INTERNATIONAL JOURNAL OF RESEARCH AND TECHNOPRENEURIAL INNOVATIONS (IJRTI)

Volume 1 Issue 1 (April 2024)

Central Instrument Laboratory, University of Port Harcourt, Nigeria
E-mail: centralinstumentlaboratory@uniport.edu.ng

Multi-Antibiotic Resistant Bacteria Associated with Bioremediated Crude Oil Polluted Soil

Uredo-Ojo Bridget Fajoyomi,[1] Chimezie Jason Ogugbue[2] and Kome Otokunefor.[2]

[1]Microbiology Technology/ School of Science Laboratory Technology/ University of Port Harcourt, Rivers State, Nigeria.
[2] Microbiology/ Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

Email address:
*Corresponding author: uredo-ojo.ogwu@uniport.edu.ng

To cite this article:
Fajoyomi, U.B., Ogugbue, C.J. and Otokunefor, k. Multi-Antibiotic Resistant Bacteria Associated With Bioremediated Crude Oil Polluted Soil. *International Journal of Research and Technopreneurial Innovations* 2024; 1(1): 102-108.

Keywords:
Multi-antibiotic resistance, Bioremediation, Crude oil.

ABSTRACT

This study was carried out to isolate and characterize multi-antibiotic resistance bacteria associated with bioremediated crude oil polluted soil. Soil samples were obtained from a bioremediated site and pristine soil of different depths in Eleme Local Government Area of Rivers state. Physico-chemical characterization of soil samples was determined. Isolation of total heterotrophic bacterial and hydrocarbon utilizing bacterial was carried out. Antibiotics susceptibility test of the isolated bacterial species was determined using the disc diffusion method. Biodegradability potential of isolates that were susceptible to antibiotics was determined using the 2,6-dichlorophenolindophenol (DCPIP) redox method. Bacterial count ranged from 1.7 x 10^5 cfu/g – 2.8 x 10^7 cfu/g. Total petroleum hydrocarbon concentration for pristine and bioremediated soil was 69.24 mg/kg and 238.9mg/kg respectively. Bacterial isolates from both soils include *Bacillus* spp., *Pseudomonas* spp., *Burkholderia* spp., *Streptococcus* spp., *Staphylococcus* spp., *Streptomyces* spp., *Cellulomonas* spp. and *Nitrobacter* spp. Isolates from bioremediated soil had multiantibiotic resistance index (MAR) of 0.1 -1 while isolates from the pristine soil had multiantibiotic resistance index (MAR) of 0.1 -0.4 with the bioremediated soil having isolates with the highest MAR index of 1. *Bacillus* spp. and *Pseudomonas* spp. isolated from the pristine soil which were susceptible to antibiotics were able to degrade crude oil. All isolates from bioremediated soil were resistant to one or more antibiotics exposed to. This poses a serious public health risk. The use of bacterial species susceptible to antibiotics and having crude-oil biodegradability potential is recommended for bioremediation to curb the spread of antibiotics resistant bacteria.

1. INTRODUCTION

The growing emergence of antibiotics resistance in bacteria is a major public health risk worldwide due to noteworthy morbidity and death [1]. The decline in the discovery of new antibiotics from the pharmaceutical industry coupled with selective pressure exerted by the excessive and indiscriminate use of antimicrobial agents such as antibiotics and sanitizers to kill or inhibit various bacterial pathogens and exposure to pollutants has

accelerated the emergence and spread of antimicrobial resistance among bacteria [2]. Bacteria have developed numerous mechanisms to evade the effects of antimicrobials and pollutants [3]. The major group of environmental pollutants worldwide are products from crude oil-based hydrocarbons. Crude oil spills occur during transportation, storage operations processing and bunkering [4]. This could lead to lethal stress on microbial fauna and flora. The majority of bacterial cells are inhibited or killed in the presence of lethal stress, a subpopulation of bacterial cells known as persister cells can survive and resume growth after relief of the imposed lethal stress [5]. The main compositions of crude oil are; Carbon, Hydrogen, Nitrogen, Sulfur, Oxygen, and Metals (Hg, Au, Cu, Al, Ca, Co, K, Mg, Si, Sr, Mo, Ti, Mn, Li, Se, Rb, Ag, Ba, Pb, As, Cd, Cr, Fe, Ni, V, Zn.) [6]. The presence of hydrocarbon especially polycyclic aromatic hydrocarbon and heavy metals in crude oil contributes to the persistence of these compounds in the environment after crude oil spills. Several studies have implicated bacteria exposure to metals and crude oil to bacteria resistance to antibiotics [7]. It has been proven that antimicrobial agents different from antibiotics can promote a co-selection process, indirectly selecting antibiotic resistance. There has been great concern about heavy metals selecting indirectly for antibiotic resistance by co-selection. This indirect selection process is due to a coupling of the resistance mechanisms against antibiotics and heavy metals [8]. These mechanisms can be coupled physiologically (cross-resistance) and genetically (co-resistance). Cross-resistance describes mechanisms that provide tolerance to more than one antimicrobial agent such as antibiotics and heavy metals. As an example, several multi-drug efflux pumps are known to mediate decreased susceptibility toward antibiotics and heavy metals by rapid extrusion of the toxins out of the cell. Co-resistance is defined as two or more genetically linked resistance genes, meaning that genes responsible for two or more resistances are located next to each other on one mobile genetic element. Due to the close arrangement of the genes, these genes are likely subject to a combined transmission in the case of a horizontal gene transfer [9]. Bioremediation is a process of using microorganisms (bacteria, fungi and algae) to break down pollutants into less harmful substances. In a way to obtain energy for their growth, microorganisms break down many organic compounds in the environment. Numerous physicochemical and biological treatment processes have been employed worldwide to eradicate crude oil spill pollution from soil and seawater. Bioremediation treatment process for the biodegradation of crude oil has received much attention because of its effectiveness and lower cost. The use of micro-organisms for the bioremediation of crude oil polluted sites is necessary to establish green technology [4]. Since bioremediation is a proven clean-up mechanism for crude oil spills thus this research was carried out to isolate multi-antibiotics resistant bacteria from a bioremediated site.

2. MATERIALS AND METHODS

2.1. Sample collection

Soil samples (bioremediated soil sample and pristine soil sample) were collected at a site in Ogale in Eleme Local Government Area of Rivers State, Nigeria where bioremediation has been carried out by Hydrocarbon Pollution Remediation Project (HYPREP) It is situated in the Niger Delta Area of Nigeria. Soil auger was used to collect soil samples from three different spots randomly at three different depths of 0-5cm, 10-15cm and 20-25cm to form a composite sample. Samples were put into polythene bags and placed inside a cooler with ice packs and then taken to the laboratory for analysis.

2.2 Determination of Total petroleum hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbons (PAHs)

This was carried out using Gas Chromatography - mass spectrophotometer (GC-MS) as described by Ehis-Eriakha [10].

2.3 Determination of metal concentration

This was done using Atomic Absorption Spectrophometer method (AAS) as described by Tanee and Albert [11].

2.4 Enumeration of Total Heterotrophic Bacterial counts (THB)

1g of soil (wet weight) was homogenized in normal saline (0.85%) with a vortexing machine. Ten-fold decimal dilutions of the suspensions were plated out on Nutrient Agar and incubated at 37°C for 24 hours.

2.5 Enumeration of Hydrocarbon Utilising Bacterial counts (HUB)

Culture enrichment was performed by adding 1g of soil into 100 ml of Bushnell Haas Mineral Salts (BHMS) amended with 0.5% crude oil (v/v) as the carbon source. The medium was incubated for 5 days at 37°C, 130 rpm in a shaker incubator. Thereafter, serial dilution was performed, and the inoculum plated out on Bushnell Haas agar amended with crude oil for 5 days. Distinct colonies were enumerated and subcultured into nutrient agar plates for further analysis. Characterization and identification of isolates was done using biochemical tests such as gram staining, glucose fermentation, motility test, indole test, triple sugar iron test, catalase and citrate test.

2.6 Determination of antibiotic susceptibility

It was carried out using the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Antibiotic discs contained the following antibiotics; Ampilclox (APX) 20ug, Chloramphenicol (CH) 30ug, Amoxil (AM) 20ug, Rifampicin (RD) 20ug, Ciprofloxacin (CPX) 10ug, Streptomycin (S)30ug, Erythromycin (E) 30ug, gentamycin (CN) 10ug, Septrin (SXT) 30ug, Pefloxacin (PEF) 10ug. The inoculum size was prepared and compared with 0.5 McFarland standard. This was done by sub-culturing pure isolates into nutrient broth and incubated at 37°C for 24 hours. McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions. The turbidity of the growth was adjusted by dilution with sterile distilled water until equal to the turbidity of a 0.5% McFarland's standard. A 0.5% McFarland standard was prepared by mixing 0.05ml of 1.175% barium chloride dehydrate (BaCl2 H20), with 9.95ml of 1% sulfuric acid (H2SO4). The commercial antibiotics sensitivity discs were aseptically placed in the Petri dishes using a pair of sterile forceps and incubated for 24 hours at 37°C. Thereafter the zones of inhibition were measured using a rule. The diameters of the zones of inhibition were measured in millimeters (mm). Isolates with antibiograms or zones of inhibition less than or equal to 12mm (≤ 12mm) were regarded as resistant to the respective antibiotics, while those greater than 12mm (> 12mm) were susceptible to the respective antibiotics. This method was adopted from [13].

2.7 Screening of crude oil degrading potential of antibiotics susceptible organisms

This was carried out using the redox (2,6-dichlorophenolindophenol (DCPIP)) method as described by [14]. Bacterial cell suspension of 3 µl, 2 µl of crude oil, 150 µl of Bushnell hass broth and 45 µl of 1.0 g/L DCPIP were put in a test tube. The degradative potential of isolates was detected from the change of colour from blue (oxidized) to colourless (reduced). Tubes were incubated at 25°C for 120 hours with colour observation at 24, 48, 72, 96 and 120 hours. Two sets of controls were also prepared to assess the interactions of the components and the redox indicator.

3. RESULTS AND DISCUSSION

3.1. Finding of research

In this study, multi-antibiotic resistant bacteria associated with bioremediated crude oil polluted soil were identified and characterized. The physicochemical parameters were determined, the concentration of TPH, PAH, metal, Nitrate, Phosphate and Potassium were higher in the bioremediated soil than in the control sample as shown in Table 1 and Figure 1-4. Total petroleum hydrocarbon for bioremediated soil ranged from 101.27 mg/kg – 421.3 mg/kg with an average of 238.9 mg/kg while the control ranged from 35.25 mg/kg – 116.15 mg/kg with an average of 69.24 mg/kg (Table 1).

3.2. **Table 1: Physicochemical parameters of bioremediated and control soil.**

S/N	Parameters	B.S (Average)	C. (Average)
1.	Ph	6.7	6.2
2.	EC (us)	69	124
3.	TOC (%)	0.4	0.53
4.	Total Nitrate (mg/kg)	21.5	9.9
5.	Phosphate (mg/kg)	14.4	11.6
6.	Potassium (mg/kg)	120.4	83.8
7.	Moisture (%)	2	5
8.	PAH (mg/kg)	57.11	5.13
9.	TPH (mg/kg)	238.9	69.24

Table 2: Metal concentration of bioremediated and control soil.

S/N	Metals	B.S (Average concentration (mg/kg))	C. (Average concentration (mg/kg))
2.	Cr	0.112	0.060
3.	As	1.081	0.183
4.	Vd	ND	ND
5.	Cu	1.476	1.333
6.	Pb	3.778	2.896

KEY- B.S: Bioremediated soil; C: Control soil; ND: Not detected

Despite the bioremediation the metal concentration, TPH and PAH were still higher than the control soil (figures 1 & 2).

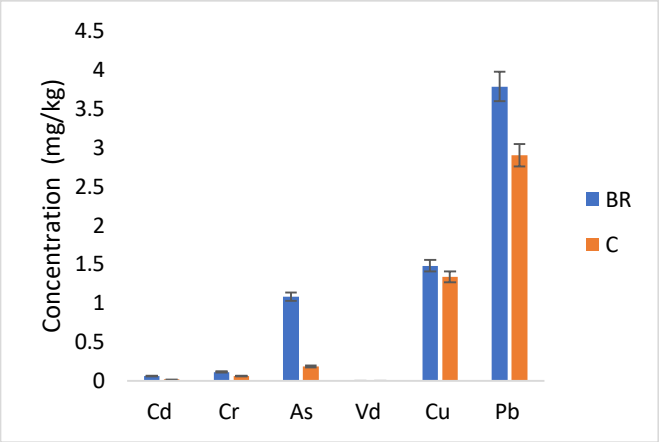

Figure 1: Metal Concentration of soil samples

BR= Bioremediated soil
C= Control soil

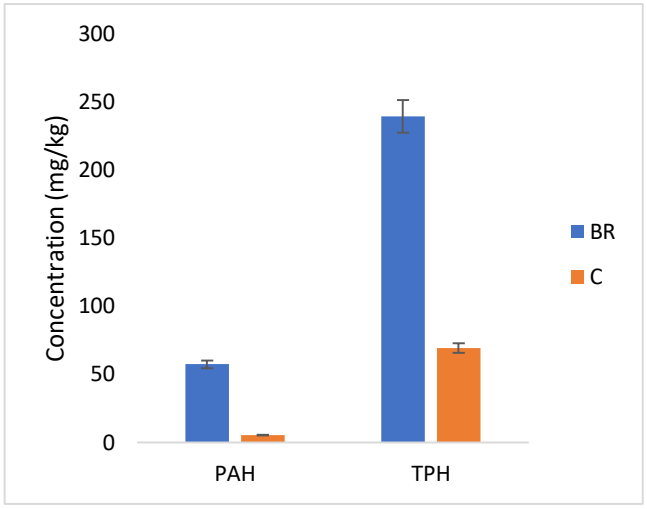

Figure 2: Concentration of Total petroleum hydrocarbon and Polycyclic aromatic hydrocarbon

The higher concentration of Nitrate, Phosphate and Potassium in bioremediated soil was observed by Tanee [11] in their study. This could be as a result of biostimulation with nutrients during the bioremediation of crude oil polluted soil (Figure 3).

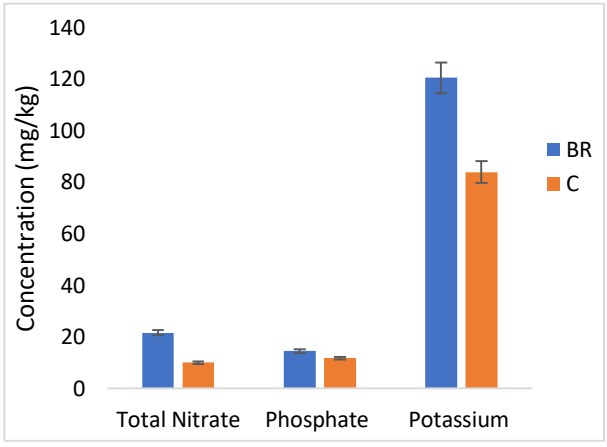

Figure 3: Concentration of Nitrate, Phosphate and Potassium

3.3 Bacterial count

The average total heterotrophic bacterial count for bioremediated soil and pristine soil were 1.9 x 10^5 cfu/g and 2.8 x 10^7 cfu/g while the average Hydrocarbon utilizing bacterial count were 2.0 x 10^7 cfu/g and 1.7 x 10^5 cfu/g for bioremediated soil and pristine respectively. The total heterotrophic bacterial count was higher in the control sample (Figure 4).

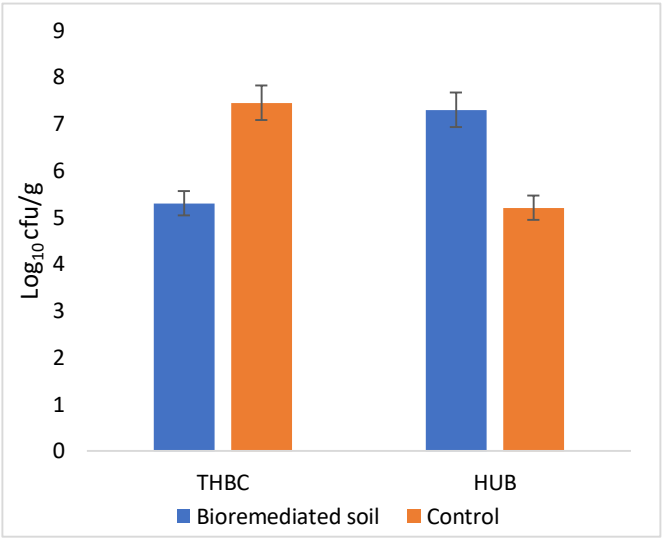

Figure 4: Total heterotrophic Bacterial count and Hydrocarbon utilizing bacterial count

Antibiotic susceptibility test carried out indicates that isolates from the bioremediated soil were all resistant to one or more antibiotics having a Multi-Antibiotics (MAR) index value of 0.1-1 (Figure 6). Isolates from pristine soil had a MAR index value of 0.1 -0.4, with 58.35% of isolate susceptible to the antibiotics exposed to and 41.65% resistant to 1 - 4 antibiotics exposed to. Out of the 41.65% resistant isolates, 36.1% and 5.6% had a MAR index of 0.1 and 0.4 respectively (Figure 5).

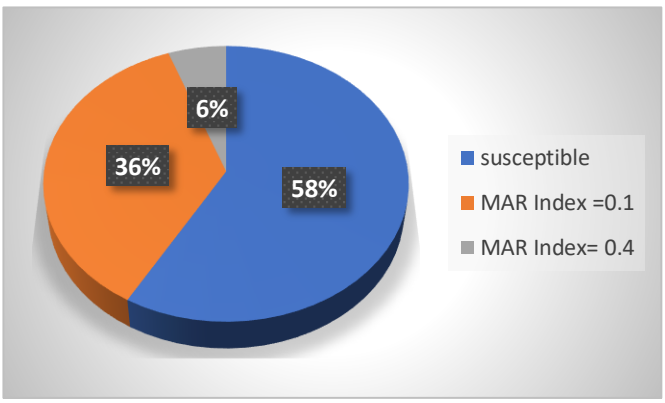

Figure 5: Percentage of Multi antibiotic resistance index in control soil

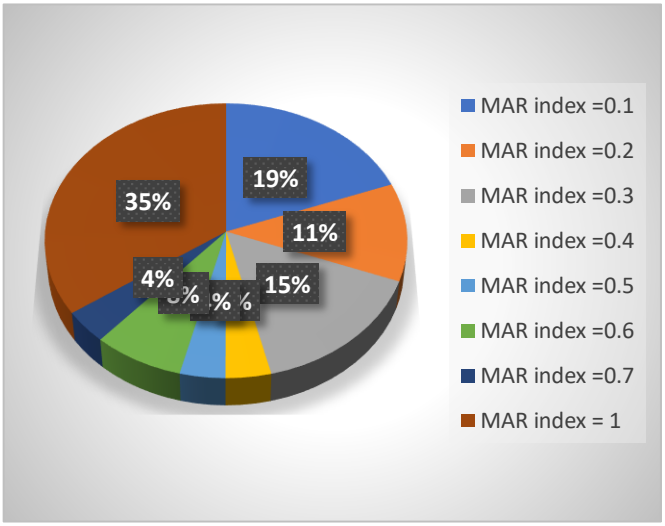

Figure 6: Percentage of Multi antibiotic resistance index in Bioremediated soil

Bacterial isolates from both soils include *Bacillus* spp., *Pseudomonas* spp., *Burkholderia* spp., *Streptococcus* spp., *Staphylococcus* spp., *Streptomyces* spp., *Cellulomonas* spp. and *Nitrobacter* spp. *Bacillus* spp. and *Pseudomonas* spp. isolated from the pristine soil which were susceptible to antibiotics were able to degrade crude oil within 48 hours and 96 hours respectively. This was observed by the colour change of the DCPIP indicator from blue to colourless.

According to [14], antibiotics resistance can occur through mutation, or antibiotics resistant genes may already be present in the environment and spread through conjugative plasmids, transformation (that is uptake of naked DNA from the environment) and transduction (Bacteriophage supported transmissio n. Antibiotics resistance caused by de novo mutation evolution is usually in response to anthropogenic selective pressures of which crude oil pollution is one of such.

4. CONCLUSION

From this study, it can be deduced that Bioremediated soil that has been contaminated with crude oil are hot spots for the accumulation of antibiotic resistant bacteria and necessitates distinct scientific attention. In recent years there has been a decline in the development of new classes of antibiotics thus prompting humans to be good stewards in preventing the spread and accumulation of antibiotics resistance organisms. As good stewards, our concern should not only be on antibiotics resistant bacteria or genes present in the environment but should focus on the proliferation and horizontal transfer of these genes to pathogenic organisms in the environment thus limiting our ability to fight infectious diseases with antibiotics.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest regarding writing of this paper.

References

[1] Abolfazl, D. and Brown, M. H. (2021). Efflux Pump Mediated Antimicrobial Resistance by Staphylococci in Health Related Environments Challenges and the Quest for Inhibition. *Antibiotics* 10: (1502) 1 – 33.

[2] Venter, H. (2019). Reversing resistance to counter antimicrobial resistance in the World Health Organisation's critical priority of most dangerous pathogens. *Bioscience Report* 39: 47- 53.

[3] Leitao, J. H. (2020). New insights into antibacterial compounds: From synthesis and discovery to molecular mechanisms of action. *Antibiotics* 9: 471- 480.

[4] Khalid, S., Lavania, B. and Naresh, K. S. (2021). Bioremediation of total petroleum hydrocarbons (TPH) by bioaugmentation and biostimulation in water with floating oil spill containment booms as bioreactor basin. *International Journal of Environmental Resea rch and Public Health* 18: (22) 1-26.

[5] Yu, T., Jiang, G., Gao, R., Chen, G., Ren, Y.,

Liu, J., Van der Mei, H. C. and Busscher, H. J. (2020). Circumventing antimicrobial-resistance and preventing its development in novel, bacterial infection-control strategies. *Expert Opinion on Drug Delivery*. 17: 1151–1164.

[6] Oti, W. J. O. (2016). Levels of Heavy Metal in Bonny Light Crude Oil. *Journal of Applied Chemistry.*9:(7) 86-88.

[7] Tiku, S. P., Antai, A. D., Asitok, A. and Ekpenyong, M. G. (2016). Hydrocarbon biodegradation, heavy metal tolerance, and antibiotic resistance among bacteria isolates from petroleum polluted and pristine soil samples in Calabar metropolis. *Imperial Journal of Interdisciplinary Research* 2: 11 -17.

[8] Li-Guan, L., Yu, X. and Tong, Z. (2017). Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection. *International Society for Microbial Ecology* 11: 651–662.

[9] Claudia, S. and Thomas U. (2012). Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture *Frontiers in Microbiology* 3: 399 1-10.

[10] Ehis-Eriakha, C. B., Chikere, C. B. and Akaranta, O. (2020). Functional gene diversity of selected indigenous hydrocarbon-degrading bacteria in aged crude oil. *International Journal of Microbiology* 20: 1-11.

[11] Tanee, F. B. G. and Albert, E., (2011). Post-remediation assessment of crude oil polluted site at Kegbara-Dere community, Gokana L.G.A. of Rivers State, Nigeria. *Journal of Bioremediation and Biodegradation* 2: (3) 1-5.

[12] Clinical and Laboratory Standards Institute (CLSI) (2017). Performance Standards for antimicrobial Susceptibility Testing; Twenty-seventh Informational Supplement. CLSI document M100-S26 ISBN 1-56238-805-3. 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA.

[13] Ataikiru, T. L, Okorhi-Damisa, F. and Onyegbunwa, A. E (2020). Hydrocarbon biodegradation and antibiotic sensitivity of microorganisms isolated from an oil polluted site in Kokori, Delta State, *Nigeria Issues in Biological Sciences and Pharmaceutical Research* .8: (2) 43- 50.

[14] Syahir, H., Wan Lutfi, W., Mohd, Y., Abdul, S. and Nur, A. Y. (2017). Screening of hydrocarbon- degrading bacterial isolates using the redox application of 2,6-DCPIP. *Bioremediation Science and Technology Research* 5: (2) 13-16.

[15] Kraemer, A. S., Arthi, R. and Gabriel, G. P. (2019). Review Antibiotic pollution in the environment: From microbial ecology to public policy *Microorganisms* 7: (180) 1 - 24.