Journal of Environment & Biotechnology Research
VINANIE PUBLISHERS
www.vinanie.com/jebr

Evaluation of the physico-chemistry, microbiology and bacterial antibiotic resistance in pharmaceutical wastewaters from South-Western, Nigeria

Avemaria Ifeoma Obasi,[1] Ogochukwu Angela Agwu,[1] Esther Obiageli Ugoji,[1] Akeem Akindele Abayomi,[2] SimonCyril Ugwumba Nwachukwu[1]

[1]Department of Microbiology, University of Lagos, Akoka-Yaba, Lagos, Nigeria
[2]Chemistry department, University of Lagos, Akoka-Yaba, Lagos, Nigeria

ORIGINAL RESEARCH ARTICLE

ABSTRACT

Understanding the components of pharmaceutical wastewaters and their potential risk as sources of pollution when discharged into the environment is imminent for environmental and public health significance. This study was aimed at evaluating untreated wastewaters from pharmaceutical industries in Nigeria. Results obtained showed that the colors of the wastewaters were mostly turbid, few in brown and only one colorless sample; revealed high turbidity (16 - 999 NTU). The pH ranged from 2.9 to 9.2, whereas phosphate and nitrate levels were between the ranges of 0.0122-15.66 mg/L and 0.18-87.02 mg/L respectively. Heavy metal analysis showed high levels of iron at 53.53 mg/L and 25.6 mg/L, with chromium at 7.190 mg/L. The BOD and COD were high for most of the wastewaters. Bacteria isolates were detected in all samples with population ranging from 3.0×10^4 cfu/mL to 2.7×10^{12} cfu/mL, while fungi was averaged at 7.0×10^5 cfu/mL and total coliform was between 2.6×10^2 to 2.7×10^7 cfu/mL. Although total susceptibility to gentamicin and sulphamethozazole/trimethoprin was observed in isolates from two industries, there was an obviously high (20 - 100%) drug resistance among the bacteria tested. The most frequently resisted drugs were ampicillin, sulphamethozazole/ trimethoprin, cefuroxime, augumentin and clindamycin at values of 90.6%, 89.3%, 85,9%, 85.9% and 83.9% respectively; while nitrofurantoin (36.9%) and chloramphenicol (32.2%) were the least resisted drugs. The discharge of wastewaters without adequate treatment into aquatic environments could result in severe pollution, hence the need for proper treatment to protect the ecosystem and public health is necessary.

KEYWORDS
antibiotic resistance, bacteria, heavy metals, pharmaceutical wastewater, pollution

1. INTRODUCTION

The production process proceeding with cleaning of equipment, pipes and floors in pharmaceutical industries usually results in generation of wastewater. The quantity of wastewater generally depends upon the industrial scale and process involved (Venkatappa et al., 2012; Idris et al., 2013; Patneedi and Prasadu, 2015; Bhatti et al., 2017). The 1990 survey conducted by U.S EPA (Environmental Protection Agency) consisting of 244 chemical facilities estimated that the average daily wastewater generation by pharmaceutical industries is about 266 million gallons (Environmental Protection Agency, 1997).

The intricate nature of the wastewater is obvious, consisting of the combination of suspended solids, nutrients, biodegradable organics, microorganisms, heavy metals, refractory organics, salts, dissolved inorganic solids together with active pharmaceutical ingredients (APIs), most especially. These wastewaters get into the environment by direct discharge or through other ways such as seepage from landfills sites, sewer lines and runoff from animal wastes. Although these components undergo various physical and biological processes in the aquatic ecosystem, however, they constantly reveal the varying concentrations which

Corresponding author: **A.I. Obasi**
Tel: +234-803- 331-3473
E. mail: avemariaobasi@yahoo.com

Received: 14-08-2019
Revised: 08-09-2019
Accepted: 13-09-2019
Available online: 01-10-2019

are still being detected in the environment. The content of the wastewater, and in some instances, their metabolites, poses high risk to aquatic life and public health (Fakorede et al., 2013; Patneedi and Prasadu, 2015; Guo et al., 2017).

Concerns over the increasing role of environmental microbes as potential reservoirs of antibiotic resistance genes are rising recently, with a high possibility of transferring these genes to pathogens (Agwu, 2013; Yakubu, 2017). Hence, considering the amount of antibiotics usually present in pharmaceutical wastes, there is the danger of occurrence of drug resistance among the microbes in the waste, and their subsequent release to natural water bodies. The discharge of wastewater into the environment without proper pre-treatment to remove pharmaceuticals may lead to contamination of water sources, particularly drinking water. Wastewater is usually generated daily, so proper disposal should be regulated by the government regulatory authorities in order to protect public health. Developed countries may be exploring new technologies in treatment plants, such as flocculation, ozonation, advanced oxidation, membrane filtration and photocatalysis to improve treatment of pharmaceutical wastes (Packer et al., 2003; Larsen et al., 2004; Zuccato et al., 2006; Placide et al., 2016). However, most developing countries such as Nigeria are yet to scrutinize the wastewater from treatment plants before releasing into the lakes and rivers (Ngwuluka et al., 2011). For that reason, this study was aimed at assessing the level of physical and chemical pollutants of environmental significance, as well as characterising the bacteria with antibiotic resistance in pharmaceutical wastewaters from South-Western, Nigeria.

2. MATERIALS AND METHODS

2.1. *Description of study area*

This study was conducted in Lagos and Ogun states in the South-Western part of Nigeria where most of the pharmaceutical industries are located. The industry samples were selected taking into due consideration that the wastewaters are untreated, and these are discharged directly into the environment, such that the contaminants and the pollution dynamics of the wastewaters seep into habitats of plants, animals as well as human ecosystems (Figure 1).

2.2. *Sampling design and operation*

Ten pharmaceuticals industries were selected randomly for sampling, of which four were from Lagos and six were from Ogun states.Wastewater discharged

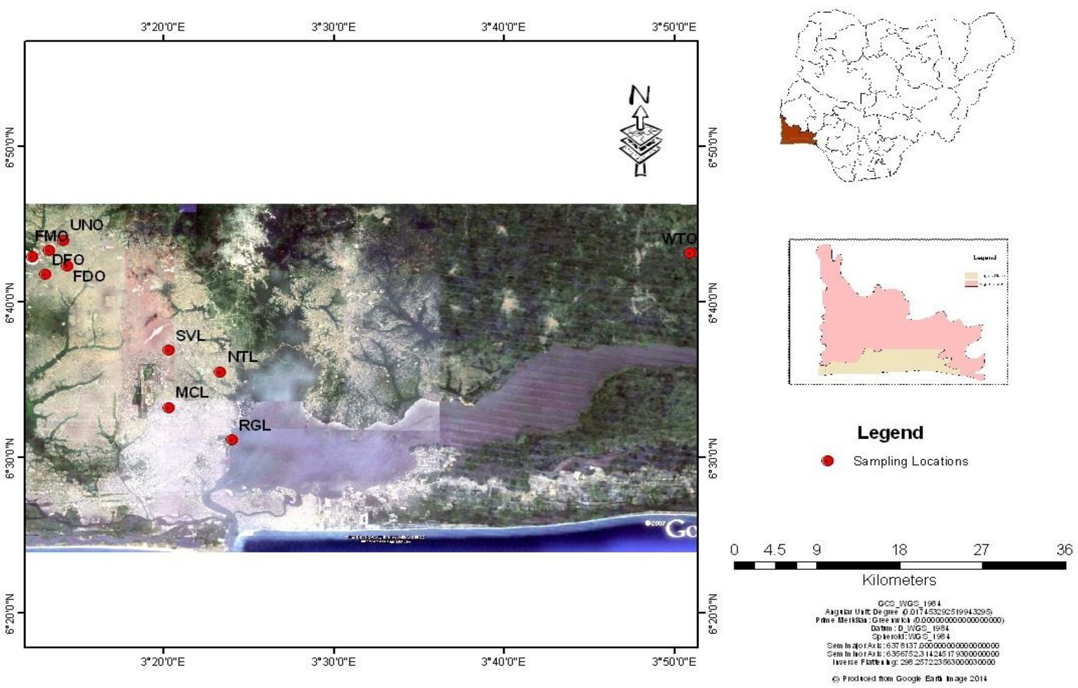

Figure 1. Map of Lagos and Ogun states showing sampling locations of wastewaters from pharmaceutical industries in South-western Nigeria

from the product manufacturing division at the time of sampling were not taken into consideration. Untreated wastewater samples (n=30) were collected from discharge points directly into 1.5 L sterile plastic bottles, preserved in cold storage and immediately taken to the laboratory for routine physico-chemical and microbiological analysis. Sampling period was 18 months; April, 2011 to October, 2012. The industries are producers of antibiotics, e.g. ciprofloxacin and cotrimoxazole; analgesic agents, cough syrups and a few other healthcare products.

2.3. Physico-chemical Analysis

The following physicochemical parameters were done in the analytical laboratory of the department of chemistry, University of Lagos, Nigeria.

2.3.1. Physical parameters

Colour, temperature, electrical conductivity, pH, turbidity (ftu), total suspended solids (TSS), total dissolved solids (TDS), and total solids (TS) were analyzed using standard methods (AOAC, 1990).

2.3.2. Chemical parameters

Chloride (mg/L), salinity (%), total alkalinity (mg/L), total acidity (mg/L), total hardness (mg/L) and oil and grease (mg/L) were analyzed using the titrimetric method (AOAC, 1990).

2.3.3. Environmental parameters

Dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD) using Winklers method (Alsterberg) (Helm et al., 2012; Hassaan, 2016; Manyuchin et al., 2018).

2.3.4. Nutrient composition

The nutrient composition of the water samples was analyzed using standard chemical methods using the spectrophotometer (model spectronic 20^{D+}) as described by the standard analytical methods for water and wastewater (Rice et al., 2012). The following nutrients are analyzed ; nitrite (mg/L), nitrate (mg/L, phosphorous (mg/L), sulphate (mg/L), potassium (mg/L), magnesium (mg/L), calcium (mg/L) and sodium (mg/L)

2.3.5. Heavy metal analysis

Water samples were digested by an oxidizing acid and the digests were analysed using appropriate lamps of flame atomic absorption spectrophotometer (AAS; model: Analyst 200 Perkins Elmer) (Campbell and Ingram, 2014). The heavy metals analyzed include: cadmium (mg/L), manganese (mg/L), zinc (mg/L), copper (mg/L), lead (mg/L), iron (mg/L) and chromium (mg/L).

2.4. Microbiological analysis: enumeration and isolation of microorganism

The standard ten-fold serial dilution of the wastewater sample was carried out and aliquots of the lower, middle and high dilutions were inoculated in duplicates into already prepared plates of Nutrient Agar (Biotec; UK) for bacteria; Potato Dextrose agar for fungi and MacConkey agar for total coliform. These were incubated aerobically at 37°C for 18-24 h for the overall bacteria count. Colonies on plates were observed and counted and the population density estimated, bacterial colonies were picked according to their cultural morphology on the plates and these were streaked on new nutrient agar plates for pure colonies (Nwachukwu and Apata, 2003; Forbes et al., 2017). Gram staining was done to determine the Gram-reaction of the bacterial isolates.

2.5. Antibiotic susceptibility tests

After proper purification of selected morphologically different bacterial isolates, the isolates were grown on nutrient broth (NB) at 37 °C for 24 h. A McFarland standard of 0.5 was achieved with an overnight culture in a tube and a sterile cotton wool swab dipped into the bacterial suspension was streaked over the entire sterile Mueller–Hinton agar plates, streaking was repeated two more times, rotating the plates approximately 60° each time to ensure even distribution of inoculums. As a final step, the rim of the agar is swabbed. The inoculated plates were allowed to dry before placing the diffusion discs containing antibiotics. Susceptibility of the isolates to 13 types of antibiotics was performed using the standard Kirby-Bauer method as described (Robert et al., 2009). Commercially available discs (Oxoid and Abtek, UK) containing ampicilline (AMP10), gentamycine (GEN10), ciprofloxacine (CPR5), ofloxacine (OFL5), augumentine (AUG30), ceftazidime (CAZ30), cefurozime (CRX30) and nitirofurantoin (NIT300), erythromycin (E15); clindamycin (DA2); nalidizic acid (NA30); chloramphenicol (C30); sulphamethozazole/trimethoprin (SXT25) were placed on the surface of the agar plates and incubated at 37 °C for 24 h. The diameters of inhibition zones formed

Table 1. Physical parameters of wastewater samples from ten pharmaceutical industries in Lagos and Ogun state environs of South-Western Nigeria

Parameter	FDO	WTO	NGO	SVL	NTL	MCL	RGL	DFO	FAO	UNO
Colour	Turbid	Brown	Tea	Turbid	Colourless	Brown	Turbid	Turbid	Turbid	Turbid
pH {6-9}	5.1	8	3.5	5.1	6.2	5.1	9.2	2.9	3.1	5
Conductivity (µS/cm) {500}	411.3	419.4	368.8	363	364.1	2997.8	760	359	674.6	320
Turbidity (NTU) {0}	122	648	457	217	0	999	20	181	65	16
TSS (mg/L) {30}	570	2270	3250	1220	0	73090	200	960	250	ND
TDS (mg/L) {2000}	720	1040	1230	260	500	22780	380	1030	840	160
TS (mg/L)	1290	3310	4480	1480	500	95870	ND	1990	1090	ND
Salinity (g/L) {0}	288.3	290.7	273.9	273.7	273.7	1064	ND	272.5	367.8	0
Alkalinity (mg/L) {50}	63.6	2650	45.8	64.6	84.8	2250	86	NIL	42.4	ND
Hardness (mg/L) {120}	630	9760	6840	5400	90	11160	400	540	6080	180

TSS, Total suspended solids; TDS, Total dissolved solids; TS, Total solids; {}: Environmental Protection Agency (EPA) standard limits for wastewater

surrounding each isolate were measured including the diameter of the discs. Results were expressed as susceptible (21 mm); intermediate (16 to 20 mm) or resistant (≤15 mm) following a standard range (Liasi et al., 2009). *Escherichia coli* and *Bacillus subtilis* served as control strains for these assays.

2.6. Frequency of antibiotic resistance

A descriptive chart obtained from GraphPad Prism (5.0 version) from windows 8.1 operating system were used to analyze the data for the frequency of antibiotic resistance of the bacterial isolates.

3. RESULTS AND DISCUSSION

The environmental impact of the physical, chemical and microbiological characteristics of wastewaters from pharmaceutical industries disposed into receiving water bodies is crucial and need to be evaluated for public health significance. The present study assessed these parameters in the pharmaceutical wastewaters from South-Western Nigeria.

3.1. Physico-chemical analysis

3.1.1. Physical parameters

The physical parameters of the wastewater samples from ten pharmaceutical industries in Lagos and Ogun state environs of South-Western Nigeria is depicted in Table 1. The colour of these wastewaters were either turbid or brown with exception that one (NTL) was colourless. The conductivity values were within the limit value (500 µS/cm), however, two industries (RGL and FAO) had slightly higher values while MCL had recorded very high value of 2997.8 µS/cm. Aside the wastewater from NTL which had zero turbidity, all the other samples had high turbidity value. A similar result was also noted for total suspended solids, while there were none detected in the samples from NTL and UNO, other samples had very high values (200 – 73,090 mg/L) beyond the EPA limits. The total dissolved solid values observed in most of the samples were within the acceptable limit (2000 mg/L) except for the sample from MCL which had 22780 mg/L. Total Solids was not detected in two samples (RGL and UNO) meanwhile the value noted in the other samples ranged between 500 and 95870 mg/L. Suspended solids are the major physical pollutants as they cause turbidity, reduce light penetration and hence hinder photosynthesis (Lester and Birkett, 2018). They are also associated with dramatic change in the concentrations of the water-quality variables (Yang et al., 2017). According to Placide et al. (2016), total suspended solids signify the total organic and inorganic particles in the wastewaters, and after observing low values (< 50 mg/L) in wastewaters from the teaching hospital of Treichville, they proposed that most of the pollutants were soluble. On the contrary, high values (≥ 200mg/L) were encountered in almost all the wastewaters in this study, suggesting that most of the pollutants are non-soluble. It is also noteworthy that high concentration of total soluble solids also resulted in reduction of oxygen in the water sample (Agwu, 2013). Furthermore, Singal and Kaur (2018) had noted that untreated effluent containing loads of suspended substances appear cloudy and exhibits either very high or low pH values. The pH of most of the samples tends towards acidic (pH 2.9 – 5.1), while a few had pH between 6 and 9 which are within the EPA acceptable limit for effluent discharge. Aquatic organisms are naturally adapted to pH values of between 6 and 8; hence any considerable change will affect their lives. Aside direct effect of pH values to living organisms in the environment, they also exert secondary effect by influencing other environmental components. For instance, the solubility and toxicity of heavy metals tend to increase at acidic media (Lester and Birkett, 2018). This suggests that the possible impact these wastewaters will have when they enter natural water bodies could be enormous and this is worrisome.

3.1.2. Chemical parameters

Parameters for the chemical attributes of the wastewaters are shown in Table 2; the level of chloride was higher than the stipulated limit of 600 mg/L, only in sample from MCL; the other samples had values ranging from 0 to 274.9 mg/L which were within the acceptable concentrations in all wastewaters except in one sample (MCL) at 2281 mg/L. This element is also known to occur in a variety of concentrations in natural water and is usually influenced by the dissolution of salts deposits and discharge of untreated wastewaters from chemical industries. It becomes injurious to human health when high concentrations are present particularly in drinking water (Venkatappa et al., 2012; Bhatti et al. 2017). The alkalinity of four samples (NGO, DFO, FAO and UNO) was within acceptable value while the other six samples were above the 50 mg/L. The alkalinity is used to support water quality metrics for additional chemical and biological parameters which is an important task for water quality regulators (Tappin et al., 2018). The salinity of two samples was at zero but the other samples had very high salinity values (273.7 - 1064 g/L). Rusydi (2018) reported that conductivity and total dissolved solids are water quality parameters which are used to describe salinity level. The value obtained for hardness ranged between 90 and 11160 mg/L and only the sample from NTL was below the acceptable limit of 120 mg/L. Hard water is classified based on the Ca^{2+} and Mg^{2+} ion concentration in the water as follows: 0-60 mg/L soft; 61-120 mg/L as moderately hard; 121-180 mg/L as hard and more than 180 mg/L as very hard water. Most water utilities consider a hardness level between 50 and 150 mg/L CaCO3 as publicly accepted (Ahn et al., 2018). Oil and grease were absent from most of the wastewater samples and reasonably low (0.1 and 1.56 mg/L) in the two samples where they were detected. Oily wastewater pollution is manifested in affecting drinking water and ground water resources, endangering human health, causing atmospheric pollution, affecting crop production and destruction of the natural landscape (Yu et al., 2017).

3.1.3. Environmental parameters

Dissolved Oxygen (DO) was not detected in five of the ten samples tested, the other five samples showed between 2.2 and 7.6 mg/L. The BOD of two samples (NTL and UNO) was below the acceptable limit while the other samples had very high values range of 56 to 976.5 mg/L. The COD of all the samples were between 72 and 8536.2 mg/L which was above the <1.0 mg/L acceptable limit. Biological oxygen demand (BOD) and Chemical oxygen demand (COD) are also recognized as crucial pollution index for industrial wastewaters. BOD measures the total amount of oxygen required by bacteria for complete decomposition of organic matters, while COD determines the oxygen needed for oxidation of both available and inert organic matters (Lokhande et al., 2011; Venkatappa et al., 2012; Rim-Rukeh and Agbozu, 2013; Lee and Nikraz, 2014; Bhatti et al., 2017; Singal and Kaur, 2018). A good percentage (70%) of the samples tested in the present study had very high levels of BOD and COD values, exceeding recommended 50 mg/L. This further supports the fact that these wastewater samples will be a source of pollution to any receiving water body if not adequately treated. In this study, considering the high levels of BOD in most of these wastewaters, it was not surprising to note that dissolved oxygen (DO) was either absent or obviously low in most of wastewater samples. Since

Table 2. Chemical parameters of wastewater samples from ten pharmaceutical industries in Lagos and Ogun state environs of South-Western Nigeria

Parameters	FDO	WTO	NGO	SVL	NTL	MCL	RGL	DFO	FAO	UNO
Chloride (mg/L){600}	45.6	52.6	4.2	3.5	3.5	2281	96	NIL	274.9	66
Sulphate (mg/L){500}	41.9	2002.1	6.6	41.1	19.7	1237.5	22.5	23.3	135.4	5
Phosphate (mg/L){5}	0.04	0.09	0.012	0.622	4.952	15.66	1.18	0.13	0.99	1.96
Nitrite (mg/L){1.0}	0.04	0.24	0.043	0.025	0.045	0.21	0.3	0.04	0.01	0.01
Nitrate (mg/L){20}	6.96	17.4	3.494	2.09	2.44	10.45	3.48	87.02	8.01	0.18
Oil and grease (mg/L){10}	NIL	NIL	NIL	NIL	NIL	NIL	1.56	NIL	NIL	0.1
DO (mg/L){4.0}	NIL	NIL	NIL	7	7.6	NIL	2.2	NIL	4.8	4.7
BOD (mg/L){50}	773.2	841.2	536.1	134.8	44.8	976.5	56	668.4	276.2	36
COD (mg/L) {<1.0}	2704.2	3450.7	2816.4	224	51.8	8536.2	100	3080.4	403.2	72
Calcium (mg/L){200}	0.846	0.824	0.781	0.668	0.794	8.61	280	0.646	0.791	120
Potassium (mg/L){200}	0.088	0.069	0.061	0.128	0.076	0.88	ND	0.088	0.106	ND
Magnesium (mg/L){200}	0.081	0.024	0.046	0.096	0.058	3.9	120	0.061	0.086	60
Zinc (mg/L){<1}	0.034	0.062	0.044	0.056	0.066	1.24	1.45	0.041	0.068	0.88
Copper (mg/L) {<1}	ND	0.002	0.302	0.208	0.153	0.28	0.86	0.048	0.126	0.82
Lead (mg/L){<1}	0.1	0.011	0.009	ND	0.061	0.14	0.12	0.009	0.014	0.22
Chromium (mg/L){<1}	ND	ND	ND	0.58	0.459	7.19	ND	ND	0.719	0.31
Iron (mg/L){20}	ND	ND	ND	0.293	0.074	53.53	25.6	1.697	ND	36.45

{}: Environmental Protection Agency (EPA) standard limits for wastewater

elevated BOD values consequently reduce the dissolved oxygen as most of the available oxygen will be used by the microbes (Al-Othman, 2015).

3.1.4. Nutrient composition

The concentrations of sulphate in eight wastewater samples tested were evidently low and within the limit; however two samples (WTO and MCL) had very high concentrations of 2002.1 mg/L and 1237.5 mg/L, exceeding the limits. Likewise, the phosphate concentrations were within the acceptable range in most of the wastewater samples except for one which had a considerable high value (15.66 mg/L). Interestingly, Nitrite concentration was tolerable in all the samples, meanwhile nitrate concentration was over the acceptable limit in DFO (87.02 mg/L), but the other samples had lower concentrations. Nitrogen and phosphorus are usually used to evaluate the nutrient status of wastewaters (Singal and Kaur, 2018). The concentrations of the nitrates, phosphates and sulphates in the wastewater tested in this study were within the acceptable limits although a few had higher concentrations. In accordance with an earlier report by James et al. (2014), they attributed the low concentration of these compounds in pharmaceutical wastewater to the absence or minimal concentration of nitrates, phosphates and sulphates in the raw materials used in drug production. Sulphate as a stable highly oxidized soluble form of sulphur serves as an alternate electron acceptor in oxygen depleted ecosystems (Venkatappa et al., 2012).

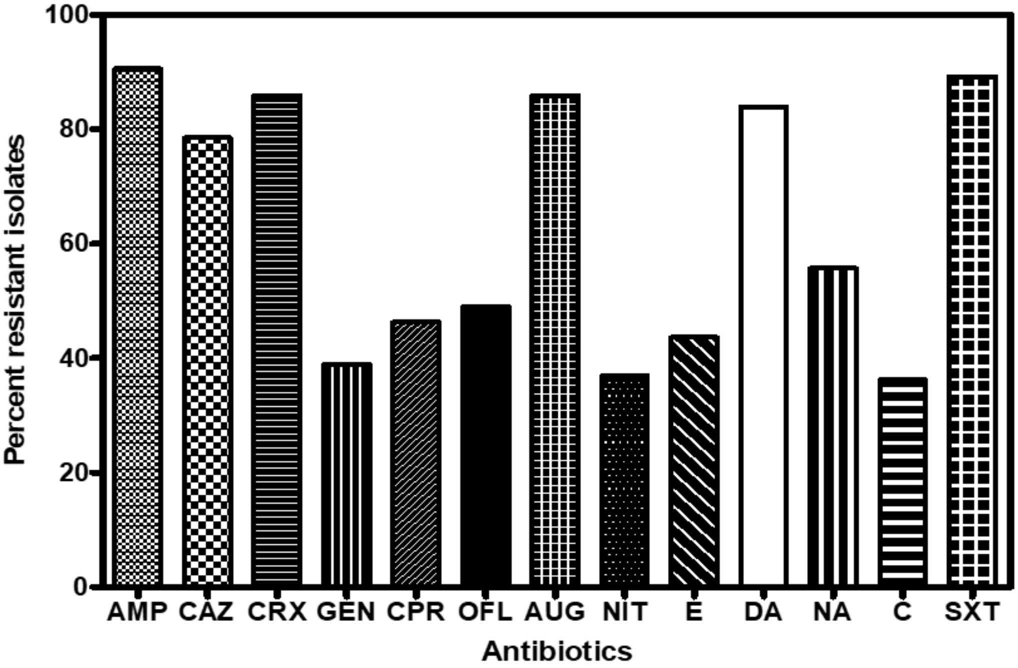

Figure 2. Frequency of antibiotic resistance of bacterial isolates from pharmaceutical wastewaters in south-western Nigeria. (AMP10, ampicillin; CAZ30, ceftazidime; CRX30, cefuroxime; GEN10, gentamycin; CPR5, ciprofloxacine; OFL5, ofloxacine, AUG30, augumentine; NIT300, nitrofurantion, E15, erythromycin; DA2, clindamycin; NA30, nalidizic acid; C30, chloramphenicol; SXT25, sulphamethozazole/trimethoprin).

3.1.5. Heavy metal analysis

Assessment of the metal components of the wastewater samples revealed that the concentration of potassium, zinc, copper and lead were below the acceptable limits. However, a high concentration (280 mg/L) of calcium was obtained in the sample from RGL. Also, MCL sample exhibited elevated concentration of chromium. On the other hand, iron concentrations of 53.53 mg/L, 25.6 mg/L and 36.45 mg/L observed in three samples (MCL, DFO and UNO, respectively) were higher than the acceptable limits. Although small amounts of heavy metals are essential for metabolic processes in living organisms, it is also pertinent to note that high concentrations of these elements can be detrimental (Singal and Kaur 2018). Therefore, determination of their concentrations in wastewater prior to disposal is also crucial. Interestingly, majority of the heavy metals tested in this study were less than the acceptable limit (< 1 mg/L). Nevertheless, the high concentrations of chromium and iron in a few of the wastewater samples require attention before discharge into the environment.

Indeed, when natural ecosystems are exposed to these multipart pharmaceutical wastes over a long period of time, the inhabitants are impacted in diverse ways including acute and chronic damages, behavioral changes, accumulation of toxic components in tissues, reproductive damages and cell multiplication inhibition (Patneedi and Prasadu, 2015; Placide et al., 2016).

3.2. Microbiological analysis: Microbial population and morphological characterization

Bacterial isolates were present in all the wastewater sample tested (Table 3). However, the population density ranged between 3.6 x 10^4 and 2.7 x10^{12} cfu/mL, with the highest value observed in UNO. Contrary to the bacterial occurrence in the wastewater samples, fungi were not detected in two samples (NTL and RGL). Meanwhile the population noted in the other samples were in the range of 1 x 10^3 to 6.3 x 10^6 cfu/mL. Similarly, coliform bacteria were not detected in three samples (FDO, FAO and UNO) while an average of 3.07 x 10^6 cfu/mL was observed in the other seven wastewater samples. Morphological characterization of bacterial isolates on plates showed that they were mostly roundish, transparent and opaque, milky in colour, raised or flat, and smooth on the surface. To be precise, 145 bacteria were isolated, of which 121 were bacilli of tiny, short, long and slender rods; 21 were cocci of various sizes and 3 were coccobacilli.

The bacterial counts corresponded with the counts earlier reported for the pharmaceutical

Table 3. Population of bacteria, fungi and total coliform in ten pharmaceutical wastewaters from Lagos and Ogun state environs of South-Western Nigeria

Pharmaceutical Industry	Bacterial (cfu/mL)	Fungi (cfu/mL)	Total coliform (cfu/mL)
FDO	3.0×10^4	2.0×10^3	ND
NGO	2.8×10^7	6.3×10^6	2.7×10^7
WTO	1.1×10^8	1.0×10^5	2.6×10^2
SVL	8.0×10^4	1×10^3	1.2×10^5
NTL	1.4×10^8	ND	5.1×10^2
MCL	3.8×10^7	1.4×10^5	4.4×10^5
RGL	6.0×10^6	ND	3.1×10^6
DFO	1.2×10^5	4.0×10^5	5.3×10^3
FAO	3.6×10^4	1.0×10^5	ND
UNO	2.7×10^{12}	4.8×10^4	ND

ND = not detected

Table 4. Percentage (%) values in antibiotic resistance of bacteria from ten pharmaceutical wastewaters discharged into the environs in South-Western Nigeria

Antibiotics	FDO	NGO	WTO	SVL	NTL	MCL	RGL	DFO	UNO	FAO
AMP10	77.78	80	83.33	97.71	80	100	87.5	93.75	81.82	100
CAZ30	77.78	75	75	80	90	75	87.5	68.75	72.73	84.62
CRX30	77.78	70	83.33	94.29	90	87.5	87.5	93.75	72.73	84.62
GEN10	0	40	41.67	51.43	70	12.5	50	56.25	18.18	23.08
CPR5	11.11	20	33.33	82.86	70	43.75	62.5	25	36.36	30.77
OFL5	11.11	35	41.67	82.86	80	37.5	75	25	36.36	23.08
AUG	66.67	80	83.33	94.29	90	93.75	87.5	87.5	81.82	69.23
NIT300	22.22	20	41.67	28.57	80	37.5	75	50	27.27	23.08
E 15	11.11	35	66.67	57.14	70	31.25	25	62.5	27.27	15.38
DA2	77.78	90	66.67	88.57	80	75	87.5	87.5	72.73	92.31
NA30	33.33	65	50	71.43	90	50	50	50	36.36	23.08
C30	0	30	41.67	68.57	60	25	12.5	43.75	0	7.69
SXT25	100	90	91.67	88.57	90	87.5	87.5	93.75	72.73	84.62

AMP10, ampicillin; CAZ30, ceftazidime; CRX30, cefuroxime; GEN10, gentamycin; CPR5, ciprofloxacine; OFL5, ofloxacine, AUG30, augumentine; NIT300, nitrofurantion, E15, erythromycin; DA2, clindamycin; NA30, nalidizic acid; C30, chloramphenicol; SXT25, sulphamethozazole/trimethoprin

wastewater (Lateef, 2014) and brewery wastewater (Fakorede et al., 2013). Also, the total coliform counts were comparable to the counts from a hospital effluent as reported by Placide et al. (2016). The high occurrence of microbes in these wastewaters is expected; owing to the high level of nutrients present, the heterotrophic nature of most microorganisms makes it possible for them to utilize the organic matter in the wastewater and subsequently proliferate profusely. Furthermore, the observed physical parameters such as pH were within the range for microbial survival. The occurrence of total coliforms in some of the wastewater samples also indicated fecal contamination of the samples. These groups of bacteria are normal flora of human intestinal organs hence it might be possible that contamination occurred during production processes by personnel, as a result of poor sanitary and handling techniques.

3.3. Antibiotic susceptibility of bacterial isolates from the different wastewaters

The bacterial isolates from the different pharmaceutical wastewaters exhibited different levels of resistance to the 13 antibiotics tested (Table 4). A total of 145 bacterial isolates from the 10 wastewater samples were used for antibiotic susceptibility studies. Antibiotic resistance for all bacterial isolates for possible detection of antibiotic resistant bacteria (ARB), were analyzed for each wastewater. All the isolates from FDO were susceptible to gentamycin and chloramphenicol, nevertheless they were all resistant to sulphamethozazole/trimethoprin with about 11 to 78% resistant to the other antibiotics. A total susceptibility to chloramphenicol was also noted in isolates from UNO, while a reasonable percentage of them (27 – 82%) resisted the other antibiotics. An outstanding percentage resistance (60≥90%) to all the tested antibiotics was observed in the isolates from NTL. The percentage susceptibility value of the isolates from the other pharmaceutical wastewaters that were resistant to the antibiotics ranged from low (< 30%) to moderate (30 ≥ 50%) and very high (55 ≥ 100%). Bacterial antibiotic resistance was high for most drugs tested in this study. The resistance to ampicillin was most prominent and it is noteworthy that Agwu (2013) had reported high resistance of this drug among heterotrophic bacteria from Lagos lagoon surface water. Indeed, resistance to beta lactam class of antibiotics to which ampicillin belongs to, has been frequently noted and attributed to the ability of many bacterial species, due to their production of the beta lactamase enzyme which destroys the beta lactam ring of the drug. Besides, there are other basic mechanisms of resistance which involve inactivation, the creation of substitute metabolic pathways, impermeability of cytoplasmic membranes, alteration in the target site and drug modification. However, inherent resistance could also be a possibility and sometimes combination of mechanisms of resistance such as impermeability and efflux (Weldhagen et al. 2003; Al Naiemi et al., 2006; Ong et al., 2011). The bacteria isolated by Lateef (2014) from pharmaceutical effluents showed higher resistance (76%) to nitrofurantoin compared to 36.9% resistance noted by the isolates of this study. Nevertheless, the percentage resistances (55.7% and 52%) to nalidixic acid in the isolates from the two studies were similar. Considering that some of the pharmaceutical industries were producing antibiotics during the period of wastewater collection, it is obvious that residues of these antibiotics will remain in the wastewater as reported in previous studies (Bhatti et al., 2017; Obayiuwana et al., 2018). Li et al. (2010) had also implied widespread of tetracycline and other antibiotic resistance gene in effluent receiving water is due to the selective pressure exerted by elevated concentration of oxytetracycline from the production plant. Furthermore, Yakubu (2017) had noted that antibiotics form a substantial component of active pharmaceutical ingredients (API) found in the environment, and various concentrations have been detected in industrial effluents.

3.4. Frequency of antibiotic resistance

The frequency at which the isolates in this study resisted the thirteen antibiotics tested, is depicted in Figure 2 utilizing the GraphPad statistical tool. The highest percentage of resistance (90.6%) was observed for ampicillin, followed by 89.3%, 85.9%, 85.9% and 83.9% for sulphamethozazole/trimethoprin, cefuroxime, augmentin, clindamycin respectively. On the other hand, the percentage resistance of bacterial isolates to the other antibiotics ranged between 36.2% to 55.7%. According to Hancock (1998), selective pressure such as antibiotic contact results in observable change in genotype because of exposure of the strains to a set of inducing conditions. These resistant strains might have resistance gene coded in the R plasmid or coded in the transposon (Herwig et al., 1997). Plasmids play a major role in microbial ecology and evolution as vehicles of lateral gene transfer and reservoirs of accessory gene functions in microbial populations (Wein et al., 2019). The R plasmid can transfer genetic material among different species through conjugation and transformation processes. Hence, when these wastewaters are discharged into water bodies, these resistance genes can be disseminated in nature and transferred to pathogenic counterparts of bacterial species by genetic mobile elements, this will further increase difficulty in the treatment of infections (Wellington et al., 2013). Thus, there is need for effective treatment of the drug wastewaters before they are discharge into the environment.

This preliminary study has been able to establish the fact that the wastewaters from pharmaceutical industries in Nigeria were polluted and the bacterial isolates showed resistance to antibiotics. There is also a possibility of co-resistance of these heavy metals and antibiotics in the bacterial isolates of this study which could have also contributed to the development of multidrug-resistance in microorganisms, even in the absence of antibiotics. It has been shown that continued exposure of microorganism to chromium in industries might contribute to the development of multidrug-resistance in microorganisms even in the absence of antibiotics (Mahmud et al., 2015). Also, it has been observed during a study on the role of intracellular iron in antibiotic-induced mutagenesis by Mehi et al. (2014) that iron uptake, storage, and metabolism have a key role in stress-induced mutagenesis during antibiotic stress in *E. coli* K-12. The development of future antimicrobial strategies was suggested, through binding unincorporated free iron. Iron chelators can slow down the development of resistance, i.e. targeting bacterial iron uptake and iron metabolism using novel compounds, as iron appears to be critical for both bacterial growth and mutagenesis (Mehi et al., 2014). Microorganisms are capable of adapting rapidly to heavy metal toxicity mostly by gaining mutations, by nonspecific adsorption phenomenon or by the spreading of the resistance factor (R-factor). Thus, future research will disclose the possible mechanism underlying the existence of such dual resistance, and also its impact on public health as well as the identity of the species of all the isolates, using the most widely known method; genetic techniques such as the 16S rRNA gene analysis for phylogenetic study.

4. CONCLUSIONS

Through this study, the pollution levels in various pharmaceutical wastewaters were evaluated using number of the physicochemical parameters, and the results revealed an elevated microbial load and presence of total coliforms, indicating fecal contamination of some wastewaters. Although high levels of chloride, sulphate, phosphate and nitrates were detected in at least one of the samples tested, most of these were within the prescribed limit for most of the samples. However, BOD and COD values detected were very high; iron levels in some of the samples were also high, as well as chromium in a sample. In addition, the frequencies of antibiotic resistance of the bacterial isolates from the industrial wastewater samples were enormous, ranging from 83.9% to 90.6% for clindamycin and ampicillin. Analyzing the antibiotic resistance profile and detected heavy metals, one could attribute the high levels of antibiotic resistance of bacteria from few wastewaters to de due to the heightening of iron in the wastewaters as observed in MCL and RGL. In addition to selective pressure in antibiotic resistance acquisition, this data further supports the role of iron in bacterial resistance from the wastewaters; revealing the fact that targeting iron acquisition could be a novel approach to explore for future antimicrobial drug development. Inappropriate disposal of chromium or chromium containing by-product into the environment causes severe pollution and these call for serious environmental and public health concerns. This study further affirms that pharmaceutical wastewaters are complex in nature with both organic and inorganic, and biological pollutants.

ACKNOWLEDGMENT

We thank Adebayo Shittu from Obafemi Awolowo University Nigeria for his mentorship; Olufunke Oyebamiji and Folashade Agbaje-Daniels for their various contributions.

REFERENCES

Agwu, O.A. (2013) Antibiotic resistance among heterotrophic bacteria in Lagos Lagoon, Nigeria. African Journal of Aquatic Science, 38, 331–336.

Ahn, M.K., Chilakal, R., Han, C. and Thenepalli, T. (2018) Removal of hardness from water samples by a Carbonation process with a closed pressure reactor. Water, 10 (1), 54.

Al Naiemi, N., Dium, B. and Bart, A. (2006) A CTX-M extended-spectrum beta-lactamase in *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. Journal of Medical Microbiology, 55, 1607-1608.

Al-Othman, A. (2015) Seasonal variations in surface water quality index of the mainstream running through Riyadh, Saudi Arabia. Research Journal of Environmental Toxicology, 9, 71–89.

AOAC, (Association of Official Analytical Chemist) (1990) Official methods of analysis of the AOAC. 15th ed. Arlington: VA, USA, 1990; Methods 932.06, 925.09., 985.29, 923.03

Bhatti, M.A., Almani, K.F., Mastoi, G.M., Qureshi, M.A. and Qureshi, M.M. (2017) Physico-chemical study of effluents of pharmaceutical industries of Hyderabad division Sindh Pakistan. International Journal of Environmental Chemistry, 1, 28–31.

Campbell, N.R. and Ingram, J.C. (2014) Characterization of 234U/238U Activity Ratios and potential Inorganic Uranium Complexation species in unregulated water sources in the southwest region of the Navajo reservation. Journal of Water Reclamation and Sustainability, 14, 77-94.

Environmental Protection Agency (EPA) office of compliance (1997) Profile of the pharmaceutical industry. EPA/310-R-97-005 SIC Code: 2833, 2834, 48-50

Fakorede, C.N., Igbeneghu, O.A. and Odeyemi, O. (2013) The microbiological and physicochemical characterization of wastewater from a brewery in southwest Nigeria: A case study. Issues in Biological Sciences and Pharmaceutical Research, 1, 001-007.

Forbes, B.A., Sahm, D.F. and Weissfeld, A.S. (2017). Bailey & Scott's Diagnostic Microbiology-E-Book, 13th ed. Elsevier Health Sciences.

Guo, Y., Qi, P.S. and Liu, Y.Z. (2017) A review on advanced treatment of pharmaceutical wastewater. IOP Conference Series Earth Environmental Science, 63, 12-25.

Hancock, R.E.W. (1998) Resistance mechanism in *Pseudomonas aeruginosa* and other non-fermentative Gram-negative bacteria. Journal of Clinical Infectious Disease, 27 , S93-S99.

Hassaan, M. (2016) Wrinkler method for DO determination. National Institute of Oceanography and fisheries.

Helm, I., Jalukse, L. and Leito, I. (2012) A highly accurate method for determination of dissolved oxygen: Gravimetric Wrinkler method. Analytica Chemica Acta. 741, 21-31

Herwig, R.P., Gray, J.P. and Weston, D.P. (1997) Antibacterial resistant bacteria in surface sediments near salmon net-cage farms in Puget Sound, Washington. Aqua, 149, 263-283.

Idris, M.A., Kolo, B.G., Garba, S.T. and Ismail, M.A. (2013) Physico-chemical analysis of pharmaceutical effluent and surface water of river Gorax in Minna, Niger State, Nigeria. Bulletin of Environment, Pharmacology and Life Sciences, 2(3), 45-49.

James, O.O., Oluwaleye, S.E., Saka, K.L.and Olabanji, D.J. (2014) Physico-chemical characteristics of pharmaceutical effluents from Sango industrial area, Nigeria. Bulletin of Environment, Pharmacology and Life Sciences, 3, 78-82.

Larsen, T., Lienert, J., Joss, A. and Siegrist, H. (2004) How to avoid pharmaceuticals in the aquatic environment. Journal of Biotechnology, 113, 295-304.

Lateef, A. (2014) The microbiology of a pharmaceutical effluent and its public health implications. World Journal of Microbiology and Biotechnology, https://doi.org/10.1023/B

Lee, A.H. and Nikraz, H. (2014) BOD : COD Ratio as an indicator for river pollution. International Proceedings of Chemical, Biological and Environmental Engineering, 88, 89–94.

Lester, J.N. and Birkett, J.W. (2018) Microbiology and Chemistry for Environmental Scientists and Engineers, Taylor and Francis. E-book. London.

Li, D., Yu, T., Zhang, Y., Yang, M., Li, Z., Qi, R. and Li, D. (2010) Antibiotic resistance characteristics of environmental bacteria from an oxytetracycline production wastewater treatment plant and the receiving river. Applied Environmental Microbiology 76, 3444–3451.

Liasi, S.A., Azmi, T.I., Hassan, M.D., Shuhaimi, M., Rosfarizan, M. and Ariff, A.B. (2009) Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish product, Budu, Malaysian. Journal of Microbiology, 5, 33-37.

Lokhande, R.S., Singare, P.U. and Pimple, D.S. (2011) Study on physico-chemical parameters of waste water effluents from Taloja industrial area of Mumbai, India. Industrial Journal of Ecosystem, 1, 1–9.

Mahmud, J., Chowdhury, M., Sami, A.B., Akhand, A.A. and Ahsan, N. (2015) Co-resistance to chromium and antibiotics in bacteria isolated from tannery wastes. Dhaka University Journal of Pharmaceutical Science, 14, 193-197.

Manyuchin, M.M., Mbohwa, C. and Muzenda, E. (2018) Anaerobic treatment of opaque beer wastewater with enhanced recovery through Actizyme bio augmentation. South African Journal of Chemical Engineering, 26, 74-79.

Mehi, O., Bogas, B., Csorgo, B., Pal, F., Nyerges, A., Papp, B. and Pal, C. (2014) Perturbation of Iron Homeostasis promotes the evolution of Antibiotic resistance. Molecular Biology and Evolution, 31, 145-145.

Ngwuluka, N.C., Ochekpe, N.A. and Odumosu, P.O. (2011) An assessment of pharmaceutical waste management in some Nigerian pharmaceutical industries. African Journal of Biotechnology, 10, 11259-11268.

Nwachukwu, S.C.U. and Apata, T.V.I. (2003) Principles of quantitative microbiology, 1st edn. University of Lagos Press, Lagos, Nigeria

Obayiuwana, A., Ogunjobi, A., Yang, M. and Ibekwe, M. (2018) Characterization of bacterial communities and their antibiotic resistance profiles in wastewaters obtained from pharmaceutical facilities in Lagos and Ogun states, Nigeria. International Journal of Environmental Research and Public Health, 15, 1365-1365.

Ong, K.S., Chin, H.S. and Teo, K.C. (2011) Screening of antibiotic sensitivity, antibacterial and enzymatic activities of microbes isolated from ex-tin mining lake. African Journal of Microbiological Research, 5, 2460-2466.

Packer, J.L., Werner, J.J., Latch, D.E., McNeill, K. and Arnold, W.A. (2003) Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. Aquatic Science and Research, 65, 342-351.

Patneedi, C.B. and Prasadu, K.D. (2015) Impact of pharmaceutical wastes on human life and environment. Rasayan Journal of Chemistry, 8, 67-70.

Placide, S.S., Barte, M., Helene, L.E.M., Thiery, A., Quand-Meme, G.C., Sanogo, I. and Lassine, O. (2016) Assessment of the physicochemical and microbiological parameters of a teaching hospital's wastewaters in Abidjan in Côte d ' Ivoire. Journal of Water Resources and Protection, 08, 1251–1265.

Rice, E.W., Rodger, B.B., Eaton, A.D. and Lenore, S.C. (2012) Standard methods for the examination of water and wastewater, 22nd edn. American Water Works Association

Rim-Rukeh, A. and Agbozu, I.E. (2013) Impact of partially treated sewage effluent on the water quality of recipient Epie Creek Niger Delta, Nigeria using Malaysian Water Quality Index (WQI) * 1 A. RIM-RUKEH ; I.E. AGBOZU. Journal of Applied Science and Environmental Management, 17, 5–12.

Robert, A.P., Lorraine, F., Walter, M. and Ronald, M.R. (2009) Laboratory exercises in Microbiology, 3rd edition, U.S: John Wiley & Sons, Inc., pp 45-76.

Rusydi, A.F. (2018) Correlation between conductivity and total dissolved solid in various types of water: A review. IOP conference Series: Earth Environmental Science, 118012019. Doi:1088?1755-1315/118/1/012019

Singal, N. and Kaur, S. (2018) Review on physico-chemical analysis of various pharmaceutical industrial effluents & its impact on the environment. International Journal of Science and Applied Research, 5, 07-11.

Tappin, A.D., Navarro-Rodriguez, A., Comber, S.D.W. and Worsfold, P.J. (2018). The role of alkalinity in setting water quality metrics: phosphorus standards in United Kingdom Rivers. Environmental Science: Processes and Impacts, 20, 1361-1372.

Venkatappa, K., Kavitha, R.V., Murthy, V.K., Makam, R. and Asith, K.A. (2012) Physico-chemical analysis of effluents from pharmaceutical industry and its efficiency study. International Journal of Engineering Research and Applications, 2, 103–110.

Wein, T., Hulter, N.F., Mizrahi, I. and Dagan, T. (2019) Emergence of plasmid stability under non-selective condition maintains antibiotic resistance. Nature Communications, 10, 2595.

Weldhagen, G.F., Poirel, L. and Nordmann, P. (2003) Ambler class A extended-spectrum β-lactamases in Pseudomonas aeuriginosa: novel developments and clinical impact. Antimicrobial Agents and Chemotherapy, 47, 2385-2392.

Wellington, E.M.H., Boxall, A.B.A., Cross, P., Fell, E.J., Gaze, W.H., hawkey, PM., Johnson-Rollings A.S., Jones, D.l., Lee, N.M., Otten, W., Thomas, C.M. and Williams, A.P. (2013) The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. Lancet Infectious Diseases, 13:155-165.

Yakubu, O.H. (2017) Pharmceutical wastewater effluent-source of contaminants of emerging concern: Phytotoxicity of metronidazole to soyben (Glycin max). Toxics, 5. https://doi.org/10.3390/toxics 5020010

Yang, G., Song, L., Lu, X., Wang, N. and Li, Y. (2017) Effect of the exposure to suspended solids on the enzymatic activity in the bivalve *Sinonovacula constricta*. Aquaculture and Fisheries, 2, 10-17.

Yu, L., Han, M. and He, F., (2017) A review of treating oily wastewater. Arabian Journal of Chemistry, 10 (2), S1913-S1922.

Zuccato, E., Castiglioni, S., Fanelli, R., reltano, G., Bagnati, R., Chiabrando, C., Pomati, F., Rossetti, C. and Calaman, D. (2006) Pharmaceuticals in the environment in Italy: Causes, occurrence, effects and control. Environmental Science Pollution Research International, 13, 15-21.