

## Prevalence and Antimicrobial Susceptibility Pattern of *E.Coli* Isolated From Raw Meat Samples in the Local Markets of Peshawar

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### ABSTRACT

**Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs. Contaminated raw meat is one of the main sources of food-borne illnesses. The present study was carried out to isolate, identify and the prevalence of *E.coli* from raw meat samples. Antibiotic susceptibility pattern of the isolated bacterial species. The study was conducted at Abasyn University, Peshawar. In the present study 50 raw meat samples were collected from local markets of Peshawar. Out of 50 samples 40 samples showed significant bacterial growth. The most common bacterial species isolated were *E. coli* and *Salmonella spp.* Among these identified isolates *E.coli* counted for 35 samples and *Salmonella spp.* counted for 5 samples. The prevalence of *E coli* was (87%) and *Salmonella spp.* was (13%). The susceptibility of *E.coli* and *salmonella spp.* isolates from meat samples against various antimicrobial agent (Cefixime, Ciprofloxacin, Cefoperazone, Ceftriaxone, Moxifloxacin, Mermopene and Vancomycin) was determined by disc diffusion method. *E.coli* was sensitive mostly to Moxifloxacin and *Salmonella spp.* were found sensitive to Cefixime and mostly resistance towards Meropenem. The results have also established retail meats represent hazards to human health and can be a threat to public health. On account of this, it is necessary that the consumers adopt the basic instructions regarding good hygienic practices,**

## INTERODUCTION

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs. Contaminated raw meat is one of the main sources of food borne illnesses. Meat surfaces are prone to colonization by a wide variety of microorganisms present in its production and processing environment. Microbial contamination of raw meat results from processing and starts during slaughter, when the carcass becomes contaminated with microorganisms residing on external surfaces, the gastrointestinal tract and lymph nodes of the animal and in the plant environment. The *Enterobacteriaceae* group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. *Salmonella*, *Escherichia coli* (*E. coli*), *Proteus* and *Klebsiella* species are the most predominant species in all food poisoning cases associated with some meat products. *E. coli* is the most important member of the family *Enterobacteriaceae* and probably the best-understood organism. It has been incriminated as the aetiological agent of food poisoning involving diverse foods such as raw milk, cream, creamed fish, dates, vegetables, uncooked or poorly cooked meat and poultry. The main source of contamination of this organism is apparently beef. Several strains of *E. coli* have emerged as the potent food-borne pathogens ( Mohammed *et al.*, 2014). Food-borne illnesses are a worldwide public health concern and a significant cause of reduced economic growth. These illnesses can result from consumption of food contaminated with pathogenic organisms or toxic chemicals. Different organisms can cause food-borne infections, the most common bacterial agents being *Salmonella*, *Campylobacter*, and diarrhea-genic *Escherichia coli*. Each year, *Salmonella* and *E. coli* O157:H7 are estimated to each cause, respectively, 1.3 million and 62,000 cases of gastroenteritis in the United States. Also in developing countries, these bacteria are a public health concern and are a common source of medical complications, especially in human immunodeficiency virus-infected patients. Contaminated raw meat and meat products are considered major vehicles for transmitting food-borne pathogens to humans ( Kagambega *et al.*,2011). Food safety is an increasingly important public health issue. Unsafe food causes many acute and life-long diseases, ranging from diarrheal diseases to various forms of cancer. Food-borne and waterborne diarrheal diseases kill round about 2.2 million people yearly; 1.9 million of them are children. Therefore, food-borne diseases impose a significant burden on society in both developed and developing countries. Meat has traditionally been viewed as a vehicle for a significant proportion of human food-borne disease. Meat can be contaminated with a variety of pathogens and spoilage bacteria and it would be

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difficult to monitor each of these organisms in a meaningful way. Indicator organisms such as *Escherichia coli* are groups of bacteria that indicate the possible presence of organisms of concern, and may point to the origins of microbial contamination (Eyy *et al.*, 2012). *E. coli* is a normal and healthy part of the intestinal micro-flora of many animals, including humans. However, some strains can cause diseases. Verocytotoxigenic *E. coli* including serotype O157:H7 are one such group, causing severe, chronic, and potentially fatal illness, related to their ability to produce one or more toxins known as verotoxin or shiga-like toxin. Consumption of raw or undercooked foods of bovine origin, especially undercooked minced beef and unpasteurized cow's milk, has been the most common means of transmitting VTEC organisms in sporadic cases and in outbreaks of VTEC infection. (Cagney *et al.*, 2004). Meat is an integral component of a balanced diet, providing proteins, essential amino acids and various micronutrients such as vitamin B12, niacin, vitamin B6, iron, zinc and phosphorous. Consequently, meat production has increased rapidly over the past 50 years worldwide. Cattle meat production has more than doubled since the 1960s, increasing from 28 million tons in 1961 to 68 million tons in 2014 (Kassem *et al.*, 2020). *E. coli* is one of the major food-borne bacterial pathogen. Majority of the *E. coli* are non-pathogenic but few of them are highly pathogenic causing watery and bloody diarrhea e.g., *E. coli* O157:H7 which is associated with life threatening disease such as hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Mohamed *et al.*, 2014). The aim of the current study was to isolate *E. coli* bacteria from raw meat samples, Prevalence and Antimicrobial Susceptibility Pattern.

## MATERIALS AND METHODS

### Samples Collection

This study was conducted at Microbiology Laboratory of the Abasyn University Peshawar from November 2020 to May 2021. A total of Fifty (50) raw meat samples were collected during this period. Samples were collected in sterile bags for culture with aseptic techniques before initiating the research work at laboratory. These samples were collected from the local markets of peshawar and transfer to the Microbiology Laboratory of Abasyn University Peshawar.

### Samples Processing

In the current study, a total of 50 raw meat sample were processed that were collected from local markets of Peshawar, Pakistan. The strains were inoculated on Eosin Methylene Blue (EMB) agar plates. The streaked plates were incubated at 37°C for 24hrs. The bacterial growth was later Gram stained. Characterization of bacterial isolates was based on standard microbiological methods. Identification of isolates were done based on colony morphology and biochemical tests like Catalase test, Oxidase test, Coagulase test, triple sugar iron sugar iron agar, Urease test, Citrate utilization test and Indole test.

### Antimicrobial Susceptibility Test

For determination of antibiotic susceptibility profile of strains, well diffusion method was adapted. Isolated bacteria were inoculated on MHA plates with the help sterile swab to create uniform bacterial lawn. The panel Antibiotics include (Cefixime, Ciprofloxacin, Cefoperazone, Ceftriaxone, Moxifloxacin, Mermopene, and Vancomycin).

### RESULTS

A total of 50 raw meat samples were collected. Among them, 40 samples positive and 10 samples were negative obtained from Peshawar local markets as shown in figure.

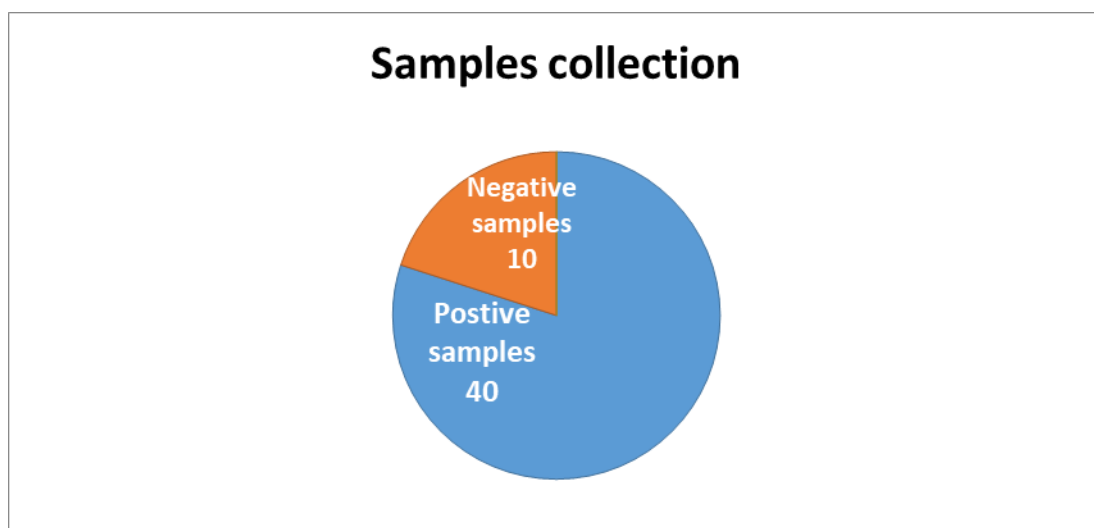


Figure1. Show the Sample collection

After cultural characteristics, a complete identification of these bacterial samples was carried out by performing biochemical tests.

Table 1 Biochemical tests of isolated bacterial species.

Isolates	Catalase	Oxidase	Coagulase	Urease	Indole	TSI test	Gas	H2S	Butt/slope	Citrate	Identified Organisms
1	+	-	-	-	+	+	+	-	Yellow	-	<i>E.coli</i>
2	+	-	-	-	-	+	-	+	Yellow	-	<i>Salmonella spp.</i>

Among the 40 positive samples that were grown a total of 2 bacterial species were identified. Among these identified isolates *E.coli* counted for 35 samples and *Salmonella spp.* counted for 5 samples

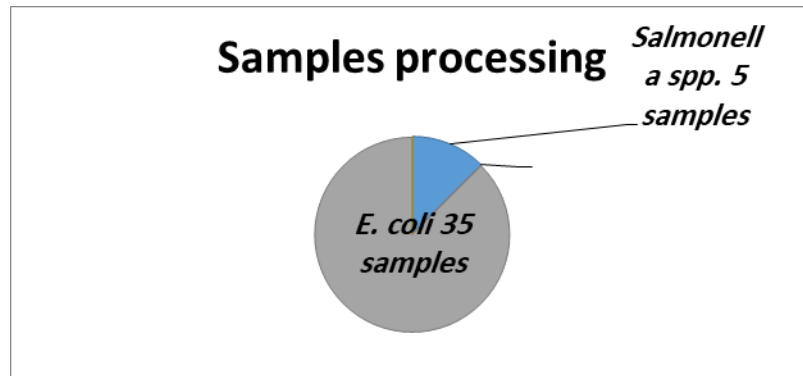


Figure 2. Show the Sample processing

Among the 40 positive samples that prevalence of 2 bacterial species were identified. Among these identified isolates *E.coli* counted for (87%) and *Salmonella* spp. counted for (13%)

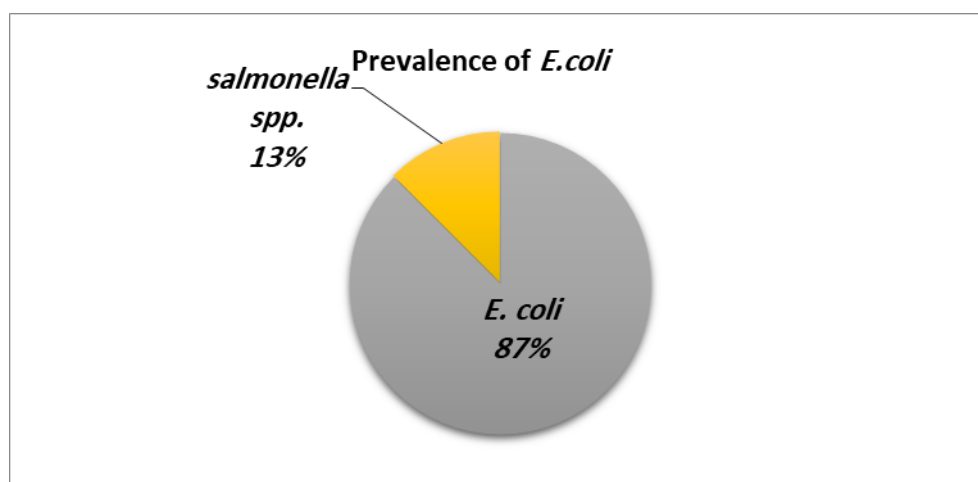
Figure 3. Show the Prevalence of *E.coli*

Table 2 Antimicrobial Susceptibility Pattern

S.NO	Antibiotics	<i>E.coli</i>	<i>Salmonella</i> spp.
1	Cefixime (CTX)	26mm	30mm
2	Ciprofloxacin (CIP)	21mm	28mm
3	Cefoperazone (CFP)	25mm	15mm
4	Ceftriaxone (CRO)	25mm	R
5	Moxifloxacin(MOX)	27mm	R
6	Meropenem (MEM)	20mm	R
7	Vancomycin (V)	18mm	R

The susceptibility of *E. coli* and *salmonella* spp. isolates from meat samples against various antimicrobial agent (Cefixime, Ciprofloxacin, Cefoperazone, Ceftriaxone, Moxifloxacin,

Mermopene, and Vancomycin) was determined by disc diffusion method. *E.coli* was sensitive mostly to Moxifloxacin and *Salmonella spp.* were found sensitive to Cefixime and mostly resistance towards Meropenem.

## DISCUSSION

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs. Contaminated raw meat is one of the main sources of food-borne illnesses. The present study was carried out to isolate, identify and prevalence of *E.coli* along with finding out the antibiotic susceptibility pattern of the isolated bacterial species.

In the current research work 50 out of 40 samples show positive growth on EMB Media. Number of 2 *Enterobacteriaceae* bacterial strains such as *E.coli* and *Salmonella* were isolated from raw meat samples which were collected from local markets of Peshawar Pakistan. The prevalence of *E.coli* was noticed to be higher (87%) which is in agreement with the report of (Eyy et al.,2012) who reported about the prevalence of *E.coli* (87.5%) and (Kagambega et al.,2011) who also reported about the prevalence of *E. coli* to be 87%. The current study showed the prevalence of *Salmonella spp.* (13%). The results are in resemblance with the work done by (Kagambega et al.,2011) who also reported the occurrence ratio for *Salmonella spp.*(9.3%). In the present study the prevalence of *E. coli* isolated from raw meat samples was higher (87%) than *Salmonella spp.* (13%) which was supported by (Davis et al.,2018) who also reported nearly the same prevalence of *E. coli* (88%) and *Salmonella spp.* (10%) in raw meat. The susceptibility of *E.coli* isolates from meat samples against various antimicrobial agent (Cefixime, Ciprofloxacin, Cefoperazone, Ceftriaxone, Moxifloxacin, Mermopene and Vancomycin) was determined by disc diffusion method. In the present study it was concluded that Moxifloxacin is the drug of choice against *E. coli* it gives (91.6%) susceptibility against *E.coli*. These finding were supported by the report of (Schedletzky et al.,1999) who also reported nearly the same susceptibility pattern (90.3%). The susceptibility of *Salmonella spp.* isolates from meat samples against various antimicrobial agent (Cefixime, Ciprofloxacin, Cefoperazone, Ceftriaxone, Moxifloxacin, Mermopene and Vancomycin) was determined by disc diffusion method. In the present study it was concluded that Cefixime is the drug of choice against *Salmonella spp.* it gives (100%) susceptibility against *Salmonella spp.* this finding is nearly supported by (Matsumoto et al.,2001) who also reported nearly the same susceptibility pattern (90%). Cefixime is the drug of choice against *E. coli* it gives (90.6%) susceptibility against *E.coli*. These finding were supported by the report of (Arshad et al.,2012) who also reported nearly the same susceptibility pattern (90 %). Mermopene is the drug choice against *Salmonella spp.* Mermopene show resistance towards *Salmonella spp.*.These finding is also nearly supported by (Jones et al.,2002) who also reported nearly the same resistance towards *Salmonella spp.* Cefoperazone is the drug choice against *E. coli* it gives (88.6%) susceptibility against *E.coli*. These finding were supported by the report of

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(Matsumoto *et al.*,2001) who also reported nearly the same susceptibility pattern (90%).Vancomycin is the drug choice against *Salmonella spp.* Vancomycin show resistance towards *Salmonella spp.* These finding is also nearly supported by (Jones *et al.*,2002) who also reported nearly the same resistance towards *Salmonella spp.* In our research study, Moxifloxacin and Cefixime both were active against *E.coli.* and *Salmonella spp.* and gives (100%) susceptibility against *E.coli.* and *Salmonella spp.*.This work is also done by (Schedletzky *et al.*,1999) who also reported nearly the same susceptibility of *E. coli* and *Salmonella spp.*

## Conclusion

The present study was carried out to isolate, identify and prevalence of *E. coli* along with finding out the antibiotic susceptibility pattern of the isolated bacterial species. The result of current work showed that the most common bacterial strains isolated from raw meat samples were *E. coli* and *Salmonella spp.* Out of 50 samples 40 samples were positive samples. Among these 40 samples, that were grown a total of 2 bacterial species were identified. Among these identified isolates *E.coli* counted for 35 samples and *Salmonella spp.* counted for 5 samples. The prevalence of *E coli* was (87%) and *Salmonella spp.* was (13%). The susceptibility of *E. coli* and *salmonella spp.* isolates from meat samples against various antimicrobial agent (Cefixime, Ciprofloxacin, Cefoperazone, Ceftriaxone, Moxifloxacin, Mermopene, and Vancomycin) was determined by disc diffusion method. *E.coli* was sensitive mostly to Moxifloxacin and *Salmonella spp.* were found sensitive to Cefixime and mostly resistance towards Meropenem.

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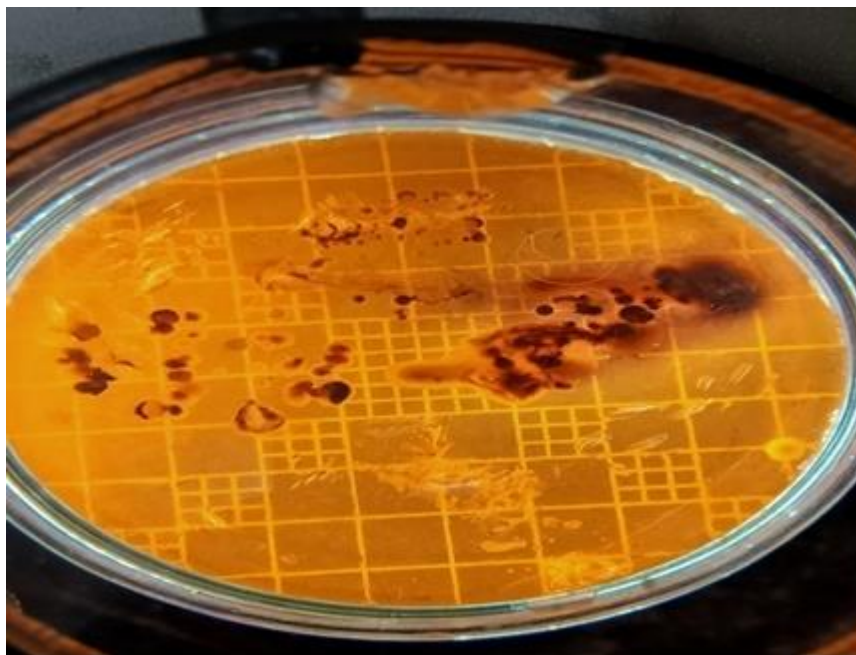
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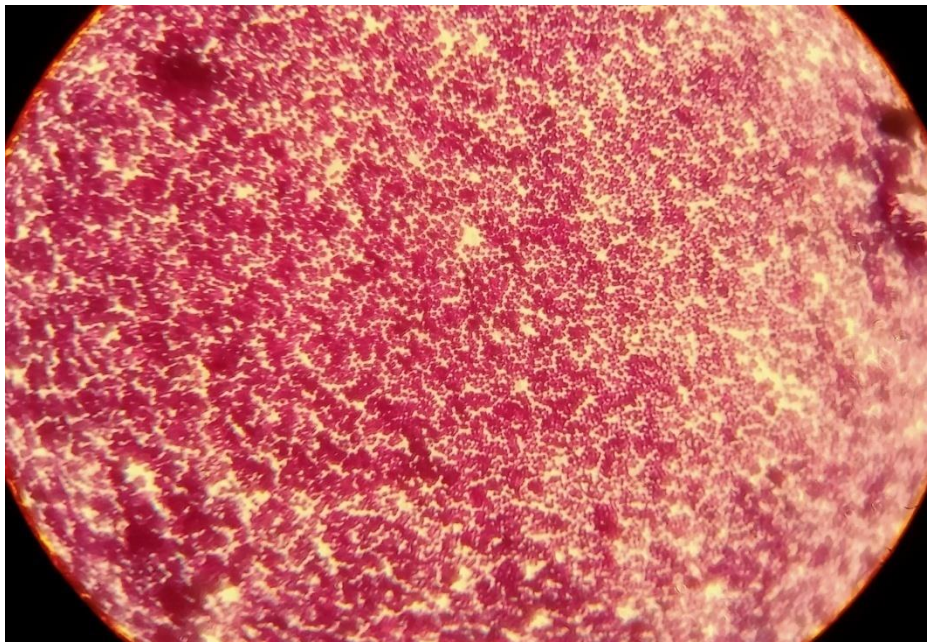
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## VIII.ANNEXURE



Growth on EMB



Gram negative



Biochemical Tests

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Citrate negative

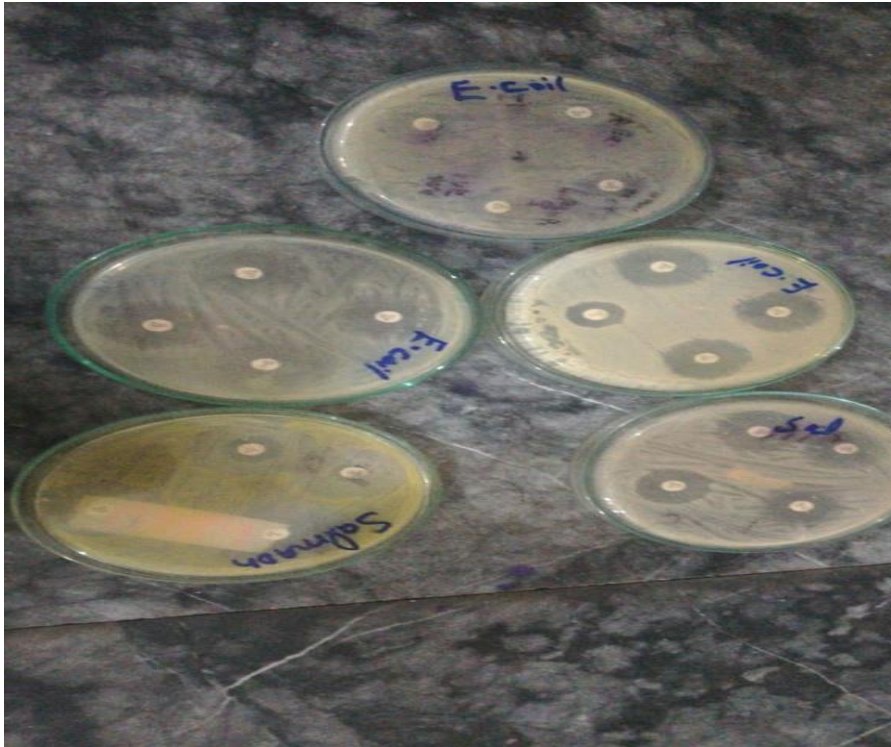


Catalase Positive



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Use of Antibiotics