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Detection of Antibacterial Activity of Apple Cider Vinegar against Escherichia Coli Isolated from Patients Visiting Tertiary Care Hospital

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ABSTRACT

Escherichia coli, a common commensal gut bacterium, can cause severe infections when found outside the digestive tract, including pneumonia, bacteremia, and UTIs. Its presence in hospitals and contaminated food highlights its role in nosocomial and foodborne diseases. The rise of multidrug-resistant (MDR) E. coli, driven by horizontal gene transfer, complicates treatment, necessitating alternative therapies to counter ineffective antibiotics and associated side effects. Apple cider vinegar (ACV) is recognized for its potential health benefits. Its organic acids, particularly acetic acid, exhibit antimicrobial properties by penetrating bacterial cell membranes and causing cell death. Hence, this study was undertaken to detect the antibacterial activity of Apple Cider vinegar with different concentrations against Escherichia coli isolated from patient vising tertiary care hospital. Total number of 250 isolates of E. coli were isolated from the patient's samples like sputum, blood, endotracheal secretions, urine, pus, tissue, wound swab, high vaginal swab and other body fluids. Patient's age group ranging from 18- 80 years were included in this study, with 113 MDR isolates of E. coli. Maximum isolates were found in ward. Out of 250 Isolates of E. coli maximum isolates showed highest susceptibility to 100% concentration of ACV with mean zone of 25.90 ± 2.41. These findings suggest that apple cider vinegar can be used as an effective adjuvant therapy.

Keywords: Multidrug-Resistant, E. coli, Apple cider vinegar

INTRODUCTION

The gram-negative bacillus Escherichia coli, or E. coli, is recognized to be a normal component of intestinal flora but can also cause intestinal and extra intestinal illnesses in people. The hundreds of E. coli strains that have been identified cause a wide range of illnesses, from septic shock and kidney failure to mild, self-limited gastroenteritis. E. coli is present in hospital and long-term care facility floors and is a component of commensal gut flora. The most prevalent gram-negative bacterium in the human digestive system, E. coli, is not virulent in this environment. Nevertheless, E. coli can cause a number of illnesses when it is discovered outside of the digestive tract, including pneumonia, bacteremia, peritonitis, and urinary tract infections (UTI). One of the main causes of

nosocomial infections, such as ventilator-associated pneumonia (VAP) and catheter associated UTIs, is E. coli. In addition, E. coli is present in water, vegetables, dirt, and undercooked foods. When consumed by humans, pathogenic strains result in intestinal disease. [1]

Escherichia coli multidrug resistance has grown to be a concerning problem in human medicine across the globe. E. coli has a high potential to acquire resistance genes, primarily by horizontal gene transfer, despite being inherently vulnerable to practically all clinically important antimicrobial treatments. [2] Multidrug resistance (MDR) has rapidly spread throughout E. coli populations, making infections more difficult and expensive to treat, which has made the problem even worse. [3] Since therapy entails several rounds of ineffective antibiotics combined with undesirable side effects, alternative therapies are essential. [4]

Apple Cider Vinegar is made from cider with a comparatively low acidity (5% acetic acid) that has undergone acetous bioconversion. Organic acids, flavonoids, polyphenols, vitamins, and minerals are also included in it. As a supplement, ACV has been praised for its ability to help with weight loss, hyperlipidemias, hypercholesterolemia, blood pressure reduction, antioxidant defense, and nutritional 2 supports. The use of organic acids as dietary supplements has been thought to be safe and effective in getting rid of dangerous gut flora. [5]

The organic acids in vinegar, particularly acetic acid, penetrate microorganisms' cell membranes and cause bacterial cell death. Temperature, pH, ionic strength, acid concentration, and bacterial strains all affect how effective organic acids are as antimicrobials. [6]

Therefore, this study was undertaken to for detection of antibacterial activity of apple cider vinegar against 250 isolates of E. coli isolated from clinical samples such as Blood, Sputum, Pus, Urine, Endotracheal secretions, wound swab, High vaginal swab and other body fluids.

MATERIALS AND METHODS

Preparation of dilutions of ACV: Twofold serial dilutions of the apple cider vinegar were prepared using sterile distilled water such as 100% (Neat), 50%, 25%, 12.5%, 6.25%, 3.12% and 1.56%.

Screening of antibacterial activity of ACV: The agar well diffusion method was used to assess the antibacterial activity of apple cider vinegar. Each isolate was added to 0.9% sterile peptone water and adjusted to 0.5 McFarland standard turbidity. The bacterial suspension was applied to sterilized Muller Hinton agar plates using a cotton swab. Sterile pipette tips were used to create wells measuring 4mm in height and 6mm in diameter [7, 8].

After that, the wells were cut and filled with different amounts of apple cider vinegar (100 microliters each). Positive and negative controls included enrofloxacin (64 µg/mL) and sterile distilled water, respectively. The plates were incubated at 37 °C for 24 hrs. After incubation, the inhibitory zone diameter was measured with a ruler. [9]

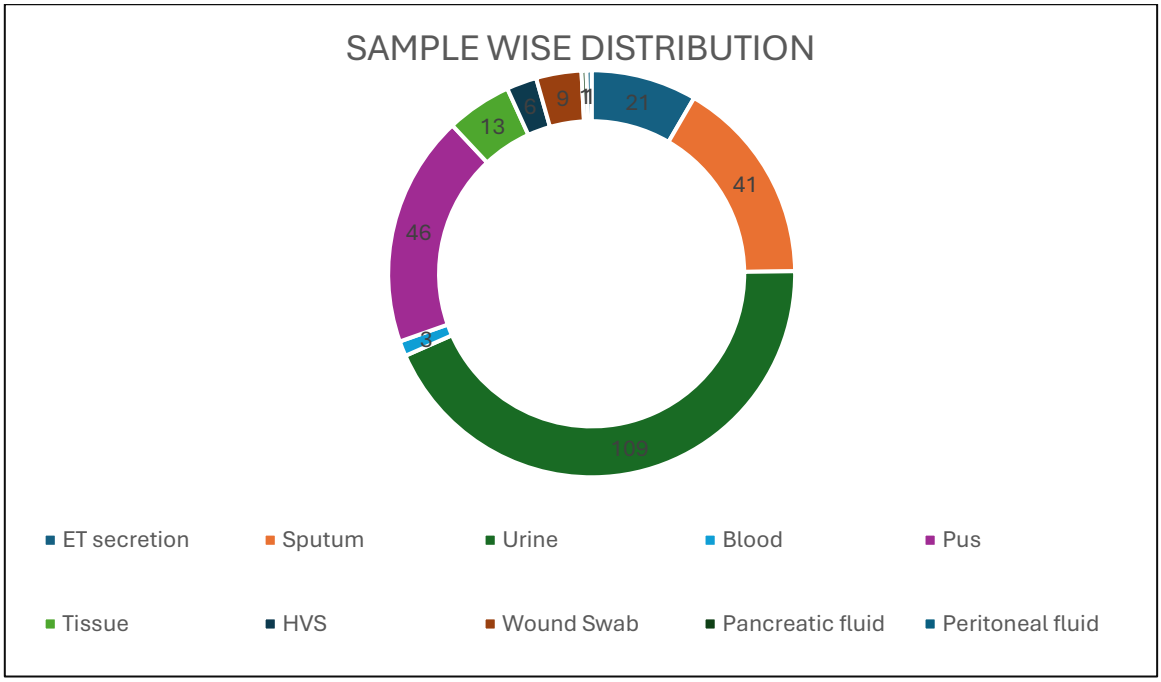
Statistical analysis: The mean diameter of zone of inhibition ± standard deviation (SD) was used to represent the results.

RESULTS

Table 1: Sample wise distribution of *E. coli* isolate

SAMPLE	NO. OF ISOLATE	PERCENTAGE
ET secretion	21	8.4%
Sputum	41	16.4%
Urine	109	43.6%
Blood	3	1.2%
Pus	46	18.4%
Tissue	13	5.2%

HVS	6	2.4%
Wound Swab	9	3.6%
Pancreatic fluid	1	0.4%
Peritoneal fluid	1	0.4%

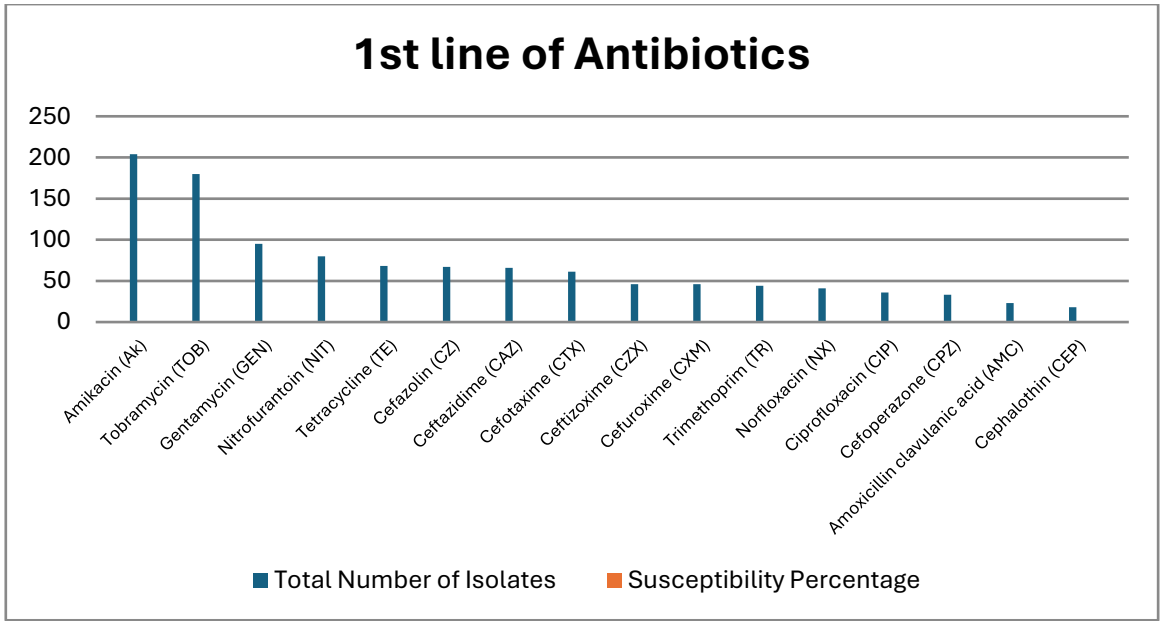


Graph 1: Sample wise distribution of *E. coli* isolate

Maximum isolates of *E. coli* were found in urine sample (43.6%)

Table 2: Antimicrobial Susceptibility Testing of *E. coli* for 1st Line Antibiotics

Antibiotics	Total Number of Isolates	Susceptibility Percentage
Amikacin (Ak)	204	81.6%
Tobramycin (TOB)	180	72%
Gentamycin (GEN)	95	38%
Nitrofurantoin (NIT)	80	32%
Tetracycline (TE)	68	27.2%
Cefazolin (CZ)	67	26.8%
Ceftazidime (CAZ)	66	26.4%
Cefotaxime (CTX)	61	24.4%
Ceftizoxime (CZX)	46	18.4%
Cefuroxime (CXM)	46	18.4%
Trimethoprim (TR)	44	17.6%
Norfloxacin (NX)	41	16.4%
Ciprofloxacin (CIP)	36	14.4%
Cefoperazone (CPZ)	33	13.3%
Amoxicillin clavulanic acid (AMC)	23	9.2%
Cephalothin (CEP)	18	7.2%

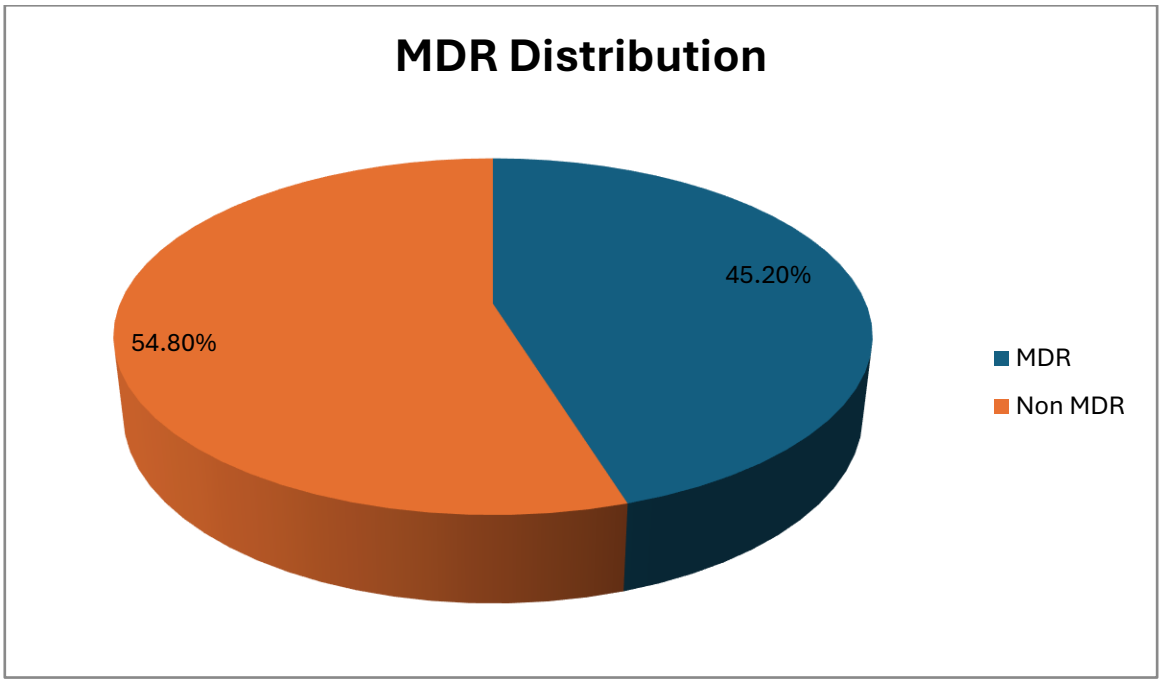


Graph 2: Antimicrobial Susceptibility Testing of *E. coli* for 1st Line Antibiotics

Maximum isolates of *E. coli* were susceptible to Amikacin (81.60%).

Table 3: MDR isolates of *E. coli* from clinical samples

Type of isolates	Number of isolates	Percentage
MDR	113	45.2%
Non MDR	137	54.8%

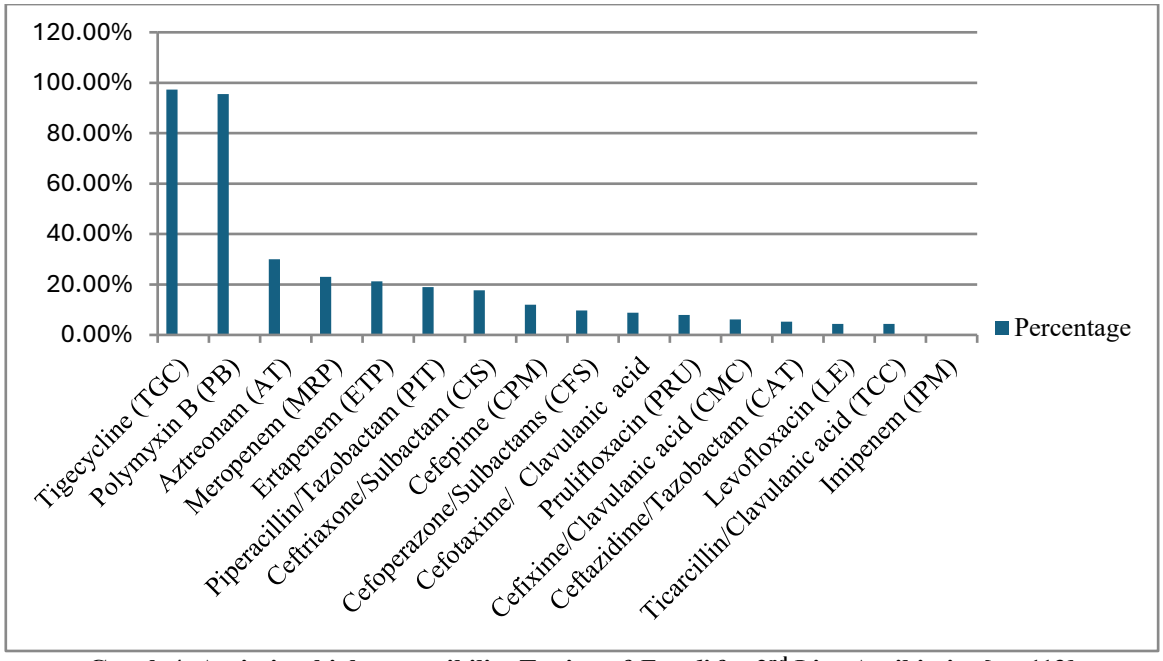


Graph 3: MDR isolates of *E. coli* from clinical samples

Maximum number of MDR isolates was (45.20%)

Table 4: Antimicrobial susceptibility Testing of *E. coli* for 2nd Line Antibiotics [n= 113]

Antibiotics	No. of Isolates Sensitive	Percentage
Tigecycline (TGC)	110	97.34%
Polymyxin B (PB)	108	95.57%
Aztreonam (AT)	34	30%
Meropenem (MRP)	26	23%
Ertapenem (ETP)	24	21.23%
Piperacillin/Tazobactam (PIT)	21	18.58%
Ceftriaxone/Sulbactam (CIS)	20	17.69%
Cefepime (CPM)	14	12.38%
Cefoperazone/Sulbactams (CFS)	11	9.73%
Cefotaxime/ Clavulanic acid (CEC)	10	8.84%
Prulifloxacin (PRU)	9	7.96%
Cefixime/Clavulanic acid (CMC)	7	6.19%
Ceftazidime/Tazobactam (CAT)	6	5.30%
Levofloxacin (LE)	5	4.42%
Ticarcillin/Clavulanic acid (TCC)	5	4.42%
Imipenem (IPM)	0	0%

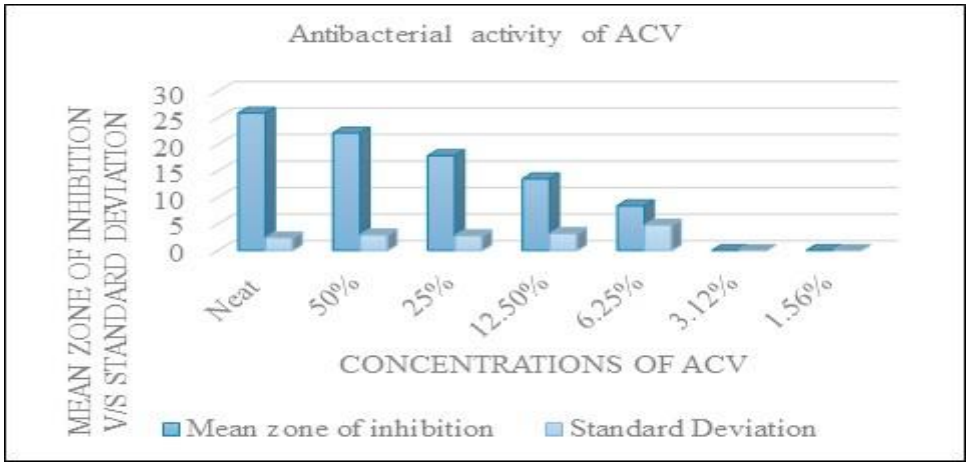


Graph 4: Antimicrobial susceptibility Testing of *E. coli* for 2nd Line Antibiotics [n= 113]

Maximum MDR isolates of *E. coli* were susceptible to Tigecycline (97.34%)

Table 5: Antibacterial activity of Apple Cider Vinegar on isolates of *E. coli* (n= 250)

Vinegar concentration	Mean zone of inhibition	Standard Deviation
100%	25.90	±2.41
50%	22.15	±2.95
25%	17.86	±2.79
12.50%	13.51	±3.20
6.25%	8.41	±4.80
3.12%	-	-
1.56%	-	-



Graph 5: Antibacterial activity of Apple Cider Vinegar on isolates of *E. coli*

Maximum isolates of *E. coli* showed susceptibility to 100% concentration of apple cider vinegar.

DISCUSSION

Several research have been conducted in an attempt to develop a treatment for MDR organisms. Despite efforts to boost the hunt for novel medicines, researchers are still unable to keep up with the rising rate of resistance. In cases of bacterial infection, this condition increases mortality and morbidity rates while also making it more difficult to determine the appropriate course of therapy. [10]

Organic acids Acetic acid, for example, has a lasting effect that stops hazardous germs from multiplying. Organic acids' ability to release protons H+ into cells reduces intracellular pH, resulting in the death of microbial membrane cells. [4,5] Vinegar's cocktail structure allows it to inhibit the growth of many organisms through the synergy of its active ingredients, demonstrating its antibacterial effectiveness. [10]

In this study the different apple cider vinegar concentrations were prepared, from which the highest susceptibility was seen to 100% with mean 25.90 and standard deviation of ± 2.41. For 3.12% and 1.56% *E. coli* were resistant. Similar results were recorded in the study by Yagnik D et al. where growth of *E. coli* was significantly inhibited at the 1/50 dilution of apple cider vinegar. [11] On the other hand, in the study by Kirtana Gopalswamy they prepared different dilutions of apple cider vinegar for MIC method, where they found no activity of apple cider vinegar. Also, they concluded that the higher concentrations of apple cider vinegar may be effective. [12]

CONCLUSION

- Total 250 non- repetitive isolates of *Escherichia coli* were obtained from the clinical samples such as Blood, Urine, Sputum, ET Secretion, High Vaginal Swab, Pus, Wound Swab, Tissue, Body fluids from the patients visiting tertiary care hospital.
- From the total 250 isolates 45.2% isolates were found to be MDR (Multi Drug Resistant).
- Among all the concentrations of apple cider vinegar, maximum isolates showed highest susceptibility to 100% concentration.
- With increase in the incidence of MDR *E. coli*, Apple Cider Vinegar can be used as an adjuvant in the treatment of infections caused by *E. coli* to minimize the burden of the disease and morbidity associated with the disease.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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