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THE THERAPEUTIC POTENTIAL OF COLD ATMOSPHERIC PLASMA AGAINST PATHOGENIC BACTERIA INHABITING DIABETIC WOUNDS

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Impaired healing process of diabetic wounds is a common problem among diabetic patients. These diabetic wounds are slow to heal and can lead to serious complications if left untreated. One of the reasons diabetic wounds are slow to heal is that pathogenic bacteria often colonize them. Cold atmospheric plasma (CAP) is a promising new technology that has been shown to effectively kill and inhibit pathogenic bacteria in diabetic wounds. In the current study, we examined by what means CAP works and its potential impact as a diabetic wound treatment. Fifty diabetic patients aged from 20 to 60 years old with diabetic wounds were included in our study using the swab method. We used general and specific culture media to isolate the pathogenic bacteria from diabetic wounds and identified the bacteria via different techniques using a light microscope (oil lens) and VITEK. Moreover, we confirmed the identification of isolated bacteria by 16S rRNA. Our results demonstrated the direct anti-bacterial effect of CAP against the isolated bacteria. The most prominent bacterial type in diabetic wounds was Staphylococcus sp. CAP demonstrated the highest effect against Bacillus sp. and Paenibacillus sp., which were responsible for relevant changes in the skin and were associated with the skin immune response and microbiota. Our results revealed the therapeutic use of CAP as a powerful strategy to overcome the invading pathogenic bacteria that colonize diabetic wounds and hence improve the healing process of diabetic wounds.

Keywords: *Bacterial colonization; cold atmospheric plasma; diabetic wounds; Paenibacillus species, pathogenic bacteria*

INTRODUCTION

Diabetic wounds are especially vulnerable to pathogenic bacterial colonization due to high glucose levels in the wound environment[1]. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* are among the bacteria most commonly found in diabetic wounds[2]. These bacteria can cause serious complications such as cellulitis, osteomyelitis, and sepsis[3]. They are also frequently resistant to antibiotics, making them difficult to treat[4]. *Staphylococcus*, *Bacillus* sp., *Paenibacillus* sp., and *Pseudomonas* sp. are commonly isolated bacteria from diabetic wounds[5-7]. These bacteria are known to cause infections that can lead to severe complications if not treated promptly[8&9]. These bacteria's pathogenicity originates from their ability to produce virulence factors such as toxins, enzymes, and adhesions, which allow them to colonize and damage tissues[10,11]. Toxins produced by *Staphylococcus* sp., and *Bacillus* sp., can destroy host cells and evade the immune system[12]. Moreover, *Paenibacillus* sp., and *Pseudomonas* sp. produce enzymes that

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degrade host tissues while promoting bacterial growth[13]. The combination of these virulence factors makes these bacteria highly pathogenic and difficult to treat[14]. To avoid further complications, it is critical to identify these bacteria early and treat them with appropriate remedies[15]. These strains' ecological and potential pathogenicity characteristics raise intriguing questions that warrant further investigation. This is where cold atmospheric plasma (CAP), as a potential solution for eradicating pathogenic bacteria, comes in.

CAP is a type of plasma that operates at or near room temperature, making it ideal for a variety of applications[16]. CAP, unlike other types of plasma, can be generated using simple devices and operated in air or other gases at atmospheric pressure[17]. Unlike traditional sterilization methods, CAP is effective at killing microorganisms and can be used on delicate surfaces and in a variety of settings[18-20]. In addition, CAP has shown promise as a treatment for chronic wounds such as diabetic ulcers and bedsores[21]. One of the most significant benefits of CAP over antibiotics is that it does not contribute to antibiotic resistance[22]. CAP can also be used to treat biofilms, which are communities of bacteria that are particularly resistant to antibiotics[18]. Several clinical trials have been carried out to assess the efficacy of CAP in the treatment of diabetic wounds. According to one study, CAP can reduce bacterial load in diabetic wounds by up to 90% after just one treatment [23]. CAP is a promising new technology that has the potential to revolutionize diabetic wound treatment. It can kill and inhibit pathogenic bacteria while not contributing to antibiotic resistance, making it an appealing option for healthcare professionals. More research is needed to fully understand its potential. Therefore, our current study aims to investigate the use of CAP as a potential therapeutic agent against pathogenic bacteria isolated from diabetic wounds.

MATERIALS AND METHODS

DBD device and instrumentation

The Dielectric Barrier Discharge (DBD) plasma device was constructed using a 50 kHz high voltage power source connected to a 1 mm thick stainless-steel electrode with an area of 1 cm^2 and isolated from the ground by a polymer plate of 0.1 mm thickness[24]. The treated sample was put between the two electrodes and the distance between the powered electrode and the sample was 1 mm[25,26].

Isolating pathogenic bacteria from diabetic wounds of diabetic patients

The ethical committee of the Faculty of Medicine, Assiut University, Egypt, approved the study (IRB no: 17400028). Informed and written consent was given by all participants (50 patients) prior to enrollment. All methods were performed in accordance with the guidelines of the Declaration of Helsinki[27]. The guidelines used for the microbial studies were those of the Clinical and Laboratory Standards Institute (CLSI)[28]. Different species of bacteria, including Gram-positive and Gram-negative bacteria, were isolated using the swab method from the diabetic foot ulcers of diabetic patients attending the Diabetes, Endocrine Center, and Vascular Surgery outpatient clinics in Assiut University Hospitals. All isolates were processed, isolated, and identified by standard methods, including Gram staining, culture, biochemical reactions, VITEK, and 16S RNA sequencing in Korea. All samples taken from diabetic wounds using swab methods[29], were transferred into a sterile Brain heart infusion (BHI) broth medium for laboratory culture. All samples were cultured for 24 hrs at 35±2 °C in Tryptic soy agar (TSA) medium. The formed isolated colonies were streaked to be purified into Mannitol salt agar (MSA) medium for detection of *Staphylococcus* species. MacConkey's agar medium for Gram negative bacteria[30]. Eosin methylene blue agar (EMB) medium for *E.coli* culture detection [31]. All of the cultural media were purchased from HIMEDIA.

The cultures were incubated under aerobic and static condition overnight at 37ºC. All single colonies formed were further purified to obtain a pure isolate for further microbiological studies[32]. The isolates used were *Staphylococcus haemolyticus* AUMC b-331 (OQ102238.1), *Bacillus safensis* strain MS1 (OQ092722.1) *Stutzerimonas kunmingensis* MS2 (OQ092246.1), *Paenibacillus* sp. MS3 (OQ093272.1), and *Stutzerimonas* sp. MS4 (OQ094294.1) Bacteria were cultured in nutrient broth (N.B), for 24 hrs at 37 °C[33]. Muller-Hinton agar medium was used for the

detection of antimicrobial activity of CAP against these pathogenic bacteria [34].

Genotypic identification using 16S rRNA

DNA isolation

The genomic DNA was extracted from the five isolates using the genomic DNA Prep kit (SolGent, Daejeon, Korea) according to the manufacturer's instructions after the glass bead to disrupt the cell walls. The extracted DNA was then used as a template for PCR to amplify the 16S rRNA gene. A universal bacterial primer set of 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3 ′) was used to amplify the nearly complete 16S rRNA gene[35,36].

PCR amplification and DNA sequencing

The PCR amplification was performed in a 25 µl reaction volume containing 10–50 ng of the template DNA, 0.4 µM of each primer, 0.75 U of EF-Taq DNA polymerase (SolGent, Daejeon, Korea), 0.2 mM of each dNTP (SolGent, Daejeon, Korea), and 1×EF-Taq reaction buffer (SolGent, Daejeon, Korea). The Thermo cycling conditions included an initial denaturation step at 95 °C for 15 min followed by 30 cycles at 95 °C for 20 seconds, 50 °C for 40 s, and 72 °C for 1.5 min with a final extension step at 72 °C for 5 min. The PCR product was separated by gel electrophoresis on 1.5 % agarose containing ethidium bromide with a 0.5 × Tris-acetate-EDTA (TAE) buffer, and visualized using a UV illuminator [37]. The PCR product was then purified using a SolGentPCR purification kit (SolGent, Daejeon, Korea) according to the manufacturer's instructions. The amplified 16S rRNA gene was sequenced using an ABI Big Dye Terminator (v 3.1) cycle sequencing kit (Applied Biosystems, Foster City, Cal., USA) and an ABI 373 0XL DNA analyzer (Applied Biosystems, Foster City, Cal., USA).

Phylogenetic analyses

The 16S rRNA dataset included 33 sequences, of which one sequence was obtained in this study for *Staphylococcus* sp. AUMC b-331, 31 sequences downloaded from GenBank for the nearest strains including the available type species, and one sequence for the outgroup. The sequences were assembled using the DNA STAR computer package (DNA star version 5.05) and Molecular Evolutionary Genetics Analysis Version 11 MEGA11 software Assembled sequence of the target isolates were aligned with those downloaded from GenBank using Multiple Alignment using Fast Fourier Transform MAFFT[38]. Alignment gaps and parsimony uninformative characters were treated by Block Mapping and Gathering with Entropy BMGE[39]. Maximum-likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed. The evolutionary history was inferred using the Neighbor-Joining method[40]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown above the branches[41]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method[42] and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1494 positions in the final dataset. Evolutionary analyses were conducted in MEGA11[43].

Elucidating the effects of CAP on isolated pathogenic bacteria

The conventional diffusion method used as a screening method to determine the anti-bacterial efficacy according to CLSI was employed for the assessment of the anti-bacterial potential of CAP[34]. The inoculum of each 18–20-hour pre-cultured pathogenic bacterial isolate to be tested, which contained 1.5 x 10^8 CFU mL^{-1}, was spread using a sterile swab moistened with the bacterial suspension on Muller-Hinton agar plates. Subsequently, the CAP was generated and exposed to specific sites within each plate for each isolate at a different time. The time of exposure was varied; we used 10, 20, 30, and 40 sec for each plate. Then, the plates were incubated at 37 °C for 24-48 hrs under aerobic conditions in an upright position [44]. The clear zone surrounding the well (microbial growth inhibition zone) was measured in millimeters (mm), and three replicates were performed against each of the pathogenic bacteria tested.

Efficacy of CAP on bacterial biofilm

For in vitro biofilm culture, strains of *Staphylococcus haemolyticus*, *Bacillus safensis* MS1, *Paenibacillus* sp. MS3, and *Stutzerimonas* sp. MS4 were used[45]. Stock cultures were kept at -20°C before being streaked onto Nutrient agar and incubated at 37°C for 48 hrs. Each microorganism culture was placed in 10 mL of Tryptic Soy Broth and incubated at 37°C for 18 –22 hrs. Cells were harvested from each microorganism culture, washed twice with 10 mL of PBS (10 mM PO4-3, 137 mM NaCl, 2.7 mL KCl), centrifuged at 4000 rpm for 5 minutes, suspended in 10 mL of RPMI medium (RPMI-1640, Sigma-Aldrich Co., USA), and standardized to 1 x 107 cells/ml. Initially, the samples were incubated in a static position within Microtitre-plate 96 wells for 24 hrs at 37°C. Non-adherent cells were then eliminated by gently washing each sample twice with 2 mL of PBS before adding 2 mL of new RPMI media. After 24 hrs of incubation at 37°C in a static incubator, 1 mL of the RPMI medium was taken out and replaced with an equivalent volume of fresh media for another 24 hrs of incubation under the identical conditions[5,46]. After 48 hrs of incubation, each disc was moved to a new well and the biofilm was gently rinsed with 2 mL of PBS[47]. The treated samples received CAP treatment, whereas the control group received no treatment. All four bacterial biofilm strains were treated with amoxicillin (50 mg/ml) as a positive control.

Statistical analysis

On normally distributed data expressed as means and standard error of the mean, GraphPad Prism software version 5 was used to perform the statistical analysis (SEM). To investigate significant differences between the three groups, a one-way ANOVA was employed, followed by a Tukey's posttest.

RESULTS AND DISCUSSION

Results

Isolation of pathogenic bacteria from diabetic wounds

The results of isolated bacteria demonstrated the patient's susceptibility to bacterial contamination due to diabetic wounds. The patients' ages ranged from 50 to 60 years showed the greatest susceptibility to *Staphylococcus* sp. contamination (70%), followed by *Pseudomonas* sp. (10%). Furthermore, *E. coli*, *Bacillus* sp., and *Enterococcus* sp. were found in nearly equal proportions as shown in **Table 1**. The most common bacteria found in the various targeted ages in this study were *Staphylococcus* species, followed by *Pseudomonas* sp., *E. coli*, *Bacillus* sp., and *Enterococcus* sp., with percentages of 60, 18, 8, 8, and 6%, respectively.

Identification and confirmation of isolated pathogenic bacteria

The biochemical test and VITEK were used to confirm the identification of the purified bacteria after the microscopic examination. The most resistant bacterial strains to antibiotics had their DNA extracted and sent to Korea for sequencing. *Staphylococcus haemolyticus* AUMC b-331, *Bacillus safensis* strain MS1, *Stutzerimonas kunmingensis* MS2**,** *Paenibacillus* sp. MS3, and *Stutzerimonas* sp. MS4 isolates were identified at the species level using the macro- and microscopic features. Additionally, all these isolates showed the highest resistance against different antibiotics using sensitivity test, so they were multi-drug resistant strains. Therefore, these isolates were subjected to confirmation of their identification using molecular techniques. The molecular typing resulted from partial 16S rRNA gene sequence of 1418, 1419, 1406, 1429, and 1394 base pair (bp) for *Staphylococcus haemolyticus* strain AUMC B-331, *Bacillus safensis* strain MS1, *Stutzerimonas kunmingensis* strain MS2, *Paenibacillus* sp. strain MS3, and *Stutzerimonas* sp. strain MS4, respectively. The results of the phylogenetic tree were obtained in **Fig.1.** The results illustrated the identified bacteria which were *Staphylococcus haemolyticus* AUMC b-331 **(Fig.1A)**, *Bacillus safensis* strain MS1 **(Fig.1B)**, *Stutzerimonas kunmingensis* MS2 **(Fig.1C)**, *Paenibacillus* sp. MS3 **(Fig.1D)** and, *Stutzerimonas* sp. MS4 **(Fig.1E).**

Table 1 : The number of patients isolates with distribution according to their ages.

Age (Years)	**Number of isolates**					
	Staphylococcus sp. (%)	*Enterococcus* sp. (%)	*Pseudomonas* sp. (%)	*Bacillus* sp. (%)	*E. coli* (%)	Total no.
20-30	1 (50)	0	0	0	1 (50)	2
30-40	3 (50)	0	1 (16.66)	1 (16.66)	1 (16.66)	6
40-50	5 (41.66)	1 (8.33)	5 (41.66)	1 (8.33)	0	12
50-60	21 (70)	2 (6.66)	3 (10)	2 (6.66)	2 (6.66)	30
Total	**30 (60 %)**	**3 (6 %)**	**9 (18 %)**	**4 (8 %)**	**4 (8 %)**	**50**

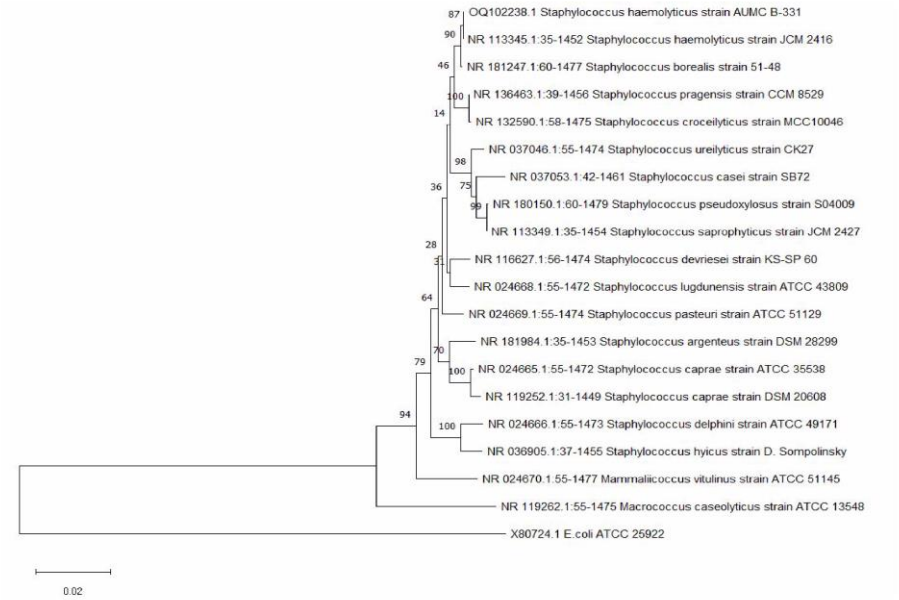

Fig. 1A: Phylogenetic tree generated from MP analysis, the neighbor-joining tree based on 16S rRNA gene sequences showing the positions of the isolated *Staphylococcus* sp. AUMC b-331 and related strains. The tree is rooted to E. coli ATCC 35922 as our group.

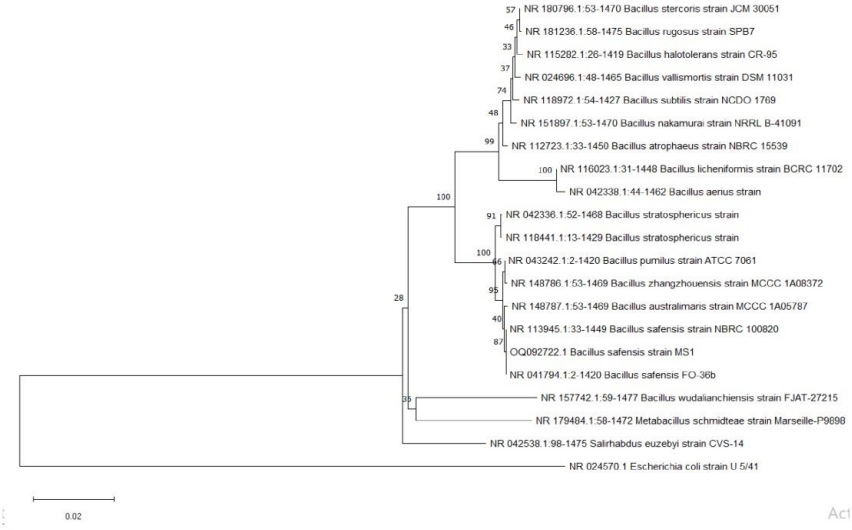

Fig. 1B: Phylogenetic tree of Bacillus sp. MS1 of 1419 base pair and reference sequences from GenBank. The tree was constructed by the neighbor-joining algorithm implemented in the MEGA-11 program.

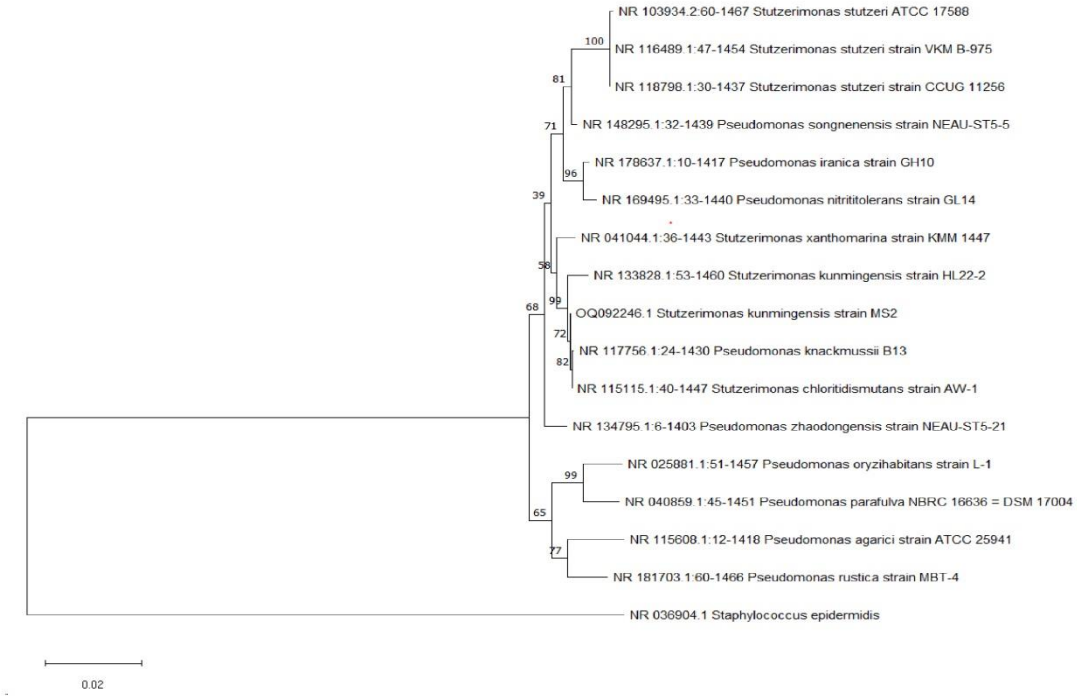

Fig. 1C: Phylogenetic tree of Pseudomonas sp. MS2 with 1394 bp and reference sequences from GenBank. The tree was constructed by the neighbor-joining algorithm implemented in the MEGA-11 program.

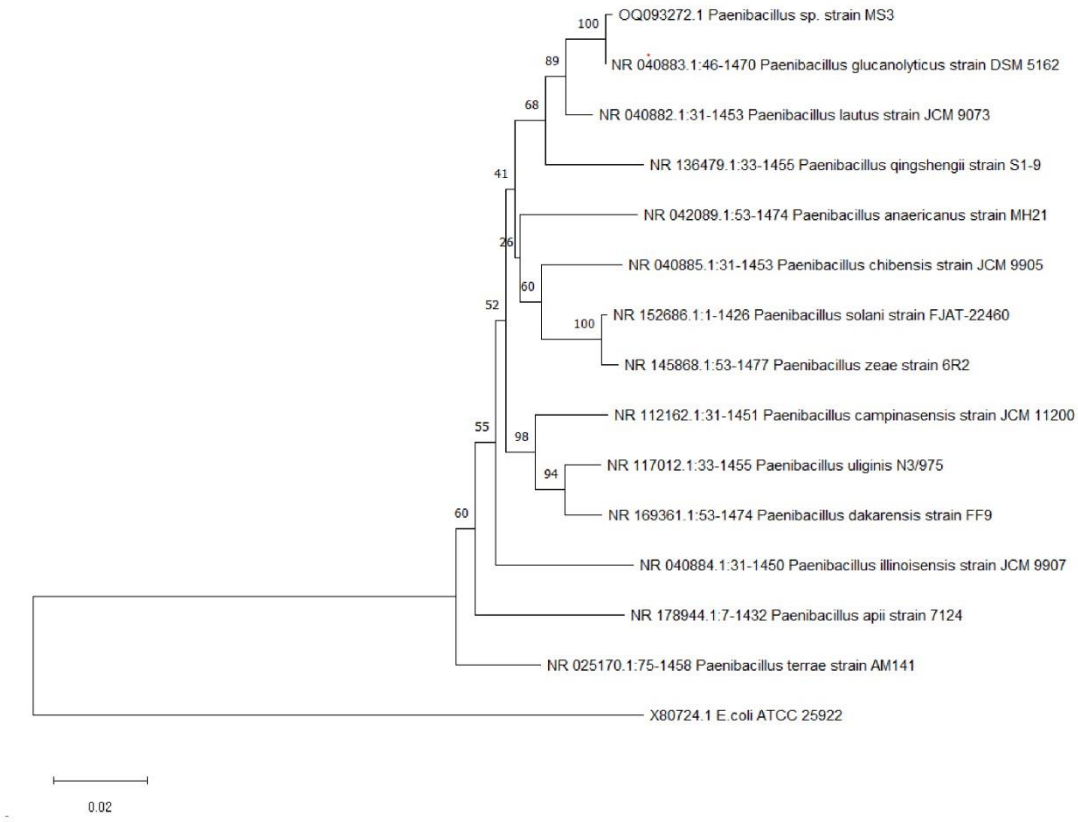

Fig. 1D : Phylogenetic tree of *Paenibacillus* sp. MS3 with 1406bp and reference sequences from GenBank. The tree was constructed by the neighbor-joining algorithm implemented in the MEGA-11 program.

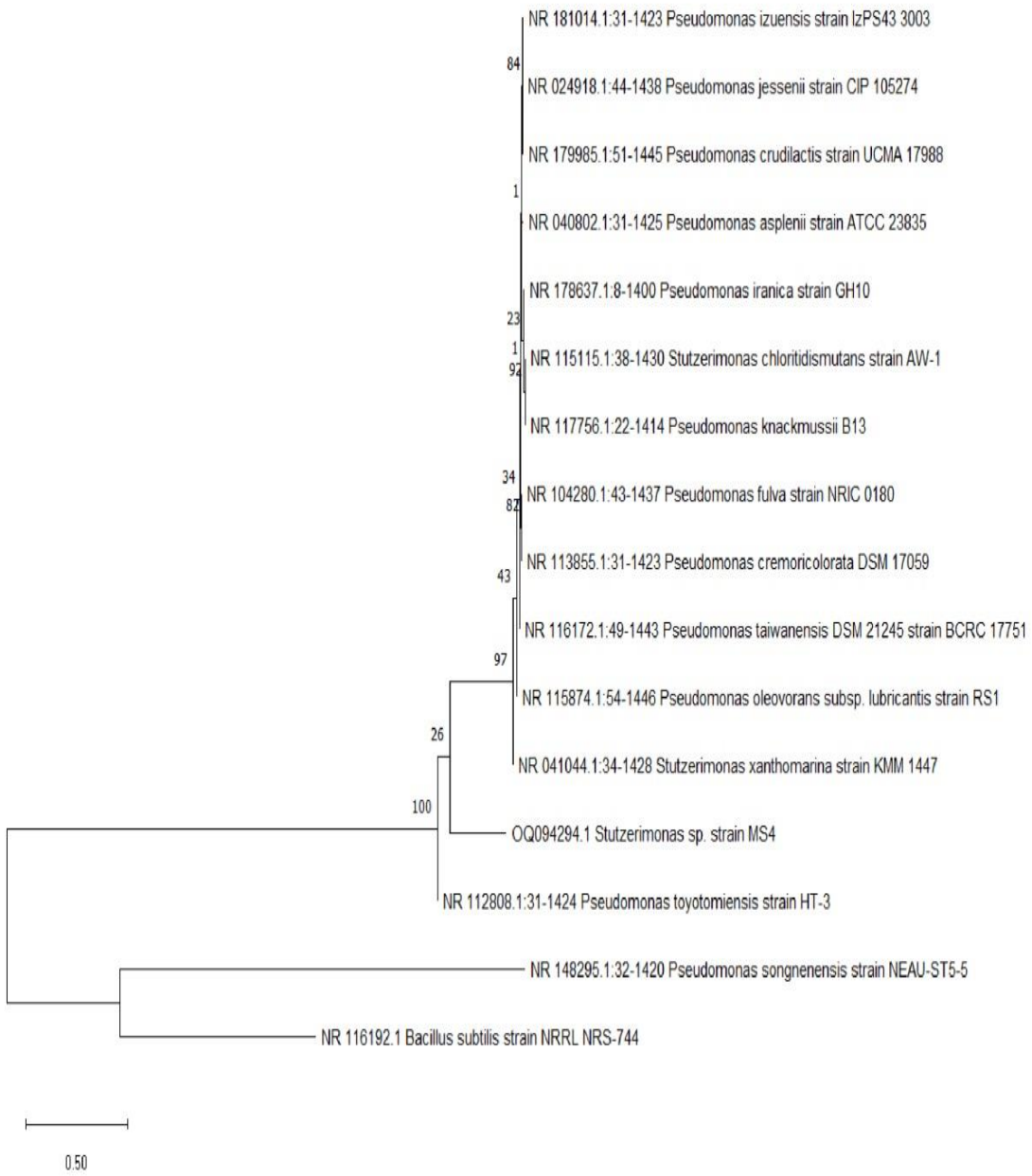

Fig. 1E: Phylogenetic tree of *Stutzerimonas* sp. MS4 with reference sequences from GenBank. The tree was constructed by the neighbor-joining algorithm implemented in the MEGA-11 program.

The potential impact of CAP against biofilm formation

CAP was tested against the most biofilm-producing strains compared to a positive control amoxicillin that usually used as a broad-spectrum antibiotic for wound infection, and the negative control is strain without receiving any treatment. The obtained data showed the great potency of CAP against the four-targeted isolates. *Staphylococcus haemolyticus* demonstrated high biofilm producing ability, followed by *Stutzerimonas* sp. MS4, *Paenibacillus* sp. MS3, and *Bacillus safensis* MS1, which in turn, showed less biofilm-producing ability among the four tested pathogenic bacterial strains. Our results demonstrated that CAP significantly reduced the bacterial biofilm as shown in **Fig.2.**

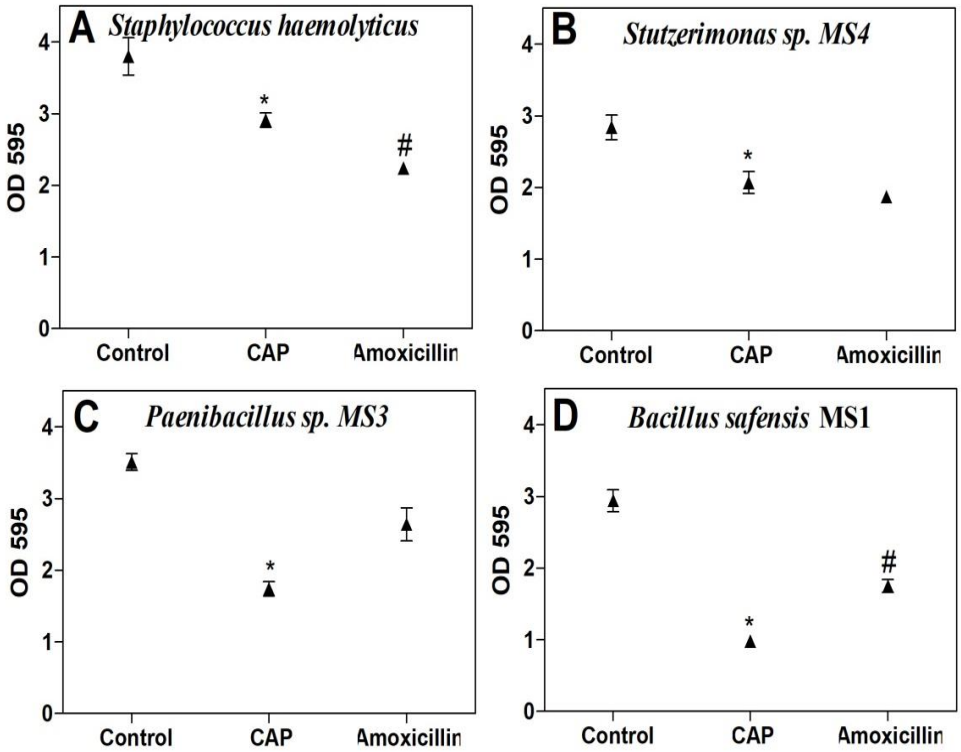

Fig. 2: The figures of A, B, C, and D were presented the four bacterial biofilm production ability and the effect of CAP to reduce the bacterial biofilm compared to Amoxicillin. OD_{595} referred to the optical density measured by spectrophotometer. The data are expressed as the mean ± SEM. $^{*}P < 0.05$, bacterial isolate treated with CAP. versus control., $^{\#}P < 0.05$, bacterial isolate treated with amoxicillin versus control.

Anti-bacterial activity of CAP

The identified pathogenic bacteria were used for investigating the anti-bacterial activity of CAP. The clear zone that appeared in the plates after the incubation period was shown in Fig.3A, which demonstrated that CAP, *in vitro*, mediated a significant reduction and growth arrest of pathogenic bacterial growth. In all tested bacteria strains, the maximal anti-bacterial effect of CAP was achieved after exposure to CAP for 40 seconds as shown in **Fig.3** (A, B, C, and D). Moreover, the effect of CAP with different exposure times demonstrated a clear anti-bacterial effect, and the means of clear zone diameter were demonstrated in **Table 2**. The data in Fig.3A illustrated the impact of CAP as an anti-bacterial against the pathogenic bacteria that were isolated from diabetic wounds. Using different exposure times of isolated bacteria to CAP showed he highest anti-bacterial effect at 40 seconds against *Bacillus safensis* MS1 with 30 mm clear zone diameter, followed by 25, 20, and 12 mm for *Paenibacillus* sp. MS3, *Stutzerimonas* sp. MS4, and *Staphylococcus haemolyticus* respectively. In contrast, exposure of pathogenic bacteria to CAP for 30 seconds illustrated that the clear zone diameters were 27, 18, 17, and 5 mm against *Bacillus safensis* MS1, *Paenibacillus* sp. MS3, *Stutzerimonas* sp. MS4, and *Staphylococcus haemolyticus* respectively. Moreover, exposure of pathogenic bacteria to CAP for 20 seconds showed clear zone diameter of 21, 16, 14, and 5 mm for *Bacillus safensis* MS1, *Paenibacillus* sp. MS3, *Stutzerimonas* sp. MS4, and *Staphylococcus haemolyticus*, respectively. Finally, when the targeted pathogenic bacteria were exposed to CAP for 10 seconds, the diameters of clear zone were 17, 11, 6, and 4 mm for *Bacillus safensis* MS1, *Paenibacillus* sp. MS3, *Stutzerimonas* sp. MS4, and *Staphylococcus haemolyticus*, respectively. Hence, our results demonstrated that exposure of pathogenic bacteria to CAP for 10 seconds exhibited less significant anti-bacterial effect than exposure for a long time until 40 seconds, which is considered the best exposure time to CAP **(Fig.3B)**.

Table 2: Anti-bacterial activity of CAP against pathogenic bacteria as expressed by the means of the clear zone diameter (mm).

	Inhibition zone diameter (mm)			
	A (40 Sec) CAP	B (30 Sec) CAP	C (20 Sec CAP	D (10Sec) CAP
Bacillus safensis MS1 (*A)*	30	27	21	17
Paenibacillus sp. MS3 *(B)*	25	18	16	11
Stutzerimonas sp. MS4 *(C)*	20	17	14	6
Staphylococcus haemolyticus D)	12	5	5	4

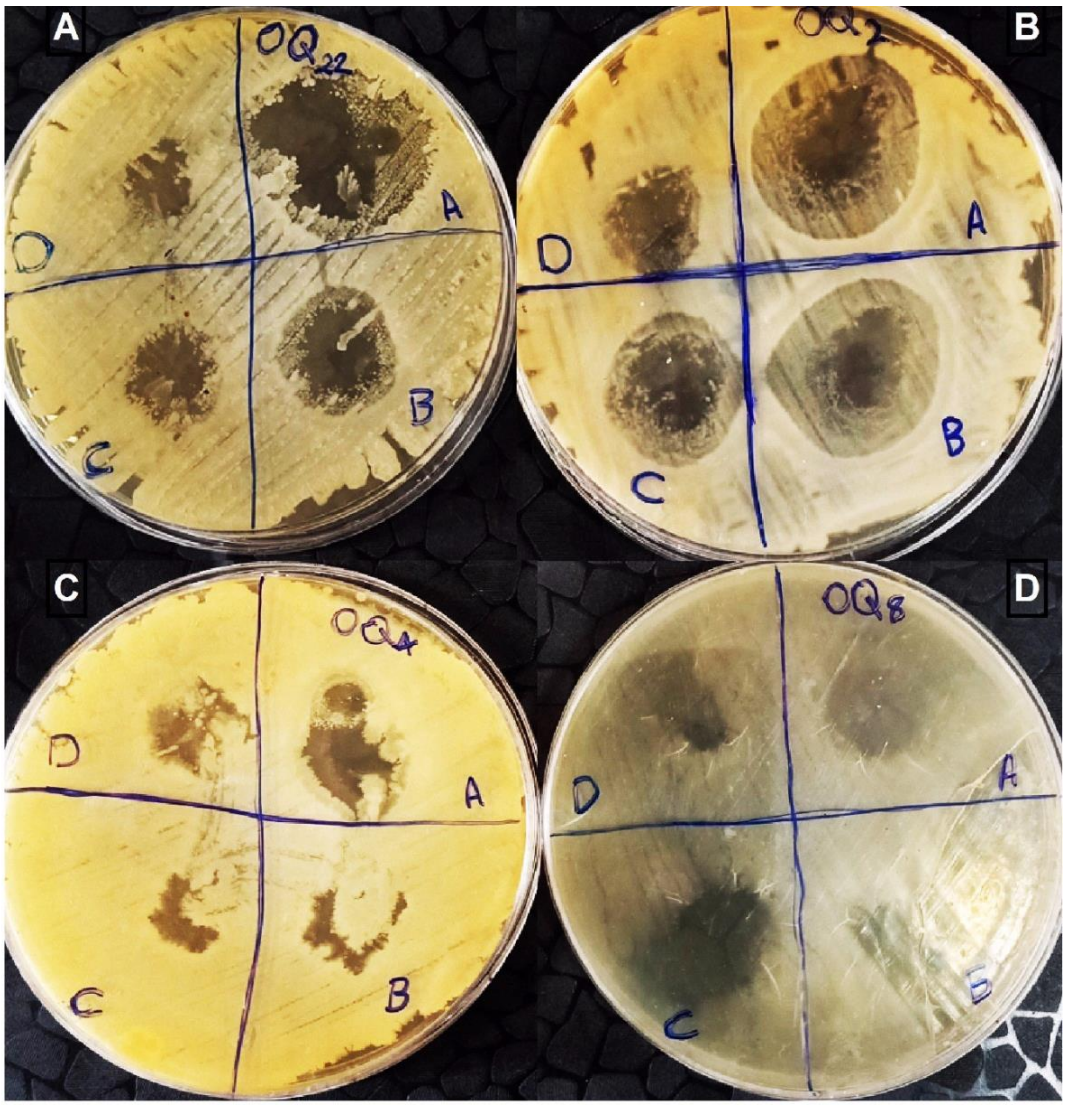

Fig. 3A: Inhibition zone showing the anti-bacterial activity of CAP on bacteria isolated from diabetic wounds. Causing a clear zone with diameter against the pathogenic bacteria, A: *Bacillus safensis* MS1, B: *Paenibacillus* sp. MS3, C: *Stutzerimonas* sp. MS4, and D: *Staphylococcus haemolyticus* AUMC b-331.

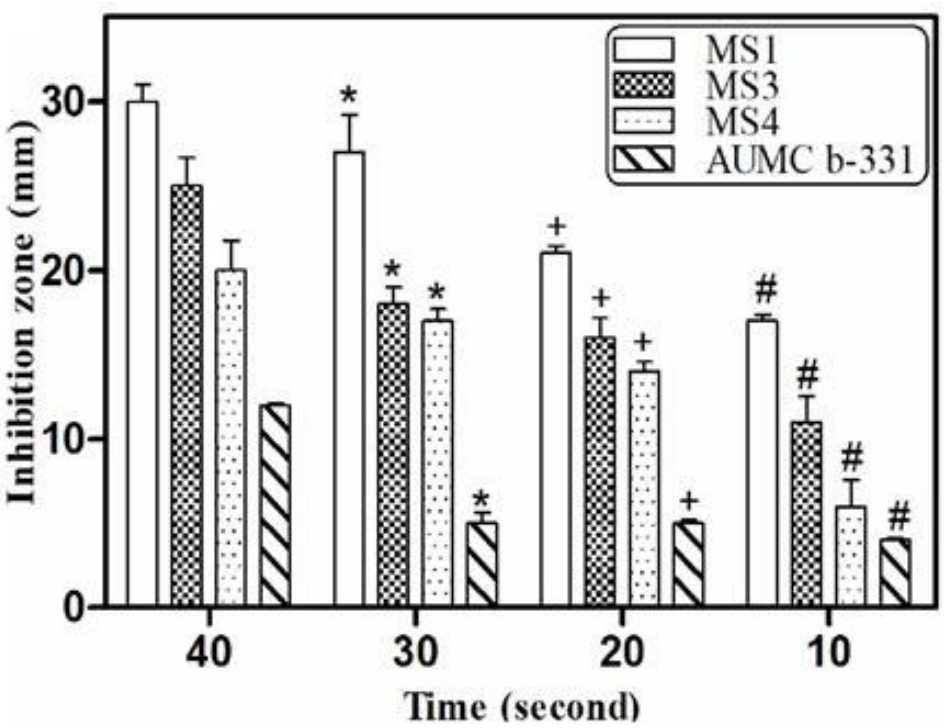

Fig. 3B: The accumulated data from triplicate test for each strain for each time (second) exposed to CAP were illustrated and the results show the significant effect of CAP on the tested pathogenic strains which indicated by the inhibition zone (mm). The data are expressed as the mean ± SEM. **P* < 0.05, 30 sec. versus 40 sec., +*P* < 0.05, 20 sec versus 30 sec., #*P* < 0.05, 10 sec versus 20 sec.

Discussion

Indeed, the coexistence of multiple bacterial species on the chronic wound, organized into patho-groups, as well as the host-related responses encountered by bacteria, alter the pathogenicity of bacteria[48]. Some investigations proved toxicogenic S. aureus strains (with exfoliatins-, EDIN-, PVL-, or TSST-encoding genes) in Diabetic Foot Infections (DFI), particularly in grade 4, with systemic consequences[49&50] . Similarly, our results of isolation demonstrated that 60 % of the entire collected samples were identified to be contaminated by *Staphylococcus* sp. Although some *Staphylococcus* sp. are considered normal skin microbiota, they could be opportunistic and become harmful, causing bacteremia and high risks at wound sites[51]. *Stutzerimonas* is a new genus within the Pseudomonadaceae that includes strains from the previously phylogenetic group of *Pseudomonas stutzeri* [52]. Nevertheless, there is no published work demonstrating the occurrence of *Stutzerimonas kunmingensis* in infected wounds; we illustrated for the first time the occurrence of this isolate, which was isolated from diabetic wounds. Moreover, they have the ability to form a biofilm. In spite of the fact that *Paenibacillus* is a rare cause of wound infection, particularly in the setting of polluted wounds and outdoor injuries[53], some literature has demonstrated the use of chestnut honey as an anti-bacterial agent against *Paenibacillus larvae*[54]. Similarly, it has been shown that *Paenibacillus* sp. is one of the pathogens that could cause wound contamination and delay the healing process of wounds. We illustrated in our study the possibility of using a new, safe, sustainable, and costless technique to overcome the bacterial virulence that gave them the ability to colonize and stand against the autoimmune system using CAP. CAP has been shown to successfully inactivate bacteria, fungus, spores, and viruses in spite of their resistance profiles to conventional antimicrobials, as well as on diverse surfaces and in biofilms[55&56]. Furthermore, CAP showed a powerful effect against the tested bacterial biofilm. Despite biofilms playing beneficial roles in a variety of

fields such as plant protection, bioremediation, wastewater treatment, and corrosion inhibition, among others[57], there are many efforts to get rid of harmful biofilms. Various techniques and approaches that interfere with bacterial attachment, bacterial communication systems (quorum sensing, QS), and biofilm matrix's have been used[5&57-59]. We demonstrated in our results that there are merits of using CAP against the pathogenic bacteria isolated from diabetic wound infection. CAP exhibited anti-bacterial effects against all the tested pathogenic bacteria *Bacillus safensis* MS1, *Paenibacillus* sp. MS3, *Stutzerimonas* sp. MS4, and *Staphylococcus haemolyticus*. Similar to several lines of evidence that proved the CAP is unique technology that could be used to overcome the wound infection[24&60&61]. CAP has shown great efforts for treating *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Enterococcus faecalis* planktonic cultures[62-66]. Therefore, CAP; which is proven to successfully inactivate bacteria, may be a potential option for use in dentistry, dermatology, and wound care medication[62]. As a consequence of the emphasis on use of CAP and the results of several studies, technical research may evolve, allowing the development of an ever-smaller device capable of delivering plasma within structures.

Conclusion

In conclusion, our results demonstrated that CAP is an effective strategy for mediating growth arrest of pathogenic bacteria invading diabetic wounds via inhibiting the bacterial colonization load. This, in turn accelerates the healing process of diabetic wounds. Although CAP showed a powerful effect against ~~the~~ pathogenic bacteria isolated from diabetic wounds, the effect of this type of plasma should be investigated in future *in vivo* studies to further investigate the role of CAP in acceleration diabetic wound healing process.

Declarations

Author contribution

Gamal Badr and F. M. El-Hossary were the main contributors to the idea of the research project; Gamal Badr put the study design, contributed to all the experiments, performed the statistical analysis, and drafted the manuscript. Fayez M. El-Hossary contributed to the drafting and revision of the manuscript. Fayez M. El-Hossary and Mohamed Khalaf built the CAP system at Sohag University for treating pathogenic bacteria. Mohamed Salah and Eman A. Sayed conducted the experiments, prepared all the figures, and helped in drafting the manuscript. Ahmed Elminshawy contributed to data processing, revision and drafted the manuscript.

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Availability of data and material

All data are available on request from corresponding author (Gamal Badr: badr73@yahoo.com and gamal.badr@aun.edu.eg).

Ethics approval and consent to participate

The ethical committee of the Faculty of Medicine, Assiut University, Egypt, approved the experiment methods (Ethics approval number: 17400028).

Competing interests

The authors declare no competing interests.

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الفعالية العلاجية لبلازما الهواء الجوي غير الحرارية ضد البكتيريا الممرضة التي تستعمر جروح السكري

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داء السكري هو مرض مزمن يصيب حوالي ١٧٠ مليون شخص في جميع أنحاء العالم ، و من المتوقع أن يتضاعف هذا العدد في السنوات القليلة المقبلة. ويزداد إنتشار هذا المرض في العديد من البلدان ، ومنها مصر ، مع استمرار تضاعف المضاعفات الحادة المرتبطة به. إن تأخر التئام الجروح هو أحد المضاعفات الخطيرة لمرض السكري والذى يؤثر سلباً على الحياة الاجتماعية والاقتصادية للمريض. ما يقرب من ٢٥ ٪ من مرضى السكري يكونوا عرضة للإصابة بقرحة القدم السكري ، الأمر الذي يتطلب غالبا طرق علاجية متقدمة للحد من هذه المضاعفات.

تعتبر إعاقة التئام جروح السكري من المشاكل الرئيسية التي تهدد حياه مرضى السكري، حيث انها تحتاج الى وقت طويل لكي تلتئم. لم تقتصر المشكلة على تلك المدة ولكن تكمن المشكلة الاساسية في المضاعفات التي تحدث نتيجة خلل في الجهاز المناعي ومستوى السكر في الدم مما يحفز استعمار البكتيريا الممرضة والإنتهازية لمواضع الجروح الأمر الذي يؤدي الى إعاقة التئام جروح السكري. يأتي من هنا أهمية استخدام أساليب حديثة للتغلب على تلك البكتيريا وتقليل استخدام المضادات الحيوية التي من خلالها تم ظهور البكتيريا المقاومة للعقاقير، ولقد تم تسليط الضوء في هذه الدراسة على استخدام بلازما الضغط الجوي غير الحرارية ضد البكتيريا الممرضة التي تسكن وتهاجم جروح مرضى السكري وتعيق التئأم الجروح. تم عزل البكتيريا بإستخدام طريقة المسحة من خمسين مريض بجروح السكري تتراوح أعمارهم من عشرين إلى ستين عاماً ، حيث تم استخدام الأوساط الغذائية لعزل وتنقية تلك البكتيريا وتم توصيفهم وتعريفهم مورفولوجياً وميكروسكوبياً بعد استخدام صباغة الجرام وبعد ذلك تم التأكيد على تعريفهم جينياً. أكدت النتائج التي تم اجرائها في المختبر على قدرة تقنية استخدام بلازما الضغط الجوي الغير حرارية في التخلص وتقليل نمو البكتيريا الممرضة التي تم استخدامها وخاصة بكتيريا المكورات العنقودية والبكتريا العصوية المكونة للجراثيم وكذلك بكتيريا العصويات القصيرة الممرضة ، هذا وقد أثبتنا عملياً قدرة هذه الاستراتيجية على تسريع التئآم جروح السكري من خلال تقليل نمو والتخلص من البكتيريا التي تعيق عملية التئآم الجروح.