

## New Virulence Factor of Normal Flora *E. Coli*

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Article History: Submitted: 10.11.2019

Revised: 05.12.2019

Accepted: 10.01.2020

### ABSTRACT

Histamine- producing bacteria may be associated with much irritable bowel disease. The present study was feces samples for histamine producing bacteria (HPB) isolation. Detection was depended on Niven's medium that out of 30 bacterial samples, only 21 isolates were positive for histamine production. The bacterial species were identified by Vitek2 –compact results. *E.coli* species were selected for present study. Vitek2 –compact device was used to study the sensitivity of bacterial isolates against 16 antibiotic. Most *E.coli* species were resistant to beta-Lactamaes (Ampicillin/Sulbactam Ampicillin and Ciprofloxacin), and sensitive to Trimethoprim (100%. The study showed that *E.coli* have histidine decarboxylase enzyme that has the ability to produce histamine and the occurrence of the disease, which is considered a virulence factor. The concentration of histamine was detected by HPLC

technique (21.01 µg/ml). There are various factors that affect the bacterial production of histamine were detected include pH, salt and temperature. *E.coli* proportion to the concentration of histamine in pH (6) 21.41µl/mg and less at a temperature 40°C was 4.82 µl/mg.

**Key word** :*Escherichia coli* , HPLC, Antibiotic, Histamine Producing Bacteria (HPB)

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DOI: [10.5530/srp.2020.2.12](https://doi.org/10.5530/srp.2020.2.12)

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### INTRODUCTION

*Escherichia coli* is a common inhabitant of the gastrointestinal tract of humans and animals. There are *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals. The pathogenic *E. coli* are divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites (1). Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. Pathogenic *E. coli* can be classified into pathotypes by their virulence factors, together with the type of disease (2). On the other hand same normal flora members might be opportunistic pathogens meaning that the organism ability producing histamine as new virulence factor. Antimicrobial drugs have played an indispensable role in decreasing illness and death associated with infectious diseases in animals and humans (3). There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. In this regard, programs for monitoring resistance have been implemented in many countries for the purpose of protecting the health of humans as well as animals (4, 5,6). Commensal bacteria from the gut microbes e.g., coli forms, may play a crucial role in the spread of resistance within a community. Surveillance data shows that resistance in *Escherichia coli* is generally consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine (7).

### MATERIALS AND METHODS

#### 1. Sample collection and identification of *E.coli*

Thirty feces sample were collected by sterile container from the feces of patients with colitis of General laboratory of General Hospital in the Basra city. The samples kept in ice box and transferred immediately to the laboratory and

cultured on Niven's agar medium. The plate were incubated at 37°C for 48hrs. Purple colony on Niven's medium is indicator of Histamine Producing Bacteria (HBP) according to (8, 9). All isolated were identified and Antibiotic susceptibility testing by Vitek2 compact system.

#### 2. Antibiotic Susceptibility Test

Antibiotic resistance of bacteria of *E.coli* was determined by Vitek2 compact system. The isolates were screened against 16 antibiotics.

3. HPLC technique for histamine Estimation and Factors affecting Histamine production. High performance liquid chromatography technique was used for histamine detection that produced by bacterial isolates. (10)

##### 3.1. PH affect

PH effect was tested by using two different Niven's medium pH (6 and 6.5). Each one inoculate with loopful of culture and Incubate at 37°C at 24 hrs. And then measured histamine production by HPLC system as (10).

##### 3.2. NaCl affect

NaCl affect was tested by using two different Niven's medium NaCl (0.25% and 0.1%). Each one inoculate with loopful of culture and Incubate at 37°C at 24 hrs. And then measured histamine production by HPLC system (10).

##### 3.3. Temperature affect

Temperature effect was tested by using two different Niven's medium temperature (35°C and 39°C) each one inoculate with loopful of culture and Incubate at 37°C at 24 hrs. And then measured histamine production by HPLC system (10).

## RESULTS AND DISCUSSION

### 1-Identification of Bacteria *E.coli*

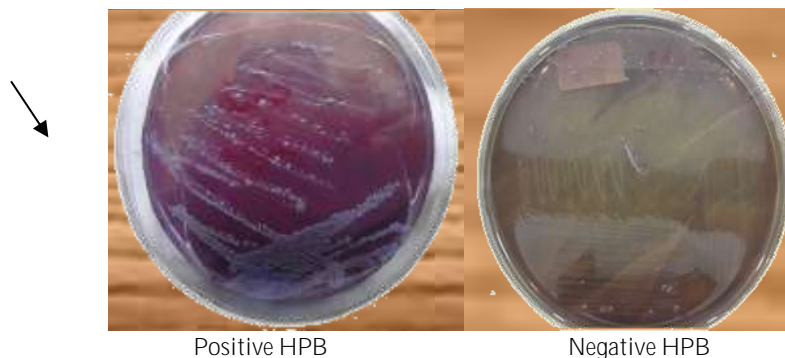


Figure (3-1): The color change on Niven's media

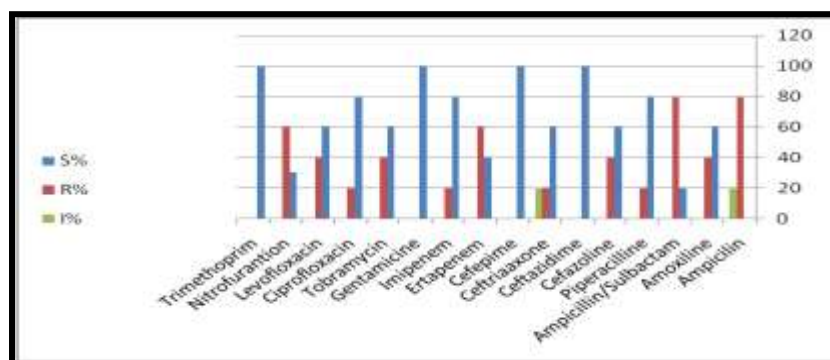
The bacterial isolates were characterized by morphological, physiological and biochemical tests of bacterial isolates by Vitek2 compact system.

This results showed that the isolates can change the color of Niven's medium, because presence Purple Bromocresol and PH change due to the activity of histidine decarboxylase enzyme that convert acidic histidine to alkaline histamine (11).

A total of 30 coliform bacteria were isolated from the studied feces samples, only *E. coli* 21 isolates were positive Histamine Producing Bacteria HPB were detected according to colorimetric change from green to violet color by Niven's medium as shown figure (1).

### Antibiotic Susceptibility Test

An antibiotic is a kind of ubiquitous contaminant in the aquatic environment with industrial effluents and sewage discharge. The bacterial isolates were exposed to 16 antibiotics for susceptibility testing. This study showed that *E. coli* highest degree of sensitivity toward Ceftazidime, Cefepime, Gentamicine and Trimethoprim (100%), while The overall resistance pattern showed high resistance against Ampicillin, Amicillin/Sulbactam(80%) and Nitrofurantion, Ertapenem (60%)as shown figure(1)



S = Sensitive, R= Resistance, I= Intermediate

Figure (3-6): Sensitivity and resistance of the *E.coli* towards various antibiotics.

To help characterize evolution of drug resistance in *E. coli* since antimicrobial drugs were first widely used, we tested existing strain collections of *E. coli* for their susceptibility to a common panel of 16 antimicrobial agents (12, 13). Marked multiple antibiotic resistances (over 70%) were observed for erythromycin, tetracycline and ampicillin, chloramphenicol and norfloxacin. Multiple antibiotic resistance refers to the resistance of two or more classes of antibiotics (14). In different parts of the world, resistance of *E. coli* to penicillins group of antibiotics have been on higher side and is

increasing day by day, but there are only few reports which indicates 100% resistance to penicillins (15). Resistance of amoxicillin and a beta lactam inhibitor Similar kinds of results, where beta lactam inhibitors increase the efficiency of penicillin group of antibiotic against *E. coli*, have been reported in previous studies(16). Multiple drug resistance (MDR) is described as resistant to at least one member from three different antibiotic classes being used for the treatment of *E. coli*.(17). Several studies also indicated high resistance

rate of avian *E.coli* against Ampicillin and Nitrofurantion ( 18,19,20,21)

3. HPLC technique for histamine Estimation and factors affecting Histamine production depending on the HPLC technique the concentration of histamine in the *E.coli* was measured (21.01 µg/ml as shown in figure (2) .

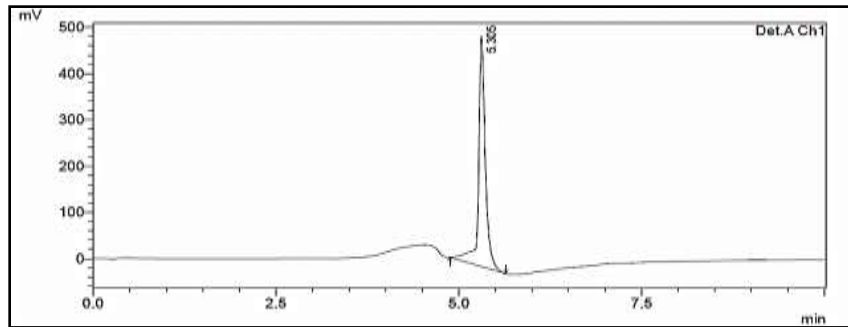


Figure (2): HPLC analysis of histamine concentration produced from *E.coli*

The genera and species of bacteria belonging to the family *Enterobacteriaceae* play a big role in the production of biogenic amines, may be this is part of the amines fed (22,23 ). The present study is agreed with the results of Koohdar V. A.1, 2011 showed that histamine producing bacteria can be divided in to two categories : those species capable of producing large quantities of histamine (> 100mg / 100 ml) example *Klebsiella pneumonia* and *Morganella morganii* and those species that produce lesser amounts of histamine (< 25

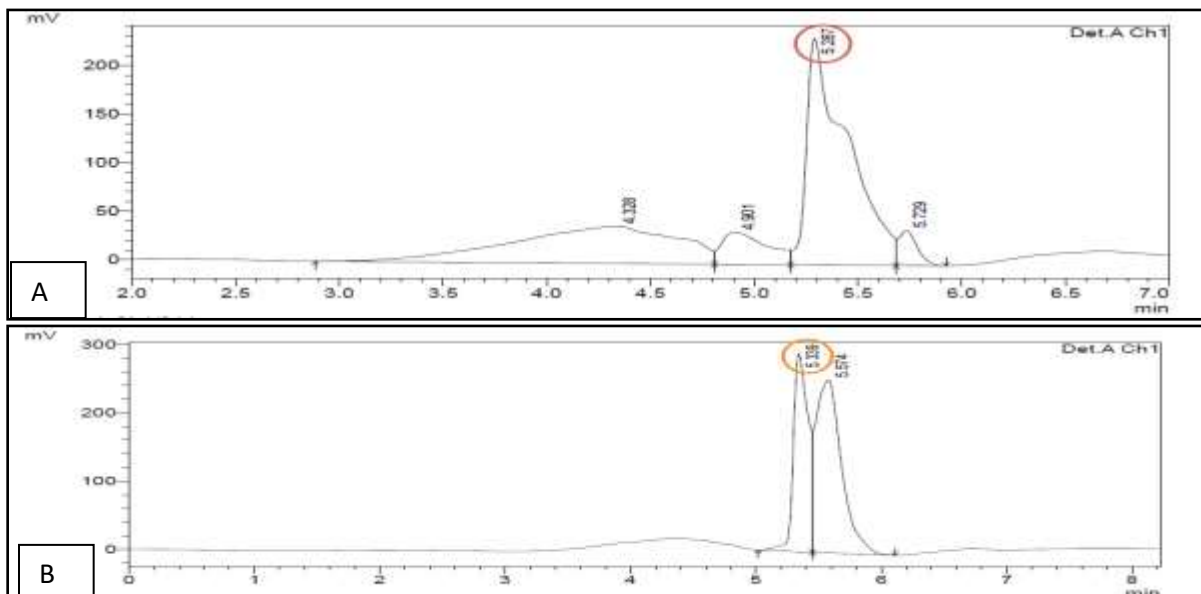
mg / 100 ml) example *Escherichia coli* and *Citrobacter freundii*. Also agreed with many research results (25, 26) the Less concentration of some microorganisms like *E.coli* and *Citrobacter freundii* to convert histidine into histamine depends strongly on their enzymatic activity and ability to grow in different environmental system. There are factors that affect the ability to produce histamine were studied like pH, NaCl and Temperature.

Table (1): Factors affecting histamine production in histamine-producing *E. coli*.

| Isolates HPB             | PH    |       | NaCl mg |       | Temperature |      |
|--------------------------|-------|-------|---------|-------|-------------|------|
|                          | 6.5   | 6     | 1       | 0.25  | 35°C        | 40°C |
| (a) <i>E. coli</i> µg/ml | 16.71 | 21.41 | 16.42   | 16.81 | 10.99       | 4.82 |

The results showed in the table (1) factors affecting the production of histamine in HPB. *E. coli* had been the highest histamine concentration 21.41 µg/ml in the pH 6 , while in

temperature 40°C had been a lower (4.82 µg/ml as shown in figure (3).



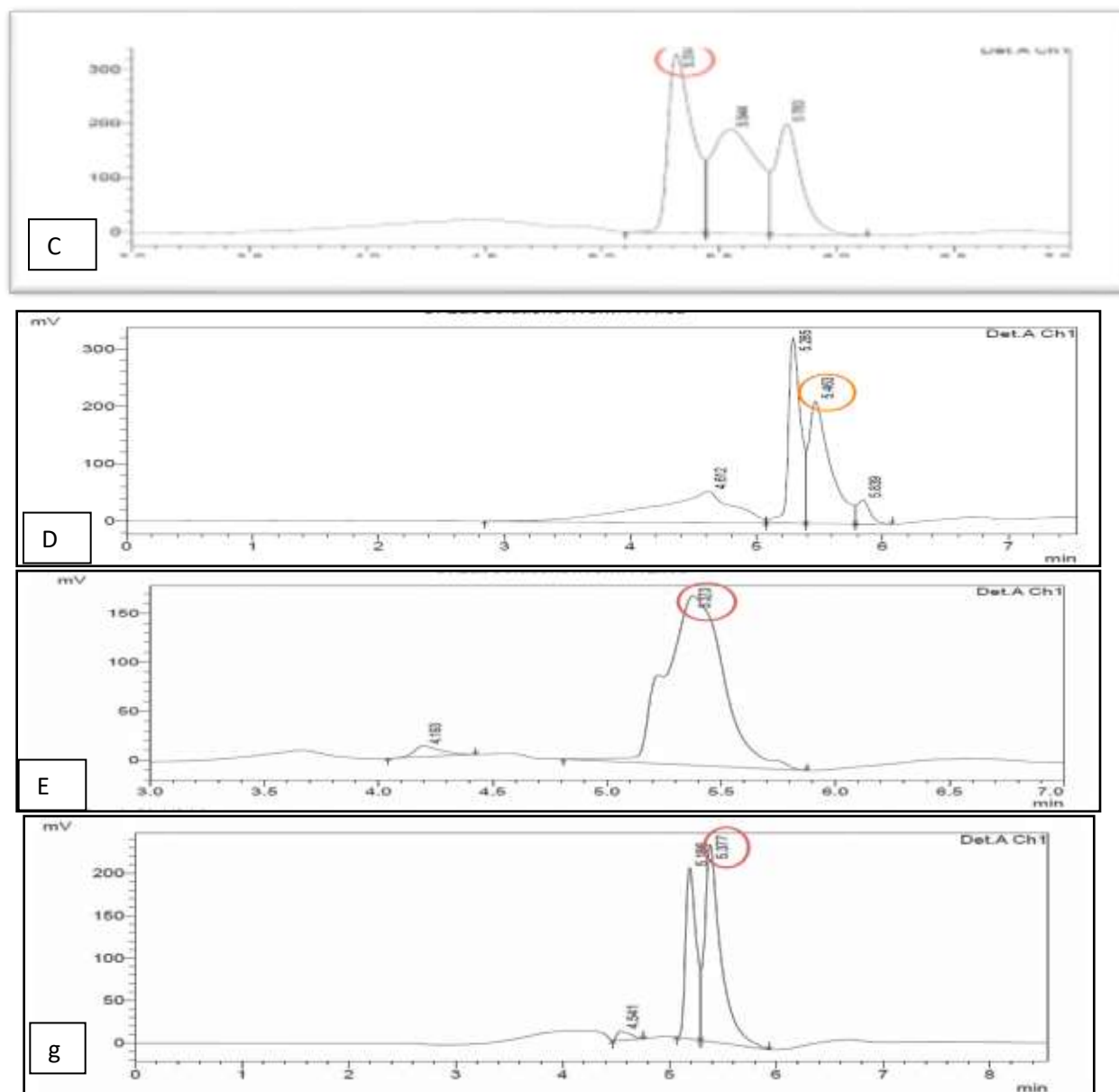


Figure (4): Factors affecting the concentration of histamine by HPLC analysis in *E. coli* a: Effect of (pH 6.5), b: Effect of (pH 6.), c: Effect salts NaCl(1 mg), d: Effect salts NaCl (0.25 mg), e: Effect temperature( 35 °C) and g: Effect temperature 40°C.

Results have agreed with many studies ( 27, 28) of that there factor affect the growth of bacteria producing histamine and the effectiveness of the enzyme to a stripper carboxyl groups and on the accumulation of histamine such as temperature incubation and pH of the medium factors. Some researchers explained that the pH primary factor affecting the effectiveness of the enzyme, and that the enzyme is usually a pH However, the rapid and sharp decline in pH leads to a decline in the growth of microorganisms is also affected by an enzyme produced from bacteria NaCl and PH ( 29). The increase in NaCl concentration of at least from the accumulation of histamine and this agreed with (30). A number of researchers found( 31,32,33) that the degree of moderate temperatures can occur for the growth of bacteria to produce histamine in less than 3–4 hrs, studies also showed that histamine to do not break degree cooking temperature.(34) found that increasing the proportion of histamine at reduced pH also found that the lower the

concentration of histamine when the product is treated concentration 5% from NaCl. Histamine producing bacteria is able to grow in wide range of temperature either high temperature or low temperature (35). Confirmed (30) stressed the oxidation reduction potential of the medium in the production of biogenic amines as stimulate the conditions conducive to the effort to reduce oxidation to produce histamine and effectiveness of the enzyme HDC seems to be ineffective or discouraging the presence of oxygen, also amines formed is strongly influenced by temperature as the temperature between 20-37°C are ideal for the growth of most bacteria containing the enzyme remover carboxylates and low temperature leads to a reduction in growth. Temperatures above 37°C depress histamine formation by all of the bacteria (36). Different species and even different strains of the same species respond differently to temperature variations. Thus, most

strains of *E. coli* have their optimum for histamine production at 31°C to 26°C., but others at 37°C (35).

## CONCLUSION

The acquisition of a new virulence factor in *E.coli* can be cause or enhance pathogenicity. The presence of new virulence agents in the bacterial normal flora is their ability to produce histamine, making it one of the causes of the disease. *E.coli* have high resistance against many antibiotics Trimethoprim, while the overall resistance pattern high resistance Ampicillin, Amicillin/Sulbactam

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