Journal of Advances in Medical and Pharmaceutical Sciences
8(3): 1-10, 2016, Article no.JAMPS.26580
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Susceptibility of Heavy Metals Tolerant Bacteria Isolated from NILEST Tannery Effluent

J. O. Oko[1,2*], M. Umar[1], D. E. Akafyi[1] and M. Abdullahi[1]

[1]Department of Science Laboratory Technology, Microbiology Division, Nigerian Institute of Leather and Science Technology Samaru, Zaria, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/26580
Editor(s):
(1) Nissar Darmani, Professor of Pharmacology, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, California, USA.
Reviewers:
(1) Adi Idris, PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Brunei.
(2) Rasha M. Fathy Barwa, Mansoura University, Egypt.
(3) Geetha Kumar-Phillips, University of Arkansas, Fayetteville, Arkansas, USA.
Complete Peer review History: http://sciencedomain.org/review-history/15054

Original Research Article

Received 24th April 2016
Accepted 10th June 2016
Published 17th June 2016

ABSTRACT

Background: The emergence of multiple antibiotics resistance among bacterial population poses a potential threat to human health. The co-existence of metal/antibiotic resistance in bacterial strains suggests the role of heavy metals as a factor which can also contribute to drug resistance phenomenon since heavy metal pollution results in selective pressure that leads to the development of multiple drug resistance among bacterial populations probably through horizontal gene transfer.

Aim: This study was aimed to isolate, identify, and determine the antibiogram profile of heavy metals tolerant bacteria from the tannery effluent at Nigerian Institute of Leather and Science Technology (NILEST), Zaria, using standard microbiological methods.

Place of Study: Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology (NILEST), Zaria, Kaduna-Nigeria.

Methodology: Tannery effluent was cultured on heavy metals incorporated nutrient agar. The resultant isolates were purified by sub-culturing on fresh heavy metals incorporated nutrient agar

**Corresponding author: E-mail: odeyadebe@yahoo.com;*

and the antibiogram profile was determined using agar diffusion method.

Results: The bacteria isolated include *Bacillus cereus* (18.75%), *Escherichia coli* (12.50%), *Klebsiella aerogenes* (18.75%), *Klebsiella pneumoniae* (6.25%), *Pseudomonas aeruginosa* (12.50%), *Proteus mirabilis* (6.25%), and *Staphylococcus aureus* (25.00%) with *Staphylococcus aureus* being the most prevalent. In this study, heavy metals tolerant bacteria isolated from the tannery effluent were multidrug resistant as each of the isolates was able to resist the activity of one or more of the antibiotics across the three classes of antibiotics studied. Gentamicin was highly resisted followed by ampicillin and amoxicillin. Kanamycin and Nalidixic acid were more effective followed by Ciprofloxacin and Streptomycin. The β-lactams/penicillins were generally not very effective as compared to the glycopeptides and the quinolones. *Staphylococcus aureus* and *Escherichia coli* were highly resistant to six (6) out of the nine (9) antibiotics tested with *Escherichia coli* showing 100% resistance to ampicillin and amoxicillin. However, *Bacillus cereus* was moderately resistant to seven (7) out of the nine (9) antibiotics tested but highly resistant to gentamicin. Ampicillin and cefixime showed no activity against *Proteus mirabilis* and *Klebsiella pneumoniae.* However, *Proteus mirabilis* and *Klebsiella aerogenes* were 100% resistant to gentamicin. Kanamycin was inactive against *Klebsiella pneumoniae*. The Multiple Antibiotic Resistance (MAR) indices of all isolates were greater than 0.2.

Conclusions: The recovery of heavy metals tolerant bacteria from the tannery effluent is an indication that the effluent is laden with heavy metals. The high antibiotic resistance profile of the isolates in this study is an indication of the correlation between heavy metals tolerance and antibiotic resistance. The identified heavy metals tolerant bacterial strains could be useful for bio-remediation of heavy metals contaminated wastewaters and soil environments even though they are potential threat to successful chemotherapy if incriminated in infections.

Keywords: Antibiotic resistance; heavy metals; tannery effluents; public health; bio-remediation; antibacterial chemotherapy.

1. INTRODUCTION

Tanning is the process of treating skins and hides of animals to produce leather, which is more durable and less susceptible to decomposition. Traditionally, tanning uses tannin, an acidic chemical compound from which the tanning process draws its name. A tannery is the term for a place where the skins are processed [1]. Tanning hides into leather involves a process which permanently alters the protein structure of skin. Tanning can be performed with either vegetable or mineral methods [2]. Before tanning, the skins are unhaired, degreased, desalted, and soaked in water over a period of 6 hours to 2 days. To prevent damage of the skin by bacterial growth during the soaking period, biocides, typically dithiocarbamates may be used. Fungicides may also be added to protect wet leathers from mold growth [3]. The tanning process is capable of generating large amount of waste ranging from solids, semi-solids, liquids, and gases. In most cases, organic matter and chemicals remain suspended or dissolved in liquids giving rise to different characteristics. This is usually referred to as tannery effluent. The characteristics of tannery effluent vary considerably from tannery to tannery depending upon the size of the tannery, chemicals used for a specific process, amount of water used and type of final product produced by a tannery. Tannery wastewater is characterized mainly by measurements of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids (SS) and Total Dissolved Solids (TDS), chromium and sulfides etc. [4].

Among the pollutants discharged into the ecosystems are heavy metals such as mercury, cadmium, chromium, lead, copper (Cu), zinc (Zn), arsenic, and other chemicals which affect aquatic life including microbial communities [5]. Pollution of environment by heavy metals represents an important ecological problem due to the negative impact of metals as heavy metals can accumulate throughout the food chain and result in serious ecological and health problems [6]. Introduction of heavy metals in various forms in the environment produces considerable modifications of microbial communities and their activities [7]. Farm animals such as pig and poultry receive additional zinc and copper in their diets via supplements of these elements in their compounded feeds as well as in medicinal remedies. The content of Zn and Cu in manure

has been shown to be especially high from pigs and poultry and from other farm animals receiving a high portion of their diet from compounded feeds. Zn and Cu are normally used in animal feeds in concentrations in excess of the nutritional requirements of the animals and for prevention of diarrheal disease, and also as an alternative to in-feed antibiotics for growth promotion [8]. The increased exposure of these trace elements in animal feeds may result in elevation in MIC-values [9]. MIC or Minimum Inhibitory Concentration is defined as the lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions. Enteric bacteria, both commensals and pathogens, in farm animals have been shown to develop resistance to trace elements such as Zn and Cu and concomitant cross-resistance to antimicrobial agents. Such bacteria may be transferred to other animals and humans [9].

Toxic metals in the environment lead to selective pressure among the microbial communities resulting in the development of metal resistant populations [10]. As a result of this selective pressure, microorganisms have evolved mechanisms to detoxify heavy metals, and some even use them for respiration. To survive under metal stressed conditions, bacteria have also evolved mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation, complexation, and reduction of the metal ions to a less toxic state [11].

Evidence support the existence of a correlation between tolerance to heavy metals and antibiotic resistance, which is a global problem threatening the treatment of infections in plants, animals, and humans [12]. Resistance genes to both antibiotics and heavy metals may be located closely together on the same plasmid in bacteria and are more likely to be transferred together in the environment [13,14]. Metal tolerance and antibiotic resistance in bacteria have been shown to increase proportionally along industrial contamination gradients [13]. It has been shown that bacterial isolates resistant to vanadium show increased resistance to quinolones (Ciprofloxacin and Norfloxacin), which are crucial in the management of *Salmonella* infections. The genes can be transferred to indigenous populations of microorganisms occurring frequently in the environment thereby enhancing the spread of antibiotic resistance [15]. Significant increase in Multiple Antibiotic Resistant bacteria are observed in various aquatic systems as well as in human infections and treatment of these bacteria are becoming more and more difficult with available drugs [16]. The resistance development may be due to nonspecific mechanism with gene regulation of plasmids and chromosomes, which may be heritable or transferable due to the presence of resistance (R-factor) factor [17].

Microbes have adapted to tolerate the presence of metals or can even use them to grow [18]. Thus, a number of interactions between microbes and metals have important environmental and health implications. Some implications are useful, such as the use of bacteria in the biogeochemical cycling of toxic metals as well as remediating metal contaminated environments [19]. Other implications are not as beneficial, as the presence of metal tolerance mechanisms may contribute to an increase in antibiotic resistance. Overall, it is most important to remember that what we put into the environment can have many effects, not just on humans, but also on the environment and on the microbial community on which all other life depends. The objective of this study therefore is to isolate and biochemically identify heavy metals tolerant bacterial isolates in NILEST tannery effluent and determine their antibiogram profile.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Tannery effluent was collected from Nigerian Institute of Leather and Science Technology (NILEST) Tannery in Zaria, Kaduna State, Nigeria. Liquid (effluent) sample was collected in sterile sample bottles and were transported to Microbiology Laboratory of the Nigerian Institute of Leather and Science Technology Samaru, Zaria for microbiological analysis. The inoculation of sample was done within one hour of collection.

2.2 Preparation and Inoculation of Sample

Salts of heavy metals (Pb^{2+}, Cr^{6+}, Hg^{2+}, and Zn^{2+}) were incorporated into nutrient agar (Oxoid, UK) media and the media was used for the selective isolation of heavy metals tolerant bacteria. The sample was diluted in a ten-fold serial dilution from 10^{-1} to 10^{-5} and 0.1 ml of the 10^{-3} dilution was used for inoculation onto the plates of

nutrient agar which was incorporated with heavy metal salt and was incubated for 24-48 hours at 37°C. The nutrient agar was prepared according to the manufacturer's instructions, sterilized and cooled to 48-50°C and the concentration of 5mg/L of the heavy metal salts was incorporated into the nutrient agar. This procedure was repeated for all the heavy metals tested in this study [20].

2.3 Isolation and Identification of Heavy Metals Tolerant Bacteria

The culture plates were observed for growth within 24-48 hours. Distinct colonies observed were sub-cultured onto fresh plates of corresponding heavy metal incorporated nutrient agar plates for purification. Gram staining as well as appropriate biochemical tests were carried out according to standard procedures [21,22]. The isolates were identified by comparing their morphological and biochemical characteristics with those of known taxa as described by Bergey's Manual for Determinative Bacteriology [23]. A confirmatory identification was done using the bioMérieux's API® identification kit.

2.4 Determination of Antibiotics Susceptibility

Nine antibiotics belonging to three classes were used in this study. The selection of the antibiotics was based on the frequency of use and popularity in Nigeria. The main classes of the antibiotics used include Beta-lactams/Penicillins (Ampicillin 25 µg, Amoxicillin 30 µg, Cefixime 30 µg), Aminoglycosides (Kanamycin 30 µg, Gentamicin 10 µg, Streptomycin 30 µg), and Quinolones (Ciprofloxacin 5 µg, Levofloxacin 5 µg, Nalidixic acid 30 µg). Antibiotic discs were purchased from Oxoid™ UK. The antibiotics susceptibility test was carried out using standard agar disc diffusion method. Sterile Mueller-Hinton agar (Oxoid, UK) plates were inoculated with each bacterial suspension, which was adjusted to a McFarland 0.5 standard and were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimetres (mm) using callipers and transparent meter rule. Strains were classified as Resistant (R), Intermediate (I) and Sensitive (S) according to the criteria recommended by the National Committee for Clinical Laboratory Standards [24]. All antibiotic sensitivity tests were performed in triplicate and average zones of inhibition were calculated. Strains of *Pseudomonas aeruginosa* were not tested against ampicillin and amoxicillin since these antibiotics do not act on *Pseudomonas.* Hence, *Pseudomonas* strains were exposed to only seven out of the nine drugs examined.

2.5 Multiple Antibiotic Resistance (MAR) Index Study

The MAR Index of each of the isolates was determined using the formula:

$$MAR\ Index = \frac{a}{b}$$

Where 'a' represents the number of antibiotics to which the isolate was resistant to and 'b' represents the number of antibiotics to which the isolate was subjected to [25]. The MAR indices of the isolates were calculated and noted.

3. RESULTS

The main goal of this study was to isolate and biochemically characterize heavy metals tolerant bacteria from tannery effluent obtained from NILEST tannery. Sixteen (16) heavy metals tolerant bacteria were isolated from the tannery effluent sample. *Bacillus cereus* (18.75%), *Escherichia coli* (12.50%), *Klebsiella aerogenes* (18.75%), *Klebsiella pneumoniae* (6.25%), *Pseudomonas aeruginosa* (12.50%), *Proteus mirabilis* (6.25%), and *Staphylococcus aureus* (25.00%) were identified with *Staphylococcus aureus* being the most prevalent (Table 1).

The results of this study indicated that heavy metals tolerant bacteria isolated from the tannery effluent were multidrug resistant as each of the isolates was able to resist the activity of one or more of the antibiotics across the three classes of antibiotics tested in this study. Gentamicin was highly resisted followed by ampicillin and amoxicillin. Kanamycin and Nalidixic acid were more effective followed by Ciprofloxacin and Streptomycin. The β-lactams/penicillins were generally not very effective as compared to the glycopeptides and the quinolones as bacterial strains were more resistant to the β-lactams/Penicillins (Fig. 1).

It was observed from this study that *Staphylococcus aureus* and *Escherichia coli* were highly resistant to six (6) out of the nine (9) antibiotics tested with *Escherichia coli* showing 100% resistance to ampicillin and amoxicillin. However, *Bacillus cereus* was moderately resistant to seven (7) out of the nine (9)

antibiotics tested but highly resistant to gentamicin. Ampicillin and cefixime showed no activity against *Proteus mirabilis* and *Klebsiella pneumoniae.* However, *Proteus mirabilis* and *Klebsiella aerogenes* were 100% resistant to gentamicin. Kanamycin was inactive against *Klebsiella pneumoniae* (Fig. 2). The MAR indices of all isolates were greater than 0.2 (Table 2).

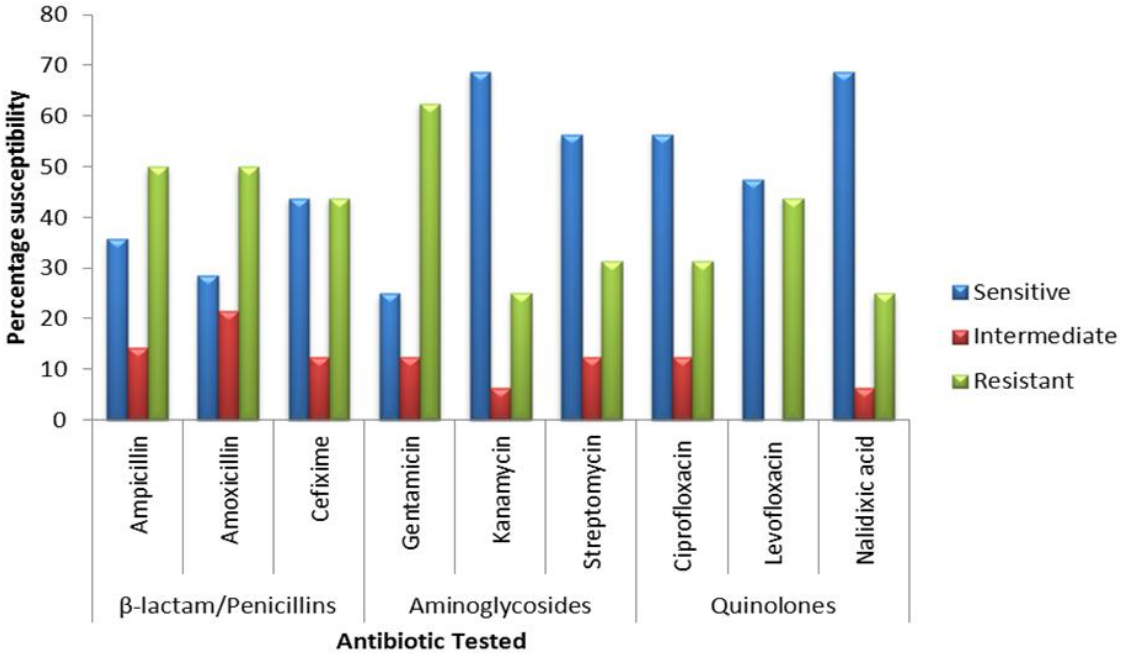

Fig. 1. Antibiotics susceptibility profile of heavy metals tolerant bacteria isolated

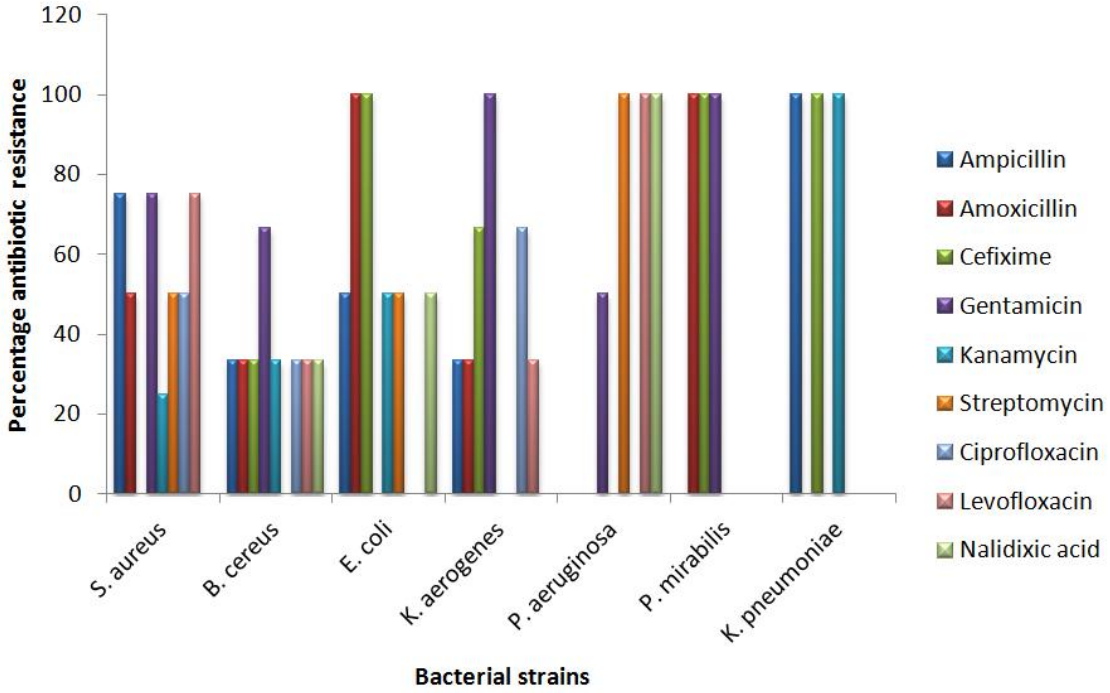

Fig. 2. Antibiotics resistance profile of heavy metals tolerant bacteria strains

Table 1. Biochemical characterization of heavy metals tolerant bacteria from NILEST tannery effluent

GRAM	SHAPE	INDO	MR	VP	CIT	CAT	COA	MOT	URE	OXI	BIL. SOL.	MAN	GLU	LAC	SUC	H_2S	CO_2	Probable organism
-	R	-	-	+	+	+	-	-	+	-	-	+	+	+	+	-	+	*Klebsiella aerogenes*
+	R	-	+	+	-	+	-	+	-	-	-	-	+	-	+	-	+	*Bacillus cereus*
+	CC	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	*Staphylococcus aureus*
-	R	-	-	-	+	+	-	+	-	+	-	+	+	+	-	-	+	*Pseudomonas aeruginosa*
-	R	+	+	-	-	-	-	+	-	-	-	+	+	+	+	-	+	*Escherichia coli*
-	R	+	+	-	-	-	-	+	-	-	-	+	+	+	+	-	+	*Escherichia coli*
-	R	-	-	+	+	+	-	-	+	-	-	+	+	+	+	-	+	*Klebsiella aerogenes*
-	R	-	-	-	+	+	-	+	-	+	-	+	+	+	-	-	+	*Pseudomonas aeruginosa*
+	CC	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	*Staphylococcus aureus*
-	R	+	+	-	+	+	-	+	+	-	-	-	+	+	-	+	-	*Proteus mirabilis*
+	CC	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	*Staphylococcus aureus*
-	R	-	+	+	-	+	-	+	-	-	-	-	+	-	+	-	+	*Bacillus cereus*
-	R	-	-	+	+	+	-	-	+	-	-	+	+	+	+	-	+	*Klebsiella aerogenes*
+	CC	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	*Staphylococcus aureus*
-	R	-	-	-	+	+	-	-	+	-	-	+	+	+	+	-	+	*Klebsiella pneumoniae*
+	R	-	+	+	-	+	-	+	-	-	-	-	+	-	+	-	+	*Bacillus cereus*

Key: INDO=Indole, MR= Methyl Red, VP= Voges Proskeuar, CIT= Citrate, CAT= Catalse, COA= Coagulase, MOT= Motility, OXI= Oxidase, URE= Urease, MAN= Mannitol, GLU= Glucose, LAC= Lactose, SUC= Sucrose, (+) = Positive, (-) = Negative, BIL. SOL. = Bile Solubility, R= Rod Shape, CC=Coccal in Clustered Shape

Table 2. Multiple antibiotics resistance (MAR) index analysis of bacterial isolates

Isolate number	Bacteria species	Antibiotics	NAR	MAR index
IS01	*Klebsiella aerogenes*	AMX, CEF, CIP, GEN, LEV	05	0.56
IS02	*Bacillus cereus*	AMP, AMX, GEN	03	0.33
IS03	*Staphylococcus aureus*	AMP, AMX, CIP, KAN, LEV	05	0.56
IS04	*Pseudomonas aeruginosa*	STR, LEV, NAL	03	0.43
IS05	*Escherichia coli*	AMP, AMX, CEF, KAN	04	0.44
IS06	*Escherichia coli*	AMX, CEF, NAL, STR	04	0.44
IS07	*Klebsiella aerogenes*	AMP, GEN	02	0.22
IS08	*Pseudomonas aeruginosa*	GEN,LEV, NAL, STR	04	0.57
IS09	*Staphylococcus aureus*	AMP, GEN, LEV, STR	04	0.44
IS10	*Proteus mirabilis*	AMX, CEF, GEN	03	0.33
IS11	*Staphylococcus aureus*	AMX, GEN, LEV, STR	04	0.44
IS12	*Bacillus cereus*	GEN, NAL	02	0.22
IS13	*Klebsiella aerogenes*	CEF, CIP, GEN,	03	0.33
IS14	*Staphylococcus aureus*	AMP, CIP, GEN,	03	0.33
IS15	*Klebsiella pneumoniae*	AMP, CEF, KAN,	03	0.33
IS16	*Bacillus cereus*	CEF, CIP, KAN, LEV	04	0.44

Key: AMP= Ampicillin, AMX= Amoxicillin, CEF= Cefixime, STR= Streptomycin, GEN= Gentamycin, KAN= Kanamycin, CIP= Ciprofloxacin, LEV= Levofloxacin, NAL= Nalidixic acid, NAR= Number of Antibiotics Resisted

4. DISCUSSION

Heavy metal contamination of the environments is a major global concern as huge amounts of heavy metals are discharged into water bodies as effluents of mining, metallurgy, and electroplating industries [26,27] as well as tanning processes. Bacterial populations isolated from heavy metal contaminated sites may have the ability to tolerate a higher concentration of metals and at the same time resist the activities of antibiotics. Hence, in the present study, heavy metals tolerant bacteria having ability to resist antibiotics were isolated and identified. The recovery of heavy metals tolerant bacteria in this study (Table 1) is an indication that the effluent is indeed polluted by the metals examined leading to selective pressure by which the tolerant bacteria have deviced means of circumventing the deleterious activity of the heavy metals. This result is similar to that of Raja et al. [20] who isolated heavy metals resistant enteric bacteria from sewage. It is also in agreement with Staehlin et al. [28] who had obtained *Enterobacteria* from a study on Copper Homeostasis and Silver Resistance Island (CHASRI) [28].

The high percentage of resistance to gentamicin (62.50%), amoxicillin, and ampicillin with resistance level of 50.00% each (Fig. 1), observed in this study may be attributed to the indiscriminate use of these drugs by patients and in animal treatments and feeds. This is in agreement with Martin et al. [29] who reported that antibiotics are administered to animals in feeds to marginally improve growth rates and to prevent infections and that there is growing evidence that antibiotic resistance in humans is promoted by the widespread use of nontherapeutic antibiotics in animals. Also Levy [30] found that drug-resistant bacteria quickly came to dominate the intestinal flora of chickens following the introduction of feed laced with oxytetracycline. Within six months, the people living on the farm also carried tetracycline-resistant coliform bacteria, which made up more than 80% of their intestinal microbes. The bacteria carried by both chickens and farmers contained plasmids that conferred traits creating resistance to multiple antibiotics, not the original drug alone. Levy's team also observed that six months after antibiotics were removed from the chicken feed; most of the workers no longer carried tetracycline-resistant bacteria [30]. The results of this current study may not be far from the views of Martin et al. [29] as the raw materials for the tannery is animals' skins and hides. However, the bacteria isolated may have contaminated the raw materials during slaughter or environmental contamination in animal houses or through feeds. However, the bacteria isolated in this present study may have acquired resistance genes to the tested antibiotics through horizontal transfer of plasmids from a donor bacterium to a recipient [17] or from mutation due to selective pressure of heavy metals on these bacteria. There is evidence of correlation between heavy metal tolerance and antibiotic resistance in this study with the high level of resistance observed. This may be because the resistance genes to both antibiotics and heavy metals are located closely together on the same plasmid in bacteria. Thus, they are more likely to be transferred together in the event of conjugation [11,14]. This phenomenon would be favourable for bacteria to survive in heavy metal contaminated environments. The microbial tolerance to heavy metals is attributed to various detoxifying mechanisms such as complexation by exopolysaccharides, binding of metal in bacterial cell envelopes, metal reduction, and metal efflux or using them as terminal electron acceptors in anaerobic respiration [31]. Previously, various studies have found significant correlation between heavy metals and antibiotics resistance. Such studies which have specifically examined the association between heavy metals and antibiotics resistance in wastewater, fresh and drinking water include those of Odjadjare et al. [32], Raja et al. [20] and Boga et al. [33] who confirm resistance of *Pseudomonas, Acinectobacter, Proteus etc.* to several heavy metals and antibiotics. Henrietta et al. [34] also observed association between chromium and ampicillin resistance and tolerance in zinc and chromium with resistance to streptomycin and tetracycline. The isolates recovered in this study are serious threats to effective chemotherapy. *Staphylococcus aureus* strains from this current study can be classified as superbug because it was able to resist the activity of seven (7) out of the nine (9) antibiotics used in this study. However, Bacillus cereus resisted eight (8) out of the nine (9) drugs. *Klebsiella aerogenes* and *Escherichia coli* showed resistance to six (6) antibiotics each (Fig. 2). This characteristic may have been acquired from exposure to chemical compounds and antimicrobial agents at one point or the other since these bacteria are predominantly native to the guts of animals or soil environment. Chromosomal mutation and plasmid carriage are very vital factors in the

resistance of bacteria and cannot be over emphasized.

In this present study, most of the isolates were resistant to at least one antibiotic across the three classes of antibiotics tested which qualify them as multiple antibiotics resistant strains of bacteria. Multiple antibiotic resistance characteristic in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype [35]. The multiple antibiotic resistance indices of the isolated bacterial strains were all greater than 0.2 (Table 2) which indicate high risk source of contamination of antibiotics [35,36]. In this case, the high pressure of heavy metals on the isolates may have been responsible for the high MAR indices. This is in line with Belliveau et al. [37] who stated that organisms undergo selective pressures in the presence of toxic compounds and develops resistance. The most common resistance is to metals and antibiotics which can be as a result of bio-essentiality or of abuse of metals and/or antibiotics. Both resistances are carried on the same plasmid and are transferable among organisms through conjugation or transduction [38]. The high incidence of resistance in bacteria isolated from sites away from human influences is possible due to the production of antibiotics by native bacteria and fungi which gives the bacteria a competitive advantage [39]. Usually essential metal tolerance genes are chromosome borne and toxic metals tolerance system are plasmid mediated [40]. Isolates observed in this study showed multiple antibiotic resistance patterns and are potential threat to successful chemotherapy. Tannery workers are at potential risk of getting infected with these pathogens. Also, tannery effluent released directly into water body may increase the dissemination of these pathogens and this poses serious threat to health of local indigenous communities.

5. CONCLUSION

The selection of heavy metals tolerant bacteria was reflected in the high degree of resistance to antibiotics tested. Multidrug resistant strains were abundant in this effluent and this may pose a potential health hazard and requires intervention as the MAR indices of all the isolates are far greater than 0.2. The resistance pattern in different isolates was more or less similar which implies that different bacterial strains carry similar antibiotic resistance determinants which have been as a result of plasmid transfer. Similar bacteria recovered in this study have been isolated in the air environment of the same tannery [41] which reflects the abundance of microbes in the processing units of this tannery. However, cross transfer of these resistance genes from donors to recipients poses great risk to workers and the general community.

6. RECOMMENDATIONS

Multiple antibiotic resistant bacteria are a public health issue. Therefore we hereby advocate for regular and consistent monitoring of tannery effluents with a view to preventing the dissemination of these pathogens into the environment where they may become clinically important in causing diseases that may be difficult to treat. Also, tannery workers should be educated on the risk and ways of preventing biohazards in the work area especially against acquisition of microbial infections from their work places.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saleem Rashid, Ahmad Adnan, Muhammad Hanif, Muhammad Saleem, Ki-Hwan Lee, Fahim Ashraf Qureshi. Synthesis and application of sulfonated adipic dihydrazide formaldehyde-based resins under different molar ratios as effective leather re-tanning agents. Iranian Polymer Journal. 2014;23(1):69-78.
2. Wemegah Rolland. Vegetable tanning in Bolgatanga: Challenges and the way forward. Arts and Design Studies. 2014;16:27-37.
3. Renner Manfred, Eckhard Weidner, Helmut Geihsler. Method for tanning animal skins. U.S. Patent 9,206,486, issued December 8, 2015.
4. Padhye, Lokesh P, Ulas Tezel. Fate of environmental pollutants. Water

Environment Research. 2014;86(10):1714-1773.

5. UNEP. UN Environmental Programme Assessment of East African Lakes. Report prepared by Lucia Peytermann and Daniel Olago for the United Nations Environmental Programme; 2006.
6. Bhojiya Ali Asger, Harshada Joshi. Heavy metal tolerance pattern of Pseudomonas putida isolated from heavy metal contaminated soil of Zawar, Udaipur (India). International Journal of Innovative Knowledge Concepts. 2016;2(1).
7. Doelman P, Jansen E, Michels M, Til M. Effects of heavy metals in soil on microbial diversity and activity as shown by the sensitivity – resistance index, an ecologically relevant parameter. Journal of Biology and Fertility of Soils. 1994;17:177–184.
8. Amachawadi RG, Shelton NW, Shi X, Vinasco J, Dritz SS, Tokach MD, et al. Selection of fecal enterococci exhibiting tcrB-mediated copper resistance in pigs fed diets supplemented with copper. Appl Environ Microbiol. 2011;77:5597–603.
9. Yazdankhah Siamak, Knut Rudi, Aksel Bernhoft. Zinc and copper in animal feed-development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microbial Ecology in Health and Disease. 2014;25.
10. Mahler I, Levinson HS, Wang Y, Halvorson HO. Cadmium and mercury resistance Bacillus strains from a salt marsh and from Boston Harbour. Applied Environmental Microbiology. 1986;52:1293-1298.
11. Nies DH. Microbial heavy-metal resistance. Appl Microbiol Biotechnol. 1999;51:730-750.
12. DeRore H, Top E, Houwen F, Mergeay M, Verestraete W. Evolution of heavy metal resistance transconjugants in a soil environment with a concomitant selective pressure. FEMS Microbial Ecology. 1994;14:263–273.
13. Osborn AM, Bruce KD, Strike P, Ritchie DA. Distribution diversity and evolution of the bacterial mercury resistance (mer) operon. FEMS Microbiological Review. 1997;19:239-262.
14. Sikuku, Mutuku Christopher. Association of heavy metal tolerance with multiple antibiotic resistance in bacteria isolated from Wetlands of Lake Victoria Basin Kenya. Diss. Kenyatta University; 2010.
15. Canadian Committee on Antibiotic Resistance. Antimicrobial resistance a deadly burden no country can afford to ignore. Report prepared by David Birnbaum for the Canadian committee on Antibiotic Resistance; 2002.
16. Lopes MFS, Riberio T, Abrantes M, Marques JJF, Tenreirom R, Crespo MTB. Antimicrobial resistance profiles of dairy and clinical isolate and type strains of entercocci. International Journal Food Microbiology. 2005;103:191-198.
17. Oko JO, Itah AY. Basic Pharmaceutical Microbiology. Ahmadu Bello University Press Ltd. Zaria, Kaduna State Nigeria; 2014. ISBN: 978-125-587-0
18. Bååth E. Effects of heavy metals in soil on microbial processes and populations (a review). Water, Air, and Soil Pollution. 1989;47(3-4):335-379.
19. Lu Mang, Zhong-Zhi Zhang, Jing-Xiu Wang, Min Zhang, Yu-Xin Xu, Xue-Jiao Wu. Interaction of heavy metals and pyrene on their fates in soil and tall fescue (*Festuca arundinacea*). Environmental Science & Technology 2014;48(2):1158-1165.
20. Raja CE, Selvam GS, Omine K. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. International Joint Symposium on Geodisaster Prevention and Geoenvironment in Asia. 2009;205-211.
21. Brown EA. Benson's microbiological applications laboratory manual in general microbiology, 9th ed. McGraw-Hill Companies, Inc., New York. 2005;230-280.
22. Oyeleke SB, Manga SB. Essentials of laboratory practicals in microbiology. Tobest publisher, Minna. Nigeria. 2008;36-75.
23. Buchanan RE, Gibbons NE. Bergey's manual of determinative bacteriology (8th Edition). William and Wilkins Co. Baltimore USA; 1974.
24. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. NCCLS approved standard M7-A5 and informational supplement M100-S19. Wayne, PA USA; 2000.
25. Subramani S, Vignesh S. MAR index study and MDR character analysis of a few golden staph isolates. Asian Journal of Pharmacy and Life Science. 2012;2(2): 151-154.

26. Mahato MK, Singh PK, Tiwari AK. Evaluation of metals in mine water and assessment of heavy metal pollution index of East Bokaro Coalfield area, Jharkhand, India. Int. J. Earth Sci. Eng. 2014;7(4): 1611-1618.
27. Tiwari AK, De Maio M, Singh PK, Mahato MK. Evaluation of surface water quality by using GIS and a heavy metal pollution index (HPI) model in a coal mining area, India. Bull. Environ. Contam. Toxicol. 2015;95:304-310.
28. Staehlin, Benjamin M, John G. Gibbons, Antonis Rokas, Thomas V. O'Halloran, Jason C. Slot. Evolution of a heavy metal homeostasis/resistance island reflects increasing copper stress in Enterobacteria. Genome Biology and Evolution. 2016;8(3): 811-826.
29. Martin Michael J, Sapna E. Thottathil, Thomas B. Newman. Antibiotics overuse in animal agriculture: A call to action for health care providers. American Journal of Public Health. 2015;105(12):2409-2410.
30. Levy, Sharon. Reduced antibiotic use in livestock: How Denmark tackled resistance. Environmental Health Perspectives. 2014;122(6):A160.
31. Haferburg G, Kothe E. Metallomics: lessons for metalliferous soil remediation. Appl. Microbiol. Biotechnol. 2010;87:1271-1280.
32. Odjadjare EE, Igbinosa EO, Mordi R, Igere B, Igeleke CL, Okoh AI. Prevalence of multiple antibiotics resistant (MAR) *Pseudomonas* species in the final effluents of three municipal wastewater treatment facilities in South Africa. Int. J. Environ. Res. Public Health. 2012;9:2092-2107. DOI: 10.3390/ijerph9062092.
33. Boga HI, Okemo PO, Mwatha WE, Muthanga J, Tsanuo MK, Ikingura JR. Heavy metal tolerance and antibiotic resistance profiles of gram negative bacteria isolated from Lake Victoria, Kenya. Journal of Tropical Microbiology and Biotechnology. 2007;3(2):20-26.
34. Henrietta C, Petitdemange E, Raval G, Gay R. Mercury reductase activity in the adaptation to cationic mercury, phenyl mercury acetate, and multiple antibiotics of gram negative population isolated from aerobic fixed bed reactor. Journal of Applied Bacteriology. 1991;71:439-444.
35. Osundiya OO, Oladele RO, Oduyebo OO. Multiple antibiotic resistance (Mar) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. Afr. J. Cln. Exper. Microbiol. 2013;14(3):164-168. Available:http://dx.doi.org/10.4314/ajcem.v14i3.8
36. Olayinka BO, Olonitola OS, Olayinka AT, Agada EA. Antibiotic susceptibility pattern and multiple antibiotic resistance index of *Pseudomonas aeruginosa* urine isolates from a University Teaching Hospital. Afr J Clin Exp Microbiol. 2004;5(2):198-202.
37. Belliveau BH, Staradub ME, Trevor JT. Occurrence of antibiotic and metal resistance in *Bacillus* strains isolated from marine sediment. Canadian Journal of Microbiology. 1991;37:13-52.
38. Nakahara H, Ishikawa T, Yasunaga S, Kondo I, Kozukue H, Silver S. Linkage of mercury, cadmium and arsenate and drug resistance in clinical isolates of *Pseudomonas aeruginosa*. Applied Environmental Microbiology. 1977;33:975-976.
39. Lancini G, Parenti P, Gallo GG. Antibiotics: A multidisciplinary approach. John Wiley and Sons Inc., New York; 1995.
40. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety. 2000;45:198–207.
41. Oko JO, Abua R. Microbiological Impact assessment of air environment of the hides and skin processing unit of NILEST Tannery, Zaria. Achieves of Current Research International. 2016;3(4):1-6. DOI: 10.9734/ACRI/2016/25951

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15054