AL-Qadisiya Journal For
Science Vol.15 No.1 Year2010

Effects of Electrical current Stimulants in Growth and Activity of Some Bacterial pathogens, *in vitro*

Amal Abdulallah sakban Medical Physics)
Department of Medical Physics
Collage of Medicine/ University of Qadissiya

Abstract :

The aim of this study was to define the effects of electrical current stimulants in growth and activity of some bacteria pathogencs, *in vitro*. Three types of bacteria pathogens were selected: *Streptococcus faecalis*, and *Staphylococcus epidermidis* isolated species from wound and stool, and spores of *Bacillus cerus*. These bacteria were exposed to levels of 125v and 175v for 5 hours to see the effects of these electrical stimulation that transfes through liquid media and has effect on growth and activity of these bacteria. The results showed that the activity of test bacteria increased at 125v, and decreased at 175v. Also, the results showed that these bacteria species were attracted to one pole, where *Streptococcus faecalis*, and *Bacillus cerus* were gathered around the positive pole, whereas *Staphylococcus epidermidis* were grouped around the negative pole.

Introduction:

Exposure of a living organism to an electric field is normally specified by the unperturbed field strength. The unperturbed field is not equal to either the electric field that actually acts on the outer surface of th body or the electric field that is induced inside the body (Schwan *et. al*. 1956, 1987).The mechanism of interaction between electric fields and biological tissues is the direct stimulation of excitable (e.g., neural) cells by the induction of voltages across their membranes sufficient to trigger their depolarization. Such stimulation underlies the physiological responses of perception (Dalziel, 1972).It is clear that the current densities directly induced in humans or other living organisms by externally applied power- frequency electric or magnetic field with magnitudes similar to environmental levels are much smaller than levels required to excite neural tissues (Kanne & Phillips, 1980).External electric fields affect cellular systems in a multitude of ways ranging from low fields with more immediate field effects associated with signaling, wound healing, cell growth, and transport to relatively large pulsed consequence on the integrity of the cell membrane. The latter effect is known as electroporation or electropermeabilization and forms the basis for several field dependent biomedical application in use today (Knne *et. al*., 1978; and Sagan *et. al*., 1987).The underlying mechanism of inactivation of

microorganisms by electric field has not been fully elucidated. The most commonly accepted theory is that local instabilities in the membranes of the microorganisms are formed by electromechanical compression and electrical field that induces a certain critical membrane potential depending on the size, the surface charge of the membrane, and the electrical conductivities of the membrane, cytoplasm, and suspending liquid medium (Patrick *et. al.*, 1999).The possible bacteria static effect of electrical stimulation was first reported over 30 years ago by Rowley & McKenna(1972,1974) Using high voltage electrical stimulation, bacteria (*E. coli*) died after a brief session of electrical stimulation. While a few studies have used similar voltages for clinical electrical stimulation of wounds [Houghton et. al., 2003] most studies that report healing use a fraction of these voltages and currents. The aim of this study was to determine the sensitivity at least in some bacterium pathogens of stimulation, *in vitro.*

Materials and Methods

Bacterial isolates :-

The bacteria *Streptococcus faecalis, Staphylococcus epidermidis*, and *Bacilli cerus* were tested. These bacteria obtained from Al-Yarmouk Teaching Hospital laboratories. *Strep. faecalis* isolated from stool and *S. epidermidis* isolated from wounds infections after cultured on MacConky and blood agar plat respectively. Spore *B. cerus* Cultured on nutrient agar These bacteria were grown at 37^{o} C in inocubator for 24- 48hr, then growing bacteria were subcultured in broth media (brain- heart infusion) at a temperature of 37^{o} C for 24hr for activation before tested and identified by the routine lab. diagnosis (Macfaddin, 2001) . All these bacteria were grown and tested for activity by microbiological light microscopy.

Electrical stimulation

The glass tube with 22cm length was designed, the tube contained 5 holes, two at its end 2.5 cm in diameter, and the other three with 1 cm width necked- holes were distributed along the tube. The ends of tube were closed by sterile rubber. Two electrode cathode and anode were passed through the sterilize closing rubbers to connect tube to electric circuit device which consists of power supply (0-250volt), a meter, voltmeter, and variable resistance (110 ohm, 2.5 amper) to control the resistance of the circuit as shown in figure (1). After The tube is filled with 50 ml of sterilized nutrient broth of these bacteria. Direct current electrical stimulation was provided by power supply 125v, and 175v with a controlled output applied for a period of 5 hours.

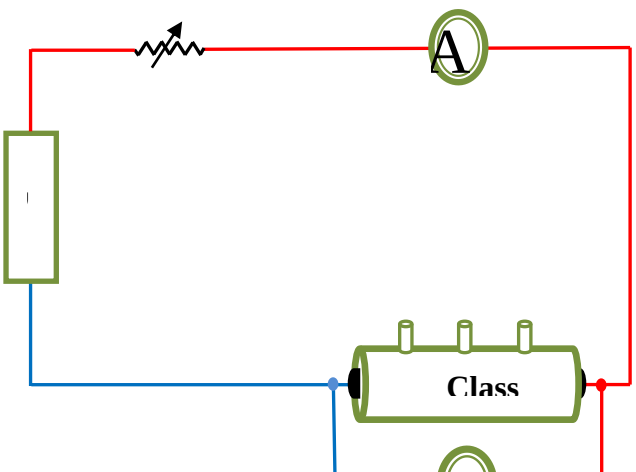


Figure 1: The schematic of electric equipment

Bacterial count

In small test tubse, o.1 ml of bacterial suspension and 4.9 ml of sterilized solution were placed and mixed thoroughly (1:50 dilution). By using leukocyte counting pipette, the suspension was drawn up. A drop of the mixture was placed in the center of the counting chamber (petroff- housser chamber). The chamber center was covered by a reinforced precision cover slip and allowed to stand 15 minutes. The number of the cells were counted in 20 squares and the total number in 20 squares was divided by 20(y) (y is the count of bacteria).(collee *el. al*., 1996).

Statistical analysis:-

Results were statisticaly analysis using means, standard error, and t-test. The level of significance was less than 0.05.(lewis, 1973).

Results :-

Table 1 shows the count of bacteria study *Strep. faecalis, S. epidermidis, and B. cerus* that exposed to electrical current at fixed voltage 125v, and 175v on both electrodes (+/- ve) for 5 hours, where the results of count of these bacteria showed significant was < o.o5. Also, this table shown the bacteria were difference with resistance of electrical current stimulants, where the *S. epidermidis more* resistance than the *Strep. faecalis* and *B. cerus.*

Table 1:the count of bacteria (Strep. faecalis, S. epidermidis, and B. cerus) (cell/ml × 10^6) in both ends of the growth tube, the time interval of exposure to electrical source (125 and 175V).

Time (hour)	Voltage at 125v		Voltage at 175v		Voltage at 25v		Voltage at 175v		Voltage at 125v		Voltage at 175v	
0	E	Count of Strep. faecalis (cell/ml ×10^6)			E	Count of S. epidermidis (cell/ml×10^6)			E	Count of B. cerus (cell/ml ×10^6)		
	+	5.029	4.552		+	5.290	4.94		+	3.20	2.953	
	-	5.036	4.431		-	5.299	4.87		-	2.55	2.653	
1	+	5.333	4.671		+	4.48	3.522		+	4.2	2.651	
	-	5.020	4.221		-	5.853	3.14		-	2.63	1.545	
2	+	6.026	4.22		+	4.30	2.553		+	7.14	2.38	
	-	4.02	3.029		-	8.2	2.99		-	2.327	1.527	
3	+	6.890	3.890		+	4.10	1.387		+	10.273	2.22	
	-	3.727	2.046		-	10.22	2.6		-	1.813	1.233	
4	+	8.877	3.267		+	3.77	1.3		+	12.14	1.42	
	-	3.220	1.923		-	13.6	2.3		-	0.99	0.812	
5	+	9.654	2.713		+	3.21	1.17		+	13.0	1.187	
	-	3.011	1.42		-	15.58	2.08		-	0.763	0.612	

Figure 2a & 2b demonstrated that the count of cells of the *Strep. faecalis* with time (hours). Shows the increase of bacterial colony growth at (+ve) electrode with a voltage 125v stimulation, where the average of increase was (6.97 × 10^6 cells/ml) for 5 hours, but the bacterial colony growth at (-ve) electrode with avoltage 125v stimulation was decreased with average (4.01× 10^6 cells/ml) for the same a period.

Figure 3a & 3b demonstrated the count of cells of the *Strep. faecalis* with time (hours). Shows the bacterial colony growth at both (-/+ve) electrodes with a voltage 175v stimulation, were reduction for 5 hours. The average of growth reduction at (-ve) was (2.85 × 10^6 cells/ml), the average reduction in growth at (+ve) was (3.89 × 10^6 cells/ml).

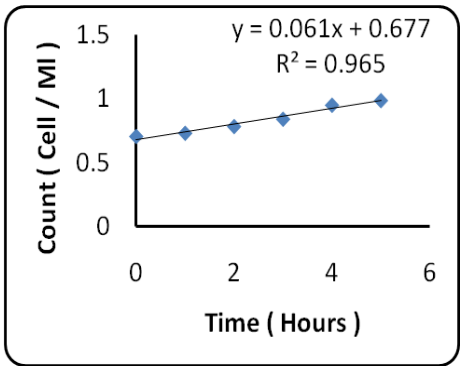
Figure 4a & 4b shows the results of the *S. epidermidis* experiments. The average change in growth for these bacteria was (9.79 × 10^6 cells/ml) after stimulation with voltage 125v for 5 hours at (-ve) electrode , but in the same period of growth bacteria at (+ve) electrode was decreased with average was (4.19× 10^6 cells/ml).

Figure 5a & 5b shows the data between the count of *S. epidermidis* cells across the time (hours). After electrical stimulation with voltage 175v for 5

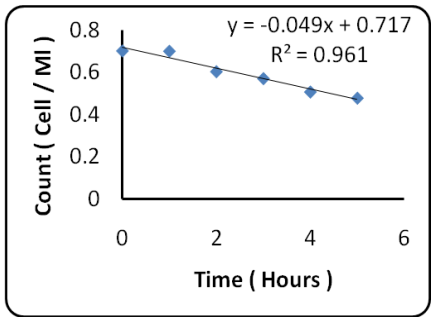
hours, the average in growth at both (-/+ve) electrode was decreased (2.99×10^6, and 2.49×10^6cells/ml) respectively.

Figure 6a & 6b shows results of count (cells/ml) of bacteria *B. cerus* versus time (hours) at two (-/+ve) electrode. After exposure the electrical stimulation with voltage 125v for 5 hours, the growth bacteria were increased with average (8.33×10^6) at(+ve) electrode, but the growth bacteria were decreased with average (1.85×10^6 cells/ml) at (- ve) electrode. The behavior of these bacteria was same the behavior of the Strep. faecalis.

Figure 7a & 7b shows results the reduction in count of bacteria through exposure of 5 hours with voltage 175v at both (-/+ve) electrode, the average reduction was (1.39×10^6 cells/ml, and 2.14×10^6 cells/ml) respectivily .



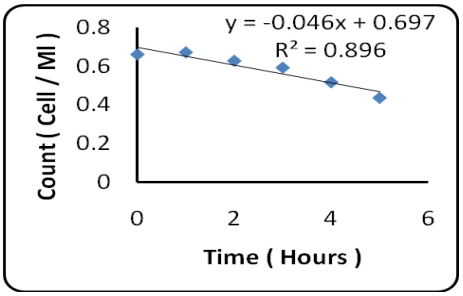
a-

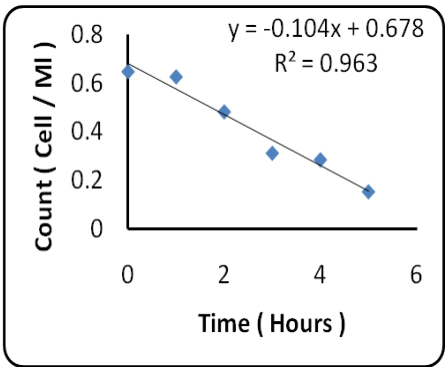


b-

Figure 2: The relationship between the a- cell count (cells/ml) and the exposure time (hour) at +ve electrode. b-cell count (cell/ml) and the exposure time at – ve electrode at a fixed potential (125 V) of media inoculated with *Strep. faecalis.*

a-

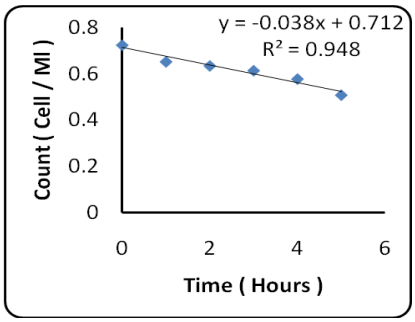


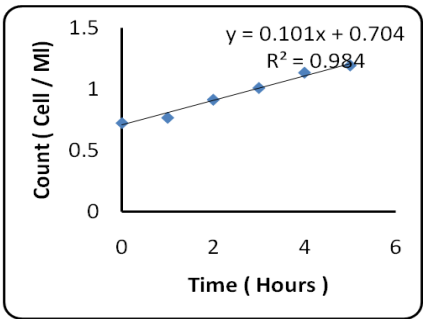


b-

Figure 3: The relationship between the a- cell count (cells/ml) and the exposure time (hour) at +ve electrode. b-cell count (cell/ml) and the exposure time at –ve electrode at a fixed potential (175 V) of media inoculated with *Strep. faecalis.*

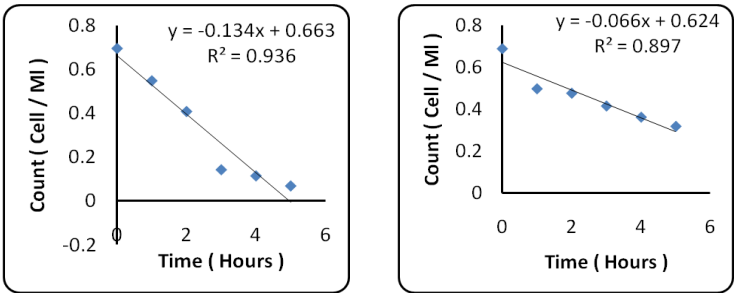
a-





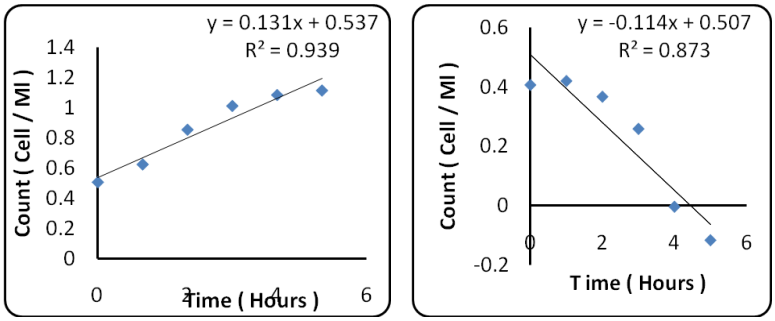
b-

Figure 4: The relationship between the a- cell count (cells/ml) and the exposure time (hour) at +ve electrode. b-cell count (cell/ml) and the exposure time at –ve electrode at a fixed potential (125 V) of media inoculated with *S. epidermidis.*



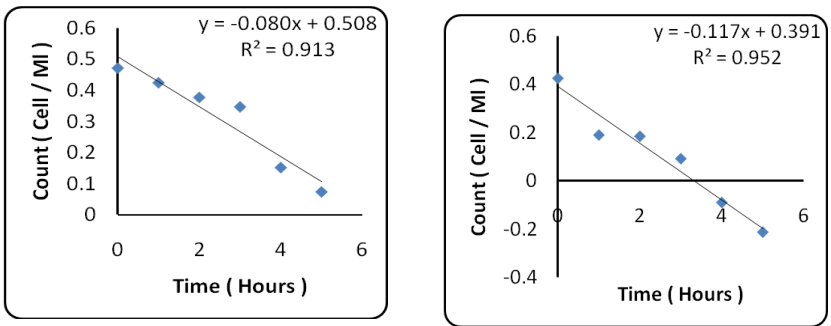
a- b-

Figure 5: The relationship between the a- cell count (cells/ml) and the exposure time (hour) at +ve electrode. b-cell count (cell/ml) and the exposure time at –ve electrode at a fixed potential (175 V) of media inoculated with *S. epidermidis*.



a- b-

Figure 6: The relationship between the a- cell count (cells/ml) and the exposure time (hour) at +ve electrode. b-cell count (cell/ml) and the exposure time at –ve electrode at a fixed potential
(125 V) of media inoculated with *B. cerus*.



a- b-

Figure 7: The relationship between the a- cell count (cells/ml) and the exposure time (hour) at +ve electrode. b-cell count (cell/ml) and the exposure time at –ve electrode at a fixed potential (175 V) of media inoculated with *B. cerus*.

Conclusion:-

Bacteria *S.epidermidis* more activity from *Strep. faecalis* and *B. cerus* when exposed to 125v, and also this bacteria is most resistance when exposed to 175v than other bacteria for extended time 5 hours.

Discussion :-

The study showed that the low electrical stimulation had the ability to increase bacterial growth. Also showed that the high electrical stimulation had inhibition in bacterial growth. These results obtained fair agreement with Barranco *et al*.,(1974); and Rowley *et al*.,(1974).

When current were applied in the experiments for a 5 hours stimulation period of electrical had effect on each of the bacterial activation. The resistant of *Strep. faecalis, S. epidermidis, and B. cerus* to electrical stimulation may due to the production of catalase, which would offer more protection to the cells from the activity of hydrogen peroxide production at the electrodes. Also, the continuous flow of electric current through the suspension liquid of microorganisms, the killing action of bacteria may be attributed to the heat produced by the current flow. These results obtained were nearly agreement with Drees *et al*. ,(2003); Gilliland & Speck (1967).

The electrical stimulation of high voltage 175v will inhibit growth in all 3 types of bacteria. While the electrical stimulation of low voltage 125v with

same bacteria did not reduce bacterial growth. The electrical stimulation may not have a direct effect on bacteria, there is evidence that bacteria were attracted on electrode as a secondary effect of the electrical stimulation, this may explain why electrical stimulation caused bacterial death. These results were fair agreement with Jass *et. al*.,(1995); Costerton *et. al*.,(1994).

In this study, the toxicity that appears when voltage applied is due to electrical ionization and it is effective in ionized medium by using electrodes made of metal, and they would inhibit cell division of bacteria Rosenberg *et. al*., (1965).

The results show the polarization of *Strep. faecalis, S. epidermidis,* and *B. cerus* at one electrode separation without the other one, its well know that the bacterial cell wall had a negative charge. where *Strep.faecalis* and *B. cerus* directed to the +ve pole; on the contrary the *S. epidermidis* directed to the –ve pole. This may be refer of the conductive of the electrical stimulation through medium, if the bacteria is more conductive with the field causing attraction, if the bacteria is less conductive than the medium causing repulsion(Pethiny, 1996).

References:

1. Barranco S., Spadero J., Berger T. (1974):" *In vitro* effect weak direct current on Staphylococcus aureus"; 100: 250-255.
2. Collee J.G., Fraster A.G., Marmion B.P. & Simmon A.S. (1996):" Practical medical Microbiology". Hunchill Livingstone.
3. Costerton J.W., Ellis B., Lam K., Johnson F., Khoury A.E.(1994):" Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria". Antimicrob Agents Chemother.; 38(12):2803-9.
4. Dalizel C.F.(1972):"electric shock hazard". IEEE spectrium: 41- 50.
5. Drees K.P., Abbaszadegan M. & Mairer R. M.(2003):"Comparative electrochemical inactivation of bacteria and bacteriophage". Water Research; 37: 229-300.
6. Gilliland S.E., and Speck M.L.(1967):"inactivation of microorganisms by electrohydroulic shock''; 15(5): 1031- 1037.
7. Houghton, P.E., Kincaid, C.B., Lovell, M., Cambell, K.E., Keast, D.H.,Woodbury, M.G., & Harris, K.A. (2003). Effect of electrical stimulation on hronic leg ulcer size and appearance. *Phys Ther,* 83, 1, 17-28.
8. Jass J., Costerton J.W., Lappin-Scott H.M.(1995):" The effect of electrical currents and tobramycin on Pseudomonas aeruginosa biofilms". J Ind Microbiol; 15(3):234-42.
9. Kanne W.T., Phillips R.D.(1980):"comparison of the coupling of grounded humans, swine, and rats to vertical, 60Hz electric fields". Bioelectromagnatics; 1: 117- 129.

10. Kanne W.T., Phillips R.D., Hjeresen D.L., Richardson R.L., eamer J.L.(1978):"a method for the exposure of miniature swine to vertical 60Hz electric fields". IEEE Trans Biomed Eng BME; 25: 276- 283.
11. Pareilleux A. & Sicard N.(1969):"lethal effects of electric current on Escherichia coli". Applied Microbiol.; 19(3): 421- 24.
12. Lewis D.G.(1973):" statical methods in education".
13. Macfaddin M.W.C.(2001):"practical medical microbiology". Lisenston. Populist N. British.
14. Park J.C., Lee D.H., Park B.J., Han D.W., Uzawa M. & Takatori K.(2003):"AEM applied and environmental microbiology inactivation of bacteria in sweater by low- amperage. Electric current"; 69(4): 2405- 08.
15. Patrick C. Wouters, Nicole Dutreux, Jan P.P.M. Smett, and Huub L.M. Lelieved(1999): "effects of pulsed electric fields on inactivation kinetics of listeria innocua". Microbiology & Preservation, Unilever research Vlaardingen, 3133 AT Vlaardingen, the Nether lands; 65(12): 5364- 5371.
16. Pethiy R.(1996):"dielectrophoresis: using inhomogeneous AC electrical fields to separate and mouipulate cells". Critical Reviews in Biotechnology; vol. 16: 331- 348.
17. Rosenberg B., Vancamp L. & Krigas T.(1965):"Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode". Nature; 205: 698-99.
18. Rowley B.A. (1972) :"Electrical current effects on E. coli growth rates". Proc Soc Exp Biol Med; 139(3): 929-34.
19. Rowley B.A., McKenna J.M., Wolocott L.E. (1974):" Proceedings:The use of low level electrical current for enhancement of tissue healing". Biomed Sci Instrum; 10: 111-4.
20. Sagan P. M., Stell M.E. Bryan G.K., Adey W.R.(1987): "detection of 60Hz vertical electric fields by rats". Bioelectromagnetics; 8: 303- 313.
21. Schwan H.P.(1977):"electrical properties of tissue and cell suspensions". Adv Biol Med Phys; 5: 147- 209.
22. Schwan H.P., Key C.F.(1956):"specific resistance of body tissue". Ciro Res; 4: 664- 670.

تأثير محفزات التيارِ الكهربائيِ في نمو ونشاط بعض البكتريا المرضية، مختبريا

أمل عبد اله صكبان
جامعة القادسية / كلية الطب

الخلاصة:

هدفت هذه الدراسة الى معرفة تأثير التيارات الكهربائية المحفزة على نمو ونشاط بعض البكتيريا المرضية، مختبريا. اذ اختيرت ثلاث انواع بكتيرية مرضية لبعض المرضى هي *(Streptococcus faecalis, Staphylococcus epidermidis, and Bacillus cerus)* المعزولة من عينات البراز والجروح على التوالي ، وابواغ بكتيريا *B. cerus* . تم تعريض هذه البكتيريا الى جهد كهربائيv 125و 175vولمدة 5 ساعات لملاحظة تأثير التيار الكهربائي المار خلال وسط النمو السائل البكتيري على نمو ونشاط البكتيريا. أظهرت نتائج البحث ان نشاط البكتيريا المختبرة قد ازداد عند التعرض الى جهد كهربائي 125v ، بينما نشاطها تناقص عند تعرضه الى جهد كهربائي 175v. كما لوحظ بأن هذه الانواع من البكتيريا انجذبت الى قطب معين ، اذ لوحظ انجذاب ال *Strep. faecalis* و *B. cerus* الى القطب الموجب، و *S.epidermidis* الى القطب السالب.