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Screening of Heavy Metals Tolerance among Beta-Lactamase Producing Bacteria from Contaminated Soil and Waste Water of Some Abattoirs in Adamawa State

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Abstract

Bacterial adaptability enables them to tolerate various stresses, including those from antibiotics and heavy metals. This study aims to investigate the tolerance to heavy metals, as well as the production of beta-lactamase and metallo-beta-lactamase, in bacterial isolates from abattoir-contaminated sites in Adamawa State. Samples were analyzed using standard microbiological techniques. Bacterial isolates with a MAR index above 0.4 were screened for tolerance to heavy metals (Cu, Zn, Fe, Co, and Pb) at concentrations of 50, 150, and 300 ppm, respectively. Extended-spectrum ß-lactamase activity (ESBL) was detected on Mueller-Hinton agar using the Kirby-Bauer double disk diffusion method. Metallo-beta lactamase activity was determined using Imipenem (IMP) – EDTA Combine disc. All isolates exhibited varying levels of growth, except for Chromobacterium spp., which showed no growth at any concentration. However, Pseudomonas aeruginosa and Escherichia coli were able to grow in the presence of all heavy metal concentrations, except for zinc at concentrations of 150 and 300 ppm. Extended-spectrum ß-lactamase (ESBL) production and the detection of metallo-ß-lactamase activity showed that the isolates were positive for the test at different intensities, with respect to the clarity of the zones; however, Pseudomonas aeruginosa and Escherichia coli had the highest zones (> 5 mm) with amoxicillin-clavulanic acid. Some were positive for metallo-ß-lactamase activity, but at different levels with respect to the clarity of the zones. E. coli showed the highest increase in zone with an Imipenem (IMP) EDTA disk of> 7 mm, followed by P. aeruginosa. These potentials can give prior information about the two isolates with respect to the hazard they present in the immediate environment.

Keywords: Heavy metals, Tolerance, ESBL, Metallo ß-Lactamase.

INTRODUCTION

Nigeria has witnessed an increase in demand for meat over the years, which is largely attributed to population growth (Emokaro and Dibiah, 2014). Abattoir activities are a source of pollution, as human activities such as animal production and meat processing have been reported to negatively impact soil and water composition, leading to pollution of the soil, natural waters, and the entire environment (Adesemoye *et al.*, 2006).

Abattoir waste or effluent can be defined as waste or wastewater from an abattoir, which consists of pollutants such as animal feces, blood, fat, animal trimmings, paunch content, and urine (Bandaw and Herago, 2019). Research has demonstrated that the characteristics of wastes or effluents from abattoirs change daily based on the quantity and type of stock being processed (Ojegunle and Lateef, 2017). Chukwu *et al.* (2008) noted that wastes from abattoirs are hazardous because they may contain varying amounts of harmful elements that could pose a risk to the environment. The liquid waste is made up of water, urine, gastrointestinal contents, dissolved particles, and blood. Significant quantities of secondary waste materials are generated from abattoirs during the process of optimizing the recovery of edible portions of meat for human consumption World Health Organization (WHO, 2010).

It has been discovered that abattoir wastewater contains variable levels of heavy metals, many of which are highly hazardous to plants and the ecosystem. The uncontrollable discharge of untreated abattoir effluents into the surrounding environment increases the concentration of heavy metals in the environment. Metals such as iron, copper, lead, chromium, and manganese play roles in the growth and development of microbes, as well as their metabolic processes. An increase in the concentration of these metals beyond a certain threshold compels microorganisms to adapt

using various biological mechanisms in order to withstand the change in concentration (Tamer *et al.*, 2013). The accumulation of heavy metals in the soil, as reported by Adekanmbi and Falodun (2015), could be toxic to the living organisms inhabiting the environment. Organisms may develop resistance to metals and antibiotics, which could pose a danger to human health (Ja'afaru *et al.*, 2021). When untreated abattoir effluent or waste water are discharged into the environment (water and soil) in particular, some elements such as iron, lead, phosphorus, calcium and zinc initially absent or present in insignificant quantities are introduced into the environment leading to the increase of these elements, thereby altering the pH and other physicochemical properties of the soil. In addition, bacteria have demonstrated an impressive capacity to generate a variety of resistance mechanisms that mediate resistance to antimicrobials initially thought to be highly effective, as well as the presence of pollutants (Ogbonna *et al.*, 2020). The capacity of bacteria to proliferate in the presence of antibiotics is known as antibiotic resistance, which can be either innate or acquired, according to the European Centre for Disease Prevention and Control (ECDC, 2013). The resistance of microbes to antimicrobial agents is a global phenomenon that spans from one environment to another, and antibiotic-resistant bacteria know no borders. This poses a significant global health challenge in the effective treatment and management of infections caused by organisms that produce these enzymes. They could compromise the effective treatment of infections caused by drug-resistant bacterial pathogens, as they harbor genes responsible for resistance. Extended-spectrum ß-lactamases (ESBLs) are beta-lactamase enzymes that hydrolyse extended-spectrum cephalosporins with an oxyimino side chain but are inhibited by clavulanic acid. This occurs mostly in Gram-negative bacteria, especially the Enterobacteriaceae, and is associated with increased morbidity and mortality (Ejikeugwu *et al.*, 2017). The increased prevalence of extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs) in resistant Gram-negative bacteria has created an urgent and important need to accurately detect these pathogens in order to keep them in check and under control. Abattoirs in Nigeria, and specifically in Adamawa State, are often located near residential areas. The effluents are uncontrollably and untreatably released into the environment, and some are perhaps used as irrigation water. These activities potentially transfer some of these resistant ESBL and MBL pathogens associated with these effluents (Ejikeugwu *et al.*, 2021; Bello *et al.*, 2023).

Furthermore, the free movement of people today conveys these microbes from one place to another, especially to abattoir environments, and this represents a major and serious problem to humans who depend on abattoirs for meat and meat products. Moreover, some organisms are multidrug-resistant in nature, exhibiting resistance to a wide variety of antibiotics used in clinical medicine (Iroha *et al.*, 2016). In countries like ours, these organisms are still not detected in hospitals because phenotypic characterization is insufficient and inappropriate for establishing the reality and presence of all these important enzymes. Therefore, direct or indirect release of abattoir waste in to the surrounding environment can cause contamination of the environment, which is a frequent practice in the developing countries like Nigeria where slaughterhouse wastewater are disposed into streams, drains on land, and the open ground, or used for irrigational purposes thereby endangering public health and polluting the environment (Bello *et al.*, 2023).

MATERIALS AND METHODS

Study Area

Adamawa State is located in the North Eastern part of Nigeria. It lies between latitudes 7 and 11°N and between Longitudes 11 and 14°E. It shares boundary with Taraba State in the south and west, Gombe State in its North-west, and Borno State to the North (Aliyu *et al.*, 2021). The three main abattoirs used in the study are the ones providing wholesome meat for the populace in those areas. Yola has the highest population of over 336,648, which makes the Jimeta abattoir the busiest, with more than 200 animals slaughtered per day, including goats, sheep, and cows. The source of water for the abattoirs is an industrial borehole in Jimeta. Locally dug boreholes in Ganye and Mubi use water from other sources, which are mostly obtained from water vendors at the time of this research.

Preparation of stock solution

Stock solutions of six different heavy metals viz; Chromium, Cobalt, Copper, Iron, Lead and Zinc were prepared from analytical grade salts of Chromium (iii) oxide, Cobaltous chloride, Copper (ii) chloride dehydrate, Iron ferric sulphate,

Lead nitrate, and Zinc sulphate heptahydrate respectively. The gram of each salt required to prepare the stock solutions of each heavy metal was determined using the formula below (Harvey, 2016).

$$W\ (g) = \frac{Conc.(ppm) \times Vol.}{1000000} \times \frac{M}{Nm}$$

Where: W, is the weight of salt required

Conc., is the stock concentration needed to be prepared in ppm (1000 ppm in this regard).

Vol., is the volume of the solution (1000 ml).

M, the molecular mass of the salt and

N, number of moles of the metal in the respective salt

m, is the molecular mass of the heavy metal.

An appropriate amount of each salt, in grams, was weighed into a 1000 cm^3 volumetric flask and filled to the mark with sterile distilled water to yield a 1000 ppm concentration.

Subsequent concentrations of 500, 300,250, 200,150, 100, and 50 ppm were prepared for each heavy metal from the stock solution using the relation,

$C_1V_1 = C_2V_2$.

Where: C_1, is the concentration of the stock (1000 ppm).

V_1 is the volume of the stock required to prepare the desired concentration.

C_2, is the final concentration needed to be prepared and

V_2 is the volume of the solution in which the second concentration is prepared.

Sample Collection:

Abattoir wastewater and soil contaminated with effluents were collected from the three abattoirs located in each of the three senatorial districts of Adamawa State, namely Jimeta, Mubi, and Ganye, respectively, in Adamawa State, North-Eastern Nigeria. Nine (9) wastewater/effluent samples were collected from the three abattoirs; three samples from each at intervals of two weeks from the months of May to June, 2023. All samples were collected at the exit points along the drainage just as it leaves the pavements of the abattoir, using sterile, wide-mouthed bottles with the grapple technique. The sample bottles were placed in an ice box and transported to the laboratory, following all aseptic guidelines (Rabah et al., 2011). A total of nine (9) soil samples were collected from the three different abattoirs mentioned in the studies. At each of these abattoirs, top contaminated soil in the slaughter house or where the abattoir wastes are disposed were collected. The contaminated soils from the abattoirs were collected after clearing waste materials from the surface of the soil in the vicinity of these abattoirs. Soil samples were collected at two-week intervals using a soil Auger to obtain soil from a depth of 0–20 cm in sterile sample bottles. Control samples were collected for all the sampling sites. Abattoir waste water and soil were collected at distances of 300- 400 meters away from the main slaughter area and the abattoir premises, where it appears not to be influenced or contaminated by the operating processes of the abattoir. Wastewater from a pond or river close to the abattoir that appeared not to be influenced by abattoir processes was collected as control.

Isolation and characterizations of Bacteria

Abattoir wastewater was analyzed using the method described by Adesemoye *et al*. (2006). A sterile pipette was used to pipette 0.1 mL of serially diluted (10^{-3}, 10^{-5}, and 10^{-7}) wastewater samples onto prepared nutrient agar plates using the plating method. The plates were incubated at 37^0C for 24 hours. After the incubation period, the colonies were sub-cultured on fresh media to obtain pure isolates. The pure isolates were maintained on agar slant for further identification

Ten grams of each soil sample were weighed and added to 90 mL of sterile distilled water to obtain an aliquot. One milliliter of the solutions (aliquot) were then serially diluted (10^{-3} 10^{-5} 10^{-7}). The plating method was used, where 0.1 mL of each of the serially diluted solutions (aliquots) was placed on Nutrient Agar (NA). The plates were incubated at 37^0C for 24 hours. After incubation, the colonies on the plates were subcultured repeatedly on fresh media to obtain pure cultures. The pure isolates were then maintained on agar slants for further identification.

Characterizations of Bacteria isolates

Bacterial isolates were characterized using microscopy and biochemical tests. These include Gram reaction, oxidase test, indole production, motility test, methyl red test, urease test, catalase test, coagulase test, citrate utilization, Voges-Proskauer test, spore staining, and triple sugar iron agar. The results of bacterial characterization were compared with those of known taxa.

Antibiogram of the bacterial isolates

Bacterial isolates were subjected to in vitro susceptibility tests against commonly used antimicrobial agents using the disc diffusion method, as recommended by the Clinical Laboratory and Standards Institute. The isolates were grown for 18 hours on nutrient agar, then suspended in 2 mL of sterile normal saline, and the turbidity was adjusted to match the McFarland Standard No. 0.5 (equivalent to 1.5×10^8 bacterial cells per mL). Bacterial suspensions of 0.1 ml were dispensed on the surface of sterile Mueller-Hinton agar plates and spread evenly using a sterile glass spreader. The sample was allowed to dry for 5 minutes, and antibiotic-impregnated discs (manufactured by Celtec Diagnostics, UK) were dispensed onto the surface of the media and incubated at 37 °C for 18 hours. The antibiotics used included the following: AUG (Amoxicillin Clavulanate 30µg), CTX (Cefotaxim 25µg), IMP (Imipenem/cilastatin 10/10µg), NF (Nitrofurantoin 300µg), CXM (Cefuroxime 30µg), CRO (Ceftriaxone Sulbactam 45µg), OFX (Ofloxacin 5µg), GN (Gentamycin 10µg), NA (Nalidixic acid 30µg), ACX (Ampiclox 10µg), ZEM (Cefexime 5µg), LBC (Levofloxacin 5µg), CIP (Ciprofloxacin 5µg) and AZN (Azithromycin 15µg). The susceptibility pattern of the isolates to different antibiotics was noted as Sensitive (S) or Resistant (R) according to the standards (Ejikeugwu *et al.*, 2018).

Multiple antibiotic resistances *(MAR) index*

Multiple antibiotic resistances (MAR) index was calculated as

$$MAR(INDEX) = \frac{a}{b}$$

Where 'a' represents the number of antibiotics to which the isolates were resistant and 'b' represents the total number of antibiotics to which the isolate was exposed. Multi- antibiotic resistance (MAR) was taken as number of antibiotics to which the isolates were resistant divided by the antibiotics tested (Ejikeugwu *et al.* (2017).

Estimation of Bacterial Tolerance to Heavy Metals

The heavy metal tolerance of the bacteria was determined using the broth dilution method. Nutrient broth medium, sterilized at 121 °C for 15 minutes, was supplemented with different concentrations (50, 100, 150, 200, 250, 300, 500 ppm) of filter-sterilized heavy metals (Zinc, Lead, Copper, Iron, and Cobalt). The amended medium was inoculated with an equal amount of bacteria isolates from the contaminated soil, wastewater, or effluent samples. Inoculated broth without heavy metal served as the control. The test and control media were incubated at 37 °C for 48 hours. After which, growth in the samples was observed and recorded (Vashishth and Khanna, 2015).

Determination of extended spectrum ß-Lactamase (ESBL) activity

This was performed on Mueller-Hinton agar using the Kirby-Bauer double disk diffusion method, as described in. The test was performed with amoxicillin-clavulanic acid and a third-generation cephalosporin disc, ceftazidime (Oxoid). Isolates were grown for 18 – 24 hours on nutrient agar, then suspended in 2 mL sterile normal saline and turbidity adjusted to match

McFarland Standard No 0.5 (equivalent to 1.5 x 10^8 bacterial density). Bacterial suspensions of 0.1 ml were dispensed on the surface of sterile Mueller-Hinton agar plates and spread evenly using a sterile glass spreader and allowed to dry for 5 minutes then a disc containing 30 µg of ceftazidime was placed on the agar at a distance of 20 mm from the amoxillin- clavulanic acid (30 µg) disc. The plates were incubated at 37°C for 24 hours. The ESBL-producing strains will show an increase in inhibition zone with the amoxillin plus clauvulanic acid disc >5 mm zone than around ceftazidime discs alone.

Detection of Metallo-beta lactamase activity by Imipenem (IMP) - EDTA Combine Disc

This was performed as described by Iroha *et al.* (2016). The imipenem combination disc was performed by inoculating the test organism onto Mueller-Hinton agar. The bacterial isolates were

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grown for 24 hours on nutrient agar. They were then suspended in 2 mL of sterile normal saline. The turbidity was adjusted to match the McFarland Standard No. 0.5. Bacterial suspensions of 0.1 ml were dispensed on the surface of sterile Mueller-Hinton agar plates and spread evenly using a sterile spreader and allowed to dry for 5 min and then two (10 µg) Imipenem discs (oxoid) was placed on the plates at a distance of 20 mm from each disc. Appropriate amount of 10 µl of EDTA solution

Table 1 Qualitative Screening of Bacterial Tolerance to heavy metals Concentrations (ppm)

S/N	Organisms	Heavy metal Conc (ppm)															
		Cu			Zn			Fe			Pb			Co			
		50	150	300	50	150	300	50	150	300	50	150	300	50	150	300	
1	*Escherichia coli*	+++	++	++	++	-	-	++	++	++	++	++	+	++	++	+	
2	*Salmonella spp*	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	
3	*Shigella spp*	++	+	+	+	++	+	+	-	-	+	+	-	++	-	-	
4	*Pseudomonas aeruginosa*	++	+	+	+	+	-	++	+	+	++	++	+	+++	++	+	
5	*Streptococcus* spp	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	
6	*Klebsiella* spp	+	++	+	-	-	+	+	+	+	++	++	+	++	+	+	
7	*Bacillus*spp	+	-	-	+	+	-	-	-	-	+	+	-	+	+	+	
8	*Proteus mirabilis*	-	-	-	+	+	-	++	+	+	+	-	-	+	-	-	
9	*Staphylococcus aureus*	++	+	+	++	+	-	++	+	+	+	+	+	++	+	-	
10	*Chromobacterium*spp	+	+	-	++	+	-	-	+	+	-	-	-	-	-	-	

KEY: + = Scanty growth, ++ = Moderate growth, +++ = Luxuriant growth, - = No visible growth

Heavy metals: Co: Cobalt Chloride ($CoCl_2$), Cu = Copper (ii) chloride dehydrate ($CuCl_2$), Fe: Iron sulphate ($FeSO_4$), Pb: Lead nitrate ($Pb(NO_3)_2$ and Zn: Zinc sulphate, ($ZnSO_4$)

was added to one of them to obtain the desired concentration (750 µg). The zones of inhibition with the imipenem and imipenem-EDTA disc were compared after 18-24 hours of incubation at 37°C. An increase in the inhibition zone with the imipenem and EDTA disc of >7 mm compared to the imipenem disc alone was considered metallo-ß-lactamase positive (Ejikeugwu *et al.*, 2014).

RESULTS

Isolation and Identification

A total of thirty-nine (39) bacterial isolates belonging to ten genera were recovered. These include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Chromobacterium* spp., *Shigella* spp., *Escherichia coli*, *Bacillus* spp., *Proteus mirabilis*, *Klebsiella* spp., *Streptococcus* spp., and *Salmonella* spp. The occurrence rate showed that *Staphylococcus aureus* had the highest frequency (23.07%), followed by *Pseudomonas aeruginosa* (15.4%). Other genera including *Shigella* spp., *Escherichia coli*, *Bacillus* spp., *Proteus mirabilis*, and *Klebsiella* spp. all had equal frequencies (7.69%), while *Streptococcus* spp. and *Salmonella* spp. had the lowest frequencies (5.12% each).

Heavy Metal Tolerance Screening

Ten (10) bacterial isolates from wastewater/effluent and soil samples with Multiple Antibiotic Resistance (MAR) indices above 0.4 were screened for heavy metal resistance. As shown in Table 1, the isolates exhibited varying growth responses at different metal concentrations (50 ppm, 150 ppm, and 300 ppm). Two isolates *Pseudomonas aeruginosa* (JS7) and *Escherichia coli* (Jww2) demonstrated the highest resistance, as evidenced by moderate to heavy growth on metal-amended media. Both organisms survived exposure to all tested metals: Cu, Zn, Fe, Co, and Pb.

Table 2. Determination of Extended spectrum-ß- lactamases (ESBL) and Metallo-ß- lactamases activities in bacterial isolates that are Heavy metal resistant and MAR index above 0.4

S/N	Organisms	Extended spectrum-ß- lactamases (ESBL)	Metallo-ß- lactamases
1	*Escherichia coli*	++	+++
2	*Salmonella* spp	+	+
3	*Shigella* spp	+	++
4	*Pseudomonas aeruginosa*	+++	++
5	*Streptococcus* spp	+	-
6	*Klebsiella* spp	+	+

KEY: +++ = very large zone, ++ = large zone, + = smaller zone, - = No zone

Determination of Extended-Spectrum ß-Lactamase (ESBL) Activity

Six isolates that were previously screened for metal tolerance were tested for ESBL production. All were positive to varying extents. However, *Pseudomonas aeruginosa* and *Escherichia coli* exhibited the highest activity, with inhibition zones exceeding 5 mm when tested with amoxicillin-clavulanic acid, as shown in Plate 1a and 1b. These results are further presented in Table 2.

Determination of Metallo ß-Lactamase (MBL) Activity

Phenotypic detection of metallo ß-lactamase activity was carried out on the same six isolates. All tested isolates were positive, though at different intensities. *Escherichia coli* showed the highest zone of inhibition with the Imipenem (IMP)–EDTA combination (>7 mm), followed by *Pseudomonas aeruginosa*. Both also showed increased zones with EDTA alone, as illustrated in Plate 2a and 2b.

DISCUSSION

The results indicate a significant diversity of bacterial genera associated with abattoir effluents, with *Staphylococcus aureus* and *Pseudomonas aeruginosa* being the most prevalent. This could be due to the indigenous nature of these bacteria in animal feces and their ability to persist in soil and water environments. Similar prevalence rates were reported by Stanley et al. (2016), who isolated *P. aeruginosa*, *Salmonella* spp., *S. aureus*, and *Klebsiella* spp. from abattoir wastewater in Ebonyi State, Nigeria. Additionally, Rabah et al. (2010) reported the presence of comparable bacterial species in soil contaminated with abattoir effluents in Sokoto metropolis, Nigeria. The frequent detection of these organisms indicates their environmental persistence and potential public health risks due to contamination.

The metal tolerance observed, particularly in *P. aeruginosa* and *E. coli*, suggests adaptation to contaminated environments, likely due to

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prolonged exposure to sublethal concentrations of metals. This finding aligns with those of Eghomwanre et al. (2016), who reported heavy metal tolerance in similar bacteria from Warri, Delta State, Nigeria.

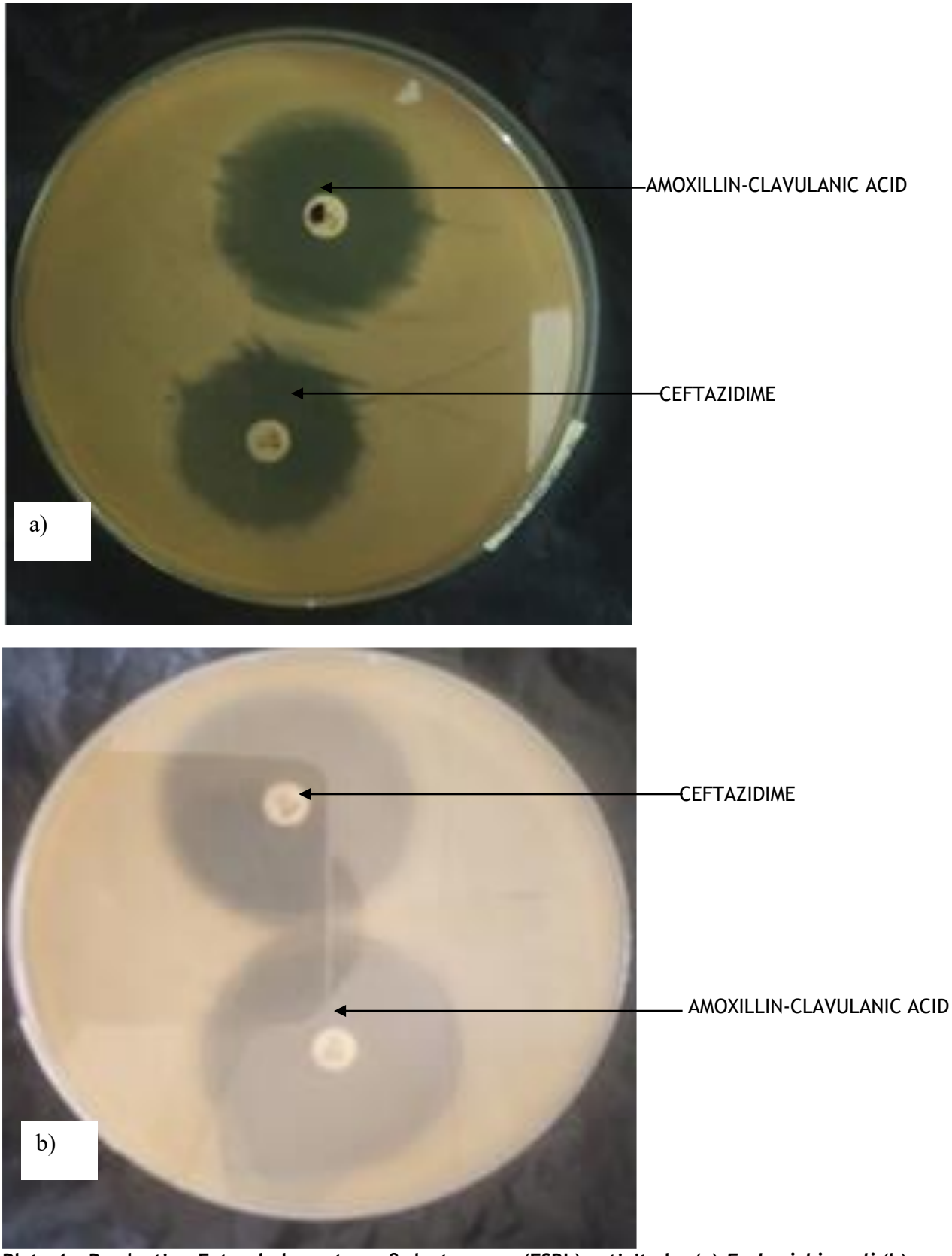

Plate 1. Production Extended spectrum-ß- lactamases (ESBL) activity by (a) *Escherichia coli* (b) *Pseudomonas aeruginosa*

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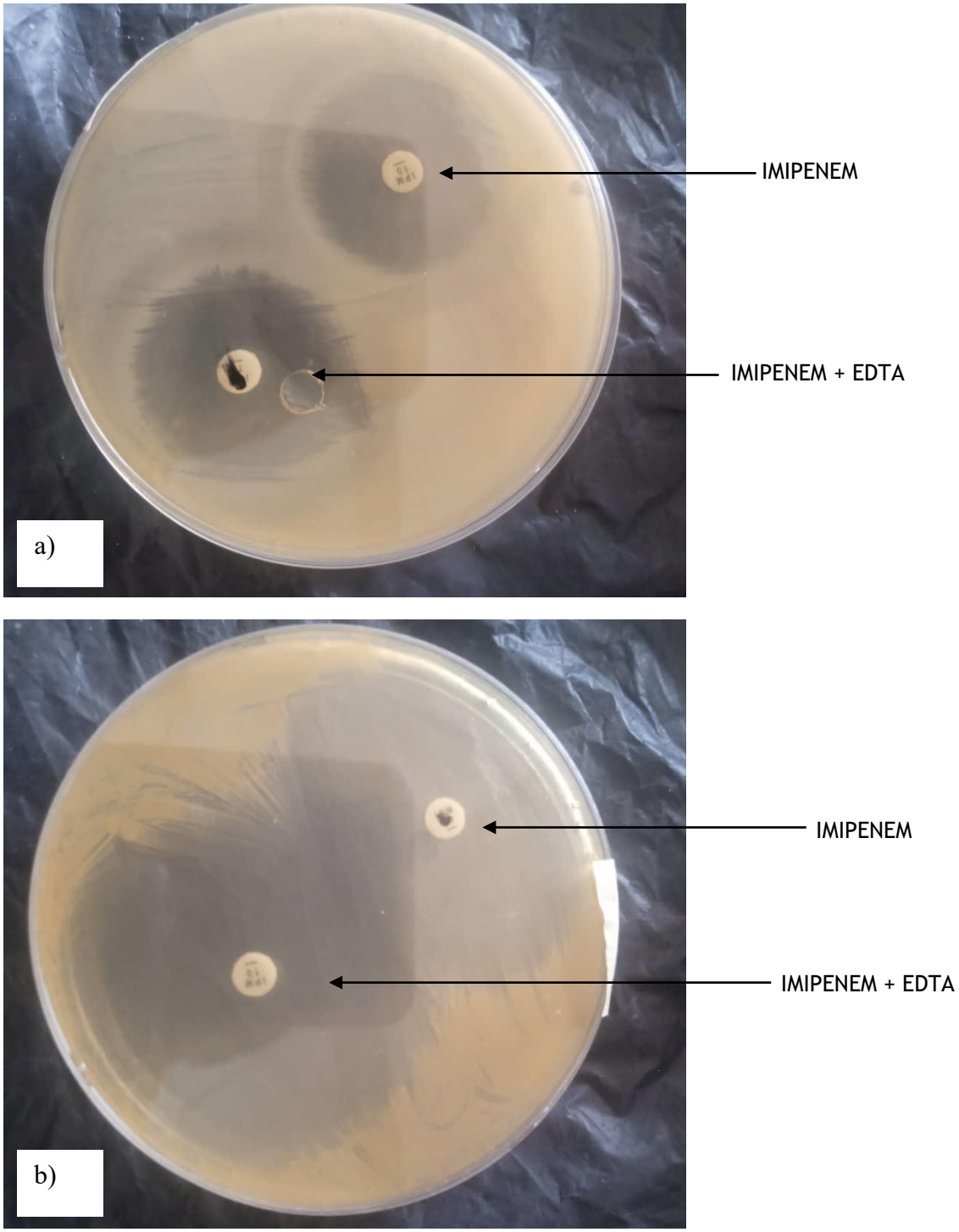

Plate 2. Production of metallo-ß-lactamase activity by (a) *Pseudomonas aeruginosa* (b) *Escherichia coli*

The ESBL activity noted, especially in *P. aeruginosa* and *E. coli*, suggests that environmental selective pressures likely induce antibiotic resistance mechanisms. Savin et al. (2020) reported similarly that E. coli from poultry slaughterhouse wastewater in Germany produced ESBLs. Ejikeugwu et al. (2015) also documented ESBL-producing *P. aeruginosa* of nosocomial origin. The detection of such resistant strains in environmental samples underscores the public health threat posed by the discharge of untreated or poorly treated effluents into the environment.

The detection of MBL activity in the isolates, particularly the high response by *E. coli* and *P. aeruginosa*, aligns with studies by Leung et al. (2013) and Chakraborty et al. (2010), who

reported MBL-producing *E. coli* in environmental samples from Australia and India, respectively. Similar findings were reported by Abd El-Baky et al. (2013), who detected MBL activity in multiple *P. aeruginosa* isolates. The co-occurrence of MBL and ESBL production among environmental bacteria signals a troubling trend of multidrug resistance. These resistance mechanisms may result from antibiotic misuse and the heavy use of antimicrobials in livestock rearing, as reported by Ejikeugwu et al. (2017). Akinduti et al. (2012) also found *E. coli*, *Klebsiella* spp., and *P. aeruginosa* producing MBL enzymes in environmental samples, supporting the data obtained in this study.

CONCLUSION

The findings from this study revealed that all the abattoirs in the study area lack proper wastewater or effluent treatment systems and harbor drug- and heavy metal-resistant pathogenic bacteria. This could constitute a major threat and can also lead to pollution of the immediate environment, posing a significant public health risk as it may facilitate the transfer of these pathogens to humans. These findings further confirm the dangers associated with discharging untreated wastewater into the environment, underscoring the need for adequate treatment to ensure decontamination. It is therefore recommended that abattoir waste waters be properly treated, and the public should be educated about the dangers of using such waste waters for irrigation and subsequent disposal on land.

COMPETING INTERESTS

The authors declare that no conflict of interest.

REFERENCES

Abd El-Baky, R. M., Abd El-Azeim, N. H., & Gad, G. F. M. (2013). Prevalence of extended spectrum beta lactamase, AmpC beta lactamase, and metallo beta lactamase among clinical isolates of *P. aeruginosa*. *Journal of Advanced Biotechnology and Bioengineering, 1*, 22–29. [Crossref]

Abdul-Gafar, H. B. (2006). *Analysis of surface and ground water pollution from abattoir waste: A case study of Minna abattoir* [Unpublished bachelor's thesis]. Department of Agricultural Engineering, Federal University of Technology Minna.

Abdullahi, I. M., Ahmed, A. A., & Dauda, T. E. (2020). Determination of levels of heavy metals and physicochemical parameters in waste water of Kasuwan shanu abattoir, Maiduguri. *Journal of Chemistry Letters, 1*, 84–88.

Adekanmbi, A. O., & Falodun, O. I. (2015). Physicochemical, microbiology and heavy metals studies on water samples and bacteria obtained from Dandaru River in Ibadan, South Western Nigeria. *African Journal of Microbiology Research, 9*, 1357–1365. [Crossref]

Adelegan, J. A. (2002). Policy and slaughterhouse waste in Nigeria. In *Proceedings of the 28th WEDC Conference* (pp. 3–6). Kolkata, India.

Adesemoye, A. O., Opere, B. O., & Makinde, S. C. O. (2006). Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria. *African Journal of Biotechnology, 5*(20), 1963–1968.

Adesina, A. O., Ogunyebi, A. L., Fingesi, T. S., & Oludoy, E. (2018). Assessment of Kara Abattoir Effluent on the Water Quality of Ogun River. *Nigerian Journal of Science and Environmental Management, 22*(9), 1465–1470. [Crossref]

Adeyemi, I. G., & Adeyemo, O. K. (2007). Waste management practices at the Bodija abattoir, Nigeria. *International Journal of Environmental Studies, 64*(1), 71–82. [Crossref]

Adeyemo, O. K., Adeyemi, I. G., & Odunsi, O. O. (2019). Physicochemical, heavy metals and microbial pollution of surface and ground water in bodija Municipal Abattoir and its Environs. *International Journal of Environment, Agriculture and Biotechnology, 4*(6). [Crossref]

Akan, J. C., Abdulrahman, F. I., & Yusuf, E. (2010). Physical and chemical parameters in abattoir wastewater sample. *Pacific Journal of Science and Technology, 11*(1), 640–648.

Akinduti, P. A., Ejilude, O., Motayo, B. O., & Adeyokinu, A. F. (2012). Emerging multidrug resistant AmpC beta-lactamase and carbapenemase Enteric isolates in Abeokuta, Nigeria. *Nature and Science, 10*, 70–74.

Aliyu, M., Ibrahim, K. H., & Abubakar, M. A. (2021). Sexual dysfunction and infertility amongst spouses in Adamawa state, Nigeria. *American Journal of Health Research, 9*, 1–8. [Crossref]

Bandaw, T., & Herago, T. (2019). Review of abattoir waste management. *Global Veterinaria, 19*(2), 517–524.

Behera, B., Das, A., Mathur, P., & Kapil, A. (2008). High prevalence of carbapenem resistant *Pseudomonas aeruginosa* at a tertiary care centre in north India. Are we under-reporting? *Indian Journal of Medical Research, 128*, 324–332.

Bello, B. V., Qadeer, M. A., & Oladapo, B. S. Effect of abattoir wastes on surface and underground water qualities in Yola Metropolis, Adamawa State, Nigeria. *FUW Trends in Science & Technology Journal, 8*(2), 184–191.

Canton, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., & Coque, T. M. (2012). Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. *Clinical Microbiology and Infection, 14*(1), 144–153. [Crossref]

Chakraborty, D., Basu, S., & Das, S. (2010). A study on infections caused by Metallo-Beta-lactamase Producing Gram-Negative Bacteria in Intensive Care Unit Patients. *American Journal of Infectious Diseases, 6*(2), 34–39. [Crossref]

Chukwu, O., Mustapha, H. A., & Abdul-Gafar, H. B. (2008). The effect of abattoir waste on surface water quality. *Environmental Research Journal, 2*(6), 334–338.

Deredjian, A., Colinon, C., Brothier, E., et al. (2011). Antibiotic and metal resistance among hospital and outdoor strains of *Pseudomonas aeruginosa*. *Research in Microbiology, 162*, 689–700. [Crossref]

Eghomwanre, A. F., Obayagbona, N. O., Osarenotor, O., & Enagbonma, B. J. (2016). Evaluation of antibiotic resistance patterns and heavy metals tolerance of some bacteria isolated from contaminated soils and sediments from Warri, Delta State, Nigeria. *Journal of Applied Science and Environment, 20*(2), 287–291. [Crossref]

Ejikeugwu, C., Duru, C., Eluu, S., Oguejiofor, B., Ezeador, C., Ogene, L., & Iroha, I. (2017). Isolation and phenotypic detection of Metallo-Beta-Lactamase (MBL)-producing *Klebsiella* species from cow anal swabs. *Global Journal of Pharmaceutical Science, 2*(3), 555–586. [Crossref]

Ejikeugwu, C., Iroha, I., Orji, J., Ugwu, M., & Okonkwo, E. (2015). Antibiogram of ESBL-producing *P. aeruginosa* isolates of nosocomial origin. *European Journal of Pharmaceutical and Medical Research, 2*, 92–99.

Ejikeugwu, C., Nworie, O., Agah, M. V., Oguejiofor, B., Ovia, K., Nworie, C. O., Iwunze, A. C., Nwambeke, A., & Edeh, C. (2018). Bacteriological and antibiogram of AmpC producing *Enterobacteriaceae* isolated from Abattoir. *Microbiology Current Research, 82*(2), 37–41.

Ejikeugwu, C., Nworie, O., Saki, M., Al-Dahmoshi, H. O., Al-Khafaji, N. S., Ezeador, C., & Adikwu, M. U. (2021). Metallo-ß-lactamase and AmpC genes in *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. *BMC Microbiology, 21*, 1–9. [Crossref]

Ejikeugwu, P. C., Ugwu, C. M., Iroha, I. R., Eze, P., Gugu, T. H., & Esimone, C. O. (2014). Phenotypic detection of Metallo-ß-Lactamase enzyme in Enugu, Southeast Nigeria. *American Journal of Biological, Chemical and Pharmaceutical Science, 2*(2), 1–6.

Emokaro, C. O., & Dibiah, O. (2014). Demand analysis for chicken meat, beef and fish among urban households in Edo and Delta states, Nigeria. *Journal of Applied and Natural Science, 6*(1), 239–245. [Crossref]

European Centre for Disease Prevention and Control. (2013). Factsheet for experts. ecdc.europa.eu

Fitsum, D. (2014). *Prevalence and antimicrobial resistance profile of Escherichia coli O157:H7 in goat slaughtered in Dire Dawa Municipal Abattoir as well as food safety knowledge, attitude and hygiene practice assessment among slaughter staff, Ethiopia* [Master's thesis]. Addis Ababa University.

Freitas, A. L., Machado, D. P., Soares, F. S. C., & Barth, A. L. (2003). Extended spectrum beta-lactamase in *Klebsiella* spp. and *Escherichia coli* obtained in a Brazilian teaching hospital: Detection, prevalence and molecular typing. *Brazilian Journal of Microbiology, 34*, 344–348. [Crossref]

Harvey, D. (2016). *Solutions manual to analytical chemistry 2.1*. McGraw-Hill. who.int

Ire, F. S., Miriam, O. A., & Ossai-Chidi, L. N. (2017). Microbiological and physiochemical assessment of abattoir effluents and receiving water bodies in Port Harcourt. *Journal of*

Pharmaceutical, Chemical and Biological Sciences, 5(1), 34–39.

Iroha, I. R., Eromonsele, O. B., Moses, I. B., Afukwa, F. N., Nwakaeze, A. E., & Ejikeugwu, P. C. (2016). In vitro antibiogram of multidrug resistant bacteria isolated from Ogbete Abattoir Effluent in Enugu State, Nigeria. *International Research Journal of Public and Environmental Health, 3*, 1–6.

Ja'afaru, M. I., Adeyemo, O. M., Okafor, C. H., & Bristone, P. (2021). Effects of abattoir effluents on heavy metal tolerance, bacteriological quality and physicochemical parameters of contaminated soil in Yola, Adamawa State, Nigeria. *Nigeria Annals of Pure and Applied Sciences, 4*(1), 1–14. [Crossref]

Leung, G. H., Gray, T. J., Cheong, E. Y., Haertsch, P., & Gottlieb, T. (2013). Persistence of related bla-IMP-4 Metallo-Beta Lactamase producing *Enterobacteriaceae* from clinical and environmental specimens within a burns unit in Australia - a six-year retrospective study. *Antimicrobial Resistance and Infection Control, 2*(1), 35. [Crossref]

Lima e Silva, A. A., Ribeiro de Carvalho, M. A., de Souza, S. A. L., Teixeira Dias, P. M., da Silva Filho, R. G., de Meirelles Saramago, C. S., de Melo Bento, C. A., & Hofer, E. (2012). Heavy metal tolerance in bacteria isolated from sewage. *Brazilian Journal of Microbiology, 43*, 1620–1631. [Crossref]

Nikaido, H. (2009). Multidrug resistance in bacteria. *Annual Review of Biochemistry, 78*, 119–146. [Crossref]

Obijiofor, O. C., Okoye, P. A. C., & Ekejiuba, I. O. C. (2018). Assessment of surface water contamination and effect of textile effluents on Ibeshe River, Ikorodu, Lagos, Nigeria. *Journal of Chemical Society of Nigeria, 43*(2), 69–79.

Ogbonna, D. N., Abu, G. O., & Okoli, C. (2020). Heavy metal contamination in abattoir wastes and soils around abattoirs in Port Harcourt, Nigeria. *International Journal of Environmental Sciences & Natural Resources, 24*(1), 1–7. [Crossref]

Ojegunle, O. Z., & Lateef, S. T. (2017). Environmental impacts of abattoir waste discharge on the quality of surface and ground water in Abeokuta. *Journal of Environmental and Analytical Toxicology, 7*(5).

Rabah, A. B., Oyeleke, S. B., Manga, T. B., Hassan, L. G., & Ijah, U. J. (2010). Microbiological and physicochemical assessment of soil contaminated with abattoir effluents in Sokoto metropolis, Nigeria. *Science World Journal, 5*, 21–42. [Crossref]

Savin, M., Bierbaum, G., Hammerl, J. A., Heinemann, C., Parcina, M., Sib, E., Voigt, A., & Kreyenschmidt, J. (2020). ESKAPE bacteria and extended-spectrum-ß-lactamase-producing *Escherichia coli* isolated from wastewater and process water from German poultry slaughterhouses. *Applied and Environmental Microbiology, 86*, e02748-19. [Crossref]

Stanley, C. O., Collins, O. O., Bruno, C. A., & Faith, C. N. (2016). Distribution of antibiotic resistant bacteria from abattoir wastes and its receiving waters at Nkwo-ezzamgbo, Ebonyi State, Nigeria. *World Journal of Medical Sciences, 13*(4), 242–250.

Tamer, A., Aysenur, K., & Sadik, D. (2013). Antibiotic levels and heavy metal resistance in gram-negative bacteria isolated from seawater, Iskenderum organized industrial zone. *Journal of Applied Biological Sciences, 7*, 10–14.

Vashishth, A., & Khanna, S. (2015). Toxic heavy metals tolerance in bacterial isolates based on their inducible mechanism. *International Journal of Novel Research in Life Science, 2*, 34–41.

World Health Organization. (2010). *The world health report - health systems financing: The path to universal coverage*. who.int

Yilmaz, E. I. (2013). Metal tolerance and bio-sorption capacity of *Bacillus circulans* strain EBT. *Journal of Research on Microbiology, 154*, 409–415. [Crossref]

Yong, D., Lee, K., Yum, J. H., Shin, H. B., Rossolini, G. M., & Cong, Y. (2002). Imipenem-EDTA disc method for differentiation of metallo-beta-lactamase producing clinical samples of *Pseudomonas* spp. and *Acinetobacter* spp. *Journal of Clinical Microbiology, 40*(10), 3798–3801. [Crossref]