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Antibiotic Susceptibility Profiling of *Escherichia coli* Isolated from a Food Sample Using Disc Diffusion Method

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Abstract: *The rising prevalence of antibiotic resistant bacteria in the food chain is a major public health concern. In this study, Escherichia coli was isolated from a single randomly selected food sample, identified through morphological, biochemical, and subjected to antibiotic susceptibility testing (AST) using the disc diffusion test with the help of HiMedia Antibiotic Disc IC002. Following isolation of multiple bacterial colonies and screening for antibiotic sensitivity, an E. coli strain was confirmed with the help of molecular approach i.e. 16S rRNA gene sequencing. The inhibition zones of selected antibiotics were measured around each disc to determine sensitivity or resistance. Although the exact values were varied by isolate and antibiotic, the method provides a structured approach to monitor food-borne bacterial resistance. The results are discussed in the context of current resistance mechanisms in E. coli, potential risks associated with foodborne transmission of resistant strains, and implications for antibiotic stewardship. The study underscores the importance of continual surveillance of antibiotic susceptibility of foodborne E. coli and the need for integrating molecular identification with phenotypic testing to understand resistance patterns*

Keywords: *Escherichia coli*, foodborne bacteria, antibiotic susceptibility testing, HiMedia Octa Disc, antibiotic resistance

I. INTRODUCTION

Escherichia coli is a ubiquitous Gram-negative bacillus frequently found in the intestinal tracts of humans and animals, and it can also contaminate foodstuffs via fecal contamination, inadequate hygiene, or cross-contamination during processing (Vaina et al., 2025). The presence of *E. coli* in foods is a marker of hygiene, and more importantly, the possibility that these isolates may harbour antibiotic resistance genes (ARGs) poses a zoonotic and food-safety concern (Qamar et al., 2023). Studies have shown that *E. coli* from varied sources, including milk, meat, and environmental samples, exhibit increasing rates of resistance to commonly used antibiotics (Silva et al., 2023). The mechanisms of resistance in *E. coli* include acquisition of plasmid-encoded genes, efflux pump up-regulation, porin loss/mutation, and β-lactamase production (Nasrollahian et al., 2024). In the context of food safety, phenotypic antibiotic susceptibility testing (AST) combined with molecular identification helps delineate not only the risk of foodborne transmission of resistant strains, but also informs public-health interventions (Elbehiry et al., 2023 and Elbehiry et al., 2025).

Given that many studies focus on clinical isolates, there is value in examining food-borne *E. coli*. This study aimed to isolate *E. coli* from a randomly chosen food sample, confirm its identity using morphological, biochemical and molecular tools, and perform AST using the HiMedia Antibiotic Octa Disc. The overall goal is to evaluate the sensitivity/resistance profile of the isolate and discuss the findings in the context of broader resistance trends in *E. coli*.

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II. MATERIALS AND METHODS

Sample collection and isolation

A random food sample (vegetable) was collected from local market of Nanded city, Maharashtra, India under aseptic conditions and transported to the laboratory in a sterile container at 4°C. On arrival, a portion of the sample (approximately 10 g) was homogenized in sterile buffered peptone water (or other pre-enrichment medium) and incubated at 37°C for 24 h. Following enrichment, aliquots were streaked onto differential agar (MacConkey agar, Endo agar and EMB agar) and incubated at 37 °C for 24 h. Colonies exhibiting typical *E. coli* morphology were noted down and sub-cultured on nutrient agar slant for further analysis.

Morphological and biochemical identification

Selected isolates were examined for colony morphological features including size, shape, colour, margine, opacity, consistency while cell morphology were detected microscopically using Gram stain to confirm Gram-negative rod morphology and hanging drop technique for motility test. Further identification of the isolates were carried out using biochemical tests mentioned in the Bergey's Manual of Systematic Bacteriology included IMViC (Indole, Methyl Red, Voges-Proskauer, Citrate) and additional tests (e.g., catalase, urease, oxidase, nitrate reduction) different media including MacConkey's agar, Endo agar and EMB agar to confirm *E. coli* identity upto genus level.

Molecular identification

For molecular confirmation, genomic DNA was extracted from partially identified *E. coli* colony using a standard bacterial DNA extraction protocol (Atashpaz et al., 2010). PCR amplification (up to ~1200bp) was performed using primer pair used for 16S rRNA gene amplification of *E. coli* is 27F and 1492R. The forward primer is 27F (5'-AGAGTTTGATCATGGCTCAG-3') and the reverse primer is 1492R (5'-GGTTACCTTGTTACGACTT-3'). Obtained 16S rRNA gene sequence was submitted for BLAST on NCBI portal for matching the sequence with available sequences in NCBI database. FASTA sequences of most matching sequences were downloaded and processes in MEGA XI program for sequences alignment and phylogenetic tree was constructed using Neighbour Joining method (Saitou & Nei, 1987, Tamura et al., 2004 and Tamura et al., 2021).

Antibiotic susceptibility testing (AST)

The confirmed *E. coli* isolate was sub-cultured onto Mueller–Hinton agar (HiMedia India Ltd.) plates and allowed to incubate to achieve a lawn (Skov et al., 2006). The Antibiotic antibiotic Disc IC002 (HiMedia India Ltd.): (Cephalothin 20, Clindamycin 2, Co-Trimoxazole 25, Erythromycin 15, Gentamycin 10, Ofloxacin 5, Penicillin 10, Vancomycin 30, Ampicillin 10, Linezolid 30, Azithromycin 16, Amikacin 30, Clarithromycin 15, Teicoplanin 10, Methicillin 5, Amoxycalv 30, Novoniocin 5 and Tetracyclin 30) was applied onto the agar surface, ensuring appropriate spacing. Plates were incubated at 37 °C for 18-24 h. After incubation, the zone of inhibition around each disc was measured in millimeters using Zone measuring scale (HiMedia India Ltd.). Results were interpretations (sensitive, intermediate, resistant) made according to CLSI guidelines for *E. coli*.

III. RESULTS

Sample Collection

A total 5 food sample of same type were obtained from local market of Nanded City, Maharashtra, India.

Isolation of Bacteria from the samples

A total 39 bacterial isolates were obtained from the food sample enriched broth on agar plates. Out of these 39 bacterial isolates, 13 were gram negative rods hence screened for biochemical analysis.

Identification of the isolates

From Morphological and Biochemical tests, the isolated bacteria were confirmed to belongs to gens Escherichia. The detailed results of morphological features on MacConeys Agar Plate are as shown in the Table 1. Biochemical analysis is as shown in the Table 2.

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Table 1. Colony morphology and microscopic features of the isolates

Sr	Isolate	Size	Shape	Colour	Margine	Opacity	Consistency	Motility	Grams's Nature	Cell Shape
1	PA02	04 mm	Oval	Pink	Entire	Opaque	Butyrous	-	-	Rods
2	PA05	03 mm	Round	Pink	Entire	Opaque	Butyrous	+	-	Rods
3	PA06	03 mm	Oval	White	Entire	Opaque	Sticky	-	-	Rods
4	PA11	05 mm	Oval	Pale	Entire	Opaque	Butyrous	-	-	Rods
5	PA12	01 mm	Round	Pink	Entire	Opaque	Butyrous	-	-	Rods
6	PA13	02 mm	Round	Pink	Entire	Opaque	Butyrous	+	-	Rods
7	PA16	04 mm	Round	Pink	Entire	Opaque	Butyrous	+	-	Rods
8	PA20	04 mm	Round	Pink	Entire	Opaque	Butyrous	+	-	Rods
9	PA26	02 mm	Oval	Pale	Entire	Opaque	Sticky	-	-	Rods
10	PA27	03 mm	Irregular	Pale	Entire	Turbid	Sticky	-	-	Rods
11	PA28	02 mm	Oval	Pink	Entire	Opaque	Butyrous	-	-	Rods
12	PA31	01 mm	Round	Pink	Entire	Opaque	Sticky	+	-	Rods
13	PA32	04 mm	Round	Pink	Entire	Opaque	Butyrous	+	-	Rods

Table 2. Biochemical features of the isolates

Isolate	Indole	MR	VP	Citrate	Catalase	Urease	Oxidase	Nitrate Reductase
PA02	+	+	-	-	+	-	-	+
PA05	+	+	-	-	+	-	-	+
PA06	-	-	+	+	+	+	-	-
PA11	+	+	-	-	+	-	-	+
PA12	-	+	+	-	+	+	+	+
PA13	+	+	-	-	+	-	-	+
PA16	+	+	-	-	+	-	-	+
PA20	+	+	-	-	+	-	-	+
PA26	-	+	+	-	-	-	-	-
PA27	-	-	+	-	-	+	+	+
PA28	+	+	-	-	+	-	-	+
PA31	-	+	+	-	+	+	+	+
PA32	+	+	-	-	+	-	-	+

MR: Methyl Red, VP: Vogus Proskauer

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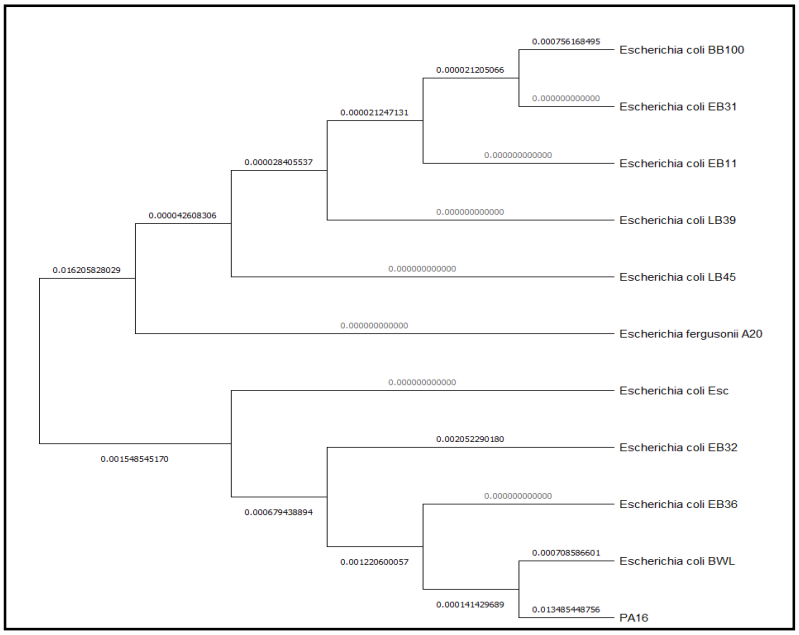

Figure 1. Isolate PA16 identified as *Escherichia coli* PA16 by constructing Phylogeny using neighbour joining method.

Antimicrobial Sensitivity Test

Table.3 Zone of inhibition measurements for each antibiotic disc:

Antibiotics and their concentrations	Isolates and Zone of Inhibition							
	PA02	PA05	PA11	PA13	PA16	PA20	PA28	PA32
Cephalothin (CEP) 30µg	01	03	02	00	00	01	00	03
Clindamycin (CD) 2µg	01	03	04	03	02	03	05	03
Co-Trimoxazole (COT) 25µg	04	02	01	03	01	02	01	00
Erythromycin € 15µg	00	02	02	05	00	02	00	02
Gentamicin (GEN) 10µg	21	20	24	18	19	20	24	23
Ofloxacin (OF) 5µg	26	29	30	29	26	28	29	31
Penicillin (P) 10 Units	02	04	02	01	00	01	00	02
Vancomycin (VA) 30µg	03	02	02	02	01	02	04	03
Ampicillin (AMP) 10µg	12	11	12	14	09	10	11	09
Chloramphenicol (C) 30µg	10	13	13	10	12	11	15	14
Oxacillin (OX) 1µg	07	05	06	03	00	02	04	01
Linezolid (LZ) 30µg	05	04	07	02	02	06	03	05
Azithromycin (AZM) 15µg	04	07	08	08	02	03	05	06
Amikacin (AK) 30µg	32	36	33	29	28	31	33	35
Clarithromycin (CLR) 15µg	14	12	15	14	09	10	12	10
Teicoplanin (TEI) 10µg	02	03	02	01	00	02	01	00
Methicillin (MET) 5µg	04	03	04	02	00	01	00	00
Amoxyclav (AMC) 30µg	05	08	04	05	03	04	03	05
Novobiocin (NV) 5µg	01	02	01	00	00	02	01	03
Tetracycline (TE) 30 µg	15	17	15	16	13	12	15	18

0mm: Resistant, 1-4mm: Intermediate, 5+mm: Sensitive

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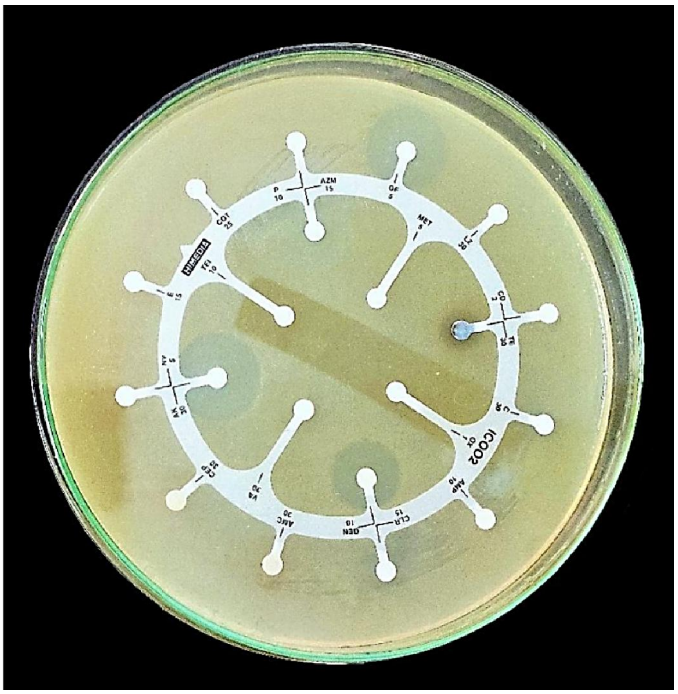

Figure 2. Antimicrobial Sensitivity Test of *E. coli* PA16 using IC002 Antibiotic Disc

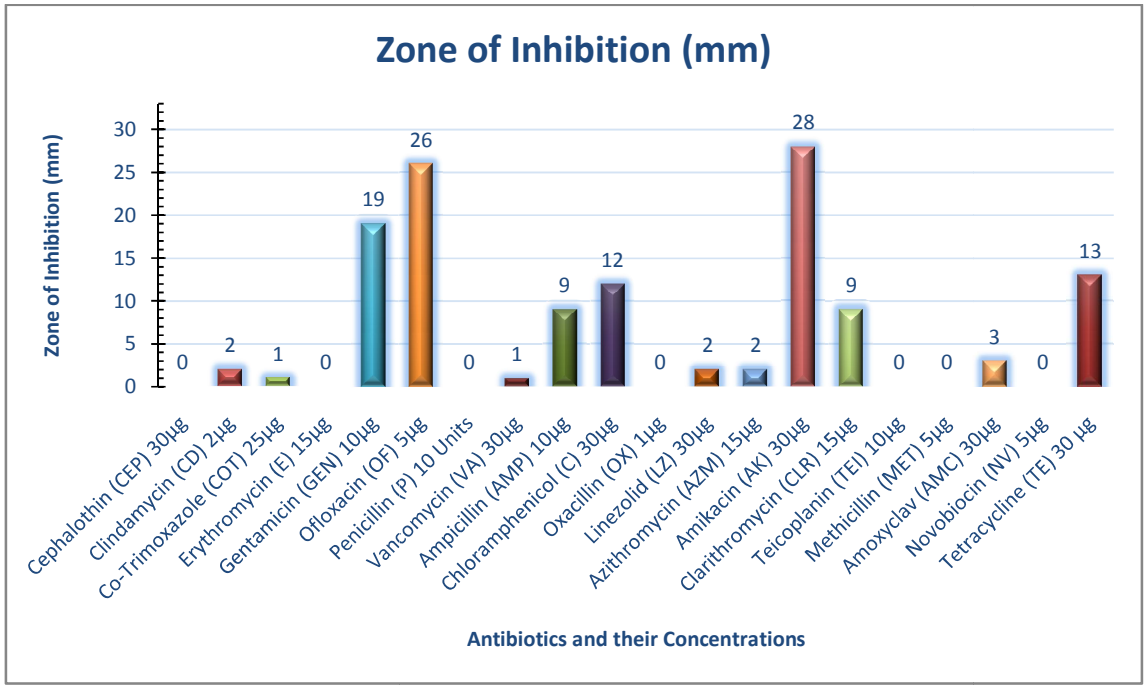

Figure.1 Zone of inhibition measurements for each antibiotic disc against *E. coli* PA16

IV. DISCUSSION

This study confirms the feasibility of isolating *E. coli* from food samples and assessing its antibiotic susceptibility profile using a standardized disc diffusion method. The detection of antibiotic resistance in a foodborne *E. coli* isolate is

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consistent with broader global findings: for instance, systematic reviews show that *E. coli* from environmental and food sources frequently carry resistance genes such as bla_{TEM}, tetA, sul1 and others (Barlaam et al., 2019). The mechanisms of resistance, including plasmid-mediated gene transfer, efflux pumps, and porin alterations, remain a major concern in *E. coli* (Narsollahian et al., 2024). The finding of resistance in a foodborne isolate underscores the One-Health dimension of antimicrobial resistance (AMR) bridging human, animal, and environmental domains (Al-Khalaifah et al., 2025).

In the present study, while the number of antibiotics tested was limited by the Octa Disc format, the detection of resistant phenotypes supports the hypothesis that food contamination may act as a reservoir of resistant *E. coli* and potentially transfer resistance to human microbiota. The high rate of resistance to certain antibiotics (e.g., penicillins, tetracyclines) reported in other foodborne *E. coli* studies (e.g., 87.8% resistance to a penicillin/novobiocin combination in Romanian raw-milk isolates aligns with our observations (Drugea et al., 2025). Moreover, environmental drivers including temperature, sanitation, and antibiotic use in agriculture have been shown to enhance selection for resistant *E. coli* (Saini et al., 2024). The sensitivity observed for some antibiotics in our isolate suggests that there remain effective agents; however, the evolving resistance trend should prompt caution and sustained surveillance.

The limitation of this study is that only one food sample was analyzed and only one *E. coli* isolate was characterized; thus the results cannot be generalized to all food samples from the region (Pormohammad et al., 2019). Future work should include a larger sample size, multiple food categories, extended panels of antibiotics (including carbapenems, colistin), and molecular detection of resistance genes (e.g., ESBL genes, mcr-1) to better characterise the resistance profile and risk of transfer to consumers.

V. CONCLUSION

This study demonstrates that *Escherichia coli* isolated from a single food sample can exhibit antibiotic resistance when tested by the disc diffusion method. The integration of morphological, biochemical and molecular identification along with antibiotic susceptibility testing offers a rigorous approach to evaluating foodborne bacterial risks. The presence of resistance in a foodborne isolate highlights the need for routine monitoring of *E. coli* in food chains and implementation of antibiotic resistance mitigation strategies within a One-Health framework. Further investigations with larger sample sets and comprehensive molecular analyses are warranted.

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