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Effects of Mobile Phone Generated High Frequency Electromagnetic Field on the Viability and Biofilm Formation of *Staphylococcus aureus*

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Abstract**—***Staphylococcus aureus*, one of the microflora in a human external auditory canal (EAC) is frequently exposed to high-frequency electromagnetic field (HF-EMF) generated by mobile phones. It is normally non-pathogenic but in certain circumstances, it can cause infections. This study investigates the changes in the physiology of *S. aureus* when exposed to HF-EMF of a mobile phone. Exponentially grown *S. aureus* were exposed to two conditions of EMF irradiation (standby-mode and on-call mode) at four durations; 15, 30, 45 and 60 min. Changes in the viability and biofilm production of the *S. aureus* were compared between the two conditions of exposure. EMF from the standby-mode has enhanced the growth of *S. aureus* but during on-call, the growth was suppressed. No significant difference in the amount of biofilm produced in both modes of exposure was observed. Thus, HF-EMF of mobile phone affects the viability of *S. aureus* but not its ability to produce biofilm.

Keywords**—**Electromagnetic field, mobile phone, biofilm, *Staphylococcus aureus*

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I. Introduction

IN the last few decades, usage of Global Systems for Mobile (GSM) communication phones has become indispensable, for the purpose of telecommunication, social interactions and information acquisitions. To date, numerous studies on the effect of electromagnetic field (EMF) of mobile phones on human and other living organisms have been reported, indicating the concern on the health effects of phone users [1]-[5]. Although many of these reports did not associate brain tumour to mobile phone usage, others relate the usage of mobile phones to neurological abnormalities such as dysaesthesiae [6], sleep disturbances [7], [8], raised blood pressure [8], [9] and cognitive effects [9]. Even if we are inevitably exposed to EMF emitted by surrounding electrical appliances, a mobile phone user in addition is exposed to significant level of electric and magnetic fields because the radiating antenna of the mobile phone operates at a very short distance from the head. The substantial number of scientific publications and reviews provided useful discussions on the health effects related to the use of mobile phones, nevertheless, uncertainties still remain due to the lack of hard evidences [10].

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During telecommunicating, mobile phones are placed in very close proximity to the ear, thus EMF is directly channeled into the external auditory canal (EAC) of the user. Since the EAC is inhabited with a diversity of commensal microorganisms, it poses a concern of whether the EMF after prolonged used of mobile phones would affect the equilibrium and the physiology of these microbes. The microbes that predominate in the EAC of healthy individuals include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Corynebacterium* species [11]-[13]. These commensals are normally non-pathogenic and acts as a deterent to colonization of EAC by pathogens, however, in events of trauma or disruption of the integrity of the epithelial lining, the EAC will be predispose to possibilities of infections leading to otitis externa [14]. In immunocompromised patients and cancer patients, failure to treat otitis externa can be life-threatening. This is demonstrated by the presence of *S. aureus* as the sole pathogen in diabetic patients with malignant otitis externa [15].

Mobile phones generate non-ionizing radiofrequency that range between 800 MHz to 1900 MHz at a maximum intensity of about 0.2 W/m^2 [16]. Radiofrequency of this range is considered to be within high frequency range (HF-EMF) [10]. Mobile phones also emit low-frequency magnetic field pulses generated by battery currents in the phone that are too weak to produce non-thermal effects [17].

Previously, there has been many reports on the effects of extremely low EMF (EL-EMF) of 0-300 Hz on bacteria, but in most cases, the EMF was generated by using parallel capacitor plates [18], Helmholtz coil ([19], [20] and solenoid coil [21], [22]. When three different bacterial strains; *Escherichia coli*, *Leclercia adecarbodxylata* and *S. aureus* were exposed to EMF of 50 Hz, the viability of *E. coli* was significantly reduced in comparison to the other two strains, with *S. aureus* being the least affected [23]. Furthermore, prolonged exposure to 50 Hz LF-EMF has also been shown to affect transposition activity in *E. coli* [19]. This finding raises a concern on the equilibrium of *S. aureus* in the EAC, which may be affected by the HF-EMF emitted by mobile phones which is >50 Hz. In addition, *S. aureus* which is capable of forming biofilm on host tissue is feared to increase biofilm formation and thus reduces the efficacy of antibiotic for treatment against infection. In this report, we investigated the responses of *S. aureus* to the EMF emitted by a GSM mobile phone. The results obtained would provide a further understanding on the biology of bacteria when exposed to HF-EMF that could be useful in formulating guidelines against the adverse effects of HF-EMF.

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II. Materials And Methods

A. Bacterial Strains and Media

Stock culture of *Staphylococcus aureus* (ATTC 11632) stored at -80°C was subcultured on blood agar (Isolac, Malaysia) to ensure its purity. Brain heart infusion (Oxoid, USA) broth was used as liquid medium and nutrient agar (Oxoid, USA) was used as the medium for the enumeration of colony forming unit (CFU). All cultures were incubated for 24 hr at 37°C.

B. Source of EMF and Temperature of Mobile Phone

A Global System for Mobile Communication (GSM) 2G mobile phone, (LG model KG 288, China) was used as the device to generate the EMF. This phone operates in dual band frequencies of 900/1800 MHz. The SAR of the phone was indicated as 0.932 W/kg. The battery of this phone was fully charged to its full capacity prior to use. The magnetic strength and the temperature emitted by the phone were measured for two hours duration, using electromagnetic field meter (Lutron model EMF-839, Taiwan) and an infrared thermometer (ScanTemp 385, Germany), respectively.

C. Exposure of Bacteria to EMF

A suspension of *S. aureus* containing 10^9 CFU/ml at logarithmic phase was seeded onto duplicate sets of nutrient agar plate using a glass spreader and was allowed to dry for at least 15 min. The mobile phone was then placed sandwiched between the two agar plates to ensure equal distance between the both agar plates and the phone. After 15 min, the agar plates were removed and incubated at 37°C for 24 h. The experiment was repeated by placing the agar plates for 30, 45 and 60 min duration. In the initial experiment, the phone was switched on but no calls (standby) were made while in the subsequent experiment, the phone was switched on and a call was made (transmission) while the bacteria were exposed to the EMF for the stipulated duration.

Viable bacteria on the agar plates were counted after 24 hr of incubation. For each of the duration, the experiment was repeated six times. Viability ratio of the organism was calculated by dividing the number of viable cells after exposure (CFU_1) to the number of viable cells of controls (CFU_0), i.e. CFU_1/CFU_0).

D. Biofilm Formation

Quantification of biofilm was carried out using 96-well flat-bottomed microtitre plate method on the treatment and control samples following the methods of [24]. The optical density of 0.25% safranin-stained biofilm was read by using a spectrophotometer at 490 nm.

E. Statistical Analysis

In every experiment, six independent trials were carried out and the mean value was used to tabulate the results. A one-way ANOVA between samples were conducted with $p<0.05$ considered as statistically significant. Post-hoc comparison using Tukey HSD test was carried out and the significance between each sample was analysed.

III. Results

A. Magnetic Field Strength and Temperature of the Mobile Phone

The strength of the magnetic fields produced by the mobile phone while in the standby mode was almost constant throughout the 2 h duration (Fig. 1). In on-call mode, the magnetic field produced was constantly fluctuating at every 15 min intervals. A slight increase in the temperature of the phone was recorded after one hour of transmission with the highest temperature being 28.6°C, an increment of 3.6°C from the standby mode.

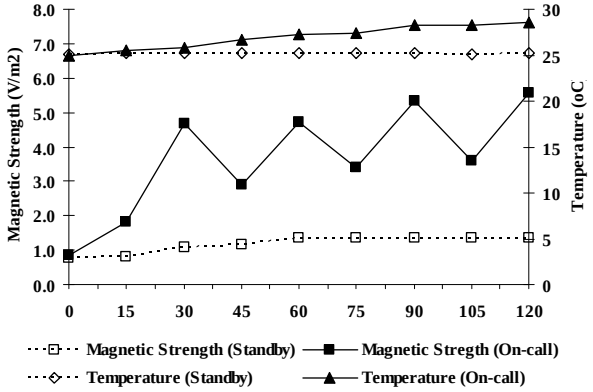

Fig. 1 Magnetic field strength and temperature of the mobile phone while on standby and on-call mode

B. Effect of EMF on the Viability of S. aureus During Standby and On-call Mode

During standby mode, the number of CFU between the exposed and the control groups were not significantly different (Fig. 2) even after prolonged exposure. The EMF during standby mode therefore, did not have any effect on the growth of the bacterium.

In the following experiment, when the bacterium were exposed to the EMF while on-call for 1 hour, the number of CFU was significantly reduced ($p<0.05$) in all the exposed groups (Fig. 3). Exposing the bacterium to the EMF during on-call for 15 to 30 min, each had reduced the number of CFU by 14%, and when the exposure time was prolonged to 45 and 60 min had resulted in the reduction in number of CFU to 27% and 33%, respectively.

During the first 30 min following exposure to the EMF of the phone, no significant difference ($p>0.05$) in the viability ratio between the standby mode and on-call mode was observed. However, after 15 min of exposure to EMF during on-call, the viability ratio of the bacterium began to decline and continued to decline much lower (Fig. 4).

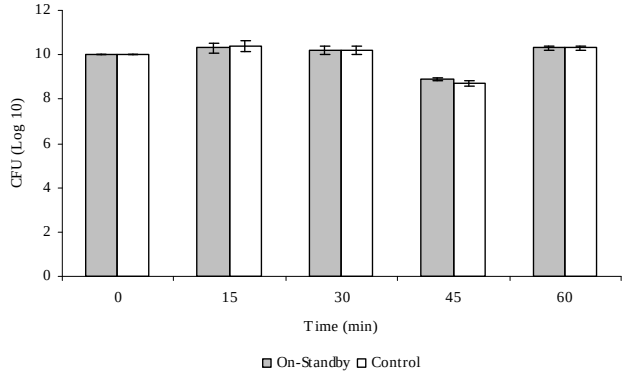

Fig. 2 Number of viable *S. aureus* cells following exposure to EMF emitted by the mobile phone during standby. Each bar represents a mean of six independent experiments performed in duplicates

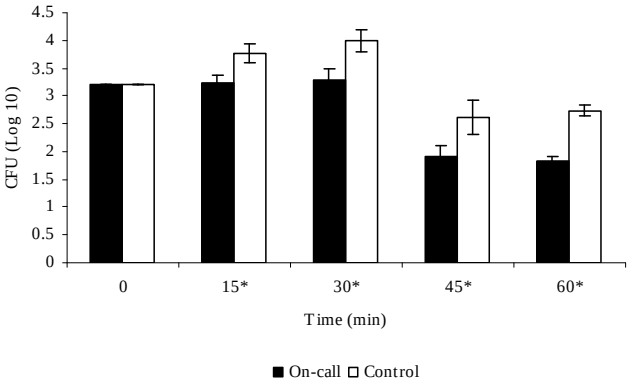

Fig. 3 Number of viable *S. aureus* cells following exposure to EMF emitted by the mobile phone during on-call mode. Each bar represents a mean of six independent experiments performed in duplicates. Asterisk (*) symbol indicates significantly different ($p<0.05$) in the number of viable cells of the exposed cells compared to the control

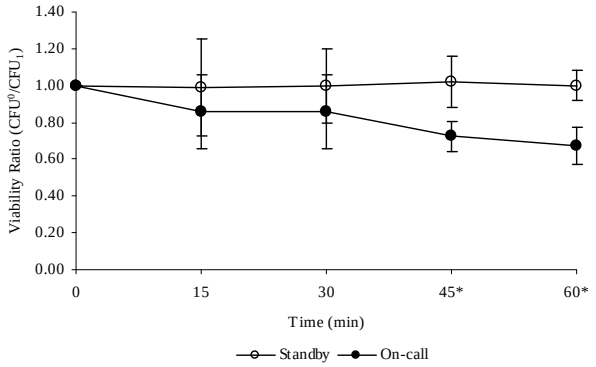

Fig. 4 Viability ratio (CFU_1/CFU_0) of *S. aureus* following exposure to EMF emitted by a mobile phone during on-call and standby modes. Each point represents a mean of six readings. A ratio >1 indicates an increased in cell viability while <1 indicates cell mortality. Asterisk (*) represents significant difference in the number of viable cells between the standby and on-call groups

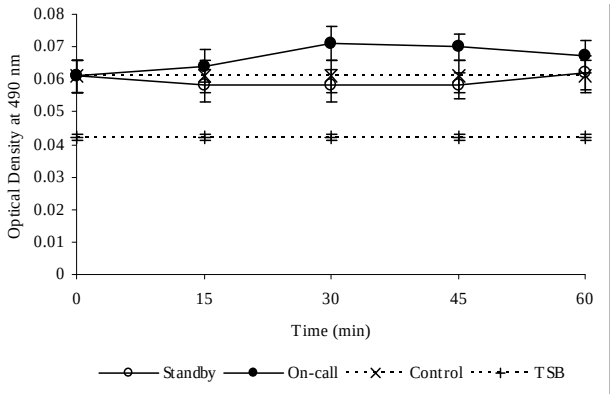

Fig. 5 The relative value of optical density reading of biofilm of all conditions measured at 490 nm. Each point represents an average of six samples. The control consists of non-exposed colonies of *S. aureus* and TSB refers to tryptose soy broth, was used as the diluent and as negative control.

IV. Discussion

The normal flora of the EAC of a mobile phone user is directly exposed to the HF-EMF of the phone. It is feared that prolonged used of mobile phones could have effects on the biology of *S. aureus* in the EAC which could lead to complications to treatment of *S. aureus* infections.

Our results showed that the growth of the *S. aureus* was not affected by the EMF that was emitted during standby, even after prolonged exposure. However, when the phone was in an on-call mode for more than 30 min, the emitted EMF was able to cause the bacterium to lose its viability. It was also observed that after 30 min of exposure, the strength of the magnetic field and the temperature of the phone had increased but the increased in the temperature was insignificantly small. This observation leads to an assumption that cell mortality was probably associated with the strength of magnetic field rather than the increased in the temperature.

Similar observation was reported by [25] that an increased in magnetic strength had caused mortality in *E. coli* whereby the bacterium lost its ability to form colonies and thus, grew at a slower rate than the controls [25]. Besides that, our results also support the report of [26] that the rate of growth retardation on *Paracoccus denitrificans* was influenced by the intensity of the magnetic field and duration of exposure of the organism to the field.

When the bacterium was suddenly exposed to high strength of magnetic field during on-call, we postulate that it experienced sudden non-thermal "heat-shock" which induced the synthesis of heat shock protein (Hsp), with the aim to protect against degradation of bacterial DNA [27], [28]. It is possible that the prolonged exposure to the magnetic field had caused over expression of Hsp which in turn potentiate DNA damage leading to cell mortality.

The frequency range of the mobile phone used in this study was 900 MHz to 1800 MHz. Within this frequency range, the EMF does not penetrate deeply into the body; instead, it is absorbed by the skin and the underlying tissues. The heat that is generated in the tissue is then channeled into blood circulatory system [29].

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Furthermore, the SAR of the phone used in this study is 0.932 W/kg, which is within the allowable limit (2 W/kg) to human tissue. To bacteria, this absorption rate could be very high. Unlike human cells, bacteria are unicellular and thus, their ultra-thin cell-wall allows the energy to penetrate deeper into its cytoplasm and cause DNA damage. Coupled with the increasing strength of magnetic field, may attribute to the further reduction in the number of viable cells.

This study demonstrates that the HF-EMF produced by the phone did not affect the ability of the *S. aureus* to produce biofilm. The strength of the magnetic field that differs during the standby and on-call did not have an influence on the biofilm formation. Although a report had shown that EL-EMF (50 Hz) exposed to *Helicobacter pylori* for 2 h was able to interfere with cell adhesion during biofilm formation [30], the effects of HF-EMF on cell adhesion of *S. aureus* has yet to be documented.

V. Conclusion

Prolonged used of a mobile phone which produced HF-EMF affects the viability of *S. aureus* but does not affect its ability to produce biofilm. Additional work need to be performed to gather more information on other biological changes in bacteria that may occur due to the exposure to HF-EMF of mobile phones.

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References

[1] H. C. Christensen, J. Schüz, M. Kosteljanetz, H. S. Poulsen, J. Thomsen, and C. Johansen, "Cellular Telephone Use and Risk of Acoustic Neuroma," *American Journal of Epidemiology*, vol. 159, pp. 277-283, February 1, 2004.

[2] T. Takebayashi, N. Varsier, Y. Kikuchi, K. Wake, M. Taki, S. Watanabe, S. Akiba, and N. Yamaguchi, "Mobile phone use, exposure to radiofrequency electromagnetic field, and brain tumour: a case-control study," *British Journal of Cancer*, vol. 98, pp. 652-659, 2008.

[3] P. D. Inskip, R. N. Hoover, and S. S. Devesa, "Brain cancer incidence trends in relation to cellular telephone use in the United States," *Neuro-Oncology*, vol. 12, pp. 1147-1151, November 1, 2010.

[4] J. E. Muscat, M. G. Malkin, S. Thompson, R. E. Shore, S. D. Stellman, D. McRee, A. I. Neugut, and E. L. Wynder, "Handheld Cellular Telephone Use and Risk of Brain Cancer," *JAMA: The Journal of the American Medical Association*, vol. 284, pp. 3001-3007, December 20, 2000.

[5] G. Oftedal, J. Wilen, M. Sandstrom, and K. H. Mild, "Symptoms experienced in connection with mobile phone use," *Occupational Medicine-Oxford*, vol. 50, pp. 237-245, May 2000.

[6] B. Hocking and R. Westerman, "Neurological abnormalities associated with mobile phone use," *Occupational Medicine (London)*, vol. 50, pp. 366-8, Jul 2000.

[7] A. A. Borbely, R. Huber, T. Graf, B. Fuchs, E. Gallmann, and P. Achermann, "Pulsed high-frequency electromagnetic field affects human sleep and sleep electroencephalogram," *Neuroscience Letters*, vol. 275, pp. 207-10, Nov 19 1999.

[8] S. Braune, C. Wrocklage, J. Raczek, T. Gailus, and C. H. Lucking, "Resting blood pressure increase during exposure to a radio-frequency electromagnetic field," *Lancet*, vol. 351, pp. 1857-8, Jun 20 1998.

[9] A. W. Preece, G. Iwi, A. Davies-Smith, K. Wesnes, S. Butler, E. Lim, and A. Varey, "Effect of a 915-MHz simulated mobile phone signal on cognitive function in man," *International Journal of Radiation Biology*, vol. 75, pp. 447-456, Apr 1999.

[10] SCEHIHR, "Preliminary opinion on possible effect of electromagnetic fields (EMF) on human health," H. a. C. P. D.-G. European Commision, Ed., ed: European Commision, 2006.

[11] I. Brook, "Microbiological studies of the bacterial flora of the external auditory canal in children," *Acta Otolaryngol*, vol. 91, pp. 285-7, Mar-Apr 1981.

[12] D. W. Stroman, P. S. Roland, J. Dohar, and W. Burt, "Microbiology of normal external auditory canal," *Laryngoscope*, vol. 111, pp. 2054-9, Nov 2001.

[13] M. N. Battikhi and S. I. Ammar, "Otitis externa infection in Jordan. Clinical and microbiological features," *Saudi Medical Journal*, vol. 25, pp. 1199-203, Sep 2004.

[14] R. Sander, "Otitis externa: a practical guide to treatment and prevention," *American Family Physician*, vol. 63, pp. 927-36, 941-2, Mar 1 2001.

[15] D. G. Keay and J. A. Murray, "Malignant otitis externa due to Staphylococcus infection," *J Laryngol Otol*, vol. 102, pp. 926-7, Oct 1988.

[16] A. Mamoon, "Biological potential hazards of electromagnetic field: The case of mobile phones," presented at the Twenteith National Radio Science Conference, Cairo, Egypt, 2003.

[17] G. J. Hyland, "Physics and biology of mobile telephony," *Lancet*, vol. 356, pp. 1833-1836, Nov 25 2000.

[18] B. Anderstam, Y. Hamnerius, S. Hussain, and L. Ehrenberg, "Studies of possible genetic effects in bacteria of high frequency electromagnetic fields," *Hereditas*, vol. 98, pp. 11-32, 1983.

[19] B. Del Re, F. Garoia, P. Mesirca, C. Agostini, F. Bersani, and G. Giorgi, "Extremely low frequency magnetic fields affect transposition activity in *Escherichia coli*," *Radiation Enviromental Biophysics*, vol. 42, pp. 113-8, Jul 2003.

[20] S. Nakasono, M. Ikehata, M. Dateki, S. Yoshie, T. Shigemitsu, and T. Negishi, "Intermediate frequency magnetic fields do not have mutagenic, co-mutagenic or gene conversion potentials in microbial genotoxicity tests," *Mutation Research*, vol. 649, pp. 187-200, Jan 8 2008.

[21] E. A. Gaafar, M. S. Hanafy, E. Y. Tohamy, and M. H. Ibrahim, "Stimulatin and control of *E.coli* by using an extremely low frequency magnetic field.," *Romanian Journal of Biophysics.*, vol. 16, pp. 283-296, 2006.

[22] L. Cellini, R. Grande, E. Di Campli, S. Di Bartolomeo, M. Di Giulio, I. Robuffo, O. Trubiani, and M. A. Mariggio, "Bacterial response to the exposure of 50 Hz electromagnetic fields," *Bioelectromagnetics*, vol. 29, pp. 302-11, May 2008.

[23] L. Fojt, L. Strasak, V. Vetterl, and J. Smarda, "Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli*, Leclercia adecarboxylata and *Staphylococcus aureus*," *Bioelectrochemistry*, vol. 63, pp. 337-41, Jun 2004.

[24] S. Turkyilmaz and O. Kaya, "Determination of some virulence factors in *Staphylococcus* spp. isolated from various clinical samples," *Turkish Journal of Veterinary and Animal Sciences*, vol. 30, pp. 127-132, 2006.

[25] L. Strasak, V. Vetterl, and J. Smarda, "Effects of low-frequency magnetic fields on bacteria *Escherichia coli*," *Bioelectrochemistry*, vol. 55, pp. 161-4, Jan 2002.

[26] L. Fojt, L. Strasak, and V. Vetterl, "Effect of electromagnetic fields on the denitrification activity of *Paracoccus denitrificans*," *Bioelectrochemistry*, vol. 70, pp. 91-5, Jan 2007.

[27] S. H. Li and K. C. Chow, "Magnetic field exposure induces DNA degradation," *Biochemical and Biophysical Research Communications*, vol. 280, pp. 1385-1388, Feb 9 2001.

[28] R. Goodman and M. Blank, "Insights into electromagnetic interaction mechanisms," *Journal of Cellular Physiology*, vol. 192, pp. 16-22, Jul 2002.

[29] M. Otto and K. E. von Muhlendahl, "Electromagnetic fields (EMF): Do they play a role in children's environmental health (CEH)?," *International Journal of Hygiene and Environmental Health*, vol. 210, pp. 635-644, Oct 2007.

[30] E. Di Campli, S. Di Bartolomeo, R. Grande, M. Di Giulio, and L. Cellini, "Effects of extremely low-frequency electromagnetic fields on *Helicobacter pylori* biofilm," *Current Microbioliolgy*, vol. 60, pp. 412-8, Jun 2010.