Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN: 2521-3520 (online)
Volume 29, Issue 3, 2018
DOI: http://doi.org/10.23851/mjs.v29i3.619

Research Article **Open Access**

Influence of Helium-Neon laser on some virulence factors of *Staphylococcus aureus* and *Escherichia coli*

Mohammed F. Abo-Ksour*, Sawsan S. Al-Jubori, Hussein A. Jawad
Department of Biology, College of Science, Mustansiriyah University, IRAQ.
**Correspondent author email: m.aboksour@uomustansiriyah.edu.iq*

ArticleInfo

Received
04/10/2018

Accepted
17/10/2018

Published
10/03/2019

Abstract

This work aims to investigate the effect of Helium-Neon laser on locally isolated Staphylococcus aureus & Escherichia coli bacteria; their resistance pattern, virulence factors, and their plasmid content. Bacteria were collected from patient suffering from urinary tract infections, septicemia, wound, and burn infections, then isolates were identified according to their characteristics, features; morphological, biochemical tests, and API systems. Out of Eighty-seven samples, twenty-seven isolates of S. aureus and thirteen isolates of E. coli were isolated. The results showed that after laser treatment, the diameter of inhibition zone increased for almost of the used antibiotics beside some isolates became sensitive especially after 2 min of laser exposure time. The hemolysin production was disappeared in (%40) of E. coli and (%20) of S. aureus after ten min of irradiation, while it decreased to half in another S. aureus isolate. All S. aureus and E. coli isolates loosed their ability to produce β-lactamase enzymes; some of these isolates loosed it after 5 minutes and the others after 10 minutes of irradiation. The results showed that laser irradiation hasn't any effect at any exposure time on adhesion factors of both S. aureus and E. coli isolates. Plasmid profile of Irradiated E. coli illustrated disappearing of DNA plasmid bands as well as RNA after ten min of irradiation.

Keywords: He-Ne laser, virulence factors, DNA plasmid, β-lactamase, hemolysin, S. aureus, E. coli..

الخلاصة

يهدف هذا البحث إلى دراسة تأثير ليزر الهليوم النيون على بكتريا Staphylococcus aureus و Escherichia coli المعزولة محليا, فضلا عن نمط المقاومة ، عوامل الفوعة ، ومحتوى البلازميد. تم جمع البكتيريا من مريض يعانون من التهابات المسالك البولية والتسمم الدموي والتهابات الجروح والحروق ، تم بعد ذلك تحديد العزلات وفقاً لصفاتها وبحسب نتائج الاختبارات المورفولوجية والكيميائية والاحيائية ونظام API. تم عزل سبعة وعشرين عزلة من بكتيريا S. aureus من أصل سبعة وثمانون عينة ، وعشرة عشر عزلة من E. coli. أظهرت النتائج أنه بعد العلاج بالليزر زاد قطر منطقة التثبيط للمضادات الحيوية المستخدمة تقريبا إلى جانب بعض العزلات التي أصبحت حساسة خاصة بعد ٢ دقيقة من زمن التعرض لليزر. اختفى إنتاج الهيموليزين في (٤٠ ٪) من بكتريا E. coli و (٢٠ ٪) من بكتيريا S. aureus بعد عشر دقائق من التشعيع ، بينما انخفض إلى نصف في عزلة أخرى من بكتريا S. aureus. جميع عزلات بكتريا S. aureus و E. coli فقدت قدرتها على إنتاج إنزيمات β-lactamase. بعض هذه العزلات فقدتها بعد ٥ دقائق والأخرى بعد ١٠ دقائق من التشعيع. وأظهرت النتائج أن التشعيع بالليزر ليس له أي تأثير على عوامل الالتصاق لكل من عزلات S. aureus وعزلات E. coli. يتضح من نتائج ترحيل البلازميد اختفاء أشرطة البلازميد DNA وكذلك RNA بعد عشر دقائق من التشعيع لبكتريا E. coli..

Introduction

Since the invention of it in 1960, laser occupied a large place of the scientist and technologist. Laser causes a series of important changes in science development, and many search in different science fields such as physics, biology, chemistry, electronics, and others were done using laser. In the last years, laser had been used in wide range in microbiology, and it solves many problems in this field. S. aureus and E. coli are the most common pathogens and have a wide distribution in all environments. They cause many diseases in various human body systems [1]. The rise of infection rates of S. aureus and E. coli come from their ability to produce

different virulence factors that capable them to avoid most of the defense mechanism of the body then cause infection; one of the most important virulence factors is the bacterial ability to produce different enzymes and toxin which play a role in invasion and tissue damages such as hemolysin toxin. In addition, bacteria can resist many different antibiotics which became a common problem in the entire world. Antibiotics resistances are attributed to many mechanisms such as decrease the permeability of outer membrane, efflux system, alteration of target site, alteration of metabolic pathways, or secretion of modifying enzymes [1][2]. On of the most important modifying enzyme is β-lactamase enzyme which capable bacteria to destroy the most important group of antibiotics that is β - lactam antibiotics, which include penicillins and cephalosporins [2]. The aim of this study is to detect the effect of He-Ne laser on the bacterial isolates; its virulence factors, antibiotic resistance, and the plasmid profile of the isolated bacteria with different exposure times.

Materials and Methods

Bacterial sampling

Eighty-seven samples were collected from many ways; urine samples of patient suffering from urinary tract infections, swab samples of wound and burn inflammations, as well as blood samples of patients suffering from septicemia. The samples were first cultured on the blood agar and MacConky agar as a differential media then incubated at 37°C for 24 hours. After that, bacterial isolates were identified according to Holt et al in (1994). The identification tests included cultural, morphological, Biochemical tests, as well as using API staph system for S. aureus isolates and api20E system for E. coli isolates. [1]

Antibiotics Tests

Susceptibility of eighteen different antibiotics was performed to the isolated bacteria. Zones of inhibition were measured and then compared with reference value of each antibiotic. [3]

Hemolysin detection

Hemolysin-produced bacteria were determined by streaking each colony of bacteria on blood agar that contained 5% of RBCs without serum or cholesterol, human blood group (AB) was used in the test because it gives clearest zone. Appearance of clear zone around the colonies due to RBCs lyses was considered as a positive result. [4]

β-lactamase detection

β-lactamase enzyme detection was prepared by using both Standard rapid iodometric (SRI) as well as Standard Nitrocefin (SN) Methods [5]

Adhesion detection.

It was performed by using epithelial cell method. [6]

Plasmid profile

Plasmid DNA isolate was done according and Neummanc at 1995.[7]

Laser Treatment

Five isolates of each species were chosen to study the laser effects on their virulence factors and antibiotics susceptibility. Each single colony of S. aureus and *E. coli* isolates was centrifuged at 6000 rpm for 10 minutes; cell pellets were washed twice with physiological saline then mixed by vortex and re-suspended in 5ml of normal saline. One milliliter of each isolate (1.5 X 108 cell/ml) was transferred to sterile ependorff tube and then treated with a 8mW He-Ne laser with 4mm beam diameter, and power density equal to 0.637 W/cm^2 at different exposure times (2, 5, and 10) minutes at room temperature in dark place. Samples were exposed to laser irradiation for different exposure time (2, 5, and 10) minutes with power density equal to (0.0637) W/cm^2, the experimental setup is shown in Figure (1). After that, the virulence factors and antibiotics sensitivity of each irradiated isolated were done separately to determine the effect of laser on each of them; non-radiated samples were used as a control group. [8]

Results and Discussion

Bacterial identification.

Results of the primary isolation illustrated the appearance of bacterial growth in seventy-one samples (81.6%). Out of seventy-one samples; twenty-seven (38.02%) and thirteen isolates of

them were identified as S. aureus and E. coli respectively.

Virulence factors results

Activities of the selected antibiotics against the bacterial isolates were ranging from the complete sensitive (such as imipenem) up to the complete resistance (such as penicillin). Generally, most of the isolates located in the range of resistance between five to nine antibiotics as shown in figure 1. While the results showed that twenty isolates (74.07%) and six isolates (46.15%) of S. aureus and E. coli were able to produce hemolysin respectively. β-lactamase results showed that twenty-five isolates of S. aureus (92.59%) were β-lactamase producer, and four isolates of E. coli (30.76%) were able to produce β-lactamase at SRI method, while the percentage raised to six isolates (46.15%) when the SN method was used in the case of E. coli isolates, the numbers of β-lactamase producer E. coli isolates at rapid iodometric method were less than that at nitrocefin method because the efficiency of nitrocefin method in detection

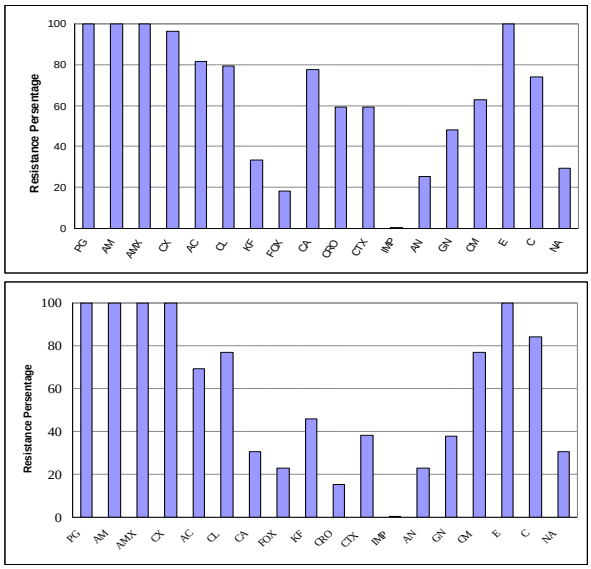

Figure (1): Antibiotic resistance of isolated bacteria. (A) S. aureus (B) E. coliPG: penicillin, AM: Ampicillin, AMX: Amoxicillin, CX: Cloxacillin, AC: Augmentine, CL: Cephalexine, KF: Cephalothin, FOX: Cefoxitin, CA: Ceftazidime, CRO: Ceftaxione, CTX: Cefotaxime, IMP: Imipenem. AN: Amikacine, GN: Gentamicin, CM: Clindomycine, E: Erythromycin, C: Chloramphenicol, NA: Nalidixic acid.

of a little concentration of β-lactamase that couldn't diagnose it in rapid iodometric method. [5] Finally the results of adhesion factors showed that fifteen isolates of S. aureus (55.56%) and eight isolates of E. coli (61.53%) were able to adhere with surface epithelial cells. The high percentage of E. coli may because her pilli which will help it to adhere with mucoid membranes that cover the urinary, respiratory, and intestinal tract systems. [9][10] While the *S. aureus* capsule and some specific surface protein may give it the adherence characteristics with epithelial cells. [11]

Laser exposure results.

Five isolates of each S. aureus and E. coli were selected to exposure to different time of He-Ne laser, the results of antibiotic sensitivity tests illustrated increasing the sensitivity of all bacterial isolates to most of the used antibiotics or some of them after bacterial irradiation.

It was clear that the diameter of inhibition zone was increased for almost of the used antibiotics beside some isolates became sensitive after laser treatment. Figure 2 shows some of the laser effects on bacterial susceptibility against antibiotics. Results also showed that the hemolysis production was lost in two isolates of E. coli (40 %) and one isolate of S. aureus (20 %) after ten minutes of irradiation.

β-lactamase production of irradiated isolates also referred to losing in all isolates. The results showed that three isolates of S. aureus and one isolates of E. coli lost their ability to produce β- lactamase after five minutes of irradiation, while the remained isolates lost it after ten minutes of irradiation. Figure 3 illustrates the laser effect on β-lactamase production of S. aureus and E. coli isolates.

The results appear that the laser have no effect on adhesion factors of irradiant bacteria, and it was clear the inability of laser to change the adherence state of any isolate of both S. aureus and E .coli at any exposure time. The results of

plasmids profile showed disappearing of the plasmid DNA of two *E. coli.* irradiated isolate after ten min of irradiation. The results also illustrated a complete disappearing of RNA of these isolates after the same time. Figure (4) shows the plasmid profile of *E. coli*. isolate before and after laser irradiation.

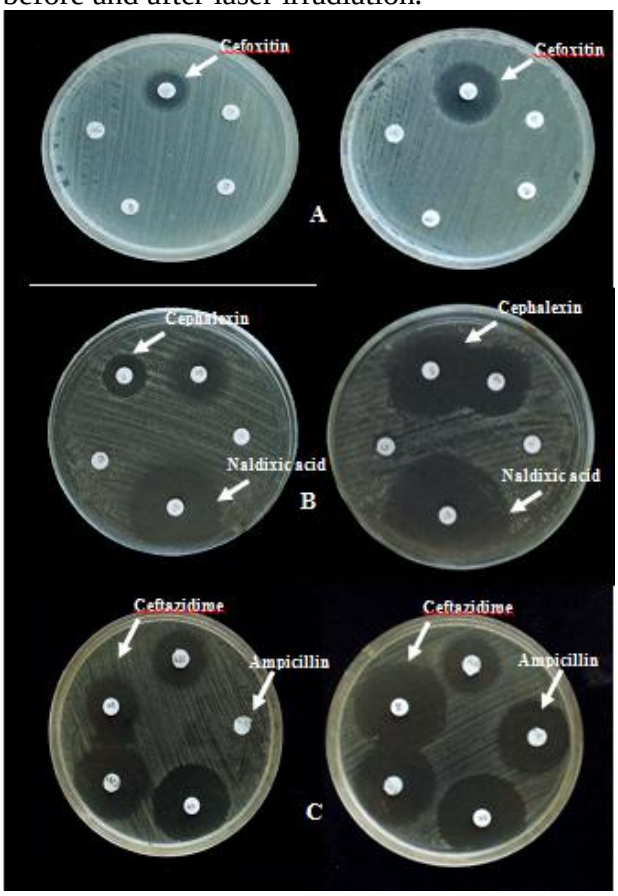

Figure2: Effect of He-Ne laser on bacterial susceptibility against antibiotics (A): S. aureus (B): E. coli (C): E. coli.

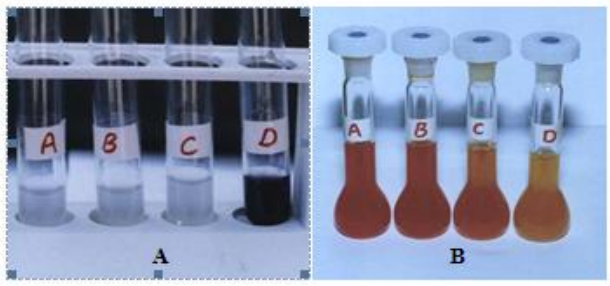

Figure 3: Effect of He-Ne laser on β- lactamase production of isolated bacteria by using SRI method (A) and by SN method (B).

(A): Bacterial suspension without irradiation (control).

(B): Irradiant bacterial suspension with 2 min.

(C): Irradiant bacterial suspension with 5 min.

(D): Irradiant bacterial suspension with 10 min.

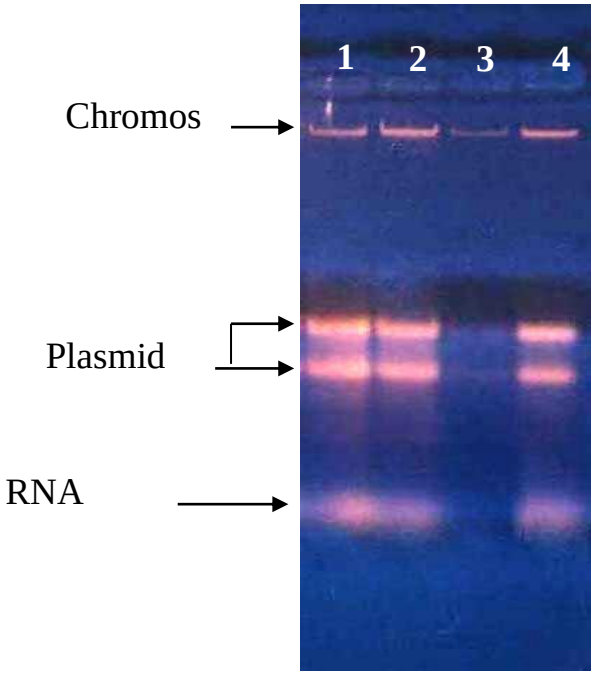

Figure 4: The plasmid profile of E. coli isolate before and after irradiation.

(1): Plasmid content after 2min of irradiation.

(2): Plasmid content after 5min of irradiation.

(3): Plasmid content after 10min of irradiation.

(4): Plasmid content without treatment

Discussion

Irreversible inhibition of plasmid DNA activity by irradiation may cause destroying of plasmid DNA and unable it to produce β-lactamase enzyme. [12] This fact explains the increasing of inhibition zones of β-lactam antibiotics (Penicillins and Cephalosporins) in bacteria after irradiation. Changing in bacterial pumping system, which mainly responsible of multi-resistance of antibiotics (such as β-lactams and aminoglycosides) also may increase the bacterial sensitivity to antibiotics. 8.In addition, increasing of bacterial sensitivity to antibiotics after irradiation may be a result of many mechanisms, which may be induced, such as cell wall and protein synthesis, membrane function, nucleic acid, and metabolic processes inhibitions [13]

Losing of the characteristic of β- lactamase production of irradiant isolates may occur due to irreversible inhibition of plasmid DNA activity that may lead to prevent bacterial from

production of plasmid-mediated β- lactamase. Malik et al noted the inability of irradiated bacteria to produce β- lactamase enzymes that encoded by plasmids. [12] Losing of β-lactamase production may explain the increasing of the sensitivity to β- lactam antibiotics. The lost hemolysin production of irradiated isolates was rare; this may come from the fact that hemolysin production in these bacteria is -mostly- encoded by chromosome. [12][14]. Many researchers noted that the genes encoding the adhesion factors in both S. aureus and E. coli, are carried on bacterial chromosome, and the last have a specific configuration bring it to withstand many environmental factors. [15]

The singlet oxygen, which generate through the irradiation process may cause plasmid destroying due to its interaction with nitrogen base of DNA. [16] The complete disappearing of RNA may regard because that RNA composed of single strip that make it easy to destroy than DNA. [17] In addition, photo lesions, which occur as a result of laser irradiation, may cause decreasing of DNA and RNA in size, and then destroy them. 17 From all the previous results, it's clear to see that the effect of He-Ne laser was mainly on plasmids without chromosome. The disappearing of plasmids was accompanied with hemolysin and β-lactamase (which are plasmid-mediated enzymes), [15][18][19] while there wasn't any effect on adhesion factors (chromosome-mediated characteristic [20].

References

[1] Holt, J.J; Krieg, N.R.; Seneath, B.H.; Staley, J.T.; and Williams, S.T. (1994). Bergey's manual determinative bacteriology 9th edition. William and Wilken. Baltimore. P: 175-248.

[2] Atlas, R. M. (1995). Principles of microbiology. 1st edition. Mosby yearbook, Inc.

[3] Maniatis, T.; Fritsch, E.; and Sambrook, J. (1988). Molecular cloning: A laboratory manual. Cold spring laboratory. Cold spring. New York.

[4] Senior, B. W.; and Hughes, C. (1997). Production and properties of hemolysin from clinical isolates of the proteae. Med. Microb. 24:17-25.

[5] WHO. (1978). Techniques for the detection of β-lactamase producing strains of Neisseria gonorrhoeae. 616:137-143.

[6] Hagberg, L.; Jodal, V.; and Lindberg, V. (1998). Adhesion, hemagglutination and virulence of E.coli causing UTI. Infect. Immune. 31: 564-570.

[7] Pospiech and Neumann (1995). Salting out procedure for the isolation of genomic DNA cited by Kieses, T. Norwich, U.K.

[8] AL-Khafaji, A.S. (2002). Bacteriocidal effect of Helium-Neon laser on the pathogenic bacteria. M.Sc.thesis, college of science. Baghdad University

[9] Martinez, J.J.; Mulvey, M.A.; and Schilling, J.D. (2000). Type1 pili- mediated bacterial invasion of bladder epithelial cells. Embo. J. 2803-2812.

[10] Sobel, J.D.; and Kaye, D. (1992). Urinary tract infections. In: principles of practice of infection disease. Mandell, G.L.; Dougles, R.G.; and Benett, J.E. Churchill Livingstone. New York P: 582-587.

[11] Robert, I.S. (1996). The biochemistry and genetic and capsular polysaccharide production in bacteria. Annu. Rev. Micrbiol. 50: 285-315

[12] Malik, Z.; Hanania, J.; and Nitzan, Y. (1990). Bacteriosidal effects at photoactivated porphyrins in alternative approach to antimicrobial drugs. J. Photochem. Photobiol. 5: 281-293.

[13] Gasha a, F. S. (2001). Study of the relationship of antibiotics resistance of *pseudomonas aeroginosa* isolated with its pyocin production. M.Sc thesis, college of science, AL-Mustanseria University.

[14] Solaga, A. Veiga M.P. Ostolaza, H. Brasseur R. (2002). Insertion of *E. coli* α – hemolysin in lipid bilayer as a non – transmemberane integral protein: production and experiment

[15] Mahfoud, N. N. (2002). Bacteriological, Molecular and cytological study on Urinary Tract Infections caused by some of the Pathogenic spp. of *Enterobacter*. PhD thesis, college of science. AL-Mustanseria University.

[16] Wilson, M; Dobson, J. and Havery, W. (1998): Sensitization of oral bacteria to killing by low power Laser radiation. Current Microbiol. 335: 1287-1291.

[17] Cheba, B.A. (2000). A study on the effect of He-Ne and Nitrogen lasers radiation on the viability of *E. coli* and its genomic content (A molecular Analysis). M.Sc. thesis, college of science / University of Baghdad.

[18] AL-Maadhidi, F. A. (1999). Comparison of the hemolysin and serological typing and antibiotic resistance of *E. coli* isolated from different infection sites. M.Sc. thesis, college of science. Mustansiriyah University.

[19]. AL-Jubori, S. S. (1997). Genetic and molecular study on β – lactamase enzyme produced by local

isolated of some gram–negative bacteria. PhD thesis, college of science. Mustansiriyah University.

[20] Jawetz, E. M.; Adelberg, E. A. (1998). Medical Microbiology, 25th edition. Appleton and Lange. USA.