



Effect of Radiofrequency Radiation from Telecommunication Base Stations on Microbial Diversity and Antibiotic Resistance

E.A, ADEBAYO¹; A.O, ADEEYO^{*1}; A.A, AYANDELE¹ I.O, OMOMOWO¹

*1 Department of Pure and Applied Biology, Ladoké Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria
eaadebayo@lautech.edu.ng; firstrebby@gmail.com, lizdeley@yahoo.com and olaiyabo@yahoo.com*

**Corresponding author: A.O ADEEYO. E-Mail: firstrebby@gmail.com*

ABSTRACT: We aimed to investigate the effects of radiofrequency radiation from telecommunication on bacteria diversity and antibiotic sensitivity of surrounding bacteria micro-flora. In all cases of bacteria isolated, results indicated that radiation majorly influences and selected bacteria of specific morphology (rod-like, gram positive and spore formers) belonging to the genera *Bacillus*, *Clostridium*, *Corynebacterium* and *Sporosarcina*. Spore formation have been associated with prevention of DNA damage, this suggest that radiation may imposes stress detrimental to various non-spore forming, gram negative bacteria of non-rod morphology. The antibiotic resistance pattern indicated resistance above 50% in three of the ten common antibiotics tested with the highest of 96% recorded against zinnacef, followed by amoxicillin (70%) and ampiclox (56%). The highest resistance recorded was attributed to *Bacillus* spp while lowest resistance was recorded in *Corynebacterium*. The two largest groups of isolates; *Bacillus* and *Clostridium* have been reported as human pathogens and found to be multidrug resistant. © JASEM

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Introduction:

Before the 1990's radiofrequencies were mainly from a few radio and television transmitters, located in remote areas and/or very high places. Since the introduction of wireless telecommunication in the 1990's the rollout of phone networks has caused a massive increase in electromagnetic pollution in cities and the countryside (Firstenberg, 1997; Galeev, 2000). Multiple sources of mobile communication result in chronic exposure of a significant part of life (and man) to microwaves at non-thermal levels (Belyaev, 2005). In recent years, life has been chronically exposed to microwaves and RFR (Radiofrequency radiation) signals from various sources, including Global system for mobile communication (GSM) and UMTS/3G wireless phones and base stations, Wireless Local Area Networks (WLAN), Wireless Personal Area Networks such as Bluetooth (WPAN), and DECT (Digital Enhanced (former European) Cordless Telecommunications) that are erected indiscriminately without studies of environmental impact assessments.

The effects of RFR on the biological functions of living organisms represent an emerging area of interest with respect to environmental influences on human health. In latest years, several studies have been performed to verify direct effects exerted by

such radiations on cell functions. Although results have been somewhat controversial, a variety of cell responses have been observed involving proliferation and differentiation (Huang *et al.*, 2006; Lisi *et al.*, 2006; Lisi *et al.*, 2008; Vianale *et al.*, 2008; Schwartz *et al.*, 2008; Foletti *et al.*, 2009), gene expression (Piacentini *et al.*, 2008; Goodman *et al.*, 2009), modulation of the membrane receptors functionality (Jia *et al.*, 2007; Ke *at al.*, 2008; De-Mattei *et al.*, 2009), apoptosis, alteration in ion homeostasis (Grassi *et al.*, 2004; Lisi *et al.*, 2006; Iorio *et al.*, 2011), and free radicals generation (Simk, 2007; Di-Loreto *et al.*, 2009). Bacteria have also been used in the studies with radiofrequency radiation (Straš'ak, *et al.*, 2005; Fojt *et al.*, 2004; Babushkina *et al.*, 2005; Justo *et al.*, 2006; Gaafar *et al.*, 2006; Cellini *et al.*, 2008; Gaafar *et al.*, 2008; Fojt., 2009; Belyaev, 2011; Inhan-Garip *et al.*, 2011, Giorgi *et al.*, 2011). In particular, it has been demonstrated that radiation can negatively (Straš'ak, *et al.*, 2005; Fojt *et al.*, 2004; Justo *et al.*, 2006) or positively (Gaafar *et al.*, 2006; Cellini *et al.*, 2008; Belyaev, 2011) affect functional parameters (cell growth and viability) and bacteria antibiotic sensitivity depending on physical parameters of the electromagnetic field (frequency and magnetic flux density) applied, the time of the exposure, and/or the type of bacteria cells used. The possibility of an effect evoked by RFR on bacterial distribution and antibiotic resistance deserves special

**Corresponding author: . E-Mail: firstrebby@gmail.com*

attention in light of the risk that antimicrobial resistance poses to public health. Bacteria are becoming increasingly resistant to almost all presently available antibiotics and this aspect is becoming a worldwide problem of highest significance (Levi, 2001; Bush *et al.*, 2011). According to these considerations, the study of effects of RFR on bacteria is essential not only for investigation of environmental stress influences on biological systems, but also to explore the antibiotic resistance pattern associated for possibility of controlling bacteria in the environment or in clinical laboratories. We have therefore attempted to investigate the possible influence of RFR on bacteria distribution and antibiotic resistance on surrounding bacteria micro-flora of telecommunication base stations located around Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

MATERIALS AND METHODS

Sample collection: Soil samples were collected from telecommunication base station located around Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria. These were collected between 0 and 100ft (0, 10, 40, 70 and 100ft) from the foot of 3 base stations. Soil samples were aseptically transferred to the laboratory for microbial analysis.

Isolation of organisms: 1 g of each soil samples was weighed into 10 ml of sterile distilled water. Subsequent transfer of 1 ml into 9 ml of sterile distilled water was engaged for serial dilution of the prepared samples followed by plating out on nutrient agar using pour plate techniques. The cultures were incubated at 37°C for 24 h from where pure cultures were prepared based on bacteria morphology using the streaking out method.

Biochemical characterization and identification of pure cultures: Pure bacterial cultures were identified on the basis of grams reaction, morphology and biochemical reactions including sugar hydrolysis, catalase, oxidase, urease, indole, citrate utilization, methyl red, nitrate reduction and voges proskauer tests. The organisms isolated were then characterized according to the taxonomic scheme of Buchanan and Gibbons (1974).

Antibiotic susceptibility tests: Susceptibility of purified bacterial cultures to a set of ten antimicrobial substances was assessed by disk diffusion on Mueller-Hinton agar plates according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). After incubation, for 24 h at 37°C, the sizes of

zones of inhibitions were measured and interpreted by comparing with the standard antibiotic sensitivity chart to determine their resistant patterns. Resistance was calculated according to the method of Adebayo *et al.* (2012).

Antimicrobial agents: Antibiotics used include Pefloxacin (PEF), Gentamycin (CN), Ampiclox (APX), Zinnacef (Z), Amoxicillin (AM), Rocephin (R), Ciprofloxacin (CPX), Streptomycin (S), Seprin (SXT), and Erythromycin (E) and were purchased through Labtrade Chemicals, Nigeria.

RESULTS AND DISCUSSION:

The result of microbial diversity and prevalence around telecommunication base stations studied is indicated in Table 1. A total of 27 bacteria isolates belonging to four genera were identified. Result show a high occurrence of *Bacillus spp* (78 %), followed by *Clostridium spp* (11 %), *Corynebacterium striatum* (7 %) and *Sporosarcina ureae* (4 %) with the lowest distribution. Result indicated that RFR majorly influences and selected bacteria of specific morphology (rod-like, gram positive and spore formers) around the three telecommunication base stations sampled. Radiation effect seems to favour majorly the growth of *Bacillus spp* between the distance of 0 and 100ft with occasional presence of *Corynebacterium striatum*, *Sporosarcina ureae* and *Clostridium tertium* at 10, and 70ft from the base station. Spore formation has been associated with prevention of DNA damage and this suggests that radiation may impose stress detrimental to various non-spore forming, gram negative bacteria of non-rod shape. The presence of limited genera which are mostly spore formers showed that these groups survived because of their ability to form spores which protected them from the negative effect of radiation while others are probably eliminated. Rifat *et al.* (2010) reported *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azospirillum*, *Enterobacter*, *Klebsiella*, and *Pseudomonas spp* as symbiotic and free living beneficial groups of bacteria commonly encountered in the soil and have potential to contribute to sustainable plant growth. However none of these were isolated. The depletion of such nitrogen fixing bacteria will likely lead to soil infertility as such organism cannot protect themselves from the effect of radiation. This may invariably lead to reduction in yield and productivity of food crops in such environment. Previous report have implicated the ability of radiation to induce changes of cell growth and antibiotic sensitivity in bacteria strains (Grossman *et al.*, 1992; Stansell *et al.*, 2001;

Table 1: Diversity and prevalence of bacteria isolates from Telecommunication base stations in LAUTECH.

Isolates	Prevalence	Gram reaction	Shape	Spore formation
<i>Bacillus spp</i>	21 (78 %)	Positive	Rods	Spore formers
<i>Clostridium spp</i>	3 (11 %)	Positive	Rods	Spore formers
<i>Corynebacterium striatum</i>	2 (7 %)	Positive	Rods	Non-Spore formers
<i>Sporosarcina ureae</i>	1 (3 %)	Positive	cocci	Spore formers

Fojt *et al.*, 2004; Justo *et al.*, 2006; Fojt *et al.*, 2007; Fojt *et al.*, 2009). It has been found that viability of different types of bacteria (*Escherichia coli*, *Leclercia adecarboxylata*, and *Staphylococcus aureus*) was affected after exposure to an RFR (Fojt *et al.*, 2004). RFR have been reported to be lethal in *Paracoccus denitrificans*, but without changes in denitrification activity (Fojt *et al.*, 2009). While RFR as reported in this work is attributed to influencing the presence of majorly rod-shaped bacteria, Fojt *et al.* (2008) do not observe any change in bacterial morphology after short period of exposure to RFR suggesting that the radiation selected and caused survival of bacteria with specific shape and adaptive feature in the environment rather than causing a shape change in such organism. On the contrary, it has been demonstrated that short-term exposure RFR with a sinusoidal waveform of amplitude ranging from 0.1 to 1mT and frequency of 50 Hz affected morphology of cultured *E. coli* ATCC 700926 (Cellini *et al.*, 2008).

The antibiotic resistant patterns of various bacteria isolated are indicated in Table 2 and Figure 1. Antibiotic resistance among the radiofrequency radiation resistant bacteria indicated that resistance was above 50% in three of the ten common antibiotics tested. Among the twenty seven isolates, 26 (96%) were resistant to zinnacef, 19 (70%) to amoxicillin, 15 (56%) to ampiclox, 5 (19%) to pefloxacin and gentamycin respectively, 3 (11%) to erythromycin, 2 (7%) to rocephin and septrin and 1 (4%) to streptomycin and ciprofloxacin. With respect to the ten antibiotics used, *Bacillus spp* has resistance to all (100%) antibiotics used, *Clostridium spp* was resistant to four (40 %) while *Corynebacterium striatum* and *Sporosarcina ureae* were resistant to two (20% resistant) each, among the ten antibiotic used. Highest resistance was recorded in *Bacillus spp* while lowest resistance was recorded in *Corynebacterium*. The two largest groups of isolates of *Bacillus* and *Clostridium* have been reported as human pathogens and found to be multidrug resistant (Conner-kerr *et al.*, 1998). *Bacillus circulans* and *Clostridium septicum* have been

Table 2: Antibiotic resistant pattern in radiofrequency radiation resistant bacteria Isolated from LAUTECH.

Isolates	Antibiotics with proportion and percentage bacteria resistance										
	Number	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
<i>Bacillus spp</i>	21	5	4	11	20	16	2	1	1	2	3
<i>Clostridium spp</i>	3	0	1	1	3	2	0	0	0	0	0
<i>Corynebacterium striatum</i>	2	0	0	2	2	0	0	0	0	0	0
<i>Sporosarcina ureae</i>	1	0	0	1	1	1	0	0	0	0	0
Total	27	5(19%)	5(19%)	15(56%)	26(96%)	19(70%)	2(7%)	1(4%)	1(4%)	2(7%)	3(11%)

Pefloxacin (PEF), Gentamycin (CN), Ampiclox (APX), Zinnacef (Z), Amoxicillin (AM), Rocephin (R), Ciprofloxacin (CPX), Streptomycin (S), Septrin (SXT), and Erythromycin (E)

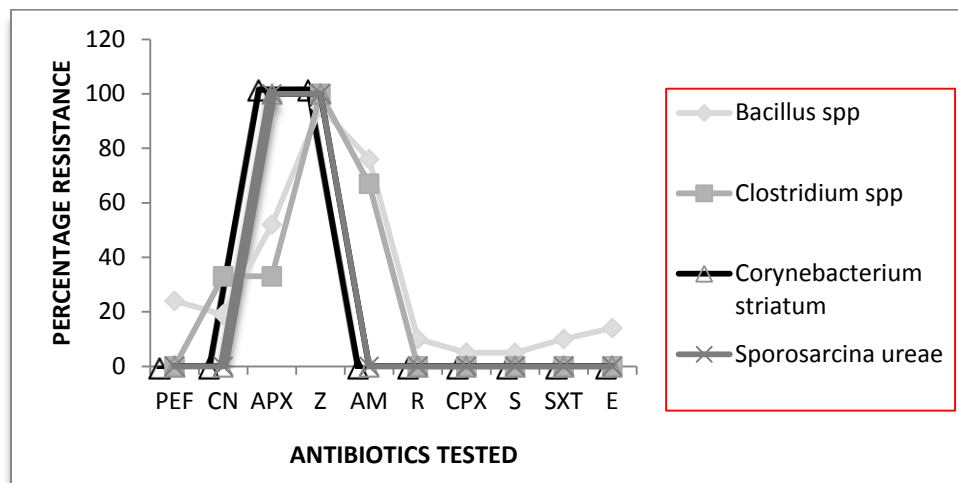


Fig 1: Percentage resistance to antibiotics among bacteria strains isolated
Pefloxacin (PEF), Gentamycin (CN), Ampiclox (APX), Zinnacef (Z), Amoxicillin (AM), Rocephin (R),
Ciprofloxacin (CPX), Streptomycin (S), Septrin (SXT), and Erythromycin (E)

associated with gas gangrene which is highly fatal and may result into death in less than 24 h (Katlic, 1980). *Clostridium septicum* is highly pathogenic in immune-compromised individuals, *Corynebacterium striatum* have been implicated in endocarditis, bacteremia and catheter infections (Coyle *et al.*, 1990). *Bacillus cereus* causes food poisoning, diarrhea, severe nausea and vomiting (McKillip, 2000). *Bacillus circulans* is a pathogen incriminated in human infection such as septicemia, mixed abscesses and wound infection. Antibiotic resistance increases in the environment as they replicate their genetic materials; this means that resistance can spread from one species of bacteria to the other enabling it to develop multiple resistances to different classes of antibiotics. Electromagnetic fields have been reported in acquisition of resistance to Cephalosporins (Cefuroxime and Ceftazidime) (Fojt *et al.*, 2007; Cellini *et al.*, 2008). Justo *et al.* (2006) reported that cell growth could be altered (stimulated or inhibited) under magnetic field effects. Exposure of bacteria to radiation have been reported to cause changes in the growth characteristic, morphology, structural properties of proteins, and the sensitivity and resistance to certain antibiotics such as amoxicillin, nalidixic acid, and erythromycin (Gaafar *et al.*, 2006; Gaafar *et al.*, 2008). These results were in agreement with the work of Stansell *et al.* (2001) who found that moderate intensity static fields were able affect antibiotic sensitivity and resistance of bacteria. Furthermore, Belyaev (2011) showed that RFR, under specific conditions of exposure (frequency ranging from 8.5Hz to 9 Hz; 0.021mT), acted as a nontoxic but cell-growth stimulating agent

on *E. coli* GE499. Again, the exposure of *E. coli* HB-101 to radiation produced a stimulation of cell growth (Babushkina *et al.*, 2005). By contrast, Grosman *et al.* (1992) found that static magnetic fields ranging from 0.5 to 4.0 T had no significant influence on the growth rate and antibiotic sensitivity of *E. coli* and *Staphylococcus aureus*. A direct comparison of these studies with results obtained in this work may be difficult because of the dissimilar experimental procedures employed. It is well known that the effects of radiation generally depend on both physical and biological parameters, including field signal characteristics (frequency, amplitude, wave shape, etc.), duration of exposure, cell metabolic state, genotype, and how long cells are allowed to grow before, during, and after exposure.

This work which aimed to investigate the effects of Radiofrequency radiation from telecommunication base stations on bacteria diversity and antibiotic resistance of surrounding bacteria micro-flora have shown that radiation influences and selected bacteria of specific morphology (rod-like and gram positive), imposes stress detrimental to various non-spore forming, gram negative bacteria of non-rod morphology and possibly may contribute to antibiotic resistance. Further research in this area is advised with the possibility of development of necessary hazard control measures for the protection of the environment from outbreak of antibiotic resistant organisms.

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