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Investigation of the effect of electric field on bacteria isolated from skin infection

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Abstract. *Al-Salami RB, Mukhaifi EA, Al-Tamimi WH. 2024. Investigation of the effect of electric field on bacteria isolated from skin infection. Biodiversitas 25: 1320-1328.* Nineteen bacterial isolates were obtained from twenty-six samples obtained from various patients with skin infections in the current study. These bacteria were identified by a genetic method depending on 16S rRNA and the molecular identification of *cna* and *fnbA* genes of *Staphylococcus aureus* isolates were detected, and an antimicrobial electric field was applied using various voltages and durations to target both Gram-positive and Gram-negative bacteria. The results showed that *Staphylococcus* sp. was the most dominant genera, followed its *Acinatobacter* sp. The high frequent species was *Staphylococcus aureus* (24%) followed by *Staphylococcus epidermidis* (21%), *Staphylococcus epidermidis* and *Staphylococcus argenteus* (10.5%), *Staphylococcus haemolyticus, Acinetobacter baumannii* and *Acinetobacter variabilis* (5.2%). Eleven new strains were identified and recorded in GenBank. A phylogenetic tree was also constructed based on 16S rRNA gene sequences of isolates to evaluate their close relationship and evolution between them. The bands of each amplified *cna* and *fnbA* gene were described at 192 bp and 191 bp respectively. The effect of the antimicrobial electric field showed an extremely high and significant decrease of the viable bacterial count of *S. haemolyticus* and *A. baumannii* after the exposure to an electric field of (1-5) V for 15 min, where the viable count of bacteria reduced sharply with percentage bacterial death (19-95%) and (16-100%), respectively, and from (66-100%) and (52-100%) after 30 min, respectively.

Keywords: 16S rRNA gene sequencing, antibiotic resistant, antimicrobial electric field, isolation, skin infection

INTRODUCTION

The human skin is the largest organ and the basic barrier in the body. It is a vital component of the immune system, serving as the first line of defense against bacterial infections and protecting against thermal, mechanical factors, and harmful radiation. It not only blocks pathogens from entering the body from the environment but also provides a large-scale biological niche for a wide range of bacteria. The bacteria, along with their genetic components and environmental interactions, make up the human skin microbiome (Ibrahim et al. 2015).

Most commonly found are Firmicutes (24.4%), Actinobacteria (51.8%), Bacteroidetes (6.3%), and Proteobacteria (16.5%). The most prevalent genera are *Propionibacterium, Corynebacterium*, and *Staphylococcus*. Various skin infections may arise from any breach of this barrier or disturbance of skin homeostasis combined with the appearance of pathogens. Abnormal conditions such as humidity, temperature, pH, and the composition of antimicrobial peptides and lipids also lead to ecological dysbiosis (Yang et al. 2022). Dysbiosis is an unbalanced microbial community in a particular location of the body that might cause the onset or development of disorders McLoughlin et al. (2021). Many skin diseases like seborrheic dermatitis and atopic, acne, alopecia areata, and psoriasis may result from dysbiosis (Silverberg 2021). McLoughlin et al. (2021) showed that dysbiosis is an unbalanced microbial community in a particular location of the body that might cause the onset or development of disorders. Many skin diseases like seborrheic dermatitis and atopic, acne, alopecia areata, and psoriasis may result from dysbiosis (Silverberg 2021).

One of the most prevalent conditions in both community and hospital settings is skin infection. These can occur in various ways, ranging from limited superficial infections that can be treated with antibiotics to severe infections in the deep tissue that lead to death if the patient stays without treatment (Lim et al. 2018). Cellulitis is a common skin disease encountered in medical emergencies in hospitals (Toh et al. 2023). It is an acute bacterial infection causing inflammation in both the deep dermis and the surrounding subcutaneous tissue appears as a poorly defined, warm, erythematous, edematous, and palpably sensitive area; the infection is not accompanied by an abscess or purulent discharge (Byrd et al. 2018).

Gram-positive bacteria, specifically *Staphylococcus aureus* and streptococcal species, are the most likely cause of soft skin tissue infections (SSTIs) (Silverberg 2021). Additionally, germs that are not pathogenic due to damage to the skin barrier might become virulent through skin injury (Zegadło et al. 2023). Antibiotic resistance is one of the most important public health challenges, and the emergence of antibiotic-resistant bacteria poses one of the biggest dangers to the healthcare sector. Deadly pathogenic multidrug-resistant bacteria (MDR) are becoming more prevalent every day and represent a serious threat to human health. Antibiotic resistance of this kind was previously

limited to nosocomial infections, but it has now become a common phenomenon (Jubeh et al. 2020). It is estimated that if new medicines are not developed or discovered, there will be no effective antibiotic available by 2050 to treat these dangerously resistant infections (Alyousif et al. 2020).

Recently, physical agents, including low electric voltages and currents, have gained attention for the treatment of antimicrobials because of their bactericidal capability (Krishnamurthi et al. 2020). It inactivates microorganisms by creating nanometer-sized membrane pores in microorganisms, a process is known as electroporation. Depending on factors such as the electric field intensity, the pulse duration, the number of pulses, and pore formation, it may be reversible or irreversible (Ohshima et al. 2021). The microampere electric currents cause membrane damage in a significant way and allow two-way leakages of proteins, ions, and small molecules (Krishnamurthi et al. 2020). So the present study aimed to investigate the effect of an electric field against bacteria isolated from bacterial skin infection.

MATERIALS AND METHODS

Clinical specimen collection

A total of twenty-six samples were collected from patients undergoing skin infections including cellulitis, erysipelas, impetigo, folliculitis, furuncles, and acne in Al-Sadr Teaching Hospital, Al-Fyhaa Teaching Hospital, Al-Mauana Teaching Hospital and Tumor Center in Basrah Province south of Iraq, during the period from the beginning of October 2022 to the end of December 2022. It should be mentioned that in the present study, all patients were selected based on clinical examination and history by specialist doctors. A cotton sterile media swab was used to collect all samples from the deepest parts of the infection (pus). The swabs were streaked on the media of blood agar, MacConkey agar, and mannitol salt agar, and incubated at 37°C for 24 hrs. Then observed the presence of isolated colonies on the plates re-cultured on nutrient agar plates for purification in subsequent experiments.

Molecular identification of bacteria

DNA extraction and amplification of 16S rRNA gene

Genomic DNA was extracted from isolates using Geneaid extraction kit according to the manufacturer's protocol. Extracted DNA was detected by 1% agarose gel electrophoresis. The bacterial 16S rRNA was amplified using the universal bacteria-specific primers, 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5'GGTTACCTTGTTACGACTT-3'), (Lane 1991). The PCR reaction system consists of a green master mix 25 µL mixed with 2 µL each, forward and reverse primers 10 pmol/ µL, 5 µL of template DNA and equal volume to 50 µL by adding nuclease-free water. The thermal cycling conditions were as follows: initial denaturation for 2 min at 94°C, 35 cycles for 40 sec of denaturation at 94°C, 30 sec of annealing at 55°C, and 1 min of elongation at 72°C. Cycling was completed by a final elongation step at 72°C for 10 min. Amplified fragments were approximately 1,500 bp (El-Sheshtawy et al. 2015). Were detected by electrophoresis 1.5% agarose with TBE buffer in conjunction with DNA ladder (100 base pair). A sequence of PCR products of 16S rRNA genes products from amplified DNA was sequenced at Macrogen company laboratories /in Korea. 16S rRNA of each isolate was purified and sequenced then aligned with known 16S rRNA sequences Gen bank using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST) at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov).

Detection of cna and fnbA genes of Staphylococcus aureus

The *cna* and *fnbA* genes were extracted according to the Geneaid`s Presto TM Mini gDNA Bacteria Kit, and genomic DNA (template) of *S. aureus* isolates was used. The *cna* gene 192 bp, was amplified by using a specific primer (F- AAAGCGTTGCCTAGTGGAGA and (R - AGTGCCTTCCCAAACCTTTT) while for the *fnbA* gene 191 bp the primer was used (F-GATACAAACCCAGG TGGTGG) and (R-TGTGCTTGACCATGCTCTTC). The thermal cycling conditions of PCR amplification are initial denaturation of 94°C for 5 min, denaturation of 94°C for 45 sec, annealing of 55°C for 45 sec, extension of 72°C for 45 sec and final extension of 72°C for 10 min (Saei 2010).

Investigation of the effect of electric field on bacterial growth

The application of AMFields (antimicrobial fields) on bacterial growth was achieved according to (Ibraheim and El-Din 2013) with some modifications.

Preparation of bacterial inoculum

The bacterial isolates that were most resistant to antibiotics were selected for the study of the effect of the electric field. A single colony of each isolate was inoculated into nutrient broth and incubated at 37°C for 24 hrs. in an orbital shaker at 180 rpm. Then activated bacterial cells were inoculated in 500 mL screw-capped flasks containing 250 mL of sterilized nutrient broth medium, the cultures allow growth by incubated at 37°C for 24 hrs. Under sterile conditions, each culture obtained was divided into two groups, one was exposed to an electric field, and the other was considered as control.

Experimental design

The electrical field was generated inside a sterile glass screw cup with the diameter 20 mm width and 80 mm length. The electric field was generated by the DC power supply (SUNSHINE P-1505TA High Precision DC Power Supply 15V 5A USB) was applied at different voltages 1, 2, 3, 4, and 5 V. 20 mL of activated culture bacteria were put under the electric field by using two electrodes of aluminum (width 8 mm, 710 mm length and thickness of 6 mm) for two periods of time 15 and 30 min. The distance between the electrodes was 1.5 cm (Figure 1). The viable bacterial count was carried out after each exposure time with a different voltage.

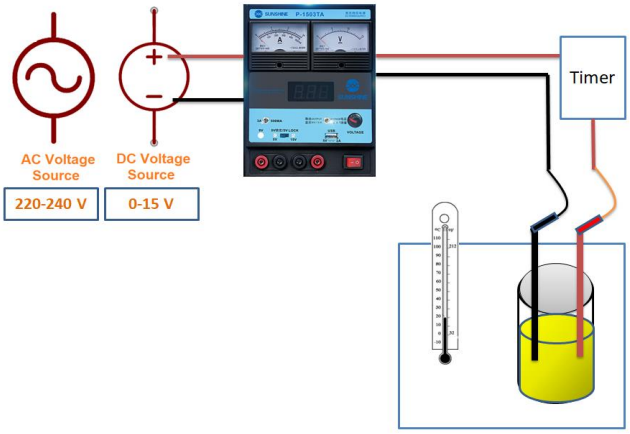


Figure 1. Experimental design of electroporation treatment with Direct Current (CD) current power supply and aluminum electrodes

Measurement of bacterial growth

Before and after exposure to an electric field, the viable count of bacteria was determined by the plate count method. Several serial 10-fold dilutions in sterile saline were performed, and 0.1 mL of the appropriate dilutions 10^{-4} of bacterial cells were used to inoculate mannitol salt agar and MacConkey agar plates. The inoculum was spread carefully, plates were incubated for 24 hrs at 37°C. The number of developed colonies was counted in replicates and multiplied by the factor of dilution, the viable count of controls was also determined, and the replicates were used in each experiment.

Determination percentage of bacterial death

According to Shawki and Gaballah (2015) The following equation was used the calculate the lethal effect of each experimental condition: Percent of bacterial death = [(CFU1 - CFU2)/CFU1] × 100 where the: CFU1 = viable count of bacteria before exposure to electric field; CFU2 = viable count of bacteria after exposure to electric field.

RESULTS AND DISCUSSION

Isolation and molecular identification of bacteria

Microbial invasion of healthy or injured skin is the cause of skin and soft tissue infections (SSTIs). they are hard to control and play a role in the development of antibiotic resistance and chronicity (Khan et al. 2023). In the current study, the total number of isolates was 19 and depending on Gram stain, 17 (89.5%) were Gram-positive whereas 2 (10.2%) were Gram-negative. The result comes in agreement with Ahmed et al. (2020). According to the genomic DNA extraction process using electrophoresis, the results showed that all isolates moved forward with 16S rRNA sequencing had pure and clear isolated DNA, furthermore, after testing for the particular amplification of 16S rRNA gene sequences using a set of universal primers, a single amplification of approximately 1500 bp was achieved for each isolate, 27F and 1492R (Figure 2). These results are compatible with previous studies (Alshami et al. 2022; Alyousif et al. 2020; Al-Zaidi et al. 2023; Aboud et al. 2021; Gmais and Burghal 2022).

The genetic identification shows that various species of bacteria were isolated from skin infections cultured on an agar medium. Based on the 19 isolates that were partially sequenced, the isolates showed a high degree of similarity (100-99.39%) to reference strains after being aligned with other 16S rRNA sequences in GenBank and based on data from BLAST at NCBI. For seven species, two genera were identified and recognized (Table 1). These genera were *Staphylococcus* sp. and *Acinetobacter* sp. *Staphylococcus* sp. were the most dominant genera. Table 2 demonstrated the results of identified isolates, *S. aureus* was recorded in high frequency (42%) meanwhile second most abundant species was *Staphylococcus epidermidis* (21%) this was agreed with previous studies (Ahmed et al. 2020; Mohanty et al. 2018; Zhao et al. 2021). *S. hominis*, *S. argenteus*, *A. variabilis* and *A. baumannii* recorded frequency between 5.2-10.5%, many studies showed the same results (Rocha et al. 2023; Mozyrska et al. 2023). A common gram-positive bacterial pathogen, *S. aureus*, can cause a broad range of clinical illnesses, from minor soft-tissue infections to potentially fatal bacteremia and endocarditis (Mohanty et al. 2018). Human skin is frequently home to the commensal bacteria *S. epidermidis*. This species is viewed as a key member of the healthy skin microbiota, involved in the fight against pathogens, influencing the immune system, and implicated in wound repair. Concurrently, it is the second source of nosocomial infections, and skin conditions including atopic dermatitis have been linked to an overgrowth of this bacteria (Landemaine et al. 2023). *A. baumannii* is becoming more linked to different epidemics, which is concerning because of its widespread antibiotic resistance and clinical symptoms. It has become a significant pathogen among susceptible and severely ill individuals over the past few decades. The most frequent presentations of this bacteria include skin and soft tissue infections, pneumonia, bacteremia, and urinary tract infections; the associated mortality rates are close to 35% (Cavallo et al. 2023).

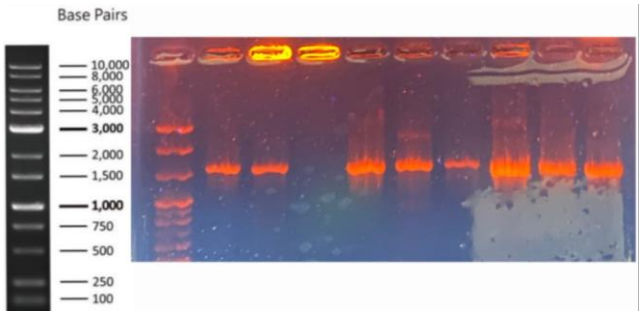


Figure 2. Agarose gel electrophoresis for PCR products of 16S rRNA approximately (1500 bp) of bacterial isolates

Table 1. BLAST results of the 16S rRNA gene sequences of bacterial isolates

Isolates code	Closet strain	Identity	Length (bp)	Accession no. of closet species
R1	*Staphylococcus epidermidis* subsp novobiosepticus	100%	930	MT585539.1
R2	*Acinetobacter variabilis* strain HNLH7X	99.93%	1405	ON680941.1
R3	*Staphylococcus haemolyticus* ZG13-26	100%	1184	OR243831.1
R4	*Staphylococcus aureus* C123	100%	500	KC212093.1
R5	*Staphylococcus aureus* 38S	100%	501	MK681352.1
R6	*Staphylococcus aureus* ATCC6538	99.89%	720	MT573388.1
R7	*Staphylococcus aureus* DSM 20231	99.86 %	1420	MN650918.1
R8	*Staphylococcus epidermidis* 2322	99.86%	1412	MT604781.1
R9	*Staphylococcus aureus* MC-CL-10	100%	768	MT132894.1
R10	*Staphylococcus aureus* strain 309-9	99.36%	620	MG557810.1
R11	*Staphylococcus aureus* strain RM_AST_SA004	99.93%	1416	MK809240.1
R12	*Staphylococcus argenteus* strain XM8	99.86%	1416	MT023385.1
R13	*Staphylococcus argenteus* strain XM8	99.93%	1414	MT023385.1
R14	*Staphylococcus aureus* OsEnb-ALM-C18	100%	1104	MN889346.1
R15	*Staphylococcus epidermidis* 72.1.1	99.84%	639	KX454153.1
R16	*Staphylococcus epidermidis* HKG218	100 %	1412	KJ741258.1
R17	*Staphylococcus epidermidis* 2322	99.8%	503	MT604781.1
R18	*Staphylococcus epidermidis* HBUM07083	100%	651	MF662509.1
R19	*Acinetobacter baumannii* strain HNKF19F	99.86%	1406	OQ569354.1

Table 2. Frequency of bacteria isolated from skin infection.

Isolation code	Bacterial isolation	Repetition	Percentage (%)	Infection
R4, R5, R6, R7, R9, R10, R11, R14	*Staphylococcus aureus*	8	42	Impetigo, Folliculitis boil (furuncle), infected asthma , cancer
R8, R15, R17, R18	*Staphylococcus epidermidis*	4	21	Chronic folliculitis boil (furuncle), chronic acne
R16, R1	*Staphylococcus epidermidis*	2	10.5	Impetigo and Chronic folliculitis
R12, R13	*Staphylococcus argenteus*	2	10.5	Impetigo and boil(furuncle)
R3	*Staphylococcus haemolyticus*	1	5.2	Cancer
R19	*Acinetobacter baumannii*	1	5.2	Chronic acne
R2	*Acinetobacter baumannii*	1	5.2	Cancer

Staphylococcus argenteus is a novel staphylococcal species closely related to *S. aureus*, and is considered as a part of *S. aureus* complex there are few reports on *S. argenteus*, Where little is known about their clinical infections (Moradigaravand et al. 2017; Olatimehin et al. 2018). Two new species, *S. argenteus* and *S. schweitzeri*, were proposed in 2015 as members of the *S. aureus* complex (Tong et al. 2015) . The main reason for the alteration was the significant genetic distance found at the whole-genome sequencing level. However, in comparison to *S. aureus*, the new species exhibits similar phenotypes, is coagulase positive, and has virtually identical rRNA 16S gene sequences. The pathogen *S. argenteus* affects humans (Chen et al. 2018). Some studies have retrospectively evaluated the presence of *S. argenteus* in collections of isolates previously identified as *S. aureus* in different countries (Tång Hallbäck et al. 2018; Kitagawa et al. 2020)

Eleven new bacterial strains were identified in this investigation, and their 16S rDNA sequences have been added to the GenBank database with accession numbers (Table 3). These strains were identified based on how similar they were to previously identified skin infection components. Any alteration to an organism's genome sequence or the process by which such changes happen is referred to as a mutation. Typically, mutations are errors that happen during DNA replication (Fitzgerald and Rosenberg 2019). They can also arise from stressful events or environmental mutations such as radiation (Shibai et al. 2017) and temperature (Chu et al. 2018). An essential evolutionary mechanism known as stress-induced mutagenesis enables bacteria to adjust to harsh situations such as osmotic pressure, chemical exposure, food shortage, and acidic environments (Gad 2021). Figure 3 shows the phylogenetic tree, it displays the connections between the nineteen different strains that were isolated from skin infections. Eleven of these nineteen strains were identified as novel strains and assigned the accession numbers (OR915998) as indicated in Table 3.

for identifying bacteria, 16S rDNA gene sequencing is the gold standard (Nayak et al. 2011). Using universal or particular primers, the PCR method is an extremely sensitive and specific way to amplify a target DNA samples, because it is present in all bacteria, its function has not changed over time, and its length is appropriate, the 16S rDNA gene is widely used to identify bacteria and is thought to be the best tool for studying bacterial phylogeny and taxonomy, where It is helpful for illustrating the evolutionary relationships among the various bacterial species, accurate and reliable analysis has also been used to

identify new bacterial strains when compared to conventional techniques (Al-Dhabaan 2019).

Detection of *cna* and *fnbA* genes by PCR technique of *S. aureus*

Numerous *S. aureus* virulence factors, such as fibronectin-binding (*cna* and *fnb A*) that are in charge of bacterial adherence, have been extensively documented (Piechota et al. 2018; Foster 2019). By comparing the band of each amplified gene to a typical molecular DNA ladder (3000 bp), the bands of each amplified *cna* and *fnbA* genes were described at 192 bp, and 191 bp respectively (Figures 4 and 5).

The result was agreed with other studies Foster et al. (2014) and Alabbosh et al. (2023). *Staphylococcus aureus* showed many associated virulence factors that are encoded by chromosome or plasmid genes, as well as the collective action of different components of the bacterial surface. These genes are particular compounds that facilitate bacterial adhesion to host cells, proper attachment and biofilm formation lead to resistance to antimicrobial agents and immune defenses. The production of microbial biofilms is regulated by specific genes linked to biofilms; in *S. aureus*, this is primarily controlled by 12 different genes. The genes produce different surface proteins that allow *S. aureus* to adhere to its host, penetrate the host, and colonize it. This process eventually results in the production of biofilms and increased virulence (Nourbakhsh and Namvar 2016; Kot et al. 2018).

Table 3. The new bacterial strains that recently recorded

Isolates code	Closet strain	Accession no.
R2	*Acinetobacter variabilis* strain RWABASR2	OR915998
R6	*Staphylococcus aureus* strain RWABASR6	OR915999
R7	*Staphylococcus aureus* strain RWABASR7	OR916000
R8	*Staphylococcus epidermidis* strain RWABASR8	OR916001
R10	*Staphylococcus aureus* strain RWABASR10	OR916002
R11	*Staphylococcus aureus* strain RWABASR11	OR916003
R12	*Staphylococcus argenteus* strain RWABASR12	OR916004
R13	*Staphylococcus argenteus* strain RWABASR13	OR916005
R15	*Staphylococcus epidermidis* strain RWABASR15	OR916006
R17	*Staphylococcus epidermidis* strain RWABASR17	OR916007
R19	*Acinetobacter baumannii* strain RWABASR19	OR916008

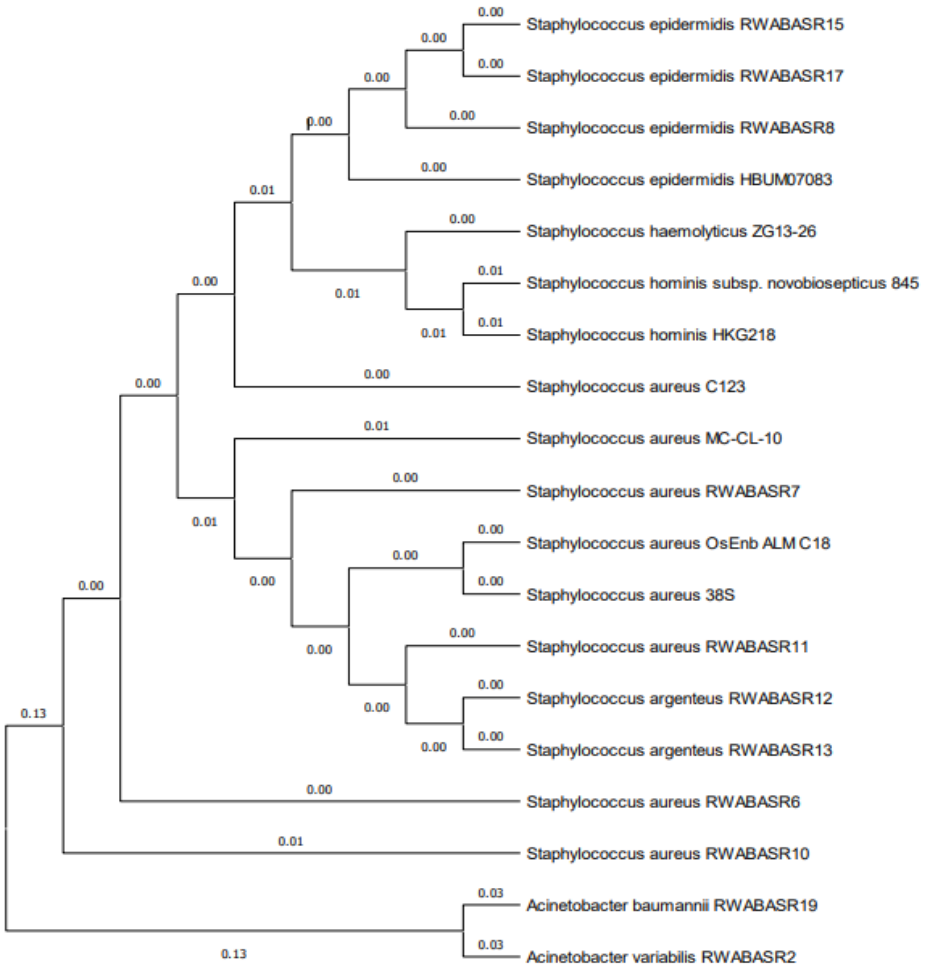


Figure 3. A phylogenetic tree showed rooted neighbor joining that was created by concatenating sequences for every strain. These sequences were obtained via an alignment of 16S rRNA gene sequences and formed through analysis using the MEGA 6 program. This N-J tree displays the distribution and evolutionary connections between the reference bacteria and the nineteen isolates from different skin infections isolated in this research

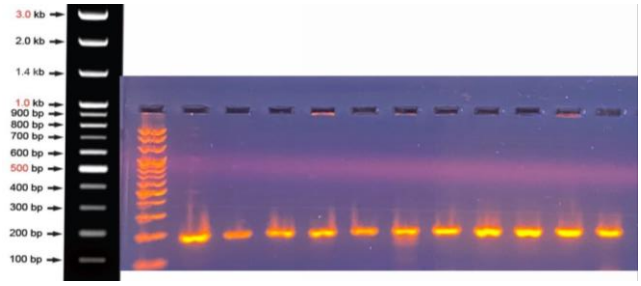


Figure 4. Agarose gel electrophoresis for PCR products of *cna* 192 bp of *S. aureus* isolates

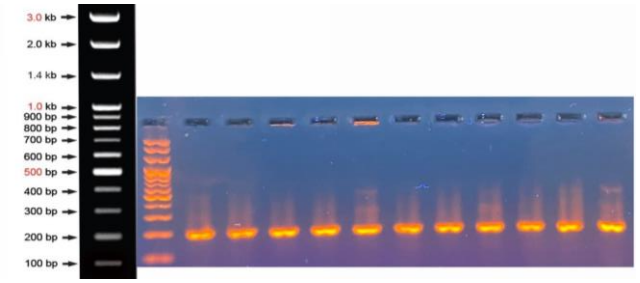


Figure 5. Agarose gel electrophoresis for PCR products of *fnbA* 191bp of *S. aureus* isolates

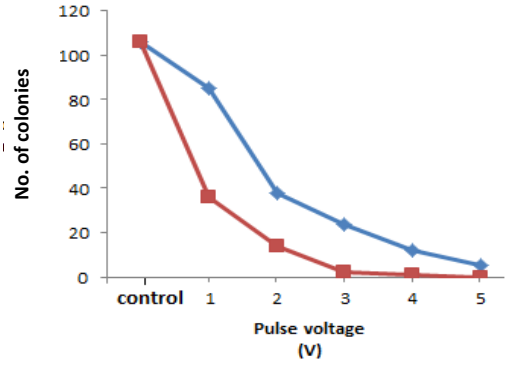


Figure 6. The effect of electrical field Voltage on the viable count of *S. haemolyticus*

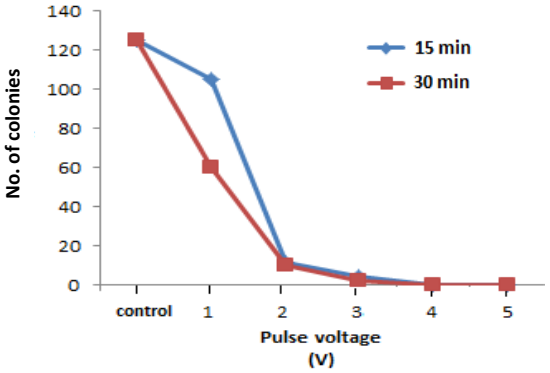


Figure 7. The effect of electrical field Voltage on viable count of *A. baumannii*

Investigation of the effect of Antimicrobial field (AMField) on bacterial growth

Exposed the bacteria to the electric field at different times and voltages 1, 2, 3, 4, and 5 V, the inactivation effect of the electric field increased as the voltage and time increased. the data in Figure 6 showed an extremely high significant decrease in viable bacterial count of *S. haemolyticus* after exposure to electric field of 5 V/cm for 15 and 30 min where the viable count reduced from $85x10^4$ to $5x10^4$ after 15 min exposure while reduced from $36x10^4$ to 0 after 30 min of exposure compared to before exposure bacterial viable count $106x10^4$ CFU/mL.

The same result was observed with an extremely high and significant decrease in viable bacterial count for *A. baumannii* after exposure to the same electric field voltage and period of time. The viable count was reduced from 105x104 to 0 after 15 min of exposure while reduced from 60x104 to 0 after 30 min compared to before exposure 125x104 CFU/mL (Figure 7).

Figures 8 and 9 show the bacterial death percentage of *S. haemolyticus* after exposure to an electric field. The percentage of lethal bacterial death significantly increased after exposure to 1-5 V for 15 and 30 min, 19-95% and 66-100%, respectively. For *A. viaribilis*, significant lethal percentages of bacteria were observed 16-100% and 52-100%, respectively, for the same period of exposure.

In the present study, as evidenced by the literature, the DC voltage energy contributed by the antibacterial field and the discharge of hazardous ions from the electrodes cooperate harmoniously for impacts on bacterial growth. Toxic ions enter the cells and cause cell death when the low-amplitude DC affect the membrane potential and creates cell holes. The result was compatible with Šalaševičius et al. (2021).

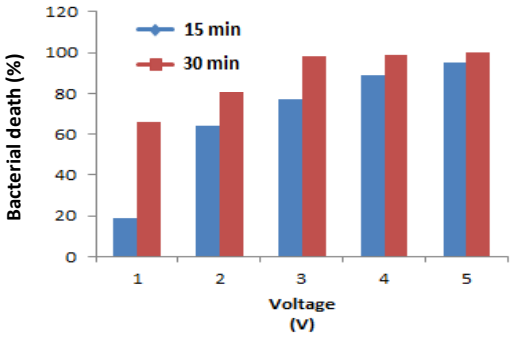


Figure 8. Percent bacterial death of *S. haemolyticus* after exposure to Direct Current (DC) electrical field

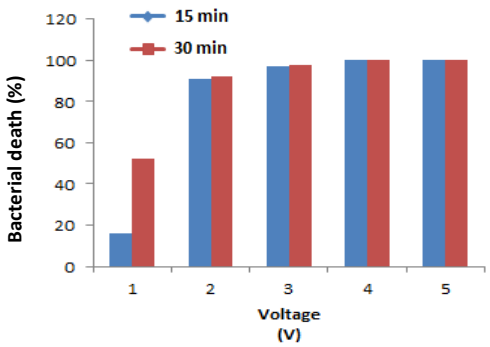


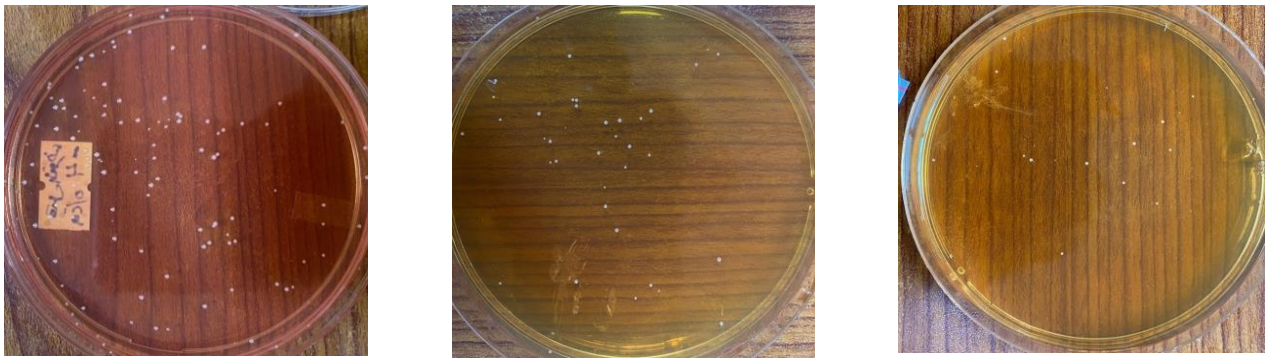
Figure 9. Percent bacterial death of *A. baumannii* after exposure to Direct Current (DC) electrical field

The bactericidal effects of electric voltages and currents are complex and involve diverse interactions between the electricity, bacteria, medium, and electrode materials (Krishnamurthi et al. 2020). Utilizing DC to kill bacteria has been done for decades. According to some theories, the mechanism underlying DC's antibacterial activity involves toxic substances (such as chlorine molecules, H_2O_2, and oxidizing radicals) created during electrolysis, enzyme and coenzyme oxidation, membrane damage that allows vital cytoplasmic components to leak out, and/or a decrease in the respiratory rate of bacteria (Pillet et al. 2016).

Glycerol-3-phosphate dehydrogenase (36) and FtsK (2, 15) are two representative proteins that are present in *pseudomonas aeruginosa* and *S. aureus* and are predicted to be affected by external electric fields. Glycerol-3-phosphate dehydrogenase is a component of glycolysis, phospholipid production, and respiration (Giladi et al. 2008).

As can be seen in Figures 10 and 11 the results showed that the effect of the DC electric field differs for individual species of bacteria. A decrease in the viable count of *A. baumannii* was recognized in a period of 5 min, and S. *haemolyticus* might be more resistant because of the variances in the morphology of bacteria; this was agreed upon (Šalaševičius et al. 2021).

The efficiency of Plused Electric Field (PEF) has been demonstrated to be significantly impacted by the size, shape, and thickness of the bacterial cell wall. Because PEF treatment induces a higher transmembrane potential, larger bacteria are more susceptible to its effects. Furthermore, PEF-induced inactivation lessens the sensitivity of bacteria with thicker cell walls. Compared to Gram-positive bacteria, Gram-negative bacteria appear to be more susceptible to electric pulses. This variation is most likely caused by variations in the thickness and makeup of the bacterial cell wall. Gram-negative bacteria have an outer membrane made of lipopolysaccharide and a thin coating of peptidoglycan. Previous findings have shown that these bacteria are better at electro-transformation than Gram-positive bacteria (Dréno et al. 2016). This is most likely due to the bacterial cell wall and envelope, which limit the quantity of outside DNA that can enter the cell. The thick layer of peptidoglycan may protect cells from lysis by preventing molecule leakage from the cell. Gram-positive and gram-negative bacteria, and even various strains within these categories, react in different ways with temperature, pH, PEF, and other combinations of the described parameters (Vitzthum 2000).



A **B** **C**

Figure 10. A. Exposure of *S. haemolyticus* to 1 volt, B. exposure of *S. haemolyticus* to 2-volt, C. exposure of *S. haemolyticus* to 3 volts for 30 min

A **B** **C**

Figure 11. A, Exposure of *A. baumannii* to 1 volt, B. exposure of *A. baumannii* to 2-volt, C. exposure of *A. baumannii* to 3 volts for 30 min

In conclusion, the predominant isolates of bacteria isolated from different skin infections belong to the genera *Staphylococcus* sp. and *Acinetobacter* sp., with the most frequent species of *S. aureus*. All isolates of these species showed virulence factors encoded by the genes *cna* and *fnbA*. Low current DC voltage can be used as a physical method to kill both gram-negative and gram-positive bacteria. significant reduction for Gram-negative and positive bacteria at different voltages and times was recorded. In the future, there are attempts to improve the process by optimizing the electric field system to treat skin infections in laboratory animals.

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