Metal-resistance encoding gene-fingerprints in some bacteria isolated from wastewaters of selected printeries in Ibadan, South-western Nigeria

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ABSTRACT
Several studies have reported the occurrence of metal-resistant bacteria and their genes in different wastewater, but there is a dearth of information on wastewater generated from printing operations as a probable source. This study aimed at fingerprinting metal-resistance encoding genes in bacteria recovered from wastewaters of selected printeries in Ibadan, Nigeria. Wastewaters from 10 selected printeries in Ibadan were collected monthly for 12 months. The metal composition of wastewater was determined using Atomic Absorption Spectrophotometry. Metal-resistant bacteria were isolated on metal-supplemented nutrient medium, and characterized using 16S rRNA gene sequencing. Metal-resistance genes were detected using specific primers and the presence of plasmids was determined using alkaline-lysis method. Forty metal-resistant bacteria belonging to six genera; Bacillus, Klebsiella, Pseudomonas, Citrobacter, Providencia and Proteus were identified. cusCBA, encoding resistance to copper and silver was detected in nine bacteria, while pbrA (encoding lead resistance) was detected in seven Pseudomonas aeruginosa isolates. chrA, encoding resistance to chromate ions, was detected in Proteus mirabilis PW3a and two isolates of Pseudomonas aeruginosa, while chrB was detected in Providencia venericola PWAP3 and Proteus mirabilis PW4c. Bacillus stratosphericus PW1b possessed the copper-resistance genes, pcoA and pcoR. Thirty-six bacteria (90%) of the total bacteria possessed plasmids larger than 10 Kb in size. In conclusion, wastewater generated from printing operations could be a potential source of metal-resistant bacteria and their genes.

1. Introduction
Printing and the processes associated with it occupy a pivotal position in everyday life as no day is complete without human beings having a direct or indirect contact with various forms of printed items. These items include packaged consumer products, books, newspapers, journal articles, computer prints, photocopies and a host of other products. Inks, the organic or inorganic pigments employed in printing process contain coloured and colourless pigment particles dispersed in suitable solvents [1]. Modern inks are very complex compounds in terms of their composition, as they contain along with pigments or dyes, other ingredients generally referred to as vehicles. These materials include: humectants (to control drying), pH modifiers, polymeric resins (for proper binding), anti-foaming agents to regulate foam efficiency, wetting agents such as surfactants (which control surface properties), biocides (to inhibit microbial growth) and thickeners (to control the application and flow of the ink) [2].

Wastewater discharged from printing processes and other related operations has been reported to contain potentially hazardous components such as residual chemicals, dyestuff, solvent residues, pigmented wiping materials, and some toxic heavy metals such as silver (Ag), copper (Cu), zinc (Zn), chromium (Cr), cadmium (Cd), lead (Pb), etc. Metals, apart from the naturally occurring ones, are products of anthropogenic activities such as chemical manufacturing, pigment and dye production, battery manufacturing, automobiles and petrochemicals. All these represent some of the major sources of input of metals into the environment [3]. Pigments used in printing operations, especially the inorganic pigments are usually metallic salts precipitated from solutions; in addition, the organic pigments also contain some metallic compounds in their chemical structures [4]. This makes it possible for bacteria in wastewater generated by printeries to develop metal resistance as a means of coping with the toxicity of metals in the wastewater. Thus wastewaters from printing operations are potential sources of metal-resistant bacteria. This feature notwithstanding has its application in the use of bacteria for metal clean up and bioremediation of metal-contaminated environments.

Although metal resistance has been widely studied in relation to industrial wastewater in Nigeria, none of...
the studies has investigated printing industry wastew-
water as a source of metal-resistant bacteria. In addition, no 
study has investigated the genes responsible for metal 
resistance in bacteria isolated from wastewater gener-
ated by printing industries. The objective of this study 
was to isolate metal-resistant bacteria from wastewater 
collected from small and medium scale printing indus-
tries in Ibadan, Southwestern Nigeria and assess the 
incidence of genes encoding resistance to metals in 
these bacteria.

2. Materials and methods

2.1. Study site

Wastewater samples were collected from 10 printeries 
located in Mokola, an area which is a hub for small and 
medium scale printing operations and the University 
of Ibadan printery, both located in Ibadan, Oyo State, 
Nigeria. The high concentration of printeries at Mokola 
was responsible for the selection of the area for sample 
collection. The University of Ibadan printery is located 
within the University of Ibadan premises. The printer-
ies collect their wastewater in holding tanks which are 
emptied into a central drainage channel which con-
nects the entire community to the Ogunpa River in 
Ibadan, Oyo state, Nigeria.

2.2. Wastewater sample collection

Wastewater samples were collected into pre-sterilized 
sample containers from the final effluent holding tanks 
of the printeries and transported in ice chests to the 
Environmental Microbiology and Biotechnology Lab-
atory, Department of Microbiology, University of 
Ibadan. Samples were analysed within six hours of col-
lection.

2.3. Metal composition of printing press industry 
                          wastewater

The metal composition of selected printing wastewa-
ter was determined using the Atomic Absorption Spect-
rophotometer (AAS) (UNICAM 929, London Atomic 
Absorption Spectrophotometer powered by SOLAAR 
software). The wastewater samples were digested using 
the nitric acid method [5]. The digested filtrates were 
than analysed using the cathode lamp of each metal.

2.4. Isolation of bacteria from printing wastewater

Aliquots of serially diluted printing wastewater were 
plated on nutrient agar (Pronadisa Laboratorios Conda, 
SA) using the standard pour plate technique. Mor-
phologically distinct colonies of bacteria growing on 
the plates were repeatedly streaked on fresh plates to 
obtain pure cultures which were stored in 15% glycerol 
stock at −80°C for further studies.

2.5. Minimum Inhibitory concentration (MIC) of 
The metals on bacteria

The isolated bacteria were subjected to increasing 
centrations of selected metals on Mueller Hinton 
agar supplemented with filter-sterilized soluble salts of 
CuSO4, PbNO3, CdCl2, K2Cr2O7, AgNO3 and ZnSO4. The 
starting concentration for each metal was 50 µg/mL. 
The culture growing on the last concentration was 
transferred to the next higher concentration until the 
isolates failed to show visible growth. The Minimum 
Inhibitory Concentration (MIC) was taken as the lowest 
centrations of the metals that prevented the growth 
of the bacteria [6,7].

2.6. Identification of the metal-resistant bacteria

The isolates were identified using PCR amplification/ 
sequencing of the 16S rRNA [8]. The PCR products 
were sequenced (Inqaba Biotech, South Africa) and the 
sequences were blasted against reference sequences 
in the GenBank for identification (http://www.ncbi.nlm.
.nih.gov/BLAST/). Extraction of the DNA was carried 
out using the ZR 96 Fungal/Bacterial DNA Kit (Zymo 
Research Corporation, USA). The sequences were sub-
mitted to the GenBank and accession numbers were 
assigned.

2.7. PCR amplification of metal-resistance 
genesis/detection of plasmids

Metal-resistance encoding genes were amplified by PCR 
with primers targeting the chromium-zinc-cadmium 
resistance genes czcA, czcB, and czcD; silver resistance 
genes silCBA, agrCBA and cusCBA; copper resistance 
genes pcoA and pcoR; chromate resistance genes chrA 
and chrB, and lead resistance gene pbrA. The reaction 
mixture in each case contained 12.5 µL of Master Mix, 
7.5 µL of PCR quality (Nuclease-free) water, 1.0 µL each 
of both forward and reverse primers and 3 µL of the 
DNA template. The annealing temperature for the PCR 
assays are as follows: 57°C (czcA, czcB, czcD, pcoA, pcoR, 
chrA and chrB), 55°C (silCBA, agrCBA and cusCBA) and 
58°C (pbrA). All reactions included a negative (ster-
ile water) control and a positive control where avail-
able. The oligonucleotide primers used in this study are 
shown in Table 1. Plasmid DNA was extracted from the 
bacteria using the alkaline-lysis method [9]. This was 
necessary because most of the genes targeted (unless 
stated otherwise) were plasmid-based. Plasmid sizes 
were determined by comparison with a DNA marker 
(Thermo Scientific).

3. Results

A total of 40 bacteria showing different levels of 
resistance to five selected metals were obtained. The
Table 1. Oligonucleotide primers used in this study.

| Target genes | Primer (Forward and Reverse) | Length of amplified region (bp) | Reference |
|--------------|------------------------------|--------------------------------|-----------|
| pbrA         | 5′-ATGAGGCAATGTTGGCTCAAG 3′  | Approx2400                      | [10]      |
| czaA         | 5′-TGAGAGCTTTGGAAGCTCCGGATG 3′ | 1885                            | [11]      |
| czbB         | 5′-ATATGGCAGGACAAACAAAGG 3′  | 1520                            | [11]      |
| czdC         | 5′-CATGTTGACAGAGTATGAGACT 3′  | 398                             | [11]      |
| chrA         | 5′-CTTATAAGCGACCCCAACTG 3′   | 1292                            | [11]      |
| chrB         | 5′-GTAAGGGCGTTGACCTGCTG 3′   | 450                             | [11]      |
| silCBA       | 5′-GGGAAACACGAGGTTACTCTT 3′  | 3562                            | [12]      |
| agrCBA       | 5′-AGCTAGCGCTGTTCTGTATTCT 3′ | 3277                            | [12]      |
| casCBA       | 5′-GATCTCTAGAAGGAGGTTGCTCC 3′ | 6413                            | [12]      |
| pcoR         | 5′-CAGGGAGTGACCCCTACCT 3′    | 636                             | [13,14]   |
| pcoA         | 5′-GGACTTCACGAAACATTCCC 3′   | 1791                            | [13,14]   |

Figure 1. Frequency of occurrence of metal-resistant bacteria from selected printneries.

16S rRNA gene sequencing showed that the isolates belonged to six genera namely: *Bacillus* (18), *Pseudomonas* (11), *Proteus* (6), *Klebsiella* (2), *Providencia* (2) and *Citrobacter* (1) as shown in Figure 1.

Table 2 shows the mean metal concentration of wastewater obtained from 3 selected printneries of the 10 sampled. The copper concentration of the wastewater was highest for PPW1 and PPW2 (3.07 and 4.52 mg/L respectively), while zinc was highest in PPW3 (2.22 mg/L) in comparison to the other metals. The least concentration of metal was silver for all the wastewater sampled. In most of the cases, the metal concentrations were more than the National Environmental Regulations (NER) limit [15], except in few instances.

3.1. Minimum inhibitory concentration (MIC) of the metals on the bacteria

The bacteria showed varying degree of tolerance to the tested metals. *Bacillus stratosphericus* PW1b showed the highest level of tolerance to copper (Cu) with an MIC of (650 µg/mL), while the MIC for all the other bacteria ranged from 100 to 500 µg/mL. In the case of lead (Pb), 5 of the isolates were resistant at concentrations < 500 µg/mL with the remaining having MIC values ranging from 500 to 550 µg/mL. At a concentration of 500 µg/mL, 37.5% (15) of the total bacteria obtained in this study were able to grow on zinc. However, the MIC range for zinc was between 100 and 400 µg/mL for the remaining isolates. Fourteen of the 40 metal-resistant bacteria, representing 35% were resistant to cadmium with MIC value of 500 µg/mL. With the exception of *Proteus mirabilis* PW4c (MIC: 0 µg/mL) and *Pseudomonas aeruginosa* PW5c (MIC: 450 µg/mL) all the other bacteria showed a MIC ranging between 100 and 400 µg/mL for cadmium. Twenty-six of the total bacteria (65%) grew in the presence of 400 µg/mL of silver, with the rest growing at MIC < 400 µg/mL (Table 3).

3.2. Detection of metal resistance genes and plasmid profile

The silver resistance genes, *silCBA* and *agrCBA*, and the chromium–zinc–copper resistance genes *czaA, czbB and czdC* were not detected in any of the isolates. However, copper resistance genes, *pcoA* and *pcoR* were both detected in *Bacillus stratosphericus* PW1b. *chrA* and *chrB* encoding chromium resistance were detected in three bacteria (*Proteus mirabilis* PW3a, PW4c, and PW5c).
Various genera of metal-resistant bacteria have been isolated from different sources notably wastewater. Several authors have reported the isolation of metal-resistant bacteria from various sources [7,16–18]. Bacteria adapt to metal stress in their environment and respond to it by developing several resistances or coping mechanisms to its toxicity [19]. This has made the study of bacteria in metal-contaminated environment an interesting one. Furthermore, the presence of metal contaminants in the immediate environment could also act as a precursor in the stimulation of resistance to metal species by bacteria. The printing press wastewater employed in this study had a considerable level of metal contaminants present, and this could have propelled the bacteria therein to develop adaptive features against those metals.

The cop resistance determinants which share a functional similarity with pco resistance determinants have been confirmed to be responsible for copper resistance in *Pseudomonas syringae* [20], whereas pco are responsible for copper resistance in *Escherichia coli*. In contrast however, other researchers reported that there is a slight difference in the mechanisms of action of cop- and pco- encoded copper resistance in bacteria. The cop genes are believed to encode the sequestration of copper and higher accumulation [21], whereas that encoded by pco is an energy-dependent export and lower accumulation of copper in the bacterial

### Table 3. Minimum Inhibitory Concentration (MIC) of the metals on the bacteria (µg/mL), metal-resistance genes detected and presence of plasmids.

| Bacterial isolate | Source | Pb | Zn | Cd | Cu | Cr | Ag | Metal-resistance genes detected | Plasmid | Accession Number |
|------------------|--------|----|----|----|----|----|----|-------------------------------|---------|-----------------|
| *Bacillus aerius* PW1a | PP1 | 500 | 500 | 400 | 500 | 200 | 400 | cusCBA | Yes | MK026845 |
| *Klebsiella oxytoca* PW1ay | PP1 | 500 | 500 | 400 | 500 | 400 | 400 | pcoA, pbrA | Yes | MK026847 |
| *Bacillus stratosphericus* PW1b | PP1 | 250 | 100 | 100 | 650 | 100 | 100 | cusCBA | Yes | MK026844 |
| *Pseudomonas japonica* PW1c | PP1 | 500 | 100 | 300 | 400 | 100 | 400 | pcoA | Yes | MK026846 |
| *Bacillus stratosphericus* PW1e | PP1 | 500 | 200 | 500 | 500 | 500 | 400 | NMGD | Yes | MK026849 |
| *Bacillus aerophilus* PW2a | UIP | 100 | 100 | 100 | 100 | 100 | 100 | cusCBA | Yes | MK026850 |
| *Providencia vermicola* PW2b | UIP | 500 | 100 | 100 | 100 | 200 | 300 | cusCBA | Yes | MK026875 |
| *Bacillus stratosphericus* PW2bb | UIP | 450 | 300 | 350 | 300 | 400 | 200 | pbrA | Yes | MK026843 |
| *Proteus mirabilis* PW3a | PP2 | 500 | 500 | 300 | 200 | 400 | 300 | chrA | Yes | MK123476 |
| *Bacillus aerophilus* PW3c | PP2 | 500 | 100 | 100 | 100 | 100 | 400 | cusCBA | Yes | MK026864 |
| *Pseudomonas aeruginosa* PW3d | PP2 | 500 | 200 | 100 | 500 | 400 | 400 | pbrA, chrA | Yes | MK026845 |
| *Klebsiella oxytoca* PW4a | PP3 | 500 | 100 | 100 | 400 | 500 | 400 | cusCBA | Yes | MK026848 |
| *Proteus mirabilis* PW4b | PP3 | 500 | 100 | 200 | 100 | 400 | 400 | NMGD | Yes | MK026861 |
| *Proteus mirabilis* PW4c | PP3 | 500 | 100 | NG | 100 | 400 | 100 | cusCBA, chrB | Yes | MK026860 |
| *Pseudomonas aeruginosa* PW4d | PP3 | 500 | 400 | 500 | 400 | 100 | 100 | pbrA | Yes | MK026858 |
| *Pseudomonas aeruginosa* PW5a | PP4 | 500 | 400 | 500 | 200 | 100 | NG | NMGD | Yes | MK026869 |
| *Bacillus cereus* PW5b | PP4 | 550 | 400 | 400 | 500 | 400 | 400 | NMGD | Yes | MK026856 |
| *Pseudomonas aeruginosa* PW5c | PP4 | 350 | 300 | 450 | 350 | 450 | 200 | pbrA, chrA | Yes | MK026867 |
| *Pseudomonas aeruginosa* PW5d | PP4 | 500 | 400 | 500 | 400 | 100 | 100 | pbrA | Yes | MK026853 |
| *Proteus mirabilis* PW5e | PP4 | 500 | 400 | 400 | 500 | NG | 400 | cusCBA | Yes | MK026870 |
| *Bacillus aerophilus* PW5f | PP4 | 500 | 500 | 100 | 500 | 500 | 300 | NMGD | Yes | MK026842 |
| *Bacillus thuringiensis* PW1A | PP5 | 500 | 200 | 400 | 100 | 400 | 400 | NMGD | Yes | MK026865 |
| *Proteus mirabilis* PW2A | PP5 | 500 | 200 | 500 | 500 | 200 | 400 | NMGD | Yes | MK026868 |
| *Bacillus cereus* PW3A | PP5 | 500 | 300 | 300 | 500 | 500 | 400 | NMGD | Yes | MK026877 |
| *Citrobacter freundii* PW4A | PP5 | 100 | 100 | 100 | 100 | 100 | - | NMGD | No | MK026871 |
| *Pseudomonas aeruginosa* PWAP1 | PP6 | 500 | 500 | 500 | 500 | 100 | 300 | pbrA | Yes | MK026872 |
| *Bacillus cereus* PWAP2 | PP6 | 500 | 500 | 500 | 500 | 200 | 400 | NMGD | Yes | MK123474 |
| *Providencia vermicola* PWAP3 | PP6 | 500 | 500 | 500 | 400 | 200 | 400 | chrB | Yes | MK026862 |
| *Bacillus aerophilus* PW1N1A | PP7 | 500 | 500 | 400 | 500 | 200 | 400 | NMGD | No | MK026857 |
| *Bacillus thuringiensis* PW1N1B | PP7 | 500 | 500 | 300 | 500 | 400 | 400 | NMGD | No | MK026866 |
| *Bacillus subtilis* PW1N1C | PP7 | 500 | 500 | 500 | 500 | 200 | 400 | NMGD | Yes | MK026874 |
| *Bacillus thuringiensis* PW1N2A | PP8 | 500 | 500 | 500 | 500 | 200 | 400 | NMGD | Yes | MK026851 |
| *Bacillus thuringiensis* PW1N2B | PP8 | 500 | 500 | 500 | 500 | 400 | 400 | NMGD | Yes | MK026873 |
| *Pseudomonas aeruginosa* PW1N2C | PP8 | 500 | 400 | 500 | 400 | 100 | 300 | pbrA | Yes | MK026852 |
| *Bacillus thuringiensis* PW1N2D | PP8 | 500 | 500 | 500 | 500 | 500 | 400 | NMGD | Yes | MK026854 |
| *Proteus mirabilis* PW1N3A | PP9 | 500 | 500 | 400 | 100 | 400 | 400 | NMGD | Yes | MK026855 |
| *Bacillus cereus* PW1N3B | PP9 | 500 | 500 | 500 | 500 | 500 | 400 | NMGD | Yes | MK026863 |
| *Pseudomonas aeruginosa* PW1N3D | PP9 | 500 | 500 | 500 | 500 | 200 | 400 | pbrA | Yes | MK026876 |
| *Pseudomonas aeruginosa* PW1N3E | PP9 | 400 | 500 | 500 | 500 | 400 | 400 | NMGD | Yes | MK026859 |
| *Pseudomonas aeruginosa* PW1N3F | PP9 | 500 | 400 | 400 | 400 | 300 | 400 | NMGD | Yes | MK026878 |

KEY: PP: Printery, UIP: University of Ibadan Printery, NG: No growth was observed after the incubation period, NMGD: No metal-resistance gene detected, Yes: Presence of plasmid, No: Absence of plasmid.
The proposed mechanism of copper resistance in Escherichia coli requires the cooperation of both the plasmid and chromosomal functions to initiate resistance in an integrated fashion [24]. Plasmid-mediated resistance to copper has been reported in several species of bacteria especially Pseudomonas syringae pv tomato and E. coli and documented by several authors [14,24–28]. On the contrary however, chromosomal resistance to copper has also been described in Enterococcus hirae [29,30]. This might be responsible for the phenotypic resistance to copper by some bacteria in this study, even without the possession of the copper resistance determinants.

The range of bacterial hosts in which the pco determinant could function, might be limited to those genera closely related to E. coli, such as Citrobacter, Salmonella, and Shigella, all Gram negative organisms. This is in sharp contrast with the findings from this study, in which Bacillus stratosphericus PW1b, a totally unrelated Gram positive organism, was found to possess the pco gene determinants. The presence of these genes in gram positive group of bacteria could be attributed to the plasmid-borne nature of the gene which might have broadened its host spectrum [24]. Though Chihomvu and his co-workers in 2015 in their study on Klip River in South Africa, reported the detection of the copper resistance gene, pcoA in Lysinibacillus sp. KR25, this study is the first report of the detection of pco genes in any Gram positive bacterium isolated from printing wastewater.

The chrBAC operon is a set of genes harboured by the pMOL28 plasmid of the multi-metal resistant Cupriavidus metallidurans CH3. chrA chromate resistance protein has been detected in strains of Pseudomonas aeruginosa [31]. Two Pseudomonas strains in this study were observed to possess the chrA gene; however Proteus mirabilis PW3a in this study was also detected to possess the same gene. Based on the literatures at our disposal, this is likely to be the first report of the detection of chrA gene in Proteus mirabilis especially from printing wastewater. In addition, it has been reported that bacterial resistance to chromium may be due to either chromosomal mutations [32] or plasmid-mediated [33,34]. The presence of plasmids encoding chromate resistance has also been reported in certain species of Pseudomonas, Alcaligenes, Salmonella, Bacillus and Escherichia coli by several authors [34–39]. In this present study, chrB which regulates the chrA transporter [40] was detected in Providencia vermicola PWAP3 and Proteus mirabilis PW4c and this corroborated the report on the possession of the chr operon on the plasmids of species of Gram negative bacteria [41]. The same authors also reported the detection of the genes in some Bacillus strains isolated from tannery effluent.

The CBA-transport systems which are involved in the export of metal ions, xenobiotics and drugs are exclusively found in Gram negative bacteria. The need for Gram negative cells to safeguard the cytoplasm and translocate metals and other toxicants across their outer membrane has necessitated this system. Contrary to this report however, the cusCBA was detected in some strains of bacteria that are not Gram negative in this study. Five of the seven bacteria possessing the cusCBA in this study were gram-positive, while the remaining belongs to the gram-negative genera. The 6413 bp gene was detected in Proteus mirabilis PW4c, Bacillus aerius PW1a, Bacillus stratosphericus PW2bb,
Bacillus aerophilus PW2a, Klebsiella oxytoca PW4a, Proteus mirabilis PW5e, Providencia vermicola PW2b, Bacillus aerophilus PW3c, and Bacillus stratosphericus PW1b. The gene which has also been detected in Escherichia coli is also carried by the pMOL30 plasmid of the well-studied, multi-metal resistant Cupriavidus metallidurans CH34 [42,43].

All the Pseudomonas strains possessing the cusCBA in this present study showed varying resistance to copper and silver as outlined by their MIC to copper and silver ions; and this corroborates the report of some authors who opined that the cus determinant is induced by copper and silver, though the inducement by silver is to a lesser extent compared to copper [44–46]; their findings were partly corroborated by other reports on the clear contribution of the cus to copper resistance under anaerobic condition [46], they went further to report that the detoxification of copper by the cus system occurred in an oxygen-rich atmosphere. Contrary to this however, it has been reported that the protein of the cus system only mediates resistance to silver and that the conferment of resistance to copper could not be ascertained, even when the copA which encodes the copper-detoxifying P-type ATPase was disrupted in a mutant background. They however showed in their finding that copper was a better inducer of the expression of cus than silver, suggesting a probable involvement of the genes in the resistance of bacteria to copper; as reported by other researchers [47,48].

The pbr proteins are a group of proteins encoded in the widely studied metal-resistant Cupriavidus metallidurans CH34, and they include; PbrT, PbrA, PbrB, PbrC, PbrD and PbrR. The pbrA, is a PIB-type ATPase in Cupriavidus metallidurans, and is the main lead efflux transporter [10]. The gene was detected in 7 of the 40 (17.5%) metal-resistant bacteria obtained in this study. The bacteria found to possess the pbrA gene were all strains of Pseudomonas aeruginosa. Strains of Pseudomonas marginalis and Bacillus megaterium have been observed to show extracellular lead exclusion and intracellular cytoplasmic lead accumulation respectively. Pb-resistant strains of other bacteria e.g. Staphylococcus aureus, Citrobacter freundii and Vibrio harveyi have also been reported [49–52].

Though it was initially thought that the Pbr efflux system was Pb(II) specific. The participation of the Pbr efflux in the protection of the cell wall against Cd (II) and Zn (II) has been reported [53]. The specific mechanisms of Pb(II) resistance require the mutual cooperation of pbrA and pbrB genes hence the two are majorly involved in mediating lead resistance in bacteria e.g. in the metal-resistant Cupriavidus metallidurans [10]. It should be stressed however that the functional roles of the other genes in the Pbr efflux system e.g. PbrT, PbrC and PbrD are still an issue of debate among researchers, because their absence does not in any way impair the ability of the system to neutralize toxic ions in Cupriavidus metallidurans [10,53] However from this present study, all the bacteria were able to tolerate different concentration of lead, even without the possession of the pbrA. This suggests that there might probably be other mechanisms of resistance to the metal possessed by the strains and the possibility of the resistance being chromosome-mediated or being mediated by other gene variants.

**Conclusion**

This study has highlighted printing industries in Ibadan, Nigeria as a potential contributor of metal-resistant bacteria and their genes into the environment, highlighting an urgent need for the enforcement of regulations regarding wastewater discharge, especially in developing countries of the world where wastewater from the manufacturing sector is discharged into the environment without prior treatment.

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