

# ANTIBACTERIAL ACTIVITY OF APPLE CIDER VINEGAR AGAINST CLINICAL ISOLATES OF ESCHERICHIA COLI

Kirtana Gopalsamy<sup>1</sup>, Dr. P. Gopinath<sup>2\*</sup>

<sup>1</sup>BDS 2nd year, Saveetha Dental College, Chennai.

<sup>2\*</sup>Senior Lecturer, Department of Microbiology, Saveetha Dental College, Chennai.

## ABSTRACT

**AIM:** To assess the antimicrobial effect apple cider vinegar against Escherichia coli

**OBJECTIVE:** To determine the antimicrobial activity by using disk diffusion and MIC method.

**BACKGROUND:** E.coli Is gram negative gamma proteobacterium which is usually found in the lower intestine of warm blooded organisms. Most of the strains are harmless but some can cause bloody diarrhoea. Some strains may cause severe anemia or kidney failure while the others may cause urinary tract infections or others. Apple cider vinegar is anti-viral, anti-microbial and anti-septic which has been used for medicinal purposes for at least 12,000 and is also known as "cure-all cleanser"

**REASON:** even though the apple cider vinegar is rich in enzymes, potassium and alkaliser. However, the FDA doesn't recognise apple cider vinegar to having any medical properties and its uses. Thus, this research aims in creating awareness and promoting the value of this substance.

## INTRODUCTION

Apple cider vinegar also known as cider vinegar is a type of vinegar made from cider or apples and has a pale to medium amber. Unpasteurised or organic apple cider vinegar contains mother of vinegar. It is made by the process of crushing apples and squeezing out the liquid. Bacteria and yeast are added to the liquid to start the alcoholic fermentation process, and the sugars are turned into alcohol. In the second fermentation process, the alcohol is mainly converted into vinegar by acetic acid-forming bacteria. Acids such as Acetic acid and malic give vinegar its sour taste.[1]

Vinegar is mainly used as a food preservative but also has high medicinal properties.

Based on the writings of US medical practitioners going back to 18th century, many ailments, from dropsy to poison ivy, and stomachache, were treated with vinegar [2]

The commercial vinegar production is done with each by fast or slow fermentation processes. For the faster fermentation processes the liquid is oxygenated by agitation and the bacteria culture is submerged permitting rapid fermentation. Slow methods are generally used for the production of the traditional wine vinegars, and the culture of acetic acid bacteria grows on the surface of the liquid and fermentation proceeds slowly over the course of weeks or months. The longer fermentation period allows for the accumulation of a nontoxic slime composed of yeast and acetic acid bacteria, this is called as the *mother* of vinegar. [3]

It has traditionally been used for cleaning and disinfecting, treating nail fungus, lice, warts and ear infections.

However, many of these applications have currently not been confirmed by research.

Hippocrates, the father of modern medicine, used vinegar for wound cleaning over two thousand years ago.

Vinegar has been used as a food preservative, and studies show that it inhibits bacteria (like E. coli) from growing in the food to spoiling it [4-7].

Apart from this they also help in lowering blood sugar levels and cure diabetes to an extent.

Escherichia coli is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. The name of a germ, or bacterium, that lives in the digestive tracts of humans and animals [8].

Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K<sub>2</sub> [11], and preventing colonisation of the intestine with pathogenic bacteria [12,13].

## MATERIALS AND METHODS:

### Bacterial isolates:

A total of 20 non repetitive urinary isolates of *Escherichia coli* were collected from Saveetha Medical College and Hospitals, Chennai. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy broth stock and were stored at 4 °C until further use.

### Antibiotic susceptibility testing:

Antibiotic susceptibility test was determined for these isolates to routinely used antibiotics such as ampicillin, amoxicillin, amikacin, norfloxacin, ceftazimide, cefotaxime, ciprofloxacin and gentamicin, imipenem as by Kirby Bauer disc diffusion method.

### Detection of antibacterial activity of apple cider vinegar against clinical isolates of *E.coli*:

- Antibacterial activity of apple cider vinegar was tested against *E. coli* isolates by minimum inhibitory concentration method. Agar dilution method was performed to attain the different concentrations of apple cider vinegar such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% in MHA. Media containing various concentrations of vinegar were poured over the sterile petridishes and allowed to dry. Media without vinegar was served as control plate. Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37°C overnight.
- The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC.

## RESULTS:

### Sample wise distribution of clinical isolates of *E.coli*:

Of the 20 clinical isolates of *E.coli*, 12/20 (60%) were from acute urinary tract infections and 8/20 (40%) were from chronic urinary tract infections. Figure 1 depicts the sample wise distribution of clinical isolates of *E.coli*.

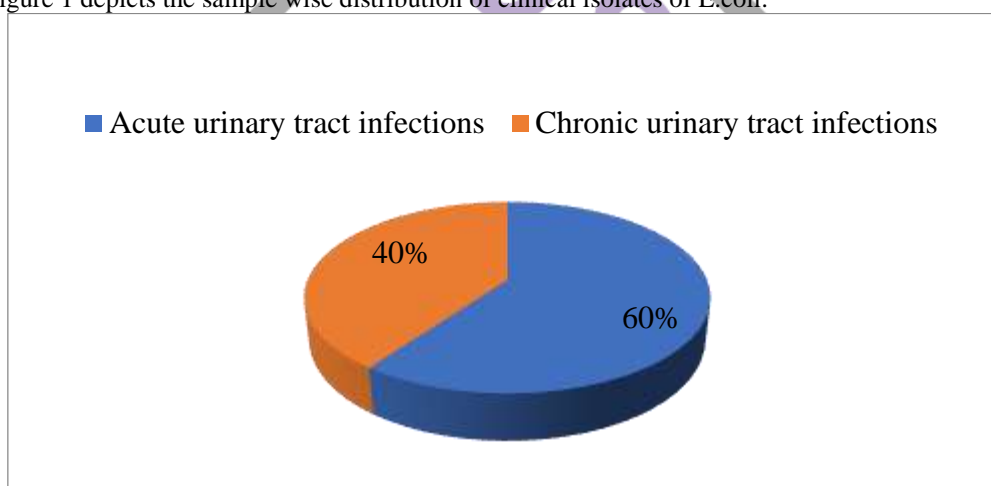


Figure 1: Sample wise distribution of urinary isolates of *E.coli*

### Antibiotic susceptibility testing:

In our isolates, we have found increased percentage 14/20 (70%) of isolates showed sensitivity to amikacin followed by gentamicin, which showed sensitivity of 9/20 (45%). 80- 90% of *E.coli* isolates showed resistance to cephalosporin group of drugs. 6/20 (30%) were found to be resistant to imipenem. However, we have observed an elevated level of resistance to other routinely used antibiotics. The detailed resistant pattern of *E.coli* isolates were showed in table 1.

Antibiotics	Sensitivity (20) (%)	Intermediate (20) (%)	Resistant (20) (%)
Ampicillin	5	0	95
Amoxicillin	5	0	95
Ceftazidime	10	10	80
Cefotaxime	5	5	90
Amikacin	70	10	20
Gentamicin	45	20	35
Norfloxacin	15	15	70
Ciprofloxacin	20	5	75
Imipenem	70	0	30

Table 1: showing antibiotic sensitivity pattern of *E.coli*

### Result of antifungal activity of apple cider vinegar against clinical isolates of E. coli:

It did not show any inhibitory activity against E. coli isolates.

### DISCUSSION

Vinegar, alone, has been used for cleaning and treating nail fungus, head lice, warts and ear infections. Consumers typically prefer natural preservative methods for inhibiting the growth of food borne pathogenic microorganisms in food. [16]

Apple cider vinegar is a commonly prescribed anti fungal agent in folk medicine for treatment of fungal skin, ear and vaginal infections. [15]

Organic acids resulting a decreasing in pH value, this may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis [17]. Other explanations have also been proposed including the membrane disruption, the interruption of metabolic reactions, and the accumulation of toxic anions. [18]

The inhibition of microbial growth increases by lowering pH of the media, and most microorganisms are susceptible to antimicrobial effects in the presence of organic acids. This phenomenon is due to the hydrophobic feature of most organic acids, which allows free diffusion of the protonized form through cell membrane. This diffusion process takes place spontaneously due to pH and osmolarity gradients that exist between the inner and outer sides of the cell. The intracellular pH is higher than the extracellular, and the acid undergoes dissociation as soon as it enters the cytoplasm and then decreases the intracellular pH by releasing the proton. To counter the decrease of cytoplasmic pH, resulting from the ionization of the entered acid, the cell allocates the main part of its energy content to eliminate these newly formed protons which results in slower growth kinetics [17].

### CONCLUSION

*Escherichia coli* is the most common urinary pathogen isolated from 50-90% of all the uncomplicated urinary tract infection [14]. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination [9]. So the usage of apple cider vinegar was used to see if it had any significant effect on it.

It did not show any inhibitory activity against E. coli isolates. May be increased dilutions are required to standardise the efficacy.

### REFERENCES

- [1] "Apple Cider Vinegar", *WebMD*, Sept. 2009
- [2] The Online Archive of American Folk Medicine. Available at: <http://www.folkmed.ucla.edu/archive.html>. Accessed March 3, 2006.
- [3] Vinegars and Acetic Acid Bacteria. International Symposium; May, 2005; Available at: [http://www.vinegars2005.com/images/Vin\\_2005\\_book.pdf](http://www.vinegars2005.com/images/Vin_2005_book.pdf). Accessed March 9, 2006.
- [4] Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. Entani E, Asai M, Tsujihata S, Tsukamoto Y, Ohta M. *J Food Prot.* 1998 Aug;61(8):953-9.
- [5] Reduction of poliovirus 1, bacteriophages, *Salmonella montevideo*, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. Lukasiak J, Bradley ML, Scott TM, Dea M, Koo A, Hsu WY, Bartz JA, Farrah SR. *J Food Prot.* 2003 Feb;66(2):188-93.
- [6] Effectiveness of household natural sanitizers in the elimination of *Salmonella typhimurium* on rocket (*Eruca sativa* Miller) and spring onion (*Allium cepa* L.). YucelSengun I, Karapinar M. *Int J Food Microbiol.* 2005 Feb 15;98(3):319-23. PMID
- [7] Evaluation of household sanitizers for reducing levels of *Escherichia coli* on iceberg lettuce. Vijayakumar C, Wolf-Hall CE. *J Food Prot.* 2002 Oct;65(10):1646-50. PMID: 12380754
- [8] Singleton P (1999). *Bacteria in Biology, Biotechnology and Medicine* (5th ed.). Wiley. pp. 444-454. ISBN 0-471-98880-4
- [9] "*Escherichia coli*". *CDC National Center for Emerging and Zoonotic Infectious Diseases*. Retrieved 2012-10-02.
- [10] Vogt RL, Dippold L (2005). "*Escherichia coli* O157:H7 outbreak associated with consumption of ground beef, June-July 2002". *Public Health Reports.* **120** (2): 174-8. PMC 149770
- [11] Bentley R, Meganathan R (Sep 1982). "Biosynthesis of vitamin K (menaquinone) in bacteria". *Microbiological Reviews.* **46** (3): 241-80. PMC 281544 PMID 6127606.
- [12] *Gut.* **49** (1): 47-55. doi:10.1136/gut.49.1.47. PMID 11413110.
- [13] Reid G, Howard J, Gan BS (Sep 2001). "Can bacterial interference prevent infection?". *Trends in Microbiology.* **9** (9): 424-428. doi:10.1016/S0966-842X(01)02132-1. PMID 11553454.
- [14] Roshene R et al / *J. Pharm. Sci. & Res.* Vol. 7(10), 2015, 878-881
- [15] Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P. 2000. Antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds. *Intl J Food Microbiol* 56:3-12.
- [16] Finkel, Richard; Clark, Michelle A.; Cubeddu, Luigi X: *Lippincott's Illustrated Reviews: Pharmacology*, 4th Edition 2009.
- [17] Hassan. R, El-Kadi, S, Sand. M. Effect of some organic acids on some fungal growth and their toxins production. *International Journal of Advances in Biology (IJAB)* Vol 2. No .1, February 2015, 1-11
- [18] Pelaez, A. M. L.; C. A. S. Catano; E. A. Q. Yepes; R. R. G. Villarreal; G. L. D. Antoni and L. Giannuzzi, Inhibitory activity of lactic and acetic acid on *Aspergillus flavus* growth for food preservation. *Food Control*, Vol. 24, pp 177-183, 2012.