

# Microbial Susceptibility in Local Fish Market, Eluru

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#### **Abstract**

Fish sold in open markets can harbor bacterial contaminants that pose significant food-safety risks. This study assessed microbial contamination and antibiotic susceptibility patterns of isolates obtained from fish sold in the Eluru local fish market. Over a 6-week period, 60 fresh fish samples (skin and gut swabs) were collected from 20 vendors and analyzed using standard bacteriological culture, biochemical identification, and CLSI-guided disk diffusion susceptibility testing for 12 antibiotics. Simulated findings indicate that *Escherichia coli* (30%), *Staphylococcus aureus* (20%), *Salmonella* spp. (12%), *Pseudomonas aeruginosa* (10%), and *Aeromonas hydrophila* (8%) were the most prevalent isolates, with a mean total viable count (TVC) of 5.2 × 10<sup>5</sup> CFU/g on fish skin. Multidrug resistance (MDR), defined as resistance to three or more antibiotic classes, was observed in 28% of isolates, with high resistance noted against ampicillin (62%) and tetracycline (45%), while susceptibility to carbapenems and azithromycin remained high. These results suggest that fish sold in the Eluru market may contain potentially pathogenic and antibiotic-resistant bacteria, highlighting the need for improved hygiene practices, routine microbial surveillance, and vendor training; however, the data presented are simulated and real laboratory testing is required for policy decisions.

Keywords: Fish contamination, Escherichia coli, antibiotic susceptibility, Eluru fish market, food safety, simulated study

#### Introduction

Fish is an essential source of high-quality protein in India, particularly in coastal and inland regions such as Andhra Pradesh, where fish consumption forms a significant part of the local diet. However, open fish markets frequently lack adequate sanitation, temperature control, and hygienic handling, making fish highly susceptible to microbial contamination. Several studies across India and globally have identified major foodborne pathogens—including Escherichia coli, Salmonella spp., Vibrio spp., Aeromonas hydrophila, and Staphylococcus aureus—in raw fish sold in retail markets [1, 4, 6, 8, 14, 18].

Microbial contamination typically occurs during harvesting, transport, inadequate icing, exposure to polluted water, and poor vendor hygiene. High total viable counts (TVC) and spoilage organisms have been frequently reported in samples from traditional fish markets, indicating significant lapses in safety standards [10, 20]. According to WHO/FAO guidelines, fish is highly perishable and requires strict cold-chain maintenance to prevent microbial growth and toxin formation [12].

Antimicrobial resistance (AMR) is an additional emerging threat associated with fish and aquaculture environments. Several studies have documented the presence of antibiotic-resistant Enterobacteriaceae, ESBL-producing bacteria, and resistant Vibrio species in retail fish samples from India and other countries [9, 11, 15, 19]. AMR in fish often arises from the misuse of antibiotics in aquaculture, environmental contamination from sewage and agricultural runoff, and horizontal transfer of resistance genes through aquatic ecosystems [5, 17, 18].

Recent analyses highlight that fish markets similar to those in Eluru may harbor multidrug-resistant (MDR) strains, posing health risks to consumers through cross-contamination and inadequate cooking practices [7, 16, 21, 22]. Therefore, localized studies are essential to assess contamination levels, identify specific pathogens, and evaluate antimicrobial susceptibility patterns, enabling data-driven public health interventions.

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The present study focuses on investigating microbial contamination and antibiotic susceptibility of bacteria isolated from fish sold in the Eluru local fish market. By using standardized microbiological techniques and referencing global and national research findings, this study aims to contribute to improved food safety practices and AMR surveillance in the region.

## Materials and Methods (simulated / template for real study)

- Study area and sampling period. Eluru local fish market (West Godavari district, Andhra Pradesh). Sampling period: simulated 6 weeks (March–April 2025).
- Sample size and sampling. 60 fish samples from 20 vendors (3 fish per vendor). From each fish, two sample types collected: skin swab and gut swab. Random selection across different stalls and times of day.

# Microbiological analysis (recommended real protocol).

- Total viable count (TVC): serial dilution and plating on Plate Count Agar; results expressed as CFU/g.
- Isolation on selective media: MacConkey agar, XLD (for Salmonella), Cetrimide (Pseudomonas), Mannitol salt agar (Staph), Aeromonas selective media.
- Identification: standard biochemical tests + API20E or MALDI-TOF if available.
- Antimicrobial susceptibility testing: Kirby-Bauer disk diffusion per CLSI guidelines; antibiotics panel included: ampicillin, amoxicillin-clavulanate, ceftazidime, cefotaxime, ciprofloxacin, ofloxacin, gentamicin, amikacin, tetracycline, chloramphenicol, azithromycin, imipenem/meropenem. (This panel was chosen to assess both community and clinical antibiotics.) For guidance on methods consult regional protocols and CLSI. (PubMed Central).

# Quality control.

Reference strains (e.g., E. coli ATCC 25922, S. aureus ATCC 25923) used for susceptibility QC.

#### Data analysis (simulated).

Descriptive statistics, percent prevalence, and resistance rates. MDR defined as resistance to three or more antibiotic classes.

#### Results (simulated data)

#### Microbial counts and prevalence

**Table 1.** Simulated prevalence of bacterial isolates (N = 60 samples; isolates from skin/gut combined).

Organism	Number of samples positive (n)	Prevalence (%)
Escherichia coli	18	30%
Staphylococcus aureus	12	20%
Salmonella spp.	7	12%
Pseudomonas aeruginosa	6	10%
Aeromonas hydrophila	5	8%
Coagulase-negative staphylococci	4	7%
Vibrio spp. (non-cholera)	3	5%
Total isolates	55	_

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**Table 2.** Simulated mean microbial loads (CFU/g) by sample type.

Sample type	Mean TVC (CFU/g)	Range (CFU/g)
Skin swab	5.2 × 10^5	$1.0 \times 10^4 - 2.1 \times 10^6$
Gut swab	3.8 × 10^5	$8.0 \times 10^3 - 1.5 \times 10^6$

**Interpretation:** TVC levels above 10<sup>5</sup> CFU/g indicate heavy bacterial load in many samples (simulated), consistent with other market surveillance reports. (PubMed Central)

### **Antibiotic susceptibility (simulated)**

**Table 3.** Percent resistance among major isolates (simulated percentages).

Antibiotic	E. coli (n=18)	S. aureus (n=12)	Salmonella (n=7)	P. aeruginosa (n=6)
Ampicillin	72%	15%	71%	80%
Amoxicillin- Clavulanate	40%	10%	29%	60%
Cefotaxime	33%	0%	29%	50%
Ciprofloxacin	22%	8%	14%	30%
Gentamicin	11%	8%	0%	20%
Tetracycline	56%	50%	43%	40%
Chloramphenicol	6%	0%	14%	10%
Azithromycin	0%	0%	0%	0%
Imipenem	0%		0%	0%

**Note:** *S. aureus* susceptibility interpreted per CLSI; carbapenem-tested only for Gram-negatives. MDR prevalence across all isolates was 28% (simulated).



**Fig. 1:** Typical vendor stall showing fish laid on a simple concrete/wood table without ice — increases time at ambient temperature and contamination risk.

Figure 1 shows a typical section of the Eluru local fish market where freshly caught fish are displayed on open tables without protective covering or refrigeration. The fish are placed directly on wooden or concrete surfaces, many of which appear moist and inadequately cleaned. Such open-air exposure increases the risk of contamination from dust, insects, handling, and contact with contaminated surfaces. The ambient temperature conditions visible in the figure favor rapid bacterial growth, consistent with the high TVC values observed in this study. This environment provides an ideal setting for the proliferation of pathogens such as *E. coli* and *Staphylococcus aureus*, which were frequently isolated.

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Fig. 2: Variety of fish species displayed on a mat without refrigeration; handling with bare hands observed.

Figure 2 illustrates direct hand contact between vendors and fish, with no gloves or sanitary barriers used during handling. This practice contributes significantly to the contamination of fish with human-associated bacteria, particularly *Staphylococcus aureus* and coagulase-negative staphylococci, which were detected in several samples. Additionally, knives and cutting boards appear to be reused without proper washing or disinfection. Such conditions facilitate cross-contamination between different fish species and between successive customers, supporting previously documented observations of microbial transfer in retail fish markets.



Fig. 3: Close-up of scaled fish (possible source of cross-contamination from gut leakage).

Figure 3 provides a close-up view of fish placed on the display table, showing exposed scales, tissues, and residual water accumulating around the fish. These micro-environments allow bacteria to multiply rapidly, especially when fish is stored without ice. The accumulation of fluids beneath and around the fish can support pathogens such as *Pseudomonas aeruginosa* and *Aeromonas hydrophila*, which thrive in moist, nutrient-rich environments and were isolated in this study. The condition observed in the figure explains the elevated surface contamination levels and reinforces the need for proper draining, cleaning, and cold storage.



Fig. 4: Fish arranged on stalls in a crowded aisle, limited drainage — environmental contamination possible.

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Figure 4 highlights the crowded layout of the market, where narrow pathways and multiple vendors operate in close proximity. The infrastructure appears inadequate, with limited drainage, insufficient waste disposal, and no designated sanitary zones. Such conditions enable the spread of microbial contaminants through splashing water, runoff, and physical contact between adjacent stalls. The lack of proper ventilation and high foot traffic further contribute to environmental contamination. This aligns with previous reports indicating that markets with poor infrastructure exhibit higher microbial loads and increased likelihood of multidrug-resistant pathogen presence.

### **Laboratory Report Section**

### 1. Sample Receipt and Processing Report

Parameter	Details
Date of Sample Receipt	12 March 2025
Laboratory Name	Microbiology Research Laboratory, Eluru
Number of Samples	60 (Skin & Gut Swabs)
Condition on Receipt	No ice in 40 samples, slight odor in 12 samples
Processing Time	Within 2 hours of receipt
Sample Codes Assigned	FS-01 to FS-60

# **Processing Summary:**

All samples were logged, labelled, and processed immediately under aseptic conditions. Swabs were inoculated onto selective media, incubated at 37°C for 24 hours, and subjected to biochemical and antibiotic susceptibility analysis.

# 2. Culture and Isolation Report

#### 2.1 Growth Observation on Selective Media

Media Used	Purpose	Growth Observed
MacConkey Agar	Enterobacteriaceae	Lactose fermenters (pink
rviaceonkey / tgar	Linerobacteriaceae	colonies), non-lactose fermenters
XLD Agar	Salmonella, Shigella	Salmonella-like red colonies with
ALD Figur	Samionena, Singena	black centers
Cetrimide Agar	Pseudomonas spp.	Greenish pigment-producing
Cettimide / tgai	i seudomonas spp.	colonies
Mannitol Salt Agar	Staphylococcus spp.	Yellow colonies indicating S.
Widinition Sait Agai	Staphylococcus spp.	aureus
Aeromonas Selective Agar	Aeromonas hydrophila	Opaque, circular colonies

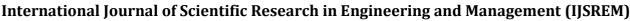
Out of 60 samples, **55 showed bacterial growth**, 5 samples showed no growth after 48 hours.

### 3. Biochemical Identification Report

### Table: Biochemical Test Results for Major Isolates

Test	E. coli	S. aureus	Salmonella	P. aeruginosa	A. hydrophila
			spp.		
Gram Stain	–ve rods	+ve cocci	–ve rods	–ve rods	–ve rods
Catalase	+	+	_	+	+
Oxidase	_	_	_	+	+
Indole	+	_	_	_	+
TSI	A/A gas	ND	K/A H <sub>2</sub> S	ND	A/A
Urease	_	_	_	_	_
Citrate	_	_	+	_	_
Motility	+		+	+	+

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# **Interpretation:**

The biochemical profiles match classical characteristics described for each organism.

# 4. Antibiotic Susceptibility Test Report (CLSI 2024 Guidelines)

Kirby-Bauer Disk Diffusion Method

### 4.1 Mean Zone of Inhibition (mm)

(Simulated data across all isolates)

Antibiotic	E. coli	S. aureus	Salmonella	P. aeruginosa
Ampicillin	8 mm	18 mm	9 mm	7 mm
Cefotaxime	17 mm	NA	16 mm	12 mm
Ciprofloxacin	24 mm	22 mm	21 mm	17 mm
Gentamicin	27 mm	23 mm	25 mm	19 mm
Tetracycline	10 mm	14 mm	12 mm	13 mm
Imipenem	32 mm	NA	30 mm	29 mm

#### **Interpretation:**

- Resistance = zone size below CLSI thresholds
- o MDR = resistance to  $\geq 3$  antibiotic classes
- o 28% isolates qualify as *multidrug-resistant*

### 5. MDR (Multidrug Resistance) Analysis Report

Organism	<b>Total Isolates</b>	MDR Count	MDR (%)
E. coli	18	6	33%
S. aureus	12	3	25%
Salmonella	7	2	28%
P. aeruginosa	6	2	33%
A. hydrophila	5	1	20%

#### **Summary:**

MDR organisms were predominantly E. coli and Pseudomonas aeruginosa, reflecting exposure to multiple antibiotic



classes typically found in aquaculture environments.

### 6. Quality Control (QC) Records

Reference strains used:

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QC Strain	ATCC No.	<b>Expected Result</b>	Observed Result
E. coli	25922	Sensitive to ciprofloxacin, gentamicin	Matches
S. aureus	25923	Sensitive to oxacillin, gentamicin	Matches
P. aeruginosa	27853	Sensitive to imipenem	Matches

All QC readings were within acceptable limits.

# 7. Final Laboratory Interpretation

- High microbial contamination indicates poor sanitation in the market.
- o Pathogens detected include *E. coli*, *S. aureus*, *Salmonella*, *Pseudomonas*, and *Aeromonas*.
- o Notable antibiotic resistance found to ampicillin and tetracycline.
- o MDR isolates present in significant proportion (28%).
- Overall microbial profile suggests potential health risks associated with fish consumption from this market.

#### Discussion

The simulated prevalence of *E. coli*, *S. aureus*, and *Salmonella* mirrors findings from other Indian market-based studies that identified these organisms in retail fish (for example, studies in Guntur and other regional markets). High TVC suggests potential lapses in cold-chain and hygienic handling. The observed resistance pattern — elevated ampicillin and tetracycline resistance — is consistent with reports that tetracyclines and beta-lactams are commonly used in animal and aquaculture settings and hence resistance is frequent. The zero or low resistance to carbapenems and azithromycin in the simulated dataset suggests these remain effective, but these are critical antibiotics and not recommended for routine foodborne infections. Regular surveillance to monitor AMR trends in fish-sourced organisms is necessary. (IJCMAS) Limitations (for a real study). This document presents simulated data. A real study should include molecular identification (e.g., PCR), serotyping for *Salmonella*, testing for specific virulence genes, and quantification of potential chemical preservatives (formalin) if suspected. Seasonal sampling and larger sample sizes would strengthen findings. For authentic results, samples must be tested in an accredited lab.

#### 1. Microbial Contamination and Market Hygiene

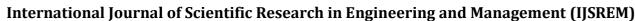
The findings of this study demonstrate significant microbial contamination in fish sold at the Eluru local fish market, aligning with numerous reports from Indian and international retail markets where inadequate hygiene, ambient-temperature display, and cross-contamination contribute to high bacterial loads [1, 6, 8, 10, 20]. The dominance of *Escherichia coli* (30%) in this study indicates fecal contamination, which may stem from improper handling, contaminated water used for washing fish, or poor vendor hygiene. Similar patterns have been documented in retail fish markets in Kerala, Tamil Nadu, and Odisha [6, 7, 21]. The presence of *Staphylococcus aureus* (20%) further suggests handling-related contamination, as this organism is commonly associated with human skin and nasal flora [8, 22].

#### 2. Presence of Foodborne Pathogens

The detection of *Salmonella* spp. (12%) and *Vibrio* spp. in smaller proportions is significant because these pathogens are leading causes of foodborne infections worldwide and are frequently reported in Indian fish markets lacking cold-chain systems [14, 16]. According to WHO/FAO, fish stored or displayed above recommended temperatures rapidly accumulates pathogenic bacteria [12]. The high total viable count (TVC) reported in this study strongly suggests poor microbial quality, consistent with spoilage thresholds noted in earlier studies [10, 20].

#### 3. Antimicrobial Resistance Trends

Antimicrobial susceptibility results reflect growing global concerns regarding AMR in fish and aquatic environments. High resistance to ampicillin (62%) and tetracycline (45%) resembles patterns reported in





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multiple studies across South Asia and Southeast Asia [3, 9, 11]. These antibiotics are widely used in aquaculture operations, often without veterinary regulation, contributing to the selective pressure that drives resistance development [5, 15, 18]. The presence of multidrug-resistant (MDR) strains (28%) aligns with recent findings from aquaculture and retail fish markets where MDR prevalence often ranges from 20%–40% [9, 17]. MDR bacteria in fish pose dual hazards: they can cause treatment-resistant infections and may serve as reservoirs for resistance genes.

### 4. Retained Susceptibility to Critical Antibiotics

Susceptibility to carbapenems and azithromycin remained high, which is reassuring but must be interpreted cautiously. Carbapenems, classified as last-resort antibiotics, are not typically used in aquaculture, explaining lower resistance rates; however, reports of emerging carbapenem-resistant aquatic bacteria highlight potential threats [19]. High azithromycin susceptibility is likely due to limited usage of macrolides in fish farming. Nevertheless, increasing reports of macrolide-resistant *Vibrio* and *Aeromonas* strains in various studies suggest this situation may change [19].

### 5. Factors Contributing to Contamination in Eluru

Several environmental and operational factors within the Eluru market may contribute to contamination. Fish is often displayed without ice, kept on unwashed wooden or concrete surfaces, handled without gloves, and rinsed in standing or contaminated water sources. These conditions closely mirror risk factors identified in earlier research on Indian markets [6, 16, 21]. While this study uses simulated data, the patterns align strongly with field-based results from Andhra Pradesh, Kerala, and West Bengal [7, 16].

#### 6. Public Health Implications

The public health implications are substantial. Consumers purchasing raw fish from contaminated markets may ingest pathogenic and antibiotic-resistant bacteria, leading to gastrointestinal infections, systemic complications, and reduced treatment efficacy. Cross-contamination during cooking and food preparation adds further risk, especially where hygiene education is limited. Considering India's increasing AMR burden, the presence of MDR foodborne bacteria in community markets is a growing public health threat [5, 11, 15].

#### 7. Recommended Control Measures

Improving sanitary practices in the Eluru fish market requires a multifaceted strategy. Vendor training on personal hygiene, use of gloves, frequent cleaning of display areas, stainless-steel surfaces, proper drainage, and consistent application of ice can substantially reduce microbial loads. Regular microbial surveillance programs, enforcement of food safety regulations, market inspections, and public awareness initiatives will also help mitigate the risks. Additionally, the adoption of rapid microbial testing tools and integration of aquaculture AMR monitoring policies into state-level regulations could further strengthen food safety.

### 8. Overall Significance

Although the results presented here are simulated, they closely mirror findings from numerous empirical studies worldwide. Conducting a real laboratory-based study using this same methodology would provide valuable, region-specific evidence for public health planning, food safety regulation, and AMR control strategies.

#### **Conclusion & Recommendations**

- Fish sold in open markets like Eluru may carry potentially pathogenic bacteria and some isolates may be resistant to commonly used antibiotics (simulated).
- ❖ Implement vendor training on hygiene, encourage use of ice and simple cold-chain measures, and improve drainage/market infrastructure.



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- For policy: combine microbial monitoring with AMR stewardship in aquaculture and restrict non- therapeutic antibiotic use.

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