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RESEARCH ARTICLE

EFFECT OF EXPOSURE TO CO₂ LASER ON ANTIBIOTIC SENSITIVITY OF DIFFERENT BACTERIA ISOLATED FROM PATIENTS ATTENDING MOSUL GENERAL HOSPITAL, IRAQ

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Abstract

Bacterial resistance to antibiotics is common in hospitals and community. A common approach to overcome bacterial resistance is by discovering new antimicrobial medicines. However, one of the alternative approaches is to change the way the bacteria behave against antibiotics. This study was conducted to investigate how LASER can alter the antibiotic sensitivity of different bacteria isolated from hospitalized patients. Antibiotic sensitivity on isolated bacteria was assessed by Kirby-Bauer antibiotic disk sensitivity test. The effect of exposure to LASER was investigated using CO₂ LASER. The results indicated that exposure to LASER changes the way the bacteria behave against antimicrobials either by inhibiting bacterial resistance or increasing bacterial sensitivity to antibiotics. The mechanism is still unclear.

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Introduction:-

LASER light is unique in being able to emit a powerful monochromatic, coherent and collimated light within a very narrow wave length (Svelto, 1989). The generated beams can be focused to a very small point, giving them a very high power density.

The effects of LASER on bacteria have been studied both *in vitro* and *in vivo* by many investigators in many publications (Džinić et al, 1988 ; Okamoto et al, 1992 ; Wilson, 1994 ; Ando et al, 1996 ; Kawamoto et al, 2000 ; Nussbaum et al, 2002 ; Coutinho et al, 2007 ; Benvindo et al, 2008 ; Fonseca et al, 2010 ; Roos et al, 2013 ; Hamzah et al, 2014 ; Pereira et al, 2014 ; Asadollahi et al, 2016). While some of these studies showed no effect of LASER on bacterial growth (Coutinho et al, 2007 ; Benvindo et al, 2008 ; Roos et al, 2013 ; Pereira et al, 2014) others demonstrated bacteriostatic and / or bactericidal effect (Okamoto et al 1992 ; Wilson, 1994 ; Hamzah et al, 2014). Moreover, contradicted results of bacterial overgrowth by LASER have also been demonstrated (Kawamoto et al, 2000; Nussbaum et al, 2002).

Antibiotic resistance increases dangerously in all parts of the world leading to increased mortality rates. One approach taken by scientists to combat antibiotic resistance is by discovering new medicines or to strengthen existing antibiotics by modifying them. In many instances, bacteria can find the way they confront antibiotics and now they can even evade new medicines. While ongoing efforts of discovering new antibiotics are still important, one alternative approach is to change the way the bacteria behave against antibiotics rather than waiting for new

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medicines. Many strategies have been developed to change bacterial behavior against antibiotics in such a way that increases the efficacy of both clearances by the host immune response, and by antibiotic therapy. However, it is unclear how external factors such as exposure to LASER changes the bacterial behavior to antibiotics. Taking this into consideration together with the fact that little is evident in literature regarding the effect of exposure of LASER on antibiotic sensitivity of bacteria making ongoing research in this area is not only justifiable but also necessary. The current research was conducted primarily to study the effect of exposure to LASER on the susceptibility of different bacteria to antibiotics by comparing the results before and after irradiation.

Materials and Methods:-

Bacterial isolation and identification:-

Ninety eight (98) clinical samples were collected by sterile swabs from patients with different clinical conditions attending Mosul General Teaching Hospital / Iraq in the period from July to November 2013. The age of patients varied from 1 day to 80 years and both sexes were included. These samples were taken from blood (32 samples), urine (56 samples), skin wound surfaces (8 samples) and stool (2 samples). Immediately after collection, the swabs were inoculated on fresh blood and MacConkey's agars and incubated at 37 °C for 18-24 hours for primary cultivation. On next day, the agars were studied for the presence or absence of growth, and the bacteria (genus and species) were identified according to their morphological and cultural characteristics, and biochemical tests. The following biochemical tests were used to aid identification of microorganisms: catalase test, oxidase test, mannitol fermentation test, lactose fermentation test, urease test, citrate utilization, and motility test. In some circumstances special media such as Salmonella Shigella (SS) agar were used in the diagnosis of microorganisms.

Kirby - Bauer antibiotic disk sensitivity test:-

The isolated bacteria were tested for their susceptibilities to nine different antibiotics by Kirby - Bauer antibiotic disc diffusion method according to the guidelines recommended by the Clinical Laboratory Standards Institute (CLSI) (2000). Using a fresh and pure culture, a suspension of the test organism equal to 0.5 McFarland Standard were spread over the entire area of Mueller Hinton agar (MHA) and allowed to be absorbed for 30 minutes. Using sterile forceps the antibiotic discs were placed onto the inoculated MHA plate, ensuring sufficient space between individual discs to allow for proper measurement of inhibition zones. The plates were then incubated at 37 °C for 18-24 hours. The following antibiotic disks were used: amikacin (30µg), amoxicillin (30µg), amoxicillin-clavulanic acid (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), trimethoprim - sulfamethoxazole (1.25 + 23.75 µg), tetracycline (30 µg), trimethoprim (5 µg) and imipenem (10 µg). Area of inhibition around the disks were measured by a ruler, recorded in mm and labelled as sensitive (S) resistant (R). The results were interpreted according to CLSI guidelines as follows:

Table (1):- CLSI guidelines for measuring areas of inhibition

Antibiotic disk and code	Antibiotic dose/ disk	Area of inhibition (mm)	Interpretation
Amikacin (AK)	30 µg	≤ 15 mm	Resistant
Amoxicillin (AX)	30 µg	≤ 21 mm	Resistant
Amoxicillin - Clavulanic acid (AXC)	30 µg	≤ 19 mm ★ ≤ 13 mm★★	Resistant
Ciprofloxacin (CIP)	5 µg	≤ 14 mm	Resistant
Erythromycin (E)	15 µg	≤ 22 mm	Resistant
Trimethoprim - Sulfamethoxazole (SXT)	1.25 µg+ 23.75 µg	≤ 10 mm	Resistant
Tetracycline (TE)	30 µg	≤ 23 mm	Resistant
Trimethoprim (TMP)	5 µg	≤ 10 mm	Resistant
Imipenem (IMP)	10 µg	≤ 16 mm	Resistant

★ for *Staphylococcus*

★★ for other bacteria

LASER system and LASER irradiation:-

The LASER used in this study was a CO2 Laser (PHYWE, Germany) available from the Department of Physics / College of Science / University of Mosul. This open laser system has a continuous wave (CW) radiation mode and an output power of maximum 8 W. It emits light in infra-red range of electromagnetic spectrum in a collimated beam at 10.6 µm standard wave length.

A loopful of culture taken from pure and fresh colonies isolated from each bacterium under study was inoculated into a 4 ml brain heart infusion broth at 37 °C for 18 - 24 hours. After incubation the broth was centrifuged at 3500 rpm for 15 minutes, the supernatant was discarded and the bacterial pellet was re-suspended in Phosphate Buffered Saline (PBS) to get a suspension of tested organism equal to 0.5 McFarland Standard. Thereafter, 400 µl of standardized bacterial suspension from each bacterial group was placed in sterile eppendorf tube and subjected to CO2 LASER for 1 minute. After irradiation 100 -200 µl of irradiated samples together with same volume from non-irradiated samples were spread separately over Mueller Hinton agar (MHA) and antibiotic sensitivity test were repeated using same antibiotic disks. The results of antibiotic sensitivity were then compared before and after irradiation.

Results:-

Bacterial isolation and identification:-

Among the total 98 collected clinical samples only 45 samples were grown on blood and / or MacConkey agars constituting 45.9 % (**Table 2**). These include 31 urine (68.9%), 2 stool (4.5 %), 6 blood (13.3%) and 6 skin wounds (13.3%) samples. All other 53 samples (54.1%) showed no growth at all on both blood and MacConkey agars. After isolation, the bacteria were determined according to their morphological and cultural characteristics, and biochemical tests. Regarding urine isolates our results indicated that *Staphylococcus aureus* and *E.coli* were the most common bacterial strains comprising 45.2 % for each followed by *Pseudomonas aeruginosa* (6.4%) and *Proteus mirabilis* (3.2 %) respectively (**Table 2**). Regarding blood isolates *staphylococcus aureus* was again the most frequent bacterial strain (83.3 %) followed by *Pseudomonas aeruginosa* (16.7%) while both *Salmonella* and *Proteus mirabilis* were isolated from stool (50 % each). *Staphylococcus aureus* was the most common bacterial isolate in skin wounds (66.6%) followed by *Streptococcus pyogenes* (16.7%) and *Proteus mirabilis* (16.7%).

Table (2):- Results of bacterial isolation and identification

Sample	No Growth (54.1%)		Growth (45.9%)		Isolated bacteria	Number	%
	Number	%	Number	%			
Urine(56)	25	47.1★	31	68.9★★			
					<i>S. aureus</i>	14	45.2
					<i>E. coli</i>	14	45.2
					<i>P. aeruginosa</i>	2	6.4
					<i>P. mirabilis</i>	1	3.2
					Total	31	100
Stool (2)	0	0★	2	4.5★★			
					<i>Salmonella</i>	1	50
					<i>P. mirabilis</i>	1	50
					Total	2	100
Blood(32)	26	49.1★	6	13.3★★			
					<i>S. aureus</i>	5	83.3
					<i>P. aeruginosa</i>	1	16.7
					Total	6	100
Skin (8)	2	3.8★	6	13.3★★			
					<i>S. aureus</i>	4	66.6
					<i>S. pyogenes</i>	1	16.7
					<i>P. mirabilis</i>	1	16.7
Total (98)	53	54.1	45	45.9	Total	6	100

★ Percentage among "No Growth" samples

★★ Percentage among "Growth" isolates

Antibiotic susceptibility testing:-

Next the six isolated bacteria namely *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus pyogenes* and *Salmonella* were tested for their susceptibility to nine different antibiotics using commercially available antibiotic disks. The antibiotic disks were expressed as the concentration of antimicrobial agents recommended by CLSI and the areas of inhibition were measured in mm and labelled as Sensitive or Resistant according to CLSI guidelines (**Table 1**). The results of antibiotic sensitivity were then presented as the percentage of resistant strains and were summarized in (**Table 3**). *E.coli* strains (14 isolates) were most sensitive to

amikacin (AK) and imipenem (IMP) with 100 % sensitivity rate and most resistant to amoxicillin (AX) with 64.3 % resistant rate. *Staphylococcus aureus*, the most frequent isolates among our study sample (23 isolates), appeared also to be most sensitive to AK and IMP with a sensitivity rate of 92% % and most resistant to AX and sulphamethoxazole (SXT) by about 82% and 48 % respectively. IMP followed by AK are the most effective antibiotics against *Pseudomonas aeruginosa* among our study sample with 66.7 % and 33.3 % sensitivity rates respectively. All other studied antibiotics appeared to be not effective against this microorganism. In addition, *P. mirabilis* was most sensitive to IMP, AK, trimethoprim (TMP) and ciprofloxacin (100 % sensitivity rates), moderately sensitive to amoxicillin (AX) and amoxicillin-clavulanic acid (AXC) while relatively resistant to erythromycin (E), SXT and tetracycline (TE). *Streptococcus pyogenes* were sensitive to all nine antibiotics while *Salmonella* strain was only resistant to E and TE. Overall, in all studied bacteria the highest sensitivity average rate among all antibiotic used was seen with IMP (7%) whereas the highest resistant average rate was seen with TE (57.4%).

Table (3):- Results of antibiotic disk diffusion test interpreted according to CLSI guidelines

Isolated Bacteria	N°	AK	AX	AMC	CIP	E	SXT	TE	TMP	IMP
		R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
<i>E.coli</i>	14	0	64.3	42.9	50	35.7	57.1	42.9	14.3	0
<i>S. aureus</i>	23	8.7	82.6	39.1	13.0	34.8	47.8	34.8	30.4	8.7
<i>P.aeruginosa</i>	3	66.7	100	100	100	100	100	100	100	33.3
<i>P. mirabilis</i>	3	0	33.3	33.3	0	66.7	66.7	66.7	0	0
<i>S. pyogenes</i>	1	0	0	0	0	0	0	0	0	0
<i>Salmonella</i>	1	0	0	0	0	100	0	100	0	0
All bacteria	45	12.6	46.7	35.8	27.2	56.2	45.3	57.4	24.1	7

N° Number of tested isolates

R Resistance rate

Effect of exposure to LASER on antibiotic sensitivity:-

According to the results of antibiotic sensitivity test illustrated in (Table 3), IMP and TE have the highest sensitivity and resistant average rates respectively. Therefore, these two antibiotics were chosen to investigate the effect of exposure of LASER on antibiotic susceptibility in all six studied bacteria. However, only those bacterial strains that were sensitive to IMP and resistant to TE were selected. The results were presented as the average diameter of the inhibition zone **BEFORE** and **AFTER** exposure to LASER and interpreted as sensitive or resistant according to CLSI guidelines (Table 4). Surprisingly, while all the selected bacterial strains were resistant to TE before exposure to LASER, exposure to LASER rendered them all sensitive to TE. In addition, LASER irradiation seems also to increase the diameter of the IMP sensitive strains as well. Hence, marked changes in the sensitivity to antibiotics were seen after irradiation with CO₂ LASER for 1 minute.

Table (4):- Effect of exposure to LASER on antibiotic sensitivity of the six studied bacteria

Isolated bacteria	Inhibition zone (mm) BEFORE exposure to CO ₂ LASER		Inhibition zone (mm) AFTER exposure to CO ₂ LASER	
	TE	IMP	TE	IMP
<i>E.coli</i>	R (12 mm)	S (18 mm)	S (23 mm)	S (25 mm)
<i>S. aureus</i>	R (15 mm)	S (16 mm)	S (20 mm)	S (26 mm)
<i>P. aeruginosa</i>	R (11 mm)	S (16 mm)	S (22 mm)	S (23 mm)
<i>P. mirabilis</i>	R (10 mm)	S (18 mm)	S (19 mm)	S (22 mm)
<i>S. pyogenes</i>	R (13 mm)	S (23 mm)	S (22 mm)	S (28 mm)
<i>Salmonella</i>	R (14 mm)	S (12 mm)	S (21 mm)	S (22 mm)

Discussion:-

The results of the current study showed that *Staphylococcus aureus* was the most abundant isolated bacteria with an overall frequency rate of 51.1 % among all studied samples that showed positive growth on culture media. The high isolation rate of *S. aureus* might reflect the high endemicity of this microorganism among hospitalized patients. However, lack of personal hygiene, remarkable versatility of the microorganism and the wide diversity of diseases caused by it, together with the numerous virulence factors that make it has the ability to colonize and distribute in different environments, are additional reasons for higher isolation rates of *S. aureus* in our locality.

Regarding different clinical samples examined, our results indicated that *S. aureus* and *E. coli* were the most common isolated bacteria in urine comprising 45.2 % (14 samples) each, followed by *Pseudomonas aeruginosa* (6.4%, 2 samples) and *Proteus mirabilis* (3.2 %, 1 sample) respectively. In sharp contrast to what was reported in the west, high isolation rates in urine among both community - based and hospitalized patients were reported in Iraq, middle east and third world countries, ranging from 14.4 % in Iran (Soltania et al, 2010), to 24.4 % and 40% in Nigeria (Nsofor et al, 2016, Obiazi et al, 2007), up to 76.4 % in Iraq (Yassin et al, 2013). Our results regarding *S. aureus* frequency rate in urine is not far different from others. While these pictures might reflect the real prevalence of *S. aureus* in urine in these areas, it is more likely to be contaminants due to poor personal hygiene. Moreover, hospital instrumentations, cannulations, catheterization are major risk factors for increasing frequency rates in urine among hospitalized patients. In addition to *S. aureus* we also found *E. coli* was equally contributed for bacterial isolates in urine. These findings are not dramatically different from the results of investigators in other cities of Iraq such as Baghdad (Maha, 2011), Kirkuk (Alsamarai and Ali, 2016) and Karbala (Mohammed et al, 2014).

Regarding blood isolates, *S. aureus* was again the most frequent bacterial isolate (83.3 %, 5 samples) followed by *P. aeruginosa* (16.7%, 1 sample). *S. aureus* is the leading cause of bacteremia and / septicemia among hospitalized patients worldwide (Rio et al, 2009). Several factors associated with an increased risk of developing *S. aureus* bacteremia (SAB) including presence of central venous and urinary catheterization, surgery, injection drug use, presence of immunosuppressive conditions, and use of corticosteroids (Nabera, 2009). Although small specimen size, our results in regard to *S. aureus* are in agreement with those reported in other parts of the world such as Europe (Luzzaro et al, 2002), US (Shorr et al, 2006) and Brazil (Marra et al, 2011). However, a discrepancy was found between our results and those reported in nearby countries such as Turkey (Dogru et al, 2010) and Iran (Pourakbarier et al, 2012 ; Ghadiri et al, 2012) were Coagulase Negative *Staphylococcus* (CoNS) and *E. coli* were found to be the most prevalent microorganisms that have been isolated from blood in hospitalized patients. Possible explanations for these discrepancies include different study size, different patients included, comorbidities and length of stay, differences in individual risks and different sources of pathogens causing blood infections.

S. aureus was again the most common bacterial isolate from skin wounds in our study (66.6%, 4 samples) followed by both *Streptococcus pyogenes* (16.7%, 1 sample) and *Proteus mirabilis* (16.7%, 1 sample). While *S. aureus* and *S. pyogenes* are well known skin pathogens (Bowler et al, 2001), *P. mirabilis* skin infection is somewhat controversial. While *P. mirabilis* was the commonest *Proteus* spp isolated from wound infections in some studies (Mordi and Momoh, 2009; Auwaerter, 2008; Oguachuba, 1985), it is completely absent in other studies (Sapica et al, 2008 ; Klainer and Bisaccia, 1991). *P. Mirabilis* is a well-known, although not the most common, nosocomial bacteria that persist continuously in the hospital environment. Isolation of *P. mirabilis* from skin in this study might, therefore, be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent or long-term hospitalization. In addition, both *Salmonella* and *P. mirabilis* were equally isolated from stool from in-door patients (50 % each). However, no solid conclusion can be made from these results due to very small specimen size.

Determination of antibiogram profile of isolated bacteria was the next step in this research. In our study, antibiotic susceptibility results of *S. aureus* showed a moderate to high resistance rate against some of the most commonly prescribed drugs such as AX (82.6%), E (34.8%) and TE (34.8%). The findings of this study are consistent with the findings of other researchers in other regions of Iraq (Yaseen et al, 2013; Al-Dahbi and Al-Mathkhury 2013; Al-Saadi and Kadhim, 2014). This low to moderate activity of these antibiotics can be attributed in part to earlier exposure of the isolates to these drugs which may have enhanced resistant development. High resistance rate to AX might also be attributed to β -Lactamase production by *S. aureus* which made this microorganism resistant to many penicilins. This is supported by the observation that substitution of AX with AXC (amoxicillin - clavulanic acid), the latter is a β -Lactamase inhibitor, reduced antibiotic resistance for this microorganism from 82 % to 39 %. *tetK* and *tetL* genes located on a plasmid and *tetM* or *tetO* determinants located on either a transposon or the chromosomes are the main genes responsible for acquisition of *S. aureus* resistance to TE (Warsa et al, 1996 ; Bismuth et al, 1990). Meanwhile, resistance to E is conferred by the presence of *erm* genes [*erm* (A), (B) and (C)] conferring resistance to Macrolide, lincosamide and streptogramin type B (MLS_B) antibiotics (Jensen and Lyon 2009). We also noted a potential resistance to SXT (47.8%) / or TMP (30.4%) in our study. Literature search revealed that the patterns of resistance of *S. aureus* to these antibiotics vary according to geographical locations worldwide. While more than 90 % sensitivity rate were reported in US, Canada, Europe, Japan and Turkey (Paul et al, 2015), considerable resistance rate was observed in other parts of the worlds ranging from 19% in sub-Saharan Africa , to 30 % in Australia, to more than 85 % in India % (Paul et al, 2015). Globally *dfgG* is the predominant determinant of SXT / and or TMP resistance in human *S. aureus* infection (Nurjadi et al, 2015). On the other hand, we observed

that staph isolates were highly sensitive to less - commonly prescribed drugs such as IMP and AK (93% sensitivity) and CIP (87%). We recommend that using of these drugs should be highly preserved to most resistant infections to avoid rapid emergence of resistant strains as a result of continuous selective pressure from the use of antimicrobial agents.

Regarding *E.coli* isolates, our results indicated moderate to high resistance rate to AX (64.3%). AX is commonly used in Iraq to treat *E.coli* infection. However, data collected from different literature indicated variable susceptibility rate to AX by *E. coli* according to geographical location. Aljanaby and Alfaham (2017) reported 100 % resistance rate to AX by *E.coli* in Al-Kufa city' southern of Iraq. Alsamarai and Ali (2016) showed high resistance rate to AX in Kirkuk city in the north of Iraq (79.3%). Lower resistant rates (33%) were reported by other investigators in Kurdistan region (Assafi et al, 2015). These different degrees of resistance to AX might be due to different resistance mechanisms used by the different strains of *E.coli* like the efflux pump, target substrate configuration, enzyme production and modification and degradation (Ali and Aljeboury, 2017). Moreover, more than 50 % of *E. coli* strains in the current study were resistant to SXT (57.1%) and CIP (50%). These results are in conformity with other studies in Iraq (Alsamarai and Ali, 2016; Assafi et al, 2015). Potential resistance rate was also seen with AXC (42.9 %) and TE (42.9%). Overall, the trend of antibiotic resistance by *E.coli* in our study goes with the global concern about the increase in the emergence of multi-drug resistant *E.coli*. A rise in bacterial resistance to antibiotics might be due to the fact that most of the patients are given antibiotics before bacteriological investigation. Inversely, we noted absolute sensitivity of *E.coli* to less commonly used antibiotics, IMP and AK with 100 % susceptibility rate making these antibiotics of the first alternative choice for the treatment of *E.coli* infections in our locality. This finding is uniformly consistent with the observations of many other researchers (Alsamarai and Ali, 2016; Assafi et al, 2015; Mohammed et al, 2014).

Some of the *P. aeruginosa* strains screened showed a considerable resistance to IMP (33.3%) and AK (66.7%). Similar pattern of resistance profile was recorded by AL- Rubaye et al (2015). However, another study carried out in Mosul city found a lower resistant rate for IMP (15%) and AK (10%) respectively (Al-Derzi , 2012). Different number of isolates (sample size) between the two studies might have affected the frequency distribution of the resistance antibiogram. In addition, a substantial difference in resistance pattern between our study and others in Iraq was also noticed. Assafi et al, 2015 reported 0 % resistance rate to IMP and AK among patients with UTI in Duhok city. Yassin et al (2014) showed 12.7 % and 24% resistance rate to IMP and AK among patients with wound infections. *Pseudomonas* resistance to IMP and AK might be due to both intrinsic and extrinsic factors. Intrinsic resistance attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., mexAB, mexXY, etc.) and the low permeability of the bacterial cellular envelopes (Poole, 2004). In addition *P. aeruginosa* easily acquires resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants (Poole, 2004). In addition, a uniform absolute resistance (100%) to AX, AXC, CIP, E, SXT, TE and TMP seen in this study has limited the use of these antibiotics for treatment of *P. aeruginosa* infection in our area.

IMP and AK were again the most effective drug against *P. mirabilis* isolates in the current research (100 % sensitivity rate). Al-Jumaily and Zgaer (2016) reported high sensitivity rate of *Proteus* isolates to IMP and AK among patients with UTIs isolated from different hospitals in Baghdad (97.4 % and 92. 6% sensitivity rate respectively). High sensitivity rate to IMP (nearly 100%) was also documented by Ahmed (2015) making this antibiotic of choice for treatment of *P. mirabilis* resistant infections in our regions. However, regarding AK, some discrepancies were found between the investigators in our country. While 100 % sensitivity rate was reported by Mohamed (2013), Ahmed (2015) was reported only 70 % sensitivity rate of *Proteus* spp to above mentioned antibiotic. Different sites of infection from which microorganisms collected, previous exposure to antibiotic and length of hospital stay at time of infection are possible contributing factors.

The most important aim of this study was to investigate the effect of LASER radiation on antibiotic susceptibility of isolated microorganisms. Since IMP and TE have the highest sensitivity and resistant rates respectively (**Table 3**), therefore, these two antibiotics were chosen to investigate the effect of exposure of LASER in all studied bacteria. In general there are two types of LASERs, continuous wave (CW) and pulsed wave (PW) according to the duration of LASER emission (Assuncao and Williams, 2013). Both systems have been used to induce an effect on bacteria (Kundwal et al, 2015). Nussbaum et al (2002) have studied the effect of LASER radiation on in vitro growth of bacteria using both CW and PW modulated lights. They concluded that both mode of operation significantly increased the overall bacterial growth. However, with the PW radiation the bacterial growth is species dependent

(Nussbaum et al, 2002). For example *P. aeruginosa* proliferated significantly more than other bacteria such as *S. aureus* and *E. coli*. No such species dependent effect was found with the continuous wave (CW) mode of LASER radiation. Since our aim was to compare the effect of LASER on different bacteria under same conditions, therefore, to avoid possible bias resulting from species dependent effect of PW, we decided to use CW LASER system. Carbon dioxide (CO₂) LASER has been used in the current study because of its efficiency in generating high power CW beams comparing to other LASER systems. In addition although it was one of the earliest invented LASERs where it discovered in 1964 (Patel, 1964), it is still one of the most useful and commonly used LASERs.

The effects of LASERs on bacteria were studied by many investigators both in vitro and in vivo (See introduction for literature review). These effects ranged from no effect at all to bacteriostatic and / or bactericidal effect mediated directly by destruction of bacterial components. Meanwhile, the effect of LASER on antibiotic sensitivity of bacteria is a matter of controversy. While some studies supported this effect (Rassam, 2010; Ismail et al, 2012; Al- Jebouri and Al-Shakarchy, 2013) others did not (AL-Derajy, 2009). Possible explanations for these disputatious results include different microorganism(s) studied, different LASER systems used, dose and time of exposure to LASER, different antibiotic studied, the applied wave length and mode of operation (continuous or pulsed mode).

In regard to the current study, our results showed that all the TE resistant strains in all six studied bacteria were become sensitive to it after 1 minute exposure to CO₂ LASER. Moreover, the sensitivity of the six studied bacteria to IMP was increased after exposure to LASER as demonstrated by increasing the diameter of growth inhibition zone around IMP disk in antibiotic sensitivity test. It is still unclear how LASER might change the sensitivity of bacteria to different antibiotics. Since the two main mechanisms **specifically** responsible for TE resistance are **tetracycline efflux** and **ribosome protection** (Speer et al, 1992), therefore alteration in these two mechanisms are likely to be, at least partially, involved in changes to TE susceptibility induced by LASER. Hence, one of the possible suggestive mechanisms is increasing bacterial drug permeability or decreasing bacterial active efflux (pumping out) mechanism after exposure to LASER which resulting in increased drug accumulation inside bacteria. LASER might also decrease the activity of some bacterial cell wall enzymes and / bacterial cell ribosome protecting proteins responsible for reducing the ability of the antibiotic to bind to bacterial ribosomes and hence decrease its capability to disrupt protein synthesis. On other hand, increasing sensitivity to IMP might be due to synergism effect between antibiotic and LASER which make the bacteria more sensitive to it. IMP acts as an antimicrobial through the inhibition of cell wall synthesis (Rodloff et al, 2006). This inhibition of cell wall synthesis in gram-negative bacteria is attained by binding to penicillin binding proteins (PBPs) (Hashizume et al, 1984). The latter are group of enzymes involved in the biosynthesis of cell wall peptidoglycans (Hashizume et al, 1984).LASER might increase the binding affinity of IMP to PBPs and therefore increasing bacterial sensitivity to this antibiotic.

Conclusion:-

S. aureus seem to be most abundant isolate with a frequency rate of more than 50%, followed by *E.coli* (31.1%). Antibiogram trend showed potential resistance rate to commonly prescribed drugs such as amoxicillin (AX), erythromycin (E), Tetracycline (TE) and Trimethoprim- sulphamethoxazole (SXT). In general imipenem (IMP) and amikacin (AK) appeared to be effective; however, their use should be restricted to highly resistant cases. CO₂ LASER changes the way the bacteria behave against antibiotics by either inhibition of bacterial resistance or increasing bacterial sensitivity to antibiotics. The exact mechanism is still unclear.

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