

Novel Clinical Trial for Treatment of Infected Subcutaneous Fistula in Female Griffon Dog using Gold Nano-Rod assisted near-Infrared Plasmonic Photothermal Therapy

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Abstract:

Raises in the occurrence of antibiotic resistant bacterial infections need novel methods for control. It is currently clear that a nanotechnology-driven method using nanoparticles to target and terminate pathogenic bacteria can be positively applied. Plasmonic photothermal therapy (PPTT) is a hopeful minimally-invasive antibacterial therapy as well as oncological treatment strategy where plasmonic nanoparticles are used to transform near infrared light to localized heat to cause cell death, mainly through apoptosis and/or necrosis. The objective of this work was to detect the clinical bactericidal activities of gold nanorods (AuNRs) using plasmonic photothermal therapy (PPTT) against pathogenic extended spectrum Beta Lactamase (ES β L) *Klebsiella pneumoniae* as well as apoptotic actions against pyogenic membrane of chronic subcutaneous fistula had no tendency to heal in clinically affected eight years old Griffon female dog. The fistula was infiltrated along its pyogenic wall and intraluminal in multiple spots with AuNR at 7.5 nM concentration and then exposed to an 808 nm diode laser with power of 5.8 W/cm² and spot size about 5.6 mm². The PPTT session was repeated every two weeks for four successive sessions. The evaluations of the curative response were done through clinical, bacteriological and histopathological repetitive examinations. Clinically, the purulent discharge decreased in the quantity until it disappeared at the third PPTT session and complete regression of the fistula occurred after the fourth PPTT application. Bacteriologically, *Klebsiella pneumoniae* isolate showed dramatic reduction in colony forming unit (CFU) count after first session and was completely absent at the fourth PPTT session. Regarding histopathology of the tissue specimens from infected fistula when the case was admitted to the clinic, there was massive liquefied necrotic area infiltrated mainly by neutrophils represented as grade +3 and after first session of PPTT, there was circumscribe necrotic area infiltrated by few numbers of neutrophils and represented as grade +1. After third session of PPTT, complete absence of necrotic tissues was recorded and new fibrous connective tissue formation was observed. Altogether, these outcomes powerfully recommend that AuNRs could be a motivating choice to control antibiotic resistant bacterial infections as well as pyogenic conditions which have no tendency to heal

Keywords: Plasmonic Photothermal Therapy (PPTT); Gold Nanorods (AUNRS); *Klebsiella Pneumoniae*, Pyogenic Fistula, Colony

Introduction:

Photodynamic therapy (PDT) is an active management to destroy both eukaryote and prokaryote creatures (Kharkwal, Sharma, Huang and Hamblin, 2011 and Sharma, Mroz, Dai, Huang, Denis and Hamblin, 2012) [1, 2]. The plasmonic photothermal therapy (PPTT) could be additional effective to eradicate microorganisms through its local hyperthermia mechanism (Fekrazad, Khoei, Hakimiha and Bahador, 2013) [3]. PPTT has been significant in the latter years in the practical medicine due to its ability of absorbing visible light (Ali, et al, 2016) [4]. At the same time, gold nanoparticles with nonspherical shape, such as gold nanorods, and polygonal particles have the ability of absorbing near-infrared (NIR) light that is within a proper wavelength field for therapeutic presentations. One of the prospective uses of gold nanoparticles with NIR absorption abilities is in the hyperthermia. Hyperthermia can be used as a potential tool to kill bacteria by joining the usage of laser and functional gold nanoparticles. Just nanorods are in contact with the bacterial cells and this is followed by laser irradiation, the hyper-thermal action can speedily terminate the bacterial cells (Huang, Tsai and Chen, 2007 and Norman, Stone, Gole, Murphy and Sabo-Attwood, 2008) [5, 6]. The situation of the plasmon maximum is associated to the particle size (larger diameter results in a longer wavelength) and the highest width to size dispersion (Kim, Yi, Kim, Lee and Kim, 2009) [7]. So, we can raise the antimicrobial potential of nanoparticles to lyse whole bacterial populations.

The growing progress of multidrug-resistant strains among pathogenic microorganisms has come to be one of the most significant difficulties in medicine worldwide (Lode, Stahlmann, and Kresken, 2013) [8]. *Klebsiella* is a gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family (Procop and Koneman, 2016) [9]. *Klebsiella pneumoniae* (*K. pneumoniae*), a member of the human intestine flora, is commonly related to hospital-acquired infection. Definite underlying syndromes such as malignancy, cirrhosis, biliary tract disorders, diabetes mellitus (DM), and alcoholism may damage an patient's defense mechanisms and raise the possibility of *K. pneumoniae* infection (Sung-Sheng Tsai, et al, 2010) [10]. *K. pneumoniae* is the second most common reason of gram-negative bacteremia after *Escherichia coli* (Bryan et al, 1983 and Yinnon, et al, 1996) [11, 12]. *K. pneumoniae* bacteremia leads to significant morbidity and mortality in common populations (Lee, et al; 1994 and Tsay, et al; 2002) [13, 14]. It has

even replaced *Escherichia coli* in some centers as a nosocomial pathogen. It causes pneumonia, urinary tract infection, other pyogenic infections, septicemia, rarely diarrhea and hospital-acquired infections (Arti Kapil, 2013) [15]. Metastatic infections, such as pyogenic brain abscess, meningitis, and endophthalmitis, are the most important features of *K. pneumoniae* infections (Wang et al, 1998 and Fang et al, 2007) [16, 17]. Biochemically typical strains of *Klebsiella pneumoniae* are resistant to a wider range of antibiotics than are most *Escherichia coli* strains. They are nearly always naturally resistant to ampicillin (Murray and Rosenthal, 2005) [18]. Resistance of *Klebsiella* to previously sensitive antibiotics is also increasing in the recent years due to overuse and misuse of antimicrobial agents and or natural causes. The existence of enzymes of extended-spectrum β -lactamases (ES β Ls) producing organism that are resistant to virtually all β -lactam antibiotics have been reported (Sung-Sheng Tsai, et al, 2010) [10]. ES β Ls are plasmid-mediated class A enzymes commonly found in the family Enterobacteriaceae, mainly *K. pneumoniae* (Lin, Chin and Lee, 2005) [19]. The increase in ES β L-producing organisms is sure to create significant therapeutic problems in the future. The available treatment regimens for infections caused by ES β L-producing bacteria are not always effective. Therefore, it is necessary to discover new antimicrobial compounds against ES β L-producing *K. pneumoniae* strains. Abundant improvements in nanotechnology have delivered a hard basis for using nanoparticles (NPs) in the battle against pathogenic microorganisms, containing multidrug resistant bacteria (Hernandez-Delgado, et al; 2013, Badireddy, et al; 2014 and Ali et al; 2016) [20, 21, 22]. Numerous works have studied the effectiveness of PDT for disinfection of oral pathogens; but the usage of PPTT against oral pathogens has not been sufficient discovered (Badireddy, et al; 2014) [21].

In this work, the clinical bactericidal activities of gold nanorods (AuNRs) plus laser (PPTT) against pathogenic antibiotic resistant *Klebsiella pneumoniae* as well as apoptotic actions against pyogenic membrane of chronic subcutaneous fistula had no tendency to heal in clinically affected five years old Griffon female dog were studied.

Materials and methods:

This study was started at January 2016 when a case of eight years old Griffon bitch admitted to the clinic of the department of surgery, anesthesiology and radiology, faculty of veterinary medicine, Cairo University suffering from chronic infected subcutaneous fistula at the region of the left stifle fold (Fig. 1).



Fig. (1) The affected eight years old bitch at the time of admission to the surgery clinic showed infected fistula at the level of the left stifle fold has no tendency to heal since about one year.

Synthesis, Conjugation and characterization of Gold Nanorods (AuNRs)

AuNRs with an average size of $25 (\pm 3) \times 5 (\pm 0.8)$ nm (length \times width) were synthesized by seedless mediated growth in school of Chemistry and Biochemistry, Georgia Institute of Technology, and Laser Dynamics Laboratory, Atlanta, USA (Ali et al; 2012) [23]. Briefly, the AuNRs growth solution was prepared, which contains 100 mL of HAuCl₄ (1.0 mM), 100 mL of CTAB (0.2 M), 5 mL of AgNO₃ (4.0 mM), HCl (160 μ L, 37%), and 1.4 mL of ascorbic acid (78.8 mM). Then, we injected 300 μ L of NaBH₄ (Sigma-Aldrich, USA) to the growth solution and kept it undisturbed for 12 h for the growth of AuNRs. After that, the extra toxic surfactant CTAB were removed by centrifugation (19,000 rcf) the AuNRs solution for 1 hour and redispersed in deionized (DI) water, followed by a second centrifugation (14,000 rcf) for 30 min. The size of the gold nanoparticles was measured by JEOL 100 CX transmission electron microscopy (TEM) (JEOL Ltd., Tokyo, Japan) images by calculating the average dimensions of 100 particles. The surface

plasmon resonance peak of the AuNRs was measured by UV-vis spectroscopy. The molar concentrations of AuNRs were calculated according to Beer's law. For conjugation of AuNPs with poly ethylene glycol thiol (SH-PEG, Mw=5000), SH-PEG solution (1 mM) was added into the AuNRs solutions (1 nM), followed by incubation for 10 h at room temperature. The excess PEG was removed by centrifugation.

Administration of PPTT

The affected case was sedated using xylazine Hcl 2% in a dose rate 1 mg/kg BWs via intramuscular route. Complete aseptic preparation of the site of the infiltrations and laser application was performed. The fistula was infiltrated along its pyogenic wall and intraluminal in multiple spots with AuNR at 7.5 nM for each 100 cm³ concentration and then exposed to an 808 nm diode laser with power of 5.8 W/cm² and spot size about 5.6 mm² (Fig. 2). The dose per injection was measured based on the volume of the fistula. The PPTT session was repeated every two weeks for four successive sessions.



Fig. (2) The steps of fistula infiltration along its pyogenic wall and intraluminal in multiple spots with AuNR at 7.5 nM for each 100 cm³ concentration and then exposed to an 808 nm diode laser with power of 5.8 W/cm² and spot size about 5.6 mm².

Clinical evaluation

Clinical evaluation was done every PPTT session for eight weeks until complete healing of the fistula to measure length and wide of the fistula in addition to presence and quantity of discharge and its physical characters.

Bacteriological evaluation

Collection of samples

The pus samples were either aspirated by disposable syringes or

collected onto sterile cotton tipped swabs. The samples were collected at the first time of the case admission for culture and antibiotic sensitivity and then collected before each PPTT session and five minutes after session for culture and total colony counts.

Characterization of bacterial isolates

Pus was aseptically inoculated on to Blood and MacConkey agar plates and incubated overnight at 37°C. Klebsiella isolates were identified by their morphology and biochemical characteristics

(Collee, et al; 2012 and Procop and Koneman, 2016) [24, 9].

Confirmatory test for ESBL-producing *K. pneumoniae* isolates

The double-disc synergy and agar diffusion tests were used as screening tools to detect ESBL-producing strains. In the double-disc synergy test, the antibiotic discs (Oxoid) used were cefotaxime (30 µg) and ceftazidime (30 µg) placed on Mueller-Hinton agar adjacent to a co-amoxiclav disc (20µg amoxicillin plus 10µg clavulanate). Reduced susceptibility to cefotaxime (30 g) and Ceftriaxone (30 g) with zone sizes 27mm and 25mm respectively were used as screening method for ESBL production (Fang, et al; 2005) [25]. The agar diffusion test was performed according to NCCLS guidelines¹². A ≤ 5 mm increase in a zone diameter for either ceftazidime/clavulanic acid (30 µg/ 10 µg) or cefotaxime/clavulanic acid (30 µg/ 10 µg) versus its zone when tested alone was taken as being indicative of ESBL-production (Fang, et al; 2007) [26].

Antimicrobial susceptibility testing

Was done for the isolates on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method according to the CLSI guidelines 2015 (CLSI, 2015) [27].

Monitoring bacterial count using “Spread Plate Technique”

Irrigation of the fistula using fixed volume (10 ml) of sterile distilled water was done before and five minutes after each session. The collected samples were subjected to series of dilution. A volume of 0.1 ml was pipetted out from the appropriate desired dilution series onto the center of each of three agar plate. Each sample was spread evenly over the surface of the agar using the sterile glass spreader and carefully the Petri dish was rotated underneath at the same time. The plate was incubated at 37°C for 24 hours. The colony forming unit (CFU) value was calculated of the sample. Once the count of the colonies was recorded, it was multiplied by the appropriate dilution factor to determine the number of CFU/mL in the original sample (Arti Kapil, 2013) [15].

Statistical analysis:

Total bacterial count results are expressed as the mean \pm SD of three petri dishes counting.

Histopathological evaluation:

Histopathological examination:

Tissue specimens from infected fistula was collected, fixed in 10 % neutral buffered formalin, processed and embedded in Paraffin wax, sectioned at 4 µm and stained with Hematoxylin and Eosin (Bancroft and Gamble, 2008) [28] and examined under an Olympus microscope (Olympus, Japan).

Morphometric measurements:

The intensity of the inflammatory response was assessed as following: +1 (inflammatory cells representing less than 10% of the cell population observed within the wound area), +2 (inflammatory

cells representing in between 10 and 50% of the cell population observed within the wound area) and +3 (inflammatory cells representing more than 50% of the cell population observed within the wound area). Light-microscopic histo-quantitation was performed by counting PMN leukocytes in tissue sections per high-power field (x40 objective, x10 ocular) with a binocular microscope in three adjacent areas (Valdinaldo et al, 2011) [29].

3. Results

The affected bitch was admitted to the surgery clinic at January 2016 with history of infected fistula at the level of the left stifle fold since about one year. At the first time of fistula evolution, the owner observed doughy swelling at the lateral aspect of the left stifle which increased in the size rapidly and then opened at the proximal level of the left stifle fold discharging serosanguinous fluid. The obtained fluid was examined for bacterial culture, isolation, identification and antibiotic sensitivity. The isolated microorganism was extended spectrum Beta Lactamase (ESBL) producing *Klebsiella pneumoniae* and was only sensitive to Imipenem antibiotic. Total blood picture evaluations showed marked neutrophilia. Serum glucose, liver and kidney functions were within normal values. The previous treatments were daily dressing using hydrogen peroxide, povidine iodine, fucidine ointment and application of drain. Systemic Imipenem antibiotic injection in a dose rate (5 mg/kg) every 8 hours intravenously was used. The dog showed mild response to the antibiotic as slight reduction in the fistula discharge. After five successive days of antibiotic administration, the affected bitch suffered from nausea, vomiting and diarrhea which recorded as antibiotic side effects. Blood analysis was performed and revealed marked neutrophilia and elevation in the serum creatinine. The veterinarian who followed the case decided to stop antibiotic and continue the topical treatment only. After two weeks of topical treatment, reexamination of the blood parameters was done which showed significant reduction of the serum creatinine and the marked neutrophilia was still present. There was no evidence of clinical improvement of the fistula after continues local daily dressing for two months. Cauterization of the fistula tract was applied using silver nitrate which resulted in slight reduction of the discharge for two weeks and then increased gradually by time. Repeating of the cauterization was done without any significant improvement of the case. The decision of application of PPTT trail was taken after agreement of the owner. Isolation and identification of the causative microorganism and antibiotic sensitivity were done before trail which revealed the same result (extended spectrum Beta Lactamase (ESBL) producing *Klebsiella pneumoniae* sensitive only to Imipenem antibiotic). Total colony count (using Spread Plate Technique) was done and used as method of monitoring the bactericidal effect of PPTT.

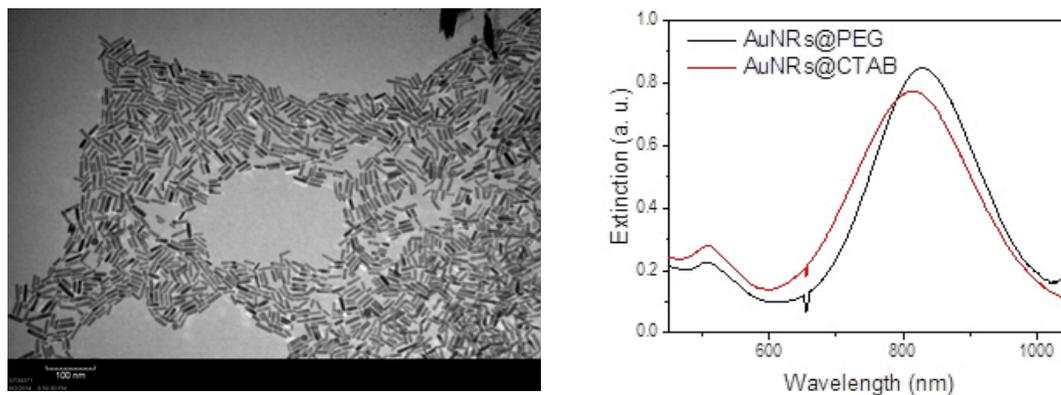


Fig. (3): Characterization of AuNRs (length 25 ± 3 , width 5 ± 0.8 nm). (A) TEM image with 100 nm scale bar. (B) UV-vis absorbance spectra showing the SPR peaks of AuNRs before and after conjugation with PEG.

Clinical evaluation:

Clinical evaluation was done every PPTT session for eight weeks until complete healing of the fistula. Length and wide of the fistula

were recorded each time (Fig. 4) (Table 1). Presence and quantity of discharge as well as its physical characters were detected (Fig. 5&6) (Table 2).

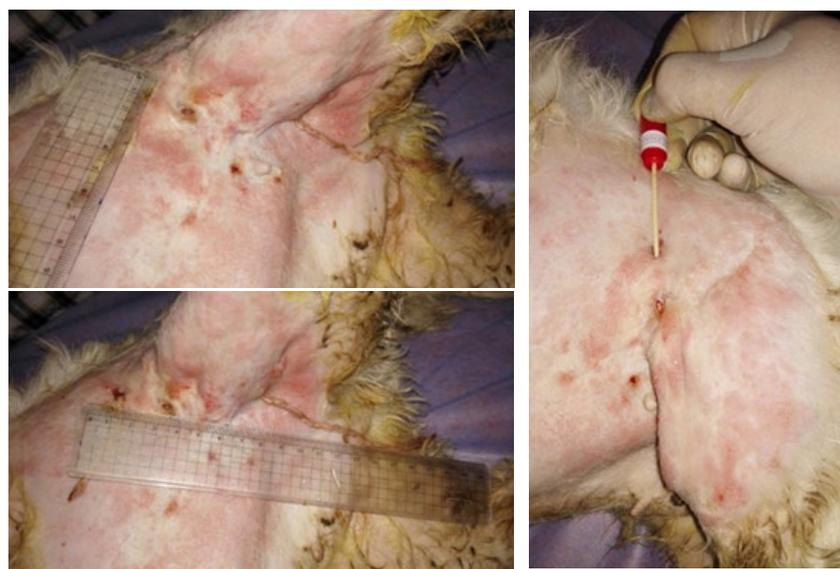


Fig. (4) Length and wide of the fistula at the time of admission.



Fig. (5) The detected quantity and physical characters of discharge A) at 2nd session and B) at 3rd session.



Fig. (6) The affected case A) at the 4th session and B) two weeks after the 4th session.

Time	Length (cm)	Width (cm)
1st session	8.00	1.20
2nd session	6.40	0.80
3rd session	4.20	0.40
4th session	0.00	0.00

Table (1): illustrated the lengths and widths of the fistula at the time of each PPTT sessions:

Time	The quantity of discharge	The physical characters of discharge
1st session	Abundant	Serosanguineous watery pus
2nd session	Low amount	Condensed pus
3rd session	Scanty	Serous fluid
4th session	No discharge	No discharge

Table (2): illustrated the quantity and physical characters of discharge at the time of each PPTT sessions:

Microbiological evaluation:

Klebsiella isolates were identified by their morphology and biochemical characteristics. Morphology of Klebsiella identified were large dome shaped colonies on blood agar and lactose fermenting mucoid colonies on MacConkey agar. In gram staining, gram negative, short, plump, straight rods were seen. The biochemical characters identified were positive Voges-Proskauer test, positive citrate utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests.

Confirmatory test for ES β L-producing *K. pneumoniae* isolates

The double-disc synergy and agar diffusion tests which were used as screening tools confirmed isolation of ES β L-producing strain.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing revealed that the isolated bacteria was sensitive only to Imipenem antibiotic.

Monitoring bacterial count using "Spread Plate Technique"

The colony forming units were recorded before and five minutes after each session as shown in figure (7) and table (3).

Time	Log10 of CFU/mL (\pm SD) at	
	Before session	Five minutes after session
1st session	11.60 (\pm 0.12)	2.45 (\pm 0.27)
2nd session	4.42 (\pm 0.08)	0.06 (\pm 0.16)
3rd session	1.33 (\pm 0.66)	0.06 (\pm 0.18)
4rd session	--	--

Table (3): illustrated the number of CFU/mL at the time of each PPTT sessions:

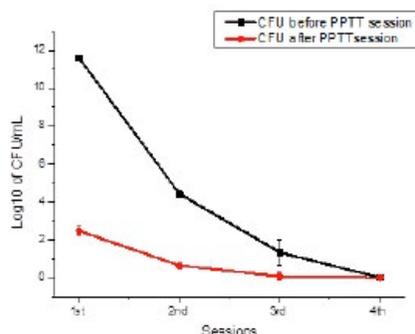


Fig. (7): Showing the total bacterial count before and after each PPTT session.

Histopathological evaluation:

The tissue specimens from infected fistula were taken before treatment revealed congested blood capillaries and massive liquefied necrotic area infiltrated mainly by neutrophils represented as grade +3 (inflammatory cells representing more than 50% of the cell population observed within the wound area) per high-power field (x40 objective, x10 ocular) Fig. (8). On other side, after treatment by laser and Nano-gold tissue section showed circumscribe necrotic area infiltrated by few numbers of neutrophils and represented

as grade +1 (inflammatory cells representing less than 10% of the cell population observed within the wound area) per high-power field (x40 objective, x10 ocular) Fig. (9). The healed wound characterized by a reconstitution of the regular epidermal lining, reduction of fibrinous exudation and a more organized and a denser type of connective tissue indicating that a new epidermis had formed completely. Large, wavy collagen fibers interspersed with blood vessels were seen. Superficial tissues had numerous small vessels, while larger blood vessels were visible in the deep tissues

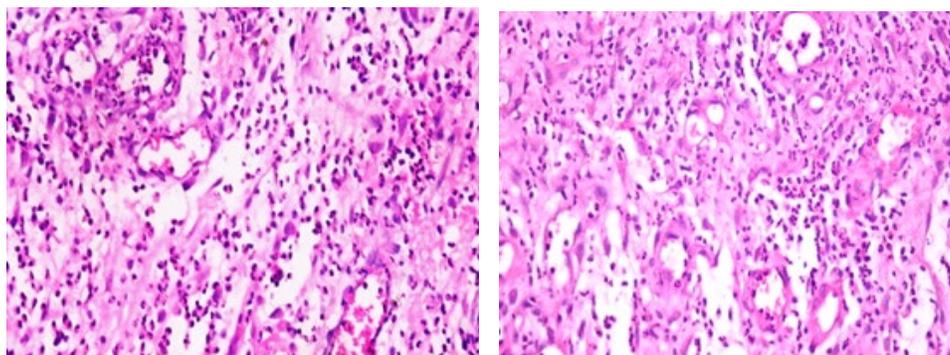


Fig. (8. A&B) Tissue sections before treatment showing massive necrosis, dilated capillaries and neutrophils infiltration- grade 3 (H&E X400)

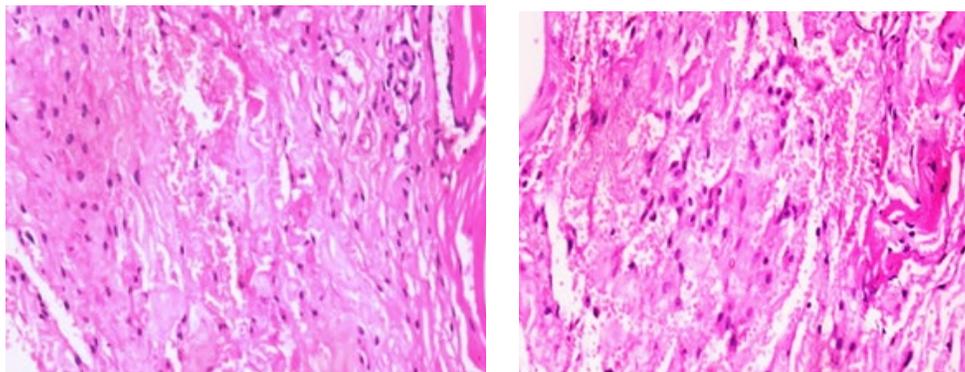


Fig. (9. A&B) Tissue sections after treatment showing circumscribe area of necrosis infiltrated with number of neutrophils –grade 1 (H&E X400)

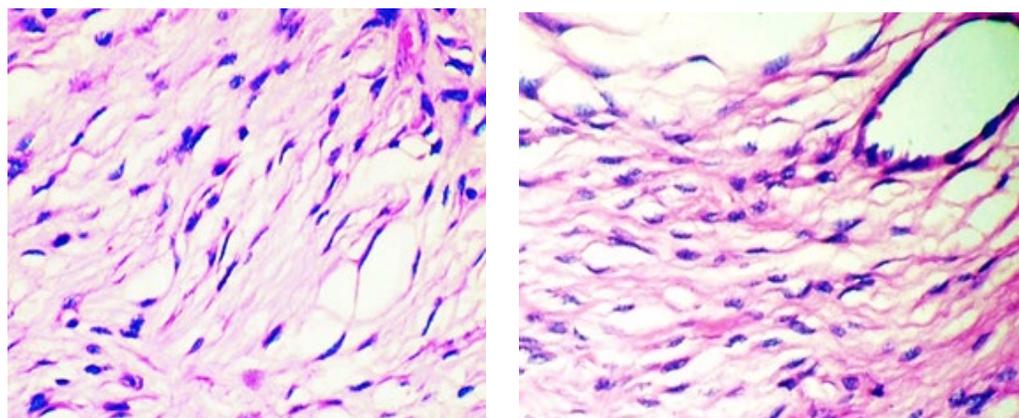


Fig. (10. A&B) Tissue sections after treatment showing organized and a denser type of connective tissue, superficial small vessels arrow and deep larger blood vessels arrow head (H&E X400)

Discussion:

In the present study, there were two challenges; the first was to achieve bactericidal effect of PPTT against ES β L *Klebsiella pneumoniae* and the second was enhancement of fistula healing without evidence of hyperthermia side effects on the tissues. Regarding first one, plasmonic photothermal therapy has been extensively investigated (in vitro) as an alternative technique in the biomedical area like determination of its bactericidal and antibiofilm activities against different strains of oral microorganisms (Castillo-Martínez, et al, 2015)[30], using of covalently primary antibodies linked gold nanorods to selectively destroy the pathogenic Gram-negative bacterium, *Pseudomonas aeruginosa* after exposure to near-infrared radiation (Norman, et al, 2008) [31], using surface plasmonic resonance-induced photo-activation of gold nanoparticles as bactericidal agents against methicillin-resistant *Staphylococcus aureus* (Mocan, et al, 2014)[32], developing a drug delivery platform where gold nanorods (AuNRs) are conjugated to rifampicin (RF), which is released after the uptake into macrophage cells (RAW264.7) (Ali, et al, 2016) [22] and studying the action of bactericidal gold nanoparticles on *Escherichia coli* (Cui, et al, 2012) [33]. In addition to that, a new anti-

bacterial gold nanorod (GNR) conjugated magnetic nanoparticle (MNP) composite (GNR–MNP) was synthesized successfully for the eradication of antibiotic resistant nosocomial pathogens in water to improve the water quality (Ramasamy, et al, 2014) [34]. There are no available lectures about using PPTT against bacterial infection in vivo.

The second challenge in this work was healing acceleration of the fistula (which had history of no tendency to heal) without risk of hyperthermia complications on the tissues. PPTT can induce cell death primarily by two pathways: necrosis and apoptosis (Pattani, et al, 2015) [35]. During necrosis, the heat induced from PPTT disrupts the plasma membrane causing the cytoplasmic components to leak out and inflammation to occur within the cell. However, apoptosis is a highly regulated cell death pathway and would thus be a cleaner way for eliminating cancer cells. Therefore, modulating PPTT to trigger apoptosis would be more favorable in clinical studies. It has been reported that different intracellular locations or shapes of NPs regulate the switch between necrosis and apoptosis (Song, et al, 2014 and Perez-Hernandez, et al, 2015) [36, 37].

From these backgrounds, the decision of application of PPTT trail for treatment of chronic extended spectrum Beta Lactamase (ESBL) *Klebsiella pneumoniae* infected subcutaneous fistula was taken after agreement of the case owner who was frustrated from the longtime of hopeless treatments.

For the bactericidal purpose, the nanorods used in the present work were of 28×8 nm in size. That was described previously as the most effective size in the plasmonic photothermal heat generation against bacterial and cancer cells (MacKey, et al, 2014 and Castillo-Martínez, et al, 2015) [38, 30]. The previous in vitro bactericidal studies irradiated 1 mL of GNP solution at various concentrations placed in a glass cuvette with approximately one cm bottom surface for 10 minutes, using an 808 or 810 nm wavelength laser with variable output power (up to 2 W/cm² diode) which was fixed above the surface, approximately 2 cm², in a vertical position and this dispersion reached a temperature up to 70°C (Mocan, et al, 2014 and Castillo-Martínez, et al, 2015) [32, 30]. These conditions are not logically acceptable among the clinical level as this temperature will lead to tissue burn.

In the present work, the fistula was infiltrated along its pyogenic wall and intraluminal in multiple spots with AuNR at 7.5 nM concentration and then exposed to an 808 nm diode laser with power of 5.8 W/cm² and spot size about 5.6 mm². Each spot irradiated for 2 minutes. In a previous study of PPTT effect on canine and feline natural mammary gland tumors, it was able to generate cancer cell apoptosis as a favorable cell death mechanism (rather than necrosis) in vitro and in vivo by optimizing AuNRs-PPTT's conditions as they directly injected the Pegylated AuNRs to the mammary tumors of each canine/feline with optimized PPTT dosages to enable a slow cancer cell apoptosis which adjusting the photothermic temperature to 44°C (Ali, et al, 2016) [39].

To achieve complete treatment of the infected fistula, the PPTT session was repeated every two weeks for four successive sessions. The evaluations of the curative response were done through clinical, microbiological and histopathological repetitive examinations. Clinically, the fistula dimensions were decreased gradually by time which indicated the good rate of granulation tissue formation filling fistula cavity. The purulent discharge decreased in the quantity and change in physical characters until it disappeared after the third PPTT session and complete regression of the fistula occurred after the fourth PPTT application.

Bacteriologically, *Klebsiella pneumoniae* isolate showed dramatic reduction in colony forming unit (CFU) count after first session and was completely absent at the fourth PPTT session. One of the most interesting finding was the aggressive reduction in CFU five minutes after each session which indicated rapid action of AuNRs-PPTT on bacterial cells.

Regarding histopathology of the tissue specimens from infected fistula when the case was admitted to the clinic, there was massive liquefied necrotic area infiltrated mainly by neutrophils represented as grade +3 and two weeks after first session of PPTT, there was circumscribe necrotic area infiltrated by few numbers of neutrophils and represented as grade +1. Two weeks after third session of PPTT, complete absence of necrotic tissues was recorded and new fibrous connective tissue formation was observed.

There were no clinical side effects recorded on the case during and after treatment. From the obtained data, the AuNRs-PPTT application gave very good bactericidal and healing responses especially when compared with specific antibiotic administration and traditional daily dressing or even cauterization.

Further studies are required on the molecular and genetic levels to detect the actual mode of action and micro-environmental changes during AuNRs-PPTT application on the clinically infected conditions.

Conclusion:

These outcomes powerfully recommend that AuNRs could be a motivating choice to control antibiotic resistant bacterial infections as well as pyogenic conditions which have no tendency to heal.

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