Prevalence of *chuA* gene virulence factor in *Escherichia Coli* isolated from clinical samples in AL-Diwaniyah province

Prof. Dr. Maitham Ghaly Yousif
Saja Salem Shahid
AL-Qadisiyah University \Collage Of Science
Sajasalim1988@gmail.com

Summary

During this study, 150 samples were collected from Al-Diwaniyah Teaching Hospitals, Children's Hospital and Al-Hussein Children's Hospital from urine and stool under from Al-Diwaniyah Hospitals, Children's Hospital and Al-Diwaniyah General Hospital from urine and faeces under the supervision of the doctor, during the period from 1-11-2021 to 01-3-2022. 20, where the results showed a return of 60 isolates of the bacteria E. coli, an examination was conducted Antibiotic sensitivity test that E. coli was multi-resistant For multidrug resistance (MDR), 50 (100%) isolates were resistant to carbenicillin 49 (98%) isolates were resistant to the antibiotic erythromycin 47 (94%) Rafampin isolate rifampin-resistant, 44 (88%) ceftazidime-resistant isolates, 38 (76%) novobiocin-resistant isolates, 37 (74%) cefotaxime-resistant isolates (66%) tetracycline-resistant 26 (52%) isolates Isolates resistant to ciprofloxacin, 15 (30%) isolates are resistant to gentamicin and two (4%) are resistant to nitrofurantoin. The results showed that 45 (90%) isolates were biofilm-forming bacteria with different degrees Different, two (4%) biofilm-forming isolates with strong adherent, 6 (12%) bio-membrane-forming isolates with moderately strong adherent, and 37 (74%) bio-film-forming isolates with weakly adherent. carbenicillin for the following antibiotics (Cefotaxime, Gentamicin, Chloramphenicol, Erythromycin Penicillin G Co-trimoxazole Tetracycline) 100% against the bacteria under study. The susceptibility of bacteria to the production of virulence factors (hemolysin, membranes) was tested.

Keywords: *E.coli*, *chuA* gene, multidrug resistance.

تأثير المضادات النانوية والمضادات الحيوية على بكتريا الاشريكية القولونية المعزولة من عينات سريرية

أ.د.ميثم غالي يوسف

سجا سالم شهيد

جامعة القادسية / كلية العلوم

Sajasalim1988@gmail.com

الخلاصة

خلال هذه الدراسة ، تم جمع 150 عينة من مستشفيات الديوانية التعليمية ومستشفى الأطفال ومستشفى الحسين للأطفال من البول والبراز أسفل مستشفيات الديوانية ومستشفى الأطفال ومستشفى الديوانية العام من البول والبراز تحت إشراف خلال الفترة من 1-11-2021 حتى 2022-3-01. 20 ، حيث أظهرت النتائج عودة 60 عزلة من بكتيريا E. coli ، تم إجراء فحص اختبار حساسية المضادات الحيوية بأن E. coli مقاومة متعددة للأدوية (MDR، 100) 50٪) عزلة مقاومة للكاربينيسيلين 49 عزلة (98٪) كانت مقاومة لمضاد الإريثروميسين 47 (94٪) عزلة رافامبين مقاومة ريفامبين ، 44 (88٪) عزلة مقاومة للسيفتازيديم ، 38 (76٪) عزلة مقاومة نوفوبيوسين ، 37 (74٪) سيفوتاكسيم- عزلات مقاومة (66٪) عزلة مقاومة للتتراسيكلين 26 (52٪) عزلات مقاومة للسيبروفلوكساسين ، 15 (30٪) عزلة مقاومة للجنتاميسين واثنتان (4٪) مقاومة للنتروفورانتوين. أظهرت النتائج أن 45 (90٪) عزلة من البكتريا المكونة للغشاء الحيوي بدرجات مختلفة ، 2 (4٪) عزلات مكونة للأغشية الحيوية ذات التصاق قوي ، 6 (12٪) عزلات مكونة لغشاء حيوي ذات قوة التصاق معتدلة ، و 37 (74٪) عزلة مكونة للفيلم الحيوي ضعيفة الالتصاق. كاربينيسيلين للمضادات الحيوية التالية (سيفوتاكسيم ، جنتاميسين ، كلورامفينيكول ، إريثروميسين بنسلين جي كو-تريموكسازول تتراسيكلين) 100٪ ضد البكتيريا قيد الدراسة. تم اختبار حساسية البكتيريا لإنتاج عوامل الضراوة (الهيموليزين ، الأغشية).

الكلمات المفتاحية: الإشريكية القولونية ، جين الضراوة ، مقاومة الأدوية المتعددة.

Introduction

Nanotechnology has emerged as a rapidly developing research field in materials science with the potential to positively affect human health (Singh et al., 2010). In nanotechnology, silver nanoparticles (AgNPs) are the most important metal element with beneficial properties. AgNPs ranging in diameter from 1 to 100 nm are an attractive research target due to their antimicrobial and cytotoxic potential imparted by their ability to easily attach to the cell wall. This attachment leads to effects on cellular respiration and permeability that result in cell death. Furthermore, AgNPs can also easily enter cells to interact with biomolecules,

including DNA and protein via their phosphorus and sulfur groups, respectively. (Elumalai et al., 2010).

Silver ions have been successfully used as antimicrobial agents, although AgNPs have shown higher antimicrobial activity in comparative studies. Very small quantities of AgNPs may have higher antimicrobial effects compared to that for their bulk material. The increased prevalence of antibiotic-resistant microbes, currently considered a widespread health problem, is likely related to intensifying antibiotic use. Decreased antibiotic efficacy has been clearly documented (El-Mokhtar and Hetta, 2018; Huh and Kwon, 2011) , triggering a surge in investigations into the antibacterial properties of other materials.

Several initiatives to develop antibiotic alternatives have stressed the potential of nanomaterials in order to prevent the development of antibiotic-resistant microbes. Silver nanoparticles are a potential strategy for fighting microbial resistance because microbes are unlikely to accumulate the numerous mutations required to develop nanoparticle resistance. Nanomaterials with dimensions ranging from 1 to 100 nm are manufactured, synthesized, and used as part of the nano drug delivery system.(Abd Ellah, et al., 2019a; Abd Ellah et al., 2019b; El-Mokhtar and Hetta, 2018) .

MDR bacteria (multidrug-resistant bacteria) continue to be the most serious hazard to public health. Diseases caused by such resistance ;resistance has become a serious concern for several antimicrobial treatments. Nanotechnology breakthroughs have opened up new opportunities for generating unique formulations based on different types of nanoparticles (NPs) of varying sizes and shapes with flexible antibacterial properties. Because NPs can both kill germs and serve as transporters for pharmaceuticals and natural antibacterial chemicals, they may be a viable choice.(Wang et al., 2017) .

The Aim of study

1- Isolation and identification of antibiotic-resistant bacteria from different clinical sources .

2- Measuring the effectiveness of antibiotics against these bacteria.

3- Using nano-antibiotics as alternatives to antibiotics and measuring their effect.

Material and methods:

(150) urine and stool samples from patients were collected from different age groups (children, young people, and adults), including males and females. Patients range in age from 1 to 60 years. Samples were obtained from patients who consulted at AL- Diwaniyah Teaching Hospital from September 2021 to March 2022. Each patient had a case sheet containing the patient name, patient sex, patient age, and appearance of the clinical signs arranged in the questionnaire in an index. All stool and urine samples were taken from patients with UTIs and diarrhea using transport medium swabs labeled with the patient's details and transferred to the laboratory as soon as possible. Occasionally, samples are kept in transit media according to the Time of collection and then sent to the laboratory for analysis. In order to determine the presence of E.Coli, all specimens were cultured based on conventional procedures on differentiation and enrichment medium (Blood, EMB, and MacConkey agar) and afterward incubated at aerobic capacity at 37 degrees Celsius for 24 hours. According to Bergy's manual for determinative bacteriology [7], Gram-negative bacteria with purple color identification was carried out using biochemical methods (oxidase, catalase, indol, Vogues–Proskauer citrate, TSI, methyl red, urease, motility, etc.).

Patients

A total of one hundred and fifty urine and stool samples from patients were collected at different age-groups (children, young people and adults) including male and female. Patients range in age from 1 to 60 years. Samples were obtained from patients who consulted at AL- Diwaniyah Teaching Hospital from 1-11-2021 to 1-3-2022. Each patient had a case sheet containing the name, age, sex, appearance of the signs and symptoms arranged in questioner in index.

Sample Collection

A total of one hundred and fifty urine and stool samples were collected from urinary tract infection patients and diarrhea patients by Amies medium swabs and labeled with the information of the patient and transported to the laboratory immediately. Sometimes the samples are preserved in a transport medium according to the time of collection until reaching the laboratory.

All samples were streaked according to standard methods on differentiating and enrichment media (Blood agar and MacConkey) to detect E.Coli and then incubated aerobically at 37°C, for 24 hours (Murray et al., 1995) .The samples processing steps were clarified in Figure (3-1).

Antibiotics resistance assay:

With the use of the disc diffusion method on Muller Hinton medium, it was possible to determine whether or not isolates were responsive to antibiotics. A panel of antibiotics was used to evaluate the antibiotic susceptibility of each isolate, with the following antibiotics included in the panel: Antibiotics such as the ones listed below are used: Amikacin AK [30g], Carbenciline PY [30g], Meropeneam MEM [10g], Ceftrixon CRO [30g], Aztreonam ATM [30g], Ciprofloxacin CIP [5g], Levofloxacine LEV [5g], Netlimicine NET [10g], Ticarciline/clavulanicacid [30g].

Small piece of bacterial suspension (made by inoculating five isolated bacteria grown on BHI media to tryptic soy broth (5) ml during 120 minutes) was added tryptic soy broth (5) ml then cultured for 120 minutes for forming the suspension with moderate turbidity (compared with McFaerland solution), bacteria were cultured on Mueller-Hinton media with discs with sufficient spacing between them to prevent overlapping of inhibition zones. Using the criteria stated by[8, after incubating the inoculation of the plates for 20 hours at 37 °C, the sensitivity and the isolates resistance against the antibiotics were examined.

. Detection of Virulence(chuA) Gene by (PCR)

Molecular detection of virulence factor (chuA) genes was performed for the presence of a gene by polymerase chain reaction on 40 isolates of bacteria, which had the character of multiple resistance(MDR) to antibiotics. The result revealed the presence of this gene in all isolates at percentage(100%) in the testes isolates, as shown in figures (4-6 , 4-7).

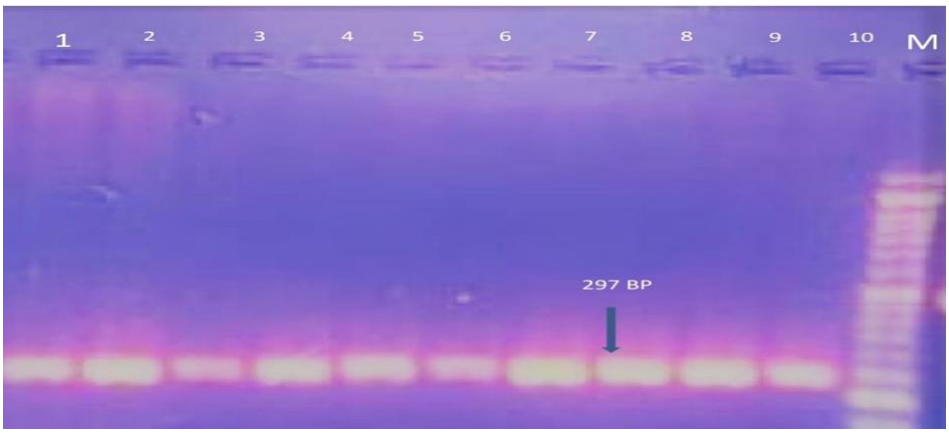


Figure (4-6): Detection of chuA gene by PCR. Lanes 1-10 represent positive isolates with bands (297 bp) in size . Lane M represents DNA markers(100-2000 bp). (1% Agarose gel ,75 volts to 1 hours).

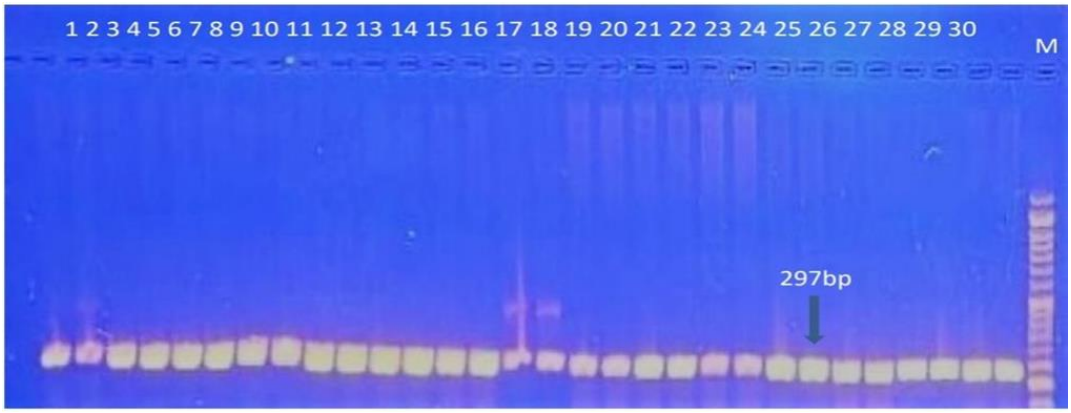


Figure (4-7): Detection of chuA gene by PCR. Lanes 1-30 represent positive isolates with bands (297 bp) in size . Lane M represents DNA markers(100-2000 bp). (1% Agarose gel ,75 volts to 1hrours).

Characterization of chemical synthesized AgNPs

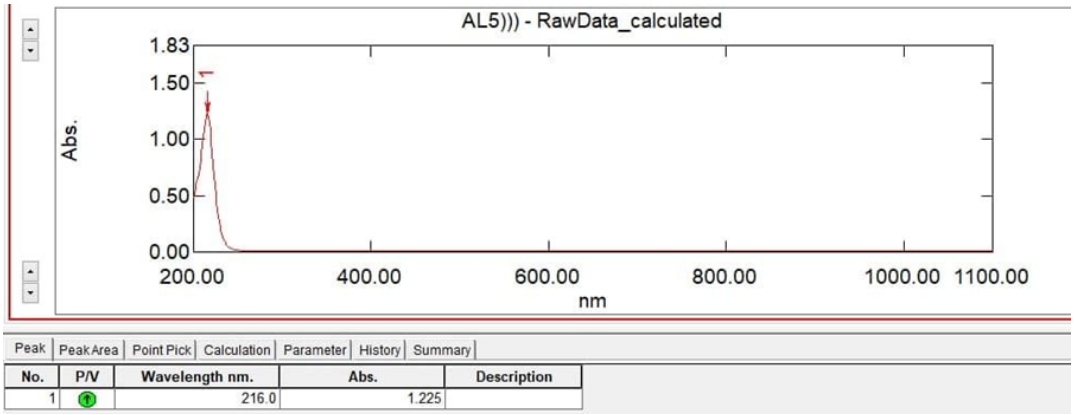
Visual inspection

At the end of reaction for synthesis silver nanoparticles. The color of the mixture was turned to yellowish brown at first and then the intensity of the color increased with the period , so the color was changed into pale yellow. on completion of the reaction with Ag+ ions. Color change was noticed only in the test flask and it is a clear indication for the formation of SNPs in the reaction mixture. As shown in figure(4-8).

Figure (4-8) Color of the AgNo3 after 30 minutes of reaction

UV-Vis spectroscopy

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs. The analysis was evaluated at different times after the start of the reaction. The λ max 216 nm(figure 4-9) was observed only in the test flask which confirmed the production and indicated the specific surface Plasmon resonance of SNPs. The scanning was continued and absorbance was recorded every 24 hours. The absorbance intensity gradually increased with time, this indicating a continuous reduction of AgNO3 and consequently, increase in SNPs concentration.



Figure(4-9) UV-Vis spectrophotometer analysis of chemically synthesized silver nanoparticles.

Conclusions and Recommendations

Conclusions

1- E. coli bacterial isolates showed multiple resistance to the antibiotics under study

2- Most of the bacterial isolates possess the ability to produce all virulence factors that were determined phenotypically (Hemolysin, biofilm), while some lack biofilm production.

3- The current study showed the possibility of biosynthesis of silver nanoparticles from the extract of the students.

4- The tests that were determined to examine the manufactured material confirmed that it is a nano-material with a spherical shape of varying sizes, which were determined between (27.2-33 nm) nm and the average size obtained is 17.95 nm.

5- Silver nanoparticles manufactured from the extract of the study showed effectiveness in inhibiting the growth of isolates E. coli compared to antibiotics that showed ineffectiveness.

6- The results of the current study showed the inability of bacteria to grow by the growth inhibition assay method at the 50% inhibitory concentration.

7- The secondary silver particles showed their ability to inhibit the virulence factors produced by E. bacterial isolates. coli is under study.

Recommendations

1- Studying the effect of silver nanoparticles on other bacterial species

2- Manufacture of secondary particles such as zinc, gold and zinc from the extract of the study plant.

3- The possibility of studying the use of silver nanoparticles as an alternative to traditional antibiotics in inhibiting bacteria.

4- The possibility of studying the effectiveness of silver nanoparticles in inhibiting other cancer cell lines.

5- The possibility of studying the effect of E. coli bacteria in other laboratory animals.

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