



THE EFFICACY OF (532nm) CW Nd:YAG LASER AND ANTIMICROBIAL AGENTS ON THE CAPSULE SIZE OF *KLEBSIELLA PNEUMONIA* ISOLATED FROM URINARY TRACT INFECTIONS (UTI)

Aunce A. Mohammed

Institute of Laser for Post-Graduate Studies – University of Baghdad/IRAQ

ABSTRACT

The aim of this study was to evaluate the effects of 532nm CW Nd:YAG laser and antimicrobial agents on capsule size reduction of *Klebsiella pneumonia*. This bacteria is a facultative anaerobic, nonmotile, rod-shaped, gram-negative with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms. *Klebsiella pneumonia* strain isolated from Urinary Tract Infections (UTI) was cultured on blood agar and incubated at 37°C for 48hrs. After incubation *Klebsiella pneumonia* suspension (10^6 cells/ml) was prepared and subjected to the following treatments: laser light using different exposure times and the number of colony-forming units (CFU) was calculated, laser light using different exposure times and capsules size was obtained and laser light using different exposure times, antibiotics sensitivity test was done then capsules size was obtained. The data was subjected to analysis of variance and means were compared by using student-t-test ($P < 0.005$). *Klebsiella pneumonia* strain was resistant to all antibiotics agents, Nd:YAG laser reduced the viable count at 5min exposure time. There was a reduction in capsule size with using (1, 3 and 5)min for *Klebsiella* (1.83 ± 0.02) as compared with control group (3.32 ± 0.14) and (0.36 ± 0.02) after treated with laser and antibiotics agents. According to the results of this study we can conclude that laser light was an effective tool in capsule size reduction of *klebsiella pneumonia*.

KEYWORDS: *Klebsiella pneumonia*, CW Nd:YAG laser, gram-negative bacteria, Urinary Tract Infections etc.

INTRODUCTION

In the recent years *Klebsiella pneumonia* has become important pathogen in nosocomial infections. *Klebsiella pneumonia* is recognized as an important opportunistic pathogen frequently causing a variety of infections including; urinary tract infections, pneumonia, septicemia and wound infections in immunocompromised individuals (Struve and Krogfelt, 2003). *Klebsiella pneumonia* is a Gram-negative, non-motile, encapsulated, rod shaped bacterium found in the normal flora of the mouth, skin and intestines (Sarathbabu et al., 2012). *Klebsiella pneumonia* is surrounded by a polysaccharide layer covering the entire bacterial surface, referred to as a capsule. The capsule is generally considered to be an important virulence factor in *Klebsiella pneumonia* (Podschun et al., 1996). The capsule shields these organisms from host defences. Typically, the larger the capsule produced, the more pathogenic the strain. Reduction or loss of capsular polysaccharide (CPS) by mutation or chemotherapeutic agents results in increased susceptibility to phagocytosis and loss of virulence (Sikarwar and Batra, 2011). Chemotherapeutic agents that reduce the production of CPS may provide a unique adjunct to therapy against infections caused by encapsulated bacteria. Antimicrobial agents with activity against *K. pneumoniae* may be more effective in the presence of fewer capsules. Moreover, are effectively. However, reducing capsule size by promoting the release of CPS from the bacterial cell, as some antibiotics have been shown to do, may do more damage. Only by reducing CPS production can these agents be benefit in vivo (Domenico et al., 1999). An increased resistance to antibiotics has been reported in *K. Pneumoniae* as the

widespread use of the third generation cephalosporins, β -lactam and broad-spectrum antibiotics (Zhou et al., 2011). Many studies have shown that capsule plays important role in pathogenicity of *Klebsiella pneumonia*. (Hassan et al., 2010) have studied the effect of diode laser 805nm on the viability of some types of Gram negative pathogenic bacteria (*Escherichia coli*, *Klebsiella pneumonia*. (Domenico et al., 1999) have shown the reduction of capsular polysaccharide production in *Klebsiella pneumonia* by sodium salicylate. (Bessom et al., 2009) have studied the role of capsule in *Klebsiella pneumonia* resistant to antimicrobial drug. (Mohammed, 2008) has studied comparison the effect of 515 nm argon laser and chlorhexidine on *klebsiella pneumoniae* isolated from root canal. The aim of the present study is to evaluate the effect of 532nm CW Nd:YAG laser and Antimicrobial agents on capsule size reduction of *Klebsiella pneumonia*.

MATERIALS AND METHODS

Bacterial isolates

Klebsiella pneumonia isolated from urinary tract infections was provided from biology department, Al-Mustansyria University and used throughout the study. The bacteria were plated on nutrient agar then incubated at 37°C for 48hrs.

Preparation of the microbial suspension for irradiation

After incubation the isolates were cultured in brain-heart infusion broth and incubated at 37°C for 18h. The cultures were centrifuged at 1300 r.p.m for 10min, and the supernatant was removed and the pellet was washed by physiological saline. This procedure was repeated twice and then the suspension was mixed by vortex and re-

suspended in 5ml physiological saline. The turbidity of the cell suspension measured by spectrophotometer at 590nm and adjusted with physiological saline to match that 0.5 McFarland which containing 5×10^6 cells per ml (Rossoni *et al.*, 2010).

One milliliter of each prepared sample was transferred to sterile endpdroff tube. Two sets of groups for each sample were made.

I-Group include the bacteria which were exposed to the laser radiation at 1, 3 and 5 minutes. After irradiation, the number of the colony forming units per milliliter (CFU/ml) was obtained. Control sample was kept without irradiation.

II-Group includes the bacteria that exposed to the laser radiation at 1, 3 and 5 minutes then the capsule size diameter was measured.

III-Group include the bacteria that exposed to the laser radiation at 1,3 and 5 then the irradiation suspension in endpdroff tubes was inoculated on Mueller Hinton agar plates then antibiotic discs were placed on media and incubated at 37°C for 24 hours, after incubation capsule size was measured.

Laser system source

light source used was Nd:YAG laser, output power 150 mW that emits; light in a collimated beam (diameter of 3mm) with a wavelength of 532 nm. The irradiation time was 1, 3 and 5min, and power density of 2.125W/cm²foreach sample.

Antibiotic Susceptibility test

Antibiotic sensitivity of clinical *K. pneumoniae* isolates was done by Bauer's and Kirby's disc diffusion method (Bauer and Kirby, 2000).Organisms were grown in BHI

broth and inoculated on Mueller Hinton agar plates by sterile swabs and then antibiotic discs were placed on media and pressed gently followed by overnight incubation. The antibiotics that weretested included Ampicillin (20mcg), Cotrimoxazole (25mcg), Piperacillin (100mcg), Gentamycin (10mcg), Amikacin (30mcg), Carbenicillin (100mcg), Cefotaxime (30mcg),Ceftizoxime (30mcg), Tetracycline (30mcg) and Ofloxacin(5 mcg).

Capsule size measurements

Capsule size was measured by light microscopy with anocular micrometer, using Nigrosin stain as a negative stain.Capsule size is expressed as the average diameter of randomly selected cells (Domenico and Schwartz,1999).

Statistical analysis

The experimental data was performed using analysis of variance and means were compared by student-T-test. The differences considered statistical significant at level ($P < 0.005$).

RESULTS

Table (1) shows antibiotics sensitivity for *K. pneumoniae*, the antibacterial activity of (10) drug was assayed by Kirby-Bauer disc diffusion method. *K.pneumoniae* showed approximately 100 % resistant activities against antibiotics. Gentamicin (10mcg), Cefotaxime (30mcg) and Tetracycline (30mcg) was showed very small inhibition zone against the growth of *K. pneumoniae*,hence considered to be resistant against these drugs, whereas no inhibition zone was seen in Azithromycin(15mcg), Ampicillin (10mcg), Lincomycin (2mcg), Co-Trimoxazole (25mcg) and Oxacillin (1mcg).

TABLE 1: Antibiotics Sensitivity test for *Klebsiella pneumonia*

Antibiotics	S/R	ZOI	S	MS
Lincomycin (2mcg)	R	-	18	16-17
Cephalexin (30mcg)	R	11mm	18	15-17
Chloramphenical(30mcg)	R	12	18	13-17
Azithromycin(15mcg)	R	-	17	13-15
Ampicillin(10mcg)	R	-	17	14-16
Co-Trimoxazole (25mcg)	R	-	16	11-15
Gentamicin (10mcg)	R	5mm	15	13-14
Cefotaxime(30mcg)	R	3mm	23	15-22
Tetracycline(30mcg)	R	3mm	19	15-18
Oxacillin(1mcg)	R	-	16	14-16

S/R=Sensitive or Resistance ;ZOI=Zone Of Inhibition,
S=Standard Sensitivity ;MS=Moderate Sensitivity

TABLE 2. Mean±standard deviation values of the colony forming units/ml (CFU/ml) was obtained after laser irradiation with different exposure times (1,3, 5)min for *Klebsiella pneumonia*

Control	1min	3min	5min
81.40±1.140	77.60±1.140*	75.60±1.94*	66.2±1.92 **

TABLE 3: Mean±Standard deviation values of the Capsule sizes (diameter in µm) was obtained after laser irradiation with different exposure times (1,3,5)min for *Klebsiella pneumonia*

Control	1min	3min	5min
3.32±0.14	2.85±0.01**	2.74±0.01**	1.83±0.02 **

TABLE 4: Mean±Standard deviation values of the Capsule sizes (diameter in μm) was obtained afterAntibiotics Sensitivity test and laser irradiation with different exposure times (1,3,5) min for *Klebsiella pneumonia*

Control	1min	3min	5min
3.32±0.14	2.66±0.02**	1.10±0.15**	0.36±0.02 **

* No Significant

** Significant ($P < 0.005$)

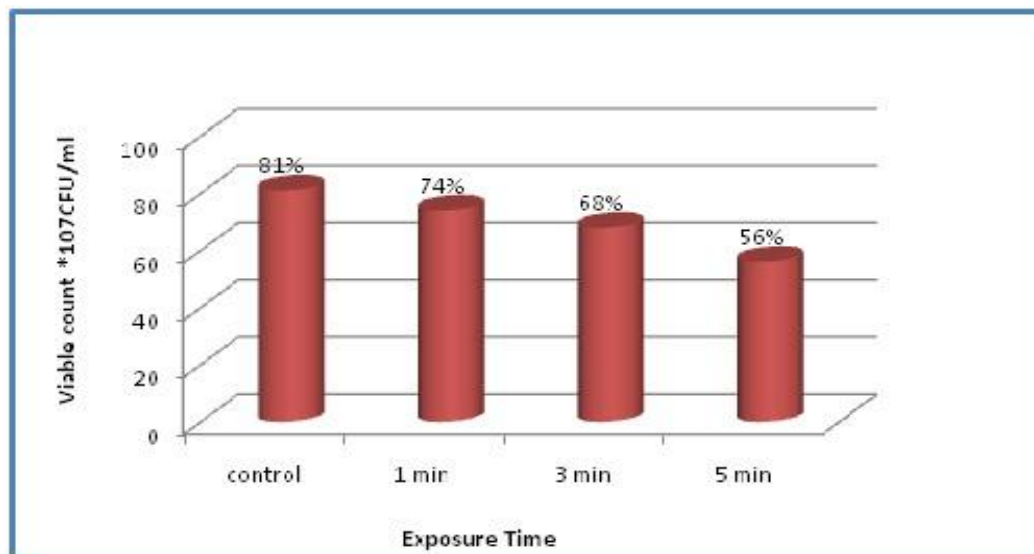


FIGURE 1: shows the effects of the laser light using different exposure times on the viable count (CFU /ml) of *Klebsiella pneumonia* cells.

Results revealed that the reduction in the numbers of viable count (CFU /ml) was not significant after irradiation for 1 and 3min exposure compared to control group. While irradiation using (5) min exposure induced a reduction in the number of (CFU /ml) of *Klebsiella pneumonia* compared to the control group.

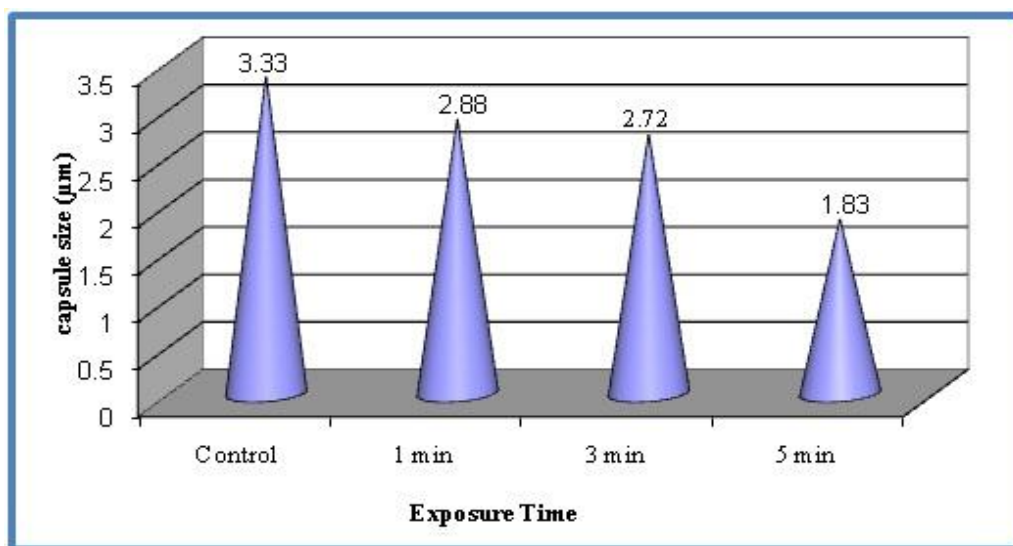


FIGURE 2: shows the effect of laser radiation using different exposure times on capsule size diameter of *Klebsiella pneumonia*.

Capsule size diameter was significantly decreased after irradiation using (1 and 3) min exposure. Furthermore, high significant decreased in capsule size has seen after using (5) min exposure.

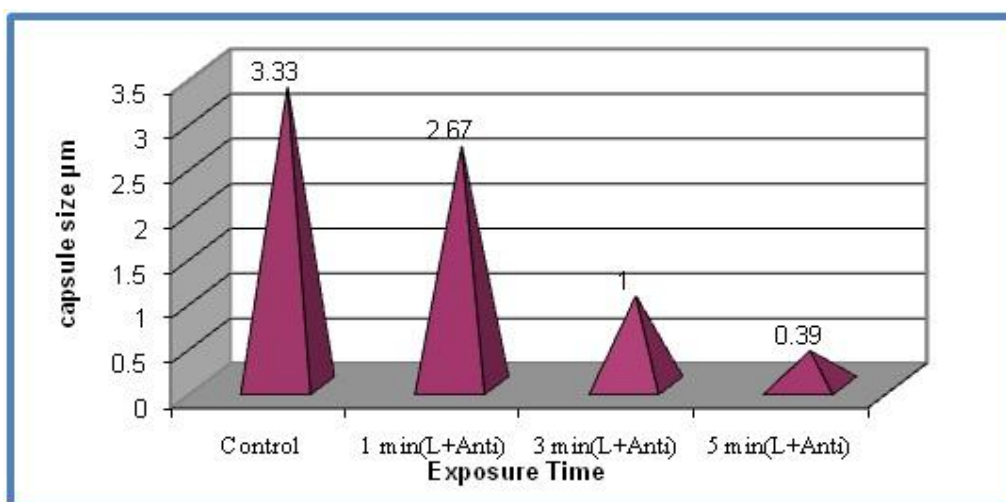


FIGURE 3: shows the effect of laser radiation on capsule size diameter after antibiotics sensitivity test using different exposure times. Significantly, there was noticeable reduction in capsule size diameter after using irradiation (1 and 3) min exposure times. Moreover, capsule size was decreased significantly after using (5) min exposure time.

DISCUSSION

The widespread use of antimicrobial agents leads to increasing the resistance of bacteria. We tested the hypothesis that the acquired bacterial resistance to these agents is due to the selection for survival of cells with larger capsules (Amin *et al.*, 2009; Sivakumari and Shanlhi, 2009).

In our study, we found that *K. pneumoniae* was also multidrug resistant bacteria to the third generation Cephalosporins, Aminoglycosides, Tetracyclines and Chloramphenicol antibiotics. Similar observations to our study demonstrated that 96%-100% *K. pneumoniae* and *P. aeruginosa* isolated from ICU patients were resistant to cephalosporins and Aminoglycosides (Ilah and Ahmed, 2009; Radji and Fauziah, 2011). This finding is related most probably due to the extensive usage of third generation cephalosporins and Aminoglycosides antibiotics at the ICU of Hospital. *K. pneumoniae* has a tendency to harbor antibiotic resistant plasmids; thus infection with multiple antibiotic-resistant strains can be anticipated (Sikarwar and Batra, 2011), of particular concern is the recent appearance of *klebsiella* strains that possess plasmids that mediate resistance to ESBL drugs. This form of resistance is due to the production of unique beta-lactamase enzymes, referred to as ESBL's. These enzymes have been seen mostly in strains of *klebsiella pneumoniae* and *E. coli*, and cause them to be resistant to most beta-lactam drugs, including the third generation cephalosporins. (Sikarwar and Batra, 2011) Bacterial resistance to antibiotics has been reviewed by (McDonnell and Russel, 1999) they suggest that a bacterial envelope could potentially be a mechanism for this resistance. They believe that bacterial envelope to which they are referring in their review is the capsule (Held *et al.*, 1995; Singh and Thakur, 2012) Gram-negative bacteria cell walls contain the lipopolysaccharide layer. This lipopolysaccharide layer is more likely to be damaged by laser radiation, thus the larger capsule would directly protect this layer. We found the bacteria that survived the agents had developed larger capsules and individual colonies had become more mucoid

capsules make bacteria more virulent and the sticky nature of capsules play a role in the development of thick biofilms (Ehrenworth and Baer, 2012). The present study extends this concept to the *klebsiella pneumoniae* when the capsule diminishes sufficiently in size after laser radiation at different exposure times; the organism largely loses its virulence for invasion (Rossoni *et al.*, 2010; Dasgupta *et al.*, 2009). Significantly at (5min) we found that highly reduction in capsules size to (1.83 ± 0.02) compared with the control group. After irradiation using (1, 3 and 5) min antibiotics Sensitivity test was done, then capsules size were obtained. We found that significant reduction in capsule size (0.36 ± 0.02) in comparing with control group (3.32 ± 0.14). Our results are in accordance with the findings of other authors, antibacterial effects of low power laser light and Volatile oil of Fennel on Gram – negative bacteria reported by El-Adly, (2007). Domenico *et al.* (1999) revealed that Sodium Salicylate was reduced the capsule size and the combined effect of Sodium Salicylate and antibiotics works additively to reduce capsular polysaccharide production.

CONCLUSION

The present results obtained that high antibiotics resistance of *klebsiella pneumoniae* towards commonly used antibiotics are the major reasons for prolonged infections in hospitals, moreover the capsules play an important role in bacterial pathogenesis primarily by shielding the microorganisms against the bactericidal of antibiotics and engulfment by phagocytic cells. Laser light was an effective tool in capsule size reduction of *klebsiella pneumoniae*.

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