

ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ISOLATED FROM SPENT ENGINE OIL CONTAMINATED SOIL

¹Ughala, E.* and ²Osaro-Matthew, R.C

¹Department of Microbiology, University of Port Harcourt, Nigeria.

²Michael Okpara University of Agriculture Umudike, Department of Microbiology, Nigeria.

*Tel: +2347034864001; Email: eziughala@gmail.com

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ABSTRACT

Spent engine oil contaminated soil not only harbors bacteria resistant to heavy metals but also to antibiotics. This study investigated the antibiotic resistant profile of bacterial isolates from spent engine oil contaminated soil. Two soil samples one each was collected from 10 years old mechanic workshop and pristine environment. Bacteria were isolated using pour plate method. The isolates were identified based on their physiological and morphological characteristics using standard microbiological techniques. Antibigram studies were carried out by disc diffusion method and results interpreted based on the CLSI guidelines. The isolates were identified as members of eleven genera (*Bacillus*, *Clostridium*, *Pseudomonas*, *Rhodococcus*, *Klebsiella*, *Escherichia*, *Providencia*, *Arthrobacter*, *Corynebacterium*, *Serratia* and *Proteus*). Results obtained revealed that all the bacterial isolates obtained from the contaminated soil exhibited antibiotics resistance, these bacterial isolates were highly resistant to cefoxatime (82.4 %) and ceftriaxone (82.4 %). Furthermore, the pristine soil isolates were highly resistant to cefoxatime (88.9 %) and ceftriaxone (88.9 %) and ceftazidime (88.9 %) these are all members of the beta-lactam class cephalosporins. The presence of antibiotics resistant bacteria in spent engine oil contaminated soil as seen in this study shows that it can be a medium of antibiotics resistance spread. Thus, stringent measures on the disposal of spent engine oil should be enforced.

Keywords: spent engine oil; antibiotics resistance, bacteria, soil.

INTRODUCTION

Soil plays a major role as a source of nutrients in the environment, it serves as a shelter for plants and other species, and works as a big bioreactor, where pollutant decomposition as well as nutrient alteration occurs (Talukder *et al.*, 2021). Over the years, soil bacteria have been incrementally antibiotic resistant although more stringent antibiotic usage laws exist in medicine and agriculture (Kumar *et al.*, 2019). The soil ecosystem which is mainly influenced by human usage, where the interaction between clinical pathogenic isolates and environmental isolates occurs, and the presence of several antimicrobial selective

pressure together, is ideal for exchanging and disseminating resistance genes (Pitondo-Silva *et al.*, 2014). Antibiotic resistance is an instance of the immense capacity for natural evolution and adaptation of bacteria to diverse ecosystems (Clarke *et al.*, 2020) While this process seems inevitable, humans have accelerated it through various anthropogenic activities (Wong, 2019). The reasons behind the increase in the number of antimicrobial-resistant bacteria in recent years include mismanagement of antibiotics in humans and animals, ineffective control of infections in hospitals and clinics or environmental sanitation (Machowska *et al.*, 2018; Wong, 2019). In addition to the causes mentioned, the

problem deteriorates as there is a draught in the discovery of new antibiotics. The increase in resistant rates of bacteria, leads to a decrease in the effectiveness of existing antibiotics, making research in this field unappealing to organizations that decide to invest in other types of fields with greater chances and benefits (Álvarez-Martínez *et al.*, 2020). Automobile mechanic workshops in developing countries contribute to the challenge of soil contamination with petroleum hydrocarbons in addition to toxic heavy metals as a result of indiscriminate disposal of spent engine oil. Such contaminated soil environments can serve as reservoirs of bacteria which possess adaptive tolerance to these metals and hydrocarbons as well as resistance to antibiotics (Onovaye *et al.*, 2022). Recently, self-employed artisan auto-mechanics are relocating to agricultural lands to set up auto-mobile mechanic workshops as a result of limited space in urban areas parts of Nigeria (Musa, 2019). Spent diesel oil are used lubricating oils obtained after servicing and subsequently draining from automobile and generator engines. This contains high percentage of aliphatic and aromatic hydrocarbons and other heavy metals such as nitrogen and sulphur. These metals are introduced into the oil as a result of wear and tear of the automobile and generator engine (Chaîneau *et al.*, 2005). Spent diesel oil causes great damage to soil and soil microflora due to poor aeration, immobilization of soil nutrients and reduction of soil pH. It also alters the physical, chemical as well as microbiological properties of the soil. At low concentrations, some of these heavy metals are essential micronutrients for plants, on the contrary, they can cause metabolic disorders and growth inhibition when the concentration is high (Musa, 2019).

Research has shown that several petroleum hydrocarbon contaminated sites are also co-contaminated with metals either as trace elements of crude oil or its derivatives. Metal contamination in soils also produces co-selection for bacteria conferring antibiotic resistance and the relationship between metal and antibiotic resistance in bacteria is also very well established (Poole, 2017). Even low concentrations of metals found in residential soils, assumed to have been free of antibiotic exposure, showed a greater relative abundance of antibiotics resistant bacteria (Knap *et al.*, 2017). In establishing the application of bacteria in the bioremediation of hydrocarbon impacted environments, there is also a need to ascertain the antibiotics susceptibility of the bacterial isolates to overcome the spread of antibiotics resistant genes in the environment. Thus, the present study aims at evaluating the antibiotic resistant propensity among bacterial isolates from hydrocarbon impacted and pristine soil samples.

MATERIALS AND METHODS

Collection and preparation samples

Ten years old mechanic workshop soil and pristine soil samples one each were collected from along East-West Road in Choba, Rivers State. Soil samples were homogenized and crushed gently multiple times with a mortar and pestle prior to passing it through a 0.5mm mesh for analysis. All the soil samples were analyzed in duplicates to minimize error.

Isolation and identification of bacteria

Soil samples were serially diluted and 0.1 mL of the dilution of choice was plated on Nutrient agar supplemented with 50 µg/ml of Nystatin (to inhibit fungal growth) and on Eosin Methylene Blue (EMB) agar and incubated for 37°C for 24 h. Distinct colonies were carefully

picked and sub-cultured in to Nutrient agar. Pure cultures were characterized using Gram reaction and biochemical tests following the standard protocols and identified using the Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

Antimicrobial susceptibility testing

Antibiogram studies were carried out according to the guidelines of the Clinical Laboratory and Standard Institute (CLSI) using Kirby-Bauer disk diffusion method. Bacterial isolates were adjusted to 0.5 McFarland turbidity standards and streaked onto Mueller-Hinton (MH) agar plates, antibiotics discs were aseptically placed on the inoculated MH agar. Plates were incubated at 37°C for 18–24h, The zone of inhibition diameter and interpreted as susceptible or

resistant based on the guidelines of CLSI (CLSI,2017).

RESULTS

A total of 28 bacteria were isolated from the two soil samples (spent engine oil contaminated soil and pristine soil), these isolates were identified to be members of 11 genera; (*Bacillus*, *Clostridium*, *Pseudomonas*, *Rhodococcus*, *Klebsiella*, *Escherichia*, *Providencia*, *Arthrobacter*, *Corynebacterium*, *Serratia*, *Proteus*) based on their physiological and biochemical characteristics. Table 1 shows the generic distribution of bacteria from the soil samples. A total of 17 (65.4%) isolates were recovered from spent oil contaminated soil, while, while 9(34.6 %) were recovered from the pristine soil sample.

Table 1: Generic distribution of bacteria from various samples

Bacteria genera	No. of isolate	SOCS	PS	Occurrence rate in both samples (%)
		No. (%)	No. (%)	
<i>Bacillus</i>	5	2(40)	3(60)	19.2
<i>Clostridium</i>	1	1(100)	0(0.00)	3.8
<i>Pseudomonas</i>	3	2(66.7)	1(33.3)	11.6
<i>Rhodococcus</i>	1	1(100)	0(0.00)	3.8
<i>Klebsiella</i>	3	2(66.7)	1(33.3)	11.6
<i>Escherichia</i>	3	1(33.3)	2(66.7)	11.6
<i>Providencia</i>	1	1(100)	0(0.00)	3.8
<i>Arthrobacter</i>	1	1(100)	0(0.00)	3.8
<i>Corynebacterium</i>	2	2(100)	0(0.00)	7.7
<i>Serratia</i>	4	3(75)	1(25)	15.4
<i>Proteus</i>	2	1(50)	1(50)	7.7
Total	26	17(65.4)	9(34.6)	100

Key: SOCS= Spent oil Contaminated soil, PS= Pristine soil

As presented in Table 2, the antibiotic resistant profile of the studied bacteria showed that the microorganisms of spent soil contaminated origin were highly resistant to cefoxatime and ceftriaxone (82.4%), followed by levofloxacin (58.8 %) and the least resistant was observed with erythromycin (5.8 %). Also in Table 3, the results from the pristine soil showed that the isolates were 100 % resistant to augumentin followed by equal resistance of 88.9 % to cefoxatime, ceftriaxone and ceftazidime while the least resistance was seen in ofloxacin (11.1 %).

Table 2: Antibiotic resistant profile of the bacterial isolates from contaminated soil

Bacteria genera	No. of isolate	CIP (%)	IMP (%)	CXM (%)	GN (%)	CTX (%)	CRO (%)	LBC (%)	AZN (%)	OFX (%)	ERY (%)	AUG (%)
<i>Bacillus</i>	2	0	50	0	0	100	100	0	0	0	0	0
<i>Clostridium</i>	1	0	0	0	0	100	100	0	0	0	0	0
<i>Pseudomonas</i>	2	0	0	100	100	100	100	0	0	0	0	0
<i>Rhodococcus</i>	1	0	0	0	0	100	100	0	0	0	0	0
<i>Klebsiella</i>	2	100	100	100	100	0	100	100	100	100	0	100
<i>Escherichia</i>	1	100	100	100	0	100	100	100	100	100	0	100
<i>Providencia</i>	1	0	100	100	0	0	100	0	0	0	0	0
<i>Arthrobacter</i>	1	0	100	100	100	100	0	0	0	100	100	0
<i>Corynebacterium</i>	2	0	100	100	50	100	0	0	100	0	0	0
<i>Serratia</i>	3	0	0	0	100	100	100	0	0	0	0	66.7
<i>Proteus</i>	1	0	0	100	0	100	100	0	0	0	0	100
Total	17	17.6	47.1	58.8	41.2	82.4	82.4	17.6	29.4	23.5	5.8	41.2

Key: CIP= ciprofloxacin, IMP =imipenem, CXM= ceftazidime, GN= gentamicin, CTX= cefoxatime, CRO= ceftriaxone, LBC= levofloxacin, AZN= azithromycin, OFX= ofloxacin, ERY= erythromycin, AUG= augumentin.

Table 3: Antibiotic resistant profile of the bacterial isolates from Pristine soil

Bacteria genera	No. of isolate	CIP (%)	IMP (%)	CXM (%)	GN (%)	CTX (%)	CRO (%)	LBC (%)	AZN (%)	OFX (%)	ERY (%)	AUG (%)
<i>Bacillus</i>	3	0	33.3	66.7	0	100	100	0	0	0	0	100
<i>Pseudomonas</i>	1	100	100	100	100	100	100	0	100	0	100	100
<i>Klebsiella</i>	1	100	100	100	0	100	100	0	100	100	0	100
<i>Escherichia</i>	2	50	100	100	0	50	100	0	100	0	100	100
<i>Serratia</i>	1	0	100	100	0	100	0	0	100	0	100	100
<i>Proteus</i>	1	0	100	100	0	100	100	0	100	0	100	100
Total	9	33.3	77.8	88.9	11.1	88.9	88.9	0	66.7	11.1	55.6	100

Key: CIP= ciprofloxacin, IMP =imipenem, CXM= ceftazidime, GN= gentamicin, CTX= cefoxatime, CRO= ceftriaxone, LBC= levofloxacin, AZN= azithromycin, OFX= ofloxacin, ERY= erythromycin, AUG= augumentin.

DISCUSSION

Microorganisms, which are important in maintaining the function and stability of the soil ecosystem, can also serve as vehicles for the dissemination of antibiotic resistance from the environment to the food chain. The generic distribution of bacteria associated with the

samples showed that the bacterial genera present in the spent crude oil contaminated soil is more diverse (11) than that of the pristine soil (6). These bacterial genera associated with the spent oil contaminated soil have been implicated in crude oil degradation and in crude oil contaminated soil by previous studies

(Gao *et al.*, 2022; Juaet *et al.*, 2016, Shi *et al.*, 2022). Contrary to the findings of most studies which suggested that crude oil contamination results to soil microbial species reduction (Abena *et al.*, 2020; Wu *et al.*, 2017), the spent crude oil contaminated soil in this study had more bacterial strains (17) than the pristine soil (9), this might be as result of some unexplained extrinsic factors not within the scope of this study.

Co-resistance to antibiotics and heavy metal is an increasing public health concern globally (Edet *et al.*, 2023; Knap *et al.*, 2017; Poole, 2017). All the bacterial strains isolated from the crude oil contaminated soil in this study which are probably resistant to some heavy metals showed resistant to one or more antibiotics. According to Edet *et al.* (2023), there appears to be a synergy between heavy metal and antibiotics resistant, as both have similar selection mechanisms (Li *et al.*, 2017; Meng *et al.*, 2022). Members of *Klebsiella* and *Escherichia* genera showed high multiple drug resistance (9 of the 10 test antibiotics) than other bacterial genera. The isolates showed high resistance (82.4 %) against ceftazidime and ceftriaxone which are both 3rd generation cephalosporins in the class of β -lactam, this is disastrous to the health sector, these results are in line with the reports of Edet *et al.* (2023) and Chen *et al.* (2020). The antibiotic resistant patterns of the bacteria from pristine soil were not different from that of the spent engine oil contaminated soil, this is pointing to the indiscriminate use and disposal of antibiotics in the environment. These bacteria can always find their way into the food chain as well as clinical settings which is a major concern.

CONCLUSION

Antibiotics resistant bacteria were found in both the spent engine oil contaminated site and the pristine soil. However, there was a greater

relative abundance of antibiotics resistant bacteria in the spent engine oil contaminated soil, this has public health implications as there could be transmission of these bacteria into the food chain or the clinical settings. There is a call for concern from the findings of this study, thus, stringent measures on the use of antibiotics and disposal of spent oil should be enforced.

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