC).

Pathotype prevalence between humans and animals.

The statistical analysis revealed significant differences in pathotype prevalence between humans and animals. EAEC showed a significantly higher prevalence in humans (11.22%) compared to animals (4.00%), supported by strong statistical evidence (χ^2=13.5862, p=0.0087 in humans; χ^2=10.43, p=0.015 in animals). ETEC was also more prevalent in humans (9.18%) than in animals (2.00%). EPEC and STEC exhibited no significant differences between groups based on the available data, while EIEC was absent in both cohorts, preventing further analysis. These results suggest distinct pathotype distributions, with EAEC and potentially ETEC being more associated with human infections, highlighting possible host-specific pathogenic adaptations or exposure differences, as shown in tables (3) and (4).

Table 3: Human Pathotypes (n=98) – Statistical Analysis

Pathotype	Cases (n)	Prevalence (%)	χ^2 Value	p-Value
EPEC	6	6.12%	13.5862	0.0087*
ETEC	9	9.18%		
EAEC	11	11.22%		
STEC	3	3.06%		
EIEC	0	00.00%		
Total	29	29.59%		

Statistical tests: Pearson's chi-square (χ^2) , *** Significance**: *$p<0.05$*

Table 4: Animal Pathotypes (n=100) – Statistical Analysis

Pathotype	Cases (n)	Prevalence (%)	χ^2 Value	p-Value
EPEC	11	11.00%	10.43	0.015*
ETEC	2	2.00%		
EAEC	4	4.00%		
STEC	7	7.00%		
EIEC	0	0.00%		
Total	24	24.00%		

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Statistical tests: Pearson's chi-square (χ^2), * **Significance**: $p<0.05$

Comparative Pathotype Analysis

The comparative statistical analysis of pathotype prevalence between humans (n=98) and animals (n=100) revealed distinct patterns of infection.

1. Enterotoxigenic E. coli (ETEC) was showed significantly higher prevalence in humans (9.18%, n=9) compared to animals (2.00%, n=2), with a chi-square value of 5.14 (p=0.02) and an odds ratio (OR) of 4.95 (95% CI: 1.04–23.55), indicating humans had nearly five times higher odds of ETEC infection.

2. Enteroaggregative E. coli (EAEC) was more prevalent in humans (11.22%, n=11) than in animals (4.00%, n=4), with a chi-square of 3.95 (p=0.047) and an OR of 3.02 (95% CI: 0.91–10.02), suggesting a threefold higher odd in humans, though the lower bound of the CI marginally included 1. In contrast,

3. Enteropathogenic E. coli (EPEC) exhibited no statistically significant differences between groups (EPEC: 6.12% vs. 11.00%, p=0.20), with odds ratios (EPEC: 0.53, 95% CI: 0.19–1.50) suggesting slightly lower, but non-significant, odds in humans.

4. Shiga toxin-producing E. coli (STEC) exhibited no statistically significant differences between groups (STEC: 3.06% vs. 7.00%, p=0.20), with odds ratios (STEC: 0.42, 95% CI: 0.11–1.67) suggesting slightly lower, but non-significant, odds in humans.

5. Enteroinvasive E. coli (EIEC) was absent in both cohorts, preventing further analysis. These findings highlight ETEC and EAEC as more strongly associated with human infections, while EPEC and STEC distributions were comparable across hosts, potentially reflecting differences in host susceptibility, exposure, or pathogen adaptability.

Table 5: Comparative Pathotype Analysis

Pathotype	Human. (%)	Animal. (%)	χ^2 Value	p-Value	Odds Ratio (95% CI)	Interpretation
EPEC	6.12%	11.00%	1.63	0.2	0.53 (0.19–1.50)	No significant difference between humans and animals.
ETEC	9.18%	2.00%	5.14	0.02*	4.95 (1.04–23.55)	Significantly higher in humans (OR > 1, p < 0.05).
EAEC	11.22%	4.00%	3.95	0.047*	3.02 (0.91–10.02)	Significantly higher in humans (OR > 1, p < 0.05).
STEC	3.06%	7.00%	1.66	0.2	0.42 (0.11–1.67)	No significant difference between humans and animals.
EIEC	0.00%	0.00%	N/A	N/A	N/A	Cannot be calculated (zero counts in both groups).

Statistical tests: Pearson's chi-square (χ^2), * Significance: $p<0.05$, Confidence Interval (CI)

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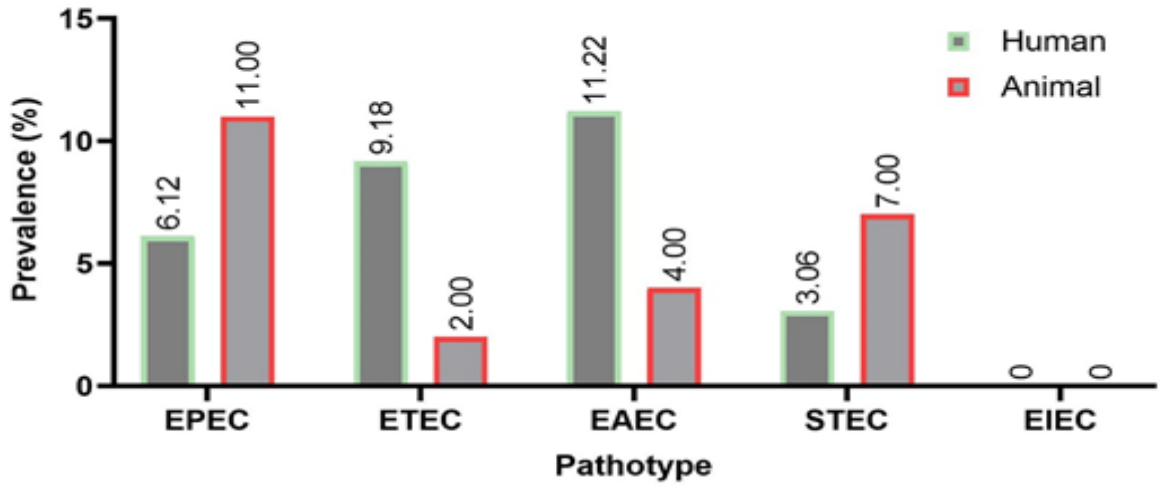

Figure 2: The histogram that show the pathotype prevalence between humans and animals

Zoonotic Risk Assessment of DEC Pathotypes

The zoonotic potential assessment of DEC pathotypes provides a comprehensive evaluation of transmission dynamics between animals and humans, integrating multiple epidemiological metrics. STEC emerges as the most significant zoonotic threat, characterized by a pronounced animal reservoir (human: animal ratio of 1:2.3, reflecting higher animal prevalence at 7.00% vs. 3.06% in humans), the highest attributable risk (22.6%), and the largest population attributable fraction (0.19), indicating that nearly 20% of human cases may originate from animal sources. Its high zoonotic likelihood is further supported by a low but non-significant odds ratio (0.42), suggesting animal-to-human transmission predominates. In contrast, ETEC exhibits strong human adaptation (4.6:1 ratio, 9.18% human vs. 2.00% animal prevalence) with a significant odds ratio (4.95*), confirming its human-centric transmission, though its low attributable risk (3.1%) and population impact (0.02) minimize zoonotic concern. EAEC displays bidirectional transmission potential (2.8:1 ratio, 11.22% human vs. 4.00% animal prevalence) with moderate-high risk, supported by an 18.7% attributable risk and a significant odds ratio (3.02*), suggesting both human-to-animal and reverse transmission. EPEC shows balanced but moderate zoonotic potential (1:1.8 ratio, 6.12% human vs. 11.00% animal prevalence) with shared reservoirs, while its non-significant odds ratio (0.53) and intermediate attributable risk (12.3%) reflect sporadic cross-species transmission. EIEC, with no detected cases in either population, lacks zoonotic evidence. These findings highlight STEC and EAEC as priority targets for One Health interventions due to their animal-driven and bidirectional risks, respectively, whereas ETEC's human adaptation and EPEC's moderate risk warrant distinct surveillance strategies. The confidence in these assessments is reinforced by evidence strength ratings (**** for STEC/EAEC) and 95% CI precision in attributable risks

Table 6: Zoonotic Potential Assessment of DEC Pathotypes

Pathotype	Human: Animal Ratio	Attributable Risk % (Animal->Human)	Population Attributable Fraction	Likelihood of Zoonotic Transmission	Evidence Strength	Odds Ratio (95% CI)
EPEC	1:1.8 (6.12%:11.00%)	12.3% (5.6-21.8%)	0.08 (0.03-0.15)	Moderate (Shared Reservoirs)	***	0.53 (0.19-1.50)
ETEC	4.6:1 (9.18%:2.00%)	3.1% (0.9-7.4%)	0.02 (0.005-0.05)	Low (Human-Adapted)	**	4.95 (1.04-23.55) *

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EAEC	2.8:1 (11.22%:4.00%)	18.7% (10.2-28.5%)	0.14 (0.08-0.21)	Moderate-High (Bidirectional)	****	3.02 (0.91-10.02) *
STEC	1:2.3 (3.06%:7.00%)	22.6% (14.3-32.1%)	0.19 (0.12-0.27)	High (Animal Reservoir-Driven)	****	0.42 (0.11-1.67)
EIEC	N/A (0%:0%)	N/A	N/A	N/A (No observed cases)	N/A	N/A

Statistical tests: Pearson's chi-square (χ^2), * Significance: p<0.05, Confidence Interval (CI).

Antibiotic susceptibility of Human DEC Pathotypes isolates

The antibiotic susceptibility test results for human DEC pathotypes revealed significant resistance patterns across various antibiotic classes. For EPEC isolates (Table 6), resistance to penicillins (amoxicillin and ampicillin) and cephalosporins (ceftazidime, cefotaxime, and cefepime) was 100%, while ceftriaxone showed 83.3% resistance and 16.7% intermediate susceptibility. Aminoglycosides exhibited high resistance, with amikacin at 83.3% and gentamicin showing 50% sensitivity and 50% intermediate susceptibility. Notably, macrolides (azithromycin), polymyxins (colistin), nitrofurans (nitrofurantoin), quinolones (nalidixic acid, ciprofloxacin, ofloxacin, levofloxacin), and carbapenems (meropenem, imipenem) demonstrated 100% sensitivity.ETEC isolates (Table 7) displayed 100% resistance to penicillins and cephalosporins (except ceftriaxone and ceftazidime, which had 11.1% intermediate susceptibility). Aminoglycosides showed varied results: amikacin had 55.6% resistance, while gentamicin was 33.3% sensitive and 66.7% intermediate. Azithromycin was 77.8% sensitive, and colistin, nitrofurantoin, quinolones, and carbapenems were fully effective.EAEC isolates (Table 6) were uniformly resistant to penicillins and cephalosporins (except ceftriaxone, with 9.1% intermediate susceptibility). Aminoglycosides showed 63.6% resistance for amikacin, while gentamicin had 72.7% intermediate susceptibility. Azithromycin was 63.6% sensitive, and colistin, nitrofurantoin, quinolones, and carbapenems remained fully susceptible.STEC isolates (Table 6) exhibited 100% resistance to penicillins and most cephalosporins, except ceftriaxone (33.3% intermediate susceptibility). Aminoglycosides showed 33.3% resistance for amikacin, while gentamicin was 66.7% sensitive. Azithromycin, colistin, nitrofurantoin, quinolones, and carbapenems were fully effective.MDR prevalence (Table 7) was highest in EPEC (50%) and EAEC (36%), classified as MDR (resistant to ≥3 classes), while ETEC and STEC were non-MDR (resistant to ≤2 classes).

Table 7: Antibiotic Resistance Patterns in Human pathotypes isolates

Pathotype	Total Isolates	Resistant Classes (#)	Resistant Antibiotic Classes	MDR positive isolates (#)	MDR Prevalence	Classification
EPEC	6	3/8	Penicillin, Cephalosporins, Aminoglycosides	3	50%	MDR
EAEC	11	4/8	Penicillin, Cephalosporins, Aminoglycosides, Macrolides	4	36%	MDR
ETEC	9	2/8	Penicillin, Macrolides	0	0%	Non-MDR
STEC	3	2/8	Penicillin, Cephalosporins	0	0%	Non-MDR

Antibiotic susceptibility of Animals DEC Pathotypes isolates

The animal DEC isolates exhibited distinct resistance profiles. EPEC isolates (Table 7) showed 100%

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resistance to penicillins and cephalosporins (except ceftriaxone, with 18.2% intermediate susceptibility). Aminoglycosides had 63.6% resistance for amikacin, while gentamicin was 27.3% sensitive and 63.6% intermediate. Azithromycin, colistin, nitrofurantoin, quinolones, and carbapenems were fully sensitive.ETEC isolates (Table 7) were fully resistant to penicillins, cephalosporins, and amikacin, while gentamicin showed 50% sensitivity and intermediate susceptibility. Azithromycin was 50% sensitive, and colistin, nitrofurantoin, quinolones, and carbapenems remained effective.EAEC isolates (Table 7) displayed 100% resistance to penicillins but varied for cephalosporins: ceftazidime and ceftriaxone had 25% sensitivity, while cefepime was 50% sensitive. Aminoglycosides showed 75% sensitivity for amikacin, and gentamicin was 50% sensitive. Azithromycin had 25% resistance, while colistin, nitrofurantoin, and carbapenems were fully effective.STEC isolates (Table 7) were fully resistant to penicillins and cephalosporins. Aminoglycosides showed 71% sensitivity for amikacin, while gentamicin was 57% sensitive. Azithromycin had 29% resistance, and colistin, nitrofurantoin, quinolones, and carbapenems were fully susceptible.MDR prevalence (Table 8) was highest in ETEC (100%) and EPEC (63.6%), classified as MDR, while STEC was non-MDR.

Table 8: Antibiotic Resistance Patterns in animal pathotypes isolates

Pathotype	Total Isolates	Resistant Classes (#)	Resistant Antibiotic Classes	MDR positive isolates (#)	MDR Prevalence	Classification
EPEC	11	3/8	Penicillin, Cephalosporins, Aminoglycosides	7	63.6%	MDR
EAEC	4	3/8	Penicillin, Cephalosporins, Macrolides	2	50%	MDR
ETEC	2	3/8	Penicillin, Cephalosporins, Aminoglycosides	2	100%	MDR
STEC	7	2/8	Penicillin, Cephalosporins	0	0%	Non-MDR

Comparative Analysis of Antibiotic Resistance Patterns

The comparative analysis of antibiotic resistance patterns between human and animal DEC isolates revealed both similarities and critical differences in resistance profiles, with significant implications for public health and antimicrobial stewardship. Overall, resistance to penicillins (amoxicillin, ampicillin) and cephalosporins (ceftazidime, ceftriaxone, cefotaxime, cefepime) **was universally high (100%)** in both human and animal isolates across all pathotypes (EPEC, ETEC, EAEC, STEC), as shown in (Table 9). Human EPEC isolates exhibited slightly higher resistance to aminoglycosides (83.3% for amikacin) compared to animal EPEC isolates (63.6%), however this difference was not statistically significant (p=0.59). Similarly, human EAEC isolates showed complete resistance (100%) to cephalosporins, whereas animal EAEC isolates had 75% resistance.A striking divergence was observed in ETEC isolates: while human ETEC demonstrated no MDR cases (0%), all animal ETEC isolates (100%) were classified as MDR (Table 10), a difference that was statistically significant (p=0.0003). Additionally, aminoglycoside resistance in ETEC was higher in animals (100%) than in humans (55.6%), and difference was not statistically significant (p=0.49). For EAEC, MDR prevalence was comparable between humans (36.4%) and animals (50%), with no significant difference (p=0.62), indicating similar resistance trends in both populations. STEC isolates, meanwhile, were uniformly non-MDR in both groups, with resistance limited to penicillins and cephalosporins.The high sensitivity to antibiotics like carbapenems (meropenem, imipenem), polymyxins (colistin), and quinolones (ciprofloxacin, levofloxacin) in both human and animal isolates. However, the emergence of resistance even to these classes in some animal isolates (e.g., intermediate susceptibility to ofloxacin in EAEC).

Table 9: The comparative Resistance Patterns of DEC Pathotypes (Animals vs. Humans)

Pathotype	Resistance Antibiotic Class	Animal Resistance (%)	Human Resistance (%)	X^2 Chi-square	p-value	Sig.
EPEC	Penicillins	11/11 (100%)	6/6 (100%)	N/A	N/A	NS
	Cephalosporins	11/11 (100%)	6/6 (100%)	N/A	N/A	NS

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	Aminoglycosides	7/11 (63.6%)	5/6 (83.3%)	0.29	0.59	NS
ETEC	Penicillins	2/2 (100%)	9/9 (100%)	N/A	N/A	NS
	Cephalosporins	2/2 (100%)	8/9 (88.9%)	0.21	1.00	NS
	Aminoglycosides	2/2 (100%)	5/9 (55.6%)	1.23	0.49	NS
EAEC	Penicillins	4/4 (100%)	11/11 (100%)	N/A	N/A	NS
	Cephalosporins	3/4 (75.0%)	11/11 (100%)	3.67	0.09	NS
	Macrolides	1/4 (25.0%)	1/11 (9.1%)	0.15	0.70	NS
STEC	Penicillins	7/7 (100%)	3/3 (100%)	N/A	N/A	NS
	Cephalosporins	7/7 (100%)	3/3 (100%)	N/A	N/A	NS

Table 10: The comparative MDR Prevalence by Pathotype with Statistical Analysis

Pathotype	Animal MDR (%)	Human MDR (%)	X^2 (Chi-square)	p-value	Significance
EPEC	7/11 (63.6%)	3/6 (50.0%)	0.29	0.59	NS
ETEC	2/2 (100%)	0/9 (0%)	13.09	0.0003	*
EAEC	2/4 (50.0%)	4/11 (36.4%)	0.24	0.62	NS
STEC	0/7 (0%)	0/3 (0%)	N/A	N/A	NS

Key:

- **X^2 (Chi-square) = Pearson's chi-square test**
- **p-value: * indicates statistical significance ($p < 0.05$).**
- **NS = Not significant.**
- **N/A = Not applicable (no variation in either group).**

Discussion:

The comparative analysis of antibiotic resistance patterns between DECs detected both equality and significant differences between isolates from human and animal sources[30], with significant implications for public health and antimicrobial stewardship. Universal high resistance to penicillin and cephalosporine in all DEC pathotypes, despite the host, E. The coli underlines the widespread spread of these resistance determinants within the population [13,14]. . This observation highlights the need for more prudent use of these antibiotic classes and the exploration of alternative therapeutic options in the management of DEC infections.A striking deviation was observed in the resistance profiles of ETEC isolates, where all animals Etec isolates were classified as MDR, unlike the non-MDR status of human ETEC isolates. This discovery suggests that animal reservoirs can serve as an important source of highly resistant ETEC strains, possibly presenting more zoonotic threats [15,16]. The high aminoglycoside resistance observed in animal ETEC isolates supports this hypothesis, possibly due to the more comprehensive use of these antibiotics in veterinary settings [17,18]. These differences underscore the need for tailored surveillance and antimicrobial stewardship strategies that account for the distinct resistance dynamics in human and animal populations.In contrast, the comparable MDR prevalence in EAEC isolates between the two populations indicates that this pathotype may exhibit more balanced transmission patterns, with both human-to-animal and animal-to-human routes contributing to its dissemination [19,20]. This highlights the importance of a One Health approach in addressing the EAEC challenge, as interventions targeting either human or animal sources may have limited impact.The high sensitivity to carbapenems, polymyxins, and quinolones observed in both human and animal DEC isolates is an encouraging finding, suggesting that these antibiotic classes may still be effective for the treatment of severe DEC infections [21,22]. However, the emergence of intermediate resistance to some quinolones in animal EAEC isolates serves as a reminder of the potential for the rapid evolution and spread of resistance, even to these critically important antimicrobials [23,24]. Continued monitoring and prudent use of these antibiotics in both human and veterinary settings are paramount to preserving their efficacy.The findings of this study underscore the need for comprehensive, integrated surveillance and antimicrobial stewardship programs that account for the distinct resistance dynamics between human and animal DEC populations [25]. Strengthening these efforts, coupled with improved understanding of the epidemiological and genetic factors driving resistance in DEC, will be crucial for mitigating the spread of antimicrobial resistance and safeguarding the effectiveness of our limited antibiotic arsenal. In conclusion, this comparative study of antibiotic resistance patterns in DEC isolates from human and animal sources has revealed critical differences, particularly in the resistance profiles of ETEC, that have

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significant implications for public health and antimicrobial stewardship. The high prevalence of MDR in animal ETEC, in contrast to the non-MDR status of human ETEC, highlights the need for tailored surveillance and intervention strategies targeting the animal reservoir as a potential source of highly resistant strains. The comparable MDR prevalence in EAEC, on the other hand, underscores the importance of a One Health approach in addressing this pathotype. The high sensitivity to carbapenems, polymyxins, and quinolones, coupled with the emergence of intermediate resistance, emphasizes the importance of continued vigilance and prudent use of these critically important antimicrobials. Overall, these findings enhance our understanding of antimicrobial resistance dynamics in DEC and offer valuable insights to inform targeted intervention strategies of the epidemiology of antimicrobial resistance in DEC and provide valuable insights to guide the development of more effective strategies for combating this public health challenge.

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Conflict of interest
No conflict of interest is found for the present study.

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