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# Effect of 532nm CW Nd:YAG Laser on Antibiotics Susceptibility of *Klebsiella Pneumoniae* Isolated from Burn and Wound Infections

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**Abstract**
This study was aims to assess the effect of laser diode light at 532nm on susceptibility of*Klebsiella pneumoniae* isolates from burn and wound infection to antibiotics. The susceptibility of three isolates of *K.pneumoniae* (T40, T92 andT105) provided from previous study were tested against nine antibiotics. All three isolates were showed sensitivity to Imipenem (IMP) and Ciprofloxacin (CIP) while it's showed resistance to the rest antibiotics. The effect of the radiation on the susceptibility to antibiotics were tested via subjecting isolates laser diode light at 532nm wavelength 3mW, for 1, 2 and 3min.Results showed that a significant increase in susceptibility for all isolates toward imipenem and ciprofloxacin.
**Key word:** Nd: YAG Laser, Antibiotic Susceptibility of *Klebsiella pneumoniae,* Burn and Wound Infections.

## INTRODUCTION

LASER (light Amplification by Stimulated Emission of Radiation) is one of the most important scientific discoveries of the previous century that has been contributed innumerous application because of its specific properties which are distinguish it from other normal light sources (Drammen, 2012). The distinctive property of laser is that its light waves propagate over long distances with a very little spreading, and such waves have fixed phase relationship (coherent) that may make laser light very intensive in bandwidth, powerful and flexible in focus on a given target (Brown and Arnold, 2010).

*K. pneumoniae* is commonly known as causes of opportunistic infections distributed in different distributed in the major systems (e.g. urinary and respiratory tracts) of healthy people (Bunyan, 2016). This microorganism consider as a hospital-acquired pathogen could be lead to pneumonia which highly risk respiratory infections (Sharma *et al.*, 2016). Furthermore, it's responsible on different range of infections such as wound, sepsis and abscesses (zedan, 2013).

According to Chiu *et al.* (2013), the dealing with *Klebsiella* infections is more complicated. This is due to present range of virulence factors (e.g. fimbriae, siderophore and capsule)in this pathogen which provide the ability to attack and survive in the host (Aher *et al.*, 2012).

In addition, *K. pneumoniae* possess distinguish structures of capsule consist of complex acidic polysaccharides (Khalid *et al.*, 2017). There is confirmed association between differences serotypes (serotype K1 and to a lesser extent K2) with highly

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severe disease (Fang *et al.*, 2012; Zedan *et al.*, 2013). According to Doud *et al* (2009), the genes cluster encoded to capsular polysaccharide synthesis consists of specific region are conserved with serotypes.

However, most reports agree that the main virulence factor in its pathogenicity is the resistant to wide range of antibiotics specially β-lactam antibiotics (Shaikh *et al.*, 2015; Al-Janaby and Alhasnawi, 2017). *K. pneumoniae* possess Carbapenemase enzymes that responsible for the Carbapenems resistant (Deshpande *et al.*, 2010). This is lead to focus more on alternative therapies to support the treatment of the highly incidence of *Klebsiella* infections (Dubey *et al.*, 2013). Therefore, in this study, the impact of LAZER will be evaluated if it enhances the antibiotic activity or not.

## MATERIALS AND METHODS

### BACTERIAL ISOLATES

The three isolates of *K. pneumoniae* are provided from previous study (Zedan, 2013). The isolation sources as following: T40 and T105 were isolated from burn infection; T92 was isolated from wound infection.

### ANTIBIOTIC SUSCEPTIBILITY TEST (DISC-DIFFUSION METHOD)

*K. pneumoniae* isolates were tested against nine antibiotics [Amikacin (AK), Amoxicillin+Clavulanic acid (AMC), Cefotaxime (CTX), Ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamicin (CN), Imipenem (IMP), Sulfamethoxazole+Trimethoprime (CXT) and Tetracyclin (TE)]. The standard disc diffusion method was used which described in following steps: The required dilution prepared by inculation 5ml of sterile (85% ) NaCl with fresh culture of the tested isolate and compared with 0.5 McFarland. A sterile cotton swab is dipped into the inoculums and swabbed across the surface of Muller-Hinton agar plate. Then, inoculated plates were incubated at room temperature for 10min. Later, the antibiotic discs were placed firmly on the inoculated plates and incubated at 37Cº for 24hrs. Second day, diameters of the inhibition zones were measured in mm. The results compared with the standards of NCCLs (the National committee for clinical laboratory standards) (Barry, 1976).

### IRRADIATION THE BACTERIA WITH DIODE PUMPED SOLID STATE (DPSS) LASERS

Nd: YAG laser was used as the Light source (conditions: output power 150mW that emits; light in a collimated beam with diameter of 3mm.); wavelength of 532nm). *Klebsiella* spp. was cultivated in brain heart infusion at 37˚Cfor 18hr.; culture was centrifuged followed for 10min. at 4000rpm. The cell pellet was diluted in sterile phosphate buffer pH=7, and adjusted to concentration of $10^6$ CFU/ml. A diluted bacterial suspension was put in tubes and exposed to laser light at different periods (1,2 and 3)min. and power density of 2.125W/$cm^2$foreach sample(AL-Aamirry, 2003).

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## STATISTICAL ANALYSIS

Data were analyzed statistically using SPSS program version 23. Results were expressed using simple statistical parameters such as mean and standard error. Differences between means were assessed by ANOVA, followed by Duncan and Tukey's tests.

## RESULTS AND DISCUSSION

### ANTIBIOTIC RESISTANCE OF *K. pneumoniae* ISOLATES

Susceptibility of the three isolates of *K. pneumoniae* was examined and the results showed that all tested isolates were sensitive to IPM and CIP and the highest sensitivity was recorded by T105 isolate to IMP (15.0±2.3)mm, on the other hand, all isolates were resistant to rest antibiotics used in this study as shown in table (3).

**Table1.Antibiotics susceptibility test of *Klebsiella pneumoniae* isolates.**

| Antibiotic symbol | Susceptibility of isolates (Mean±SE)Diameter (mm.) | | |
|---|---|---|---|
| | T40 | T92 | T105 |
| (CR) | R | R | R |
| AK | R | R | R |
| IMP | 11.6±1.2 | 10.6±1.7 | 15.6±2.3 |
| CIP) | 10.0±1.1 | 9.0±2.0 | 10.0±1.1 |
| TE | R | R | R |
| SXT | R | R | R |
| CTX | R | R | R |
| CN | R | R | R |
| AMC | R | R | R |

CR(ceftriaxone); AK(amikacin); IMP(imipenem); CIP(ciprofloxacin); TE(tertracyclin); SXT(sulfamethoxazole); CTX(cefotaxime); CN(gentamycin); AMC (Amoxicin and clavulanic acid).

This result was in agreement with (Amin *et al.* 2009) in Pakistan who noticed that the percentage of resistance to cefotaxime and ceftriaxone were 82.5% and 85%, respectively. However, (Nasehi *et al.*, 2010) reported that *K. pneumoniae* isolates were 27% resistant to ceftriaxone and this may be related to possessing of β-lactamase enzymes (cephalosporinase) which are able to the inactivate cephalosprins through cleavage β-lactam ring of the drug.

While the resistance to the penicillin group included ampicillin, amoxicillin+clvulanic acid and piperacillin were 97.5% for both ampicillin and amoxicillin+clavulanic acid and 95% for piperacillin. Resistance of *K. pneumoniae* to the cephalosporin group and penicillin group may be related to possessing of β-lactamase enzymes (cephalosporinase and penicillinase) which are able to the inactivate pencillins and cephalosprins through cleavage β-lactam ring of the drug (Stock and Wiedmenn, 2001; Pagani *et al.*, 2006). The resistance of testedisolates to the amikacin and gentamicin (aminoglycosids group) were 17.5% and 30%, respectively. Similarly,

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Nasehi *et al.* (2010) found that resistance to amikacin was 17.5 %.In this regard, Feizabadi *et al.*, (2007) found a relation between resistant to aminoglycosids and producing extended spectrum β-lactamase enzymes in *Klebsiella* isolates.

*Klebsiella* isolates showed 20% resistance to ciprofloxacin as one of quinolones group. Fluit *et al.* (2001) mentioned that the resistance to quinolones is associated with modification in antibiotic-enzyme (GyrA) binding site.

The resistance to Trimethoprim+Sulphamethoxazolewhich belongs to Sulfonamide group was 77.5%. This combination has bactericidal effect through blocks two steps in bacterial biosynthesis of essential nucleic acids and proteins (Fluit *et al*., 2001).

Study conducted by Feizabadi *et al*. (2008) revealed that *K. pneumoniae* isolated from respiratory tract showed sensitivity against imipenem. The susceptibility of thetested isolates against imipenem is agreed with study conducted by Lim et al. (2009) and Nasehi *et al.* (2010). This result might be support that imipenem is most effective treatment against *K. pneumonia* comparing with other tested antibiotics.

Results revealed that susceptibility of T40 to IMP was significantly higher after of radiation compared with before radiation (12.6, 24, and 17 *vs.* 11.6)mm. respectively. Susceptibility of T92 was (10.6, 17.6 and 10.6)mm. respectively compared with before radiation (10.6) mm. Susceptibility of T105 was (16, 24 and 14)mm. respectively in comparison with results before radiation 15.6mm. The highest susceptibility of all isolates after radiation was recorded after 2min (Table,2).

**Table2.Susceptibility of *Klebsiella pneumonia* isolates against Imipenem before and after radiation with laser diode**

| Isolate | Before radiation | Treatment (Mean ±SE)Diameter(mm.) | | |
|---|---|---|---|---|
| | | 1 min. | 2 min. | 3 min. |
| T40 | 11.6±1.2 b | 12.6±1.7 b | 24.0±2.3 a | 17.0±1.5 b |
| T92 | 10.6±1.7 b | 10.6±1.7 b | 17.6±1.4 a | 10.6±1.7 b |
| T105 | 15.6±2.3 b | 16.0±2.0 b | 24.0±1.1 a | 14.0±1.1 b |

Different letters mean significant differences between means of before and after radiations ($p<0.05$).

As shown in Table (3) results exhibited a significant increase in susceptibility of all isolates against CIP after radiation in comparison with before radiation. Susceptibility of T40 (19.6, 20.3 and 27.3 *vs*. 8)mm. The inhibition zone of T92 was (19.3, 22.6 and 26.6 *vs.* 9)mm. Susceptibility of T105was (25.6, 24.3 and 26 vs. 10)mm. Also results showed that the highest sensitivity after 3min. of radiation.

**Table3.Susceptibility of *Klebsiella pneumoniae* isolates against Ciprofloxacin before and after radiation with laser diode**

| Isolate | Before radiation | Treatment (Mean ±SE)Diameter(mm.) | | |
|---|---|---|---|---|
| | | 1 min. | 2 min. | 3 min. |
| T40 | 8.0±1.1 c | 19.6±2 b | 20.3±2.1 b | 27.3±1.7 a |

| | | | | |
|---|---|---|---|---|
| T92 | 9.0±2 b | 19.3±1.7 b | 22.6±2.4 ab | 26.6±1.4 a |
| T105 | 10.0±1.1 b | 25.6.0±1.2 a | 24.3±1.2 a | 26.0±2.3 a |

Different letters mean significant differences between means of before and after radiations ($p<0.05$).

Changes in sensitivity of bacterial isolates to the antimicrobial agents after treatment with diode laser is may be due to the combination effect of laser and antimicrobial agent making the bacterial cell more sensitive to the antimicrobial agents. The available explanation behind the sensitivity of bacteria to antibiotic may be due to the modification in bacterial efflux pump as pumping system which is that essentially responsible on bacterial resistance to antibiotics such as (B-lactams, aminoglycoside)(Al-Jailawi*et al.*, 2014). Failure of bacteria to produce specific enzymes that chemically modify specific antibiotic also may be increased the bacterial sensitivity to the antibiotics. (Karu*et al.*, 1993).Another explanation for bacterial resistance to antibiotics is related to bacterial envelope as suggested by McDonnel and Russell(1999). It believed that they are referring to capsule in their reports (Singh *et al.*, 2012). The lippolysaccharide layer present inGram-negative bacteria cell walls is more likely to be affected or impairedwhen it exposure laser radiation activity.

**Conclusions:** The effect of at 532nm wavelength 3mW diode laser increases the susceptibility of *K. pneumoniae* T40, T92 and T105 isolates to imipenem and Ciprofloxacin. The exposure time 2min gave the highest susceptibility for IMP for all isolates and exposure time 3min gave the highest susceptibility for CIP for all isolates.

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