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RESEARCH ARTICLE

Screening for Heme Oxygenase Enzyme among Clinical Isolates of Escherichia Coli Isolated from Urine Sample

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Abstract

A total of (150) urine sample were obtained from different patient which is (male, female and pediatric) who are suffering from urinary tract infection and are using catheter indwelling, who are admitted to the merjan teaching hospital in Hilla city for a period rang from (July to September 2018), only 36 isolates (24%) were diagnosis as *E. coli*. Three genes related to heme oxygenase production and transported are studied. These genes are (*chuA, chuS* and *chuT*) and the result showed that 26 isolates (90%) from total sample carry *chuA* in genetic marker and 25 isolates (85%) from total sample carry *chuS* in genetic marker and only 18 isolates (58%) from total sample carry *chuT* in genetic marker, and the total positive results of isolates which carry all these three genetic marker in genomic material is only 15 isolates. These (15) isolates were undergone for induction and production of HO-1 enzyme, through detection of beliverdin in chochlate agar and also through estimation free iron spectrophotometrically. The results showed that all isolates gave positive results for enzyme production through production of biliverdin and high levels of free iron when compared to control groups. Moreover, two different antibiotics are used to show their effect on enzyme activity, and the results revealed that neomycin and ciprofloxacin have an effect on enzyme activity in the presence of imidazole buffer.

Introduction

Escherichia coli is one of the most important pathogen in urinary tract infections and according to the phylogeny classification, E.coli which is isolated from UTIs is considered as extra intestinal isolates which may cause a wide range of diseases such as asymptomatic bacteriuria, cystitis, pyelonephritis and urosepsis, [1]. These bacteria have the ability to degrade RBCs through its ability to produce many proteins and enzymes such as hemolysin and some proteolytic enzymes, [2], also, some strains of *E.coli* have the ability to produce specific enzymes for degradation of heme which produce as a result of RBCs destruction, [3].

Sometimes, this heme that is appeared as a result of destruction of the red blood cells, could become toxic for the bacteria and for other organisms, but the bacteria who has ability to degrade this compound, through its ability to synthesize heme oxygenase enzyme (HOs) which can evade from its toxicity, through the enzyme ability to degrade heme into free iron, carbon monoxide (CO) and beliverdin, [4].

At molecular level the main genes associated with production of heme oxygenase (HO) are the gene clusters related to the (*chu*) genes which include (*chuS, chuA, chuT, chuY, chuZ, chuX* and *chuW*) as gene cluster, [5].However, the main gene associated with production of heme oxygenase (HO) is the (*chuS*) which is responsible for synthesis of this enzyme with the assistance of (*chuT*) which is responsible for the transport of iron after heme degradation into the cytoplasm of bacteria,[6].Many studies confirmed the presence of HO-1 in Enterobacteriacea such as *E.coli, salmonella* and *shigella* etc [7].

The Aim of this Study is to Investigation the HO-1 among Clinical Isolates of *E.coli*

Material and Method

A total of 150 sample of urine are collected from patient suffering from urinary tract infection including pyelonephritis and other infection of UTI. And were submitted to merjan teaching hospital in Hilla city rang

from (July to September 2018), and the patient's age range from 16 to 55 years. The specimens were generally collected from patients suffering from UTIs. Mid-stream urine samples were collected in sterilized screw-cap containers, and then the urine samples were directly inoculated on culture media (MacConkey agar) and incubated aerobically at 37°C for 24h.

Investigation on HO-1 among *E. coli* Isolates

The method of detection of HO-1 is prepared in this study as the following.

The *E. coli* isolate strain was grown in Luria-Bertani medium containing 100 mg of ampicillin per liter, overnight at 37°C and then the cells were subsequently sub cultured into fresh LB-ampicillin medium (100 ml) and grown at 37°C to mid-log phase, the cells were then sub cultured (10 ml) into Luria Bertani LB-ampicillin medium (1 liter), after 5 hours of in incubation the enzyme is induced by addition of (isopropyl-1-thiol-(D)-galactopyranoside) to a final concentration of 1 mM,

Cell growths were continued for 4 to 5 h at 30°C, and then were harvested by centrifugation (10,000 r.p.m for 20 min). And then, cells were lysed by sonication in 50mM Tris-HCl (pH 7.8) containing 1 mM EDTA and 1 mM phenylmethysulfony fluoride. The cell suspension was then centrifuged at 27,000 r.p.m for 40 min and the extract is then kept at (- 20°C) and the cells are removed.

In Vitro Detection of HO-1 by biliverdin Production

Chochlate agar is prepared and few drops of fresh blood are added before its being solid. Wells are done and the enzyme extract in added and then the plates are incubated at (37 °C). The presence of halo around the well is positive results for biliverdin production.

Estimation of Iron Concentration

Chochlate broth free of cells and deprise is used for enzyme activity through estimation of free iron spectrometerically, at absorbance (600 nm) as mention by iron kit, (Biolab company). Also, two antibiotics (Neomycin and ciprofloxacin) are used in the presence of imidazole buffer to show their effect on enzyme activity, and the solutions are added at a ratio (1:1).

Results and Discussion

Detection of Heme uptake gene (*chuS*) by PCR:

All *E.coli* pathogenic isolates are subscribes for detection of *ChuS* genes by using PCR technique at molecular size 119 bp as shown in Figure 01 which the result showed that (25) DNA sample gave positive results which is approximately about (85%) for this gene and only (5) samples (15%) which gave negative results for this gene.

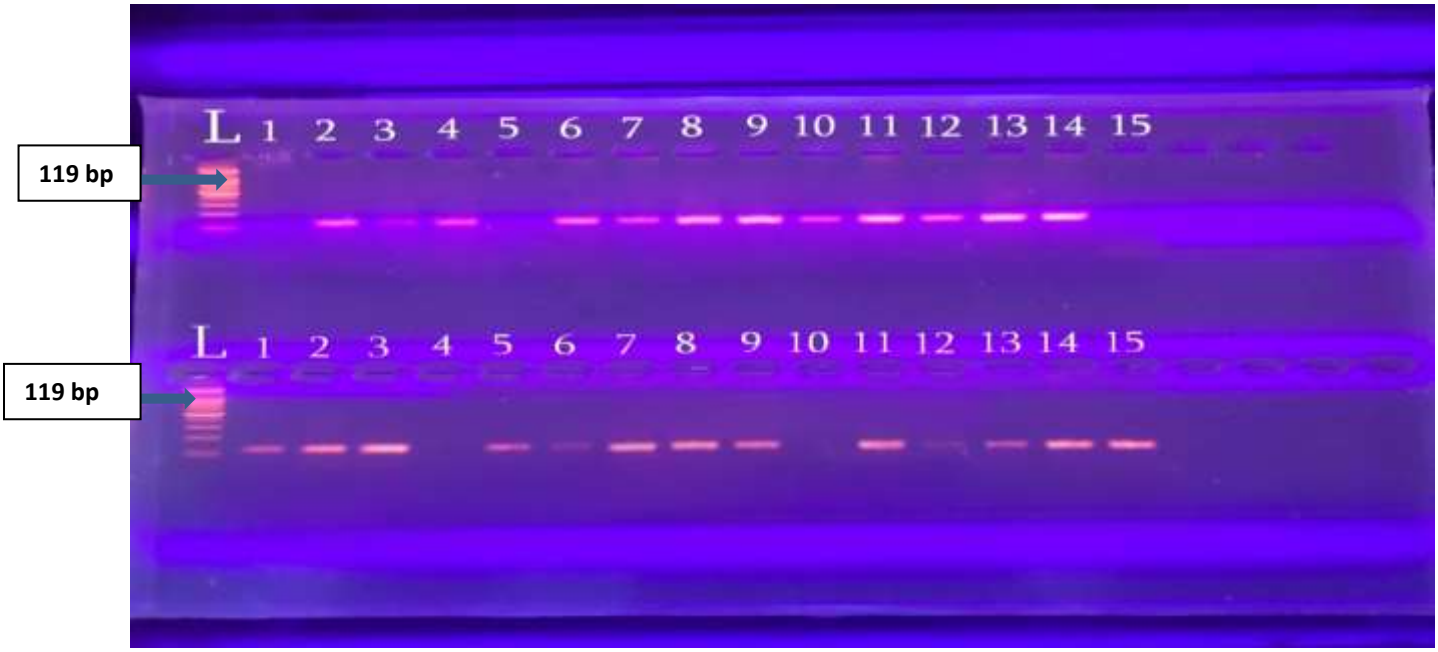

Fig A:-Gel electrophoresis of PCR for *chuS* amplicon product. L: ladder; sample (1-30) No. of isolates obtained from urine samples, the size of ladder is 100 bp (Bioneer). The size of product is 119 bp at volte 70 V.

Moreover, molecular investigation for *chuA* gene was carried out by using specific primer for PCR technique at molecular size (279 bp). The results was showed that only (17) isolates (85%) which gave positive results, and only (3) isolates (15%) that gave negative results for their possession of this gene, as shown in Figure 2.

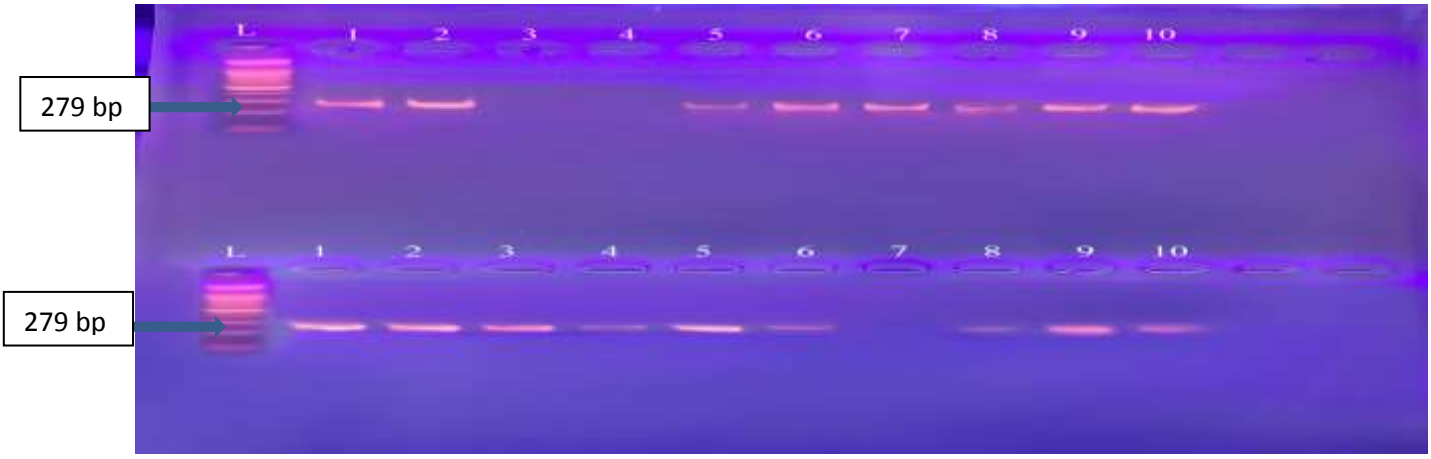

Figure 2: Gel electrophoresis of PCR of *chuA* amplicon product. L: ladder; sample (1-20) No. of isolates obtained from urine samples, the size of ladder is 100 bp (Bioneer). The size of product is 279 bp at volte 70 V.

On other hand, *chuT* gene was detected in all *E.coli* isolates strains which is (30 isolates) by using PCR at a molecular size (1000 bp). Show only 18 isolates (58%) that gave positive results in PCR technique, whereas 12 isolates (42%) that gave negative results as shown in Figure 3.

Fig. 3: Gel electrophoresis of PCR of *chuT* amplicon product. L: ladder; sample (1-30) No. of isolates obtained from urine samples, the size of ladder is 100 bp (Bioneer). The size of product is 1000 bp at volte 70 V

These results which obtained from total 30 samples isolated from patient suffering from several type of urinary tract infections, and it was found that only (15) isolates carried the all these three genes. This will confirm that not all *chu* genes may be present at the same time, so the next experiments will depend mainly on these (15) isolates for production of HO-1 enzyme.

HO-1 Production

A total of (15) isolates subjected for HO-1 production, and these established by using two tests for investigation the enzymes. At first, none of the isolates that gave positive results for extracellular enzymes, but after ultrasonication to the growth of bacteria the enzyme is released and two modified methods are used (special communication, 2019). In the first method, chocolate agar is used and the supernatant is put in the wells, and after incubation for (5) hours, the results are read. If green halo is seen, this will indicate the presence of biliverdin as a result of degradation of heme which that indicates is present in the Chocolate agar. Biliverdin halo is measured as diameter and (15) isolates are giving positive results for this test and only one isolate failed to degrade heme which as in control group. The second test used for determination heme degradation through measuring free iron in the media (chocolate broth) after incubation for (5) hours.

The results show that the same (15) isolate gave positive results and the level of iron is calculated according to the kit. Iron values are ranged from (675mg/dl -1.43 g/dl), and show these values are higher than the five

types of controls groups, and the negative control groups which is (chocolate broth before adding the supernatant) which score (68.7) mg/dl. The presence of high levels of iron came from the action of (HO-1) enzyme on the heme molecule which give indicate to release iron from this heme degradation. So, these two methods are rapidly used for determination the activity of this enzyme that is previously not used in diagnostic microbiology which depends mainly on CO levels entirely.

The method of determination of HO-1 activity may have many advantages in screening the isolates which have ability to produce it, and those which are unable to synthesize intracellularly and inducibly. The presence of catalase enzyme in the blood may enhance the activity of this enzyme, [8].

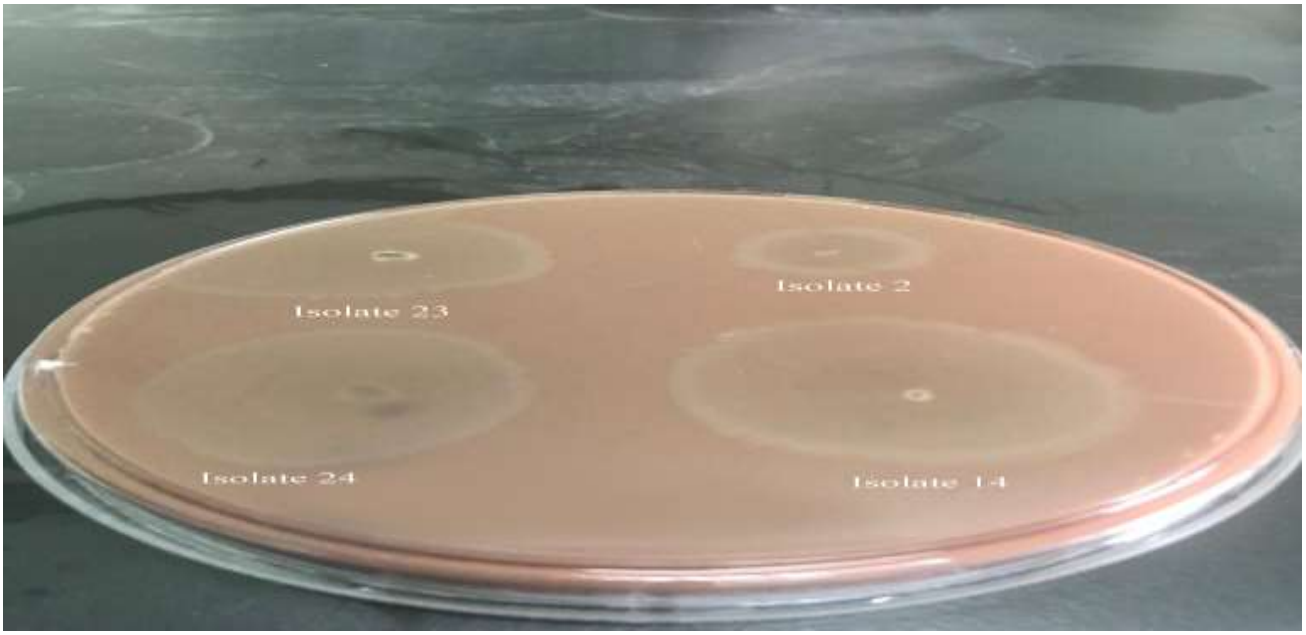

Figure 4: shown the green zone formation on chochlate agar indicates on heme degradation to biliverdin by HO-1

Detection of HO-1 by Estimation Iron Concentration in Spectrophotometer

This experiment is designed to represented second procedure to estimate the level of heme oxygenase in bacteria isolate by used iron kit and the result summarized in Table (1).

Table 1: Mean values of free iron in (15) test groups and (5) controls

groups	Iron con. Mean ± SD	P value
Tests group (15)	820.6 ± 293.7	< 0.001
Controls group (5)	115.8 ± 57.7	

In this experiment was gave (15 samples) to estimate the concentration of iron on optical density (600 µm) and used stander (26.5) and control (28).By addition (2ml iron solution + 400 µm)

Effect of Some Antibiotics on HO-1 activity

Tow type of antibiotics are used to show their effect on enzyme activity through using (Imidazole buffer) containing (2 mg/ml) of ciprofloxacin and neomycin separately and addition in ratio (1:1).

The results showed that enzyme activity is inhibited completely after one hour of incubation at (37ºC). The inhibition is seen through the absence of the beliverdin halo which make on chocolate agar.

Many studies indicate on the effect of imidazole combined with other compounds such as (dioxolanes and metalloporphyrin) on HO species activity but no previous studies indicate on the relationship between the enzyme activity and antibiotics, [9].

However, two different antibiotics which are easily dissolved in imidazole buffer, are used and the presence of antibiotics which is like (neomycin and ciprofloxacin) combined with imidazole give rise to entire inhibition of enzyme, whereas there was no effect of imidazole alone on enzyme activity.

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