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Original Article

Antimicrobial Susceptibility for Bacterial Isolates from Abattoir Effluent of Damaturu-Nigeria

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Abstract

Hazards caused by effluent into the environment are enormous and may lead to several complications in living forms. Our present research studies were conducted to determine antimicrobial susceptibility and resistance for bacterial isolates collected from abattoirs effluent in Damatruru, Nigeria against commercially available antibiotics. Temperature and pH of the effluent were determined. Bacterial isolates were identified by biochemical method and antimicrobial susceptibility was determined using disc and well diffusion method. The initial temperature of abattoir effluent were 25^{o}C, initial pH of abattoir were 6.7.The bacterial isolates from abattoir effluent were identified as *Escherichia coli*, *Shigella sp.* and *Staphylococcus aureus*. *Escherichia coli* were resistant to Chloramphenicol(Ch), Sparfloxacin(SP), Ciprofloxacin(CPX), Amoxicillin(AM), Agumentin (AU), Gentamycin(GN), Pefloxacin(PCF) and Streptomycin(S). *Shigella sp.* showed resistance against Sparfloxacin, Ciprofloxacin, Amoxicillin, Augumentin, Gentamycin, and Streptomycin, whereas *Shigella sp.* was sensitive to Septrin (Co-trimoxazole), Chlorophenicol, Pefloxacin and Tarivid (Ofloxacin). The *Staphylococcus aureus* were resistant to Ampiclox (Ampicillin and Cloxacillin), Zinacef (Cefuroxime), Amoxicillin and Erythromycin, whereas it was sensitive to Pefloxacin, Gentamycin, Ciprofloxacin, Streptomycin and Septrin. For, Well diffusion method, *Escherichia coli* were resistance to Septrin and Amoxicillin, whereas sensitive to Ciprofloxacin and Tetracycline .*Shigella sp*. were resistant to Septrin, Amoxicillin whereas, sensitive to Ciprofloxacin and Tetracycline. *Staphylococcus aureurs* were resistant to Septrin, Amoxicillin. Other showed the susceptibility to antibiotics.

Keywords: *Abattoir effluent, Escherichia coli, Shigella sp., Staphylococcus aureus, antibiotics***.**

Introduction

Environmental problems have increased over the last four decades with improper management practices being largely responsible for the gross production of aquatic environment with contaminant increase in water borne diseases especially *typhoid fever*, *diarrhoea*, *cholera* and *dysentery*. Effluent is an out flowing of water from a natural body of water or from a human made structure. Effluent is defined by the United States Environmental Protection Agency (USEPA) as "waste water-treated or untreated that flows out of a treatment plant, sewage or industrial outfall. Generally, refers to wastes discharged into surface waters '' (USEPA, 2006).

Bacteria associated with effluents has been extensively studied, *lactobacillus, Baccilus, pseudomonas, Azobacter, Arthrobacter, zoogloea, Mycobacterium, Staphylococcus, Micrococcus, Escherichia and Eikenia* (Selvi *et al*.,2012).*Escherichia coli*, *Bacillus species, Pseudomonas species*, *Flavobacterium species* and *Alcaligene species* (Krishnaveni *et al.,* 2013) *Pseudomonas species*, *Bacillus species*, *Arthrobacter species* and *Micrococcus species*.

In the livestock sector different types of farm animals are capable of carrying a wide range of zoonotic pathogens(Swai and Schoonman, 2012) Moreover, animals brought for slaughter into urban areas more often come from village where pathogens control regimens are weak, un-coordinated and often not available. Lack of veterinary service in these livestock rearing areas poses a substantial risk of widespread occurrence of diseases in the livestock population and community of human exposure to these zoonotic diseases agent (Swai and Schoonman, 2012).

In Nigeria the situation in no better by the activities of most industries and populace towards waste disposal and management which usually leads to the environment. Effluent is a major menace which is compounded in areas where they are discharge without adequate treatment. This current investigation is aimed to study the effect of antibiotics on bacteria isolated from abattoir water site.

According to the (WHO, 2013) approximately 1,1 billion people lack access to high quality water supply sources resulting in diarrhea, which is the cause to 4% of the human deaths in the world. According to (Black *et al*.,2010) 16 % of the deaths among children under five years in Uganda during 2008 were caused by gastro-intestinal diseases associated with diarrhea and in the Uganda Demographic and Health Survey for 2011 it was found that 23 % of the children that were included in the study with an age under five years had *diarrhea* two weeks prior to the start of the survey. Water contaminated with faeces from animals can cause *diarrhea* because animal faeces can contain *diarrhea* -causing microorganisms (WHO 2013). As an example animal faeces can contain pathogens such as *Escherichia coli* and *Salmonella spp*., which can infect humans (Berger and Oshiro 2002). It has been suggested that waterborne zoonosis can be a bigger problem in developing countries because of the lack of water treatment facilities and use of untreated wastewater (Dufour *et al*., 2012).

Several studies have revealed that abattoirs in developing countries have an unhygienic environment (Adeyemo 2002: Onunkwo and Ezenduka, 2010) and detected the presence of pathogens that are known causes of *diarrheal* diseases and a possible hazard to human health in the abattoir waste and water contaminated by abattoir waste (Benka-Coker and Ojior 1995; Abiade-Paul *et al*., 2005; Nwanta, Onunkwo and Ezenduka 2010). It has also been suggested that scavengers feeding on abattoir waste can spread pathogens from the waste to new locations (Adeyemi and Adeyemo 2007).

Considering above facts, research study was carried out on antimicrobial susceptibility for bacterial isolates from abattoir effluent of Damaturu, Yobe State, Nigeria.

Materials & Methodology

Preparation of Culture Media

The media used for cultivation, identification, and isolation were Nutrient agar, Eosin Methylene Blue agar, Xylose Lysine Deoxychocolate agar, Mac'Conkey agar, Mueller Hinton agar, Urea agar and Simmon's Citrate agar

Study Area

The study was conducted in Damaturu local government area Yobe State, Nigeria.

Source of Samples

Samples were collected from Damaturu modern abattoir.

Collection of Samples

Study samples were collected from Damaturu Modern abattoir, which was built in 2004. At the abattoir, waste from the

slaughtering processes are washed out in a drainage channel without any regulated process (APHA,1980). The abattoir has daily slaughter effluents from approximately 150 cattle and 100 goat and sheep.

Bacterial Isolation

Serial Dilution

The procedure from APHA (1985) was applied in carrying out serial dilution. 9ml of normal saline was poured into the test tube and 1ml of effluents sample (stock solution) was transferred into the test tube containing normal saline and test tubes were used are 10^{-1}, 10^{-2},10^{-3} ,10^{-4} and 10^{-5} respectively. Then 1ml to the next again up to 10^{-5} (10-fold dilution).

Culture

0.2 ml of diluted microbial mixture was transferred to the nutrient agar plate spread over the surface with a sterile inoculation loop. The plate was incubated at 37^0C for 24 hours. The dilution factors used for the inoculation were10^{-1}, 10^{-2}, 10^{-3} 10^{-4}, and 10^{-5} respectively.

Coliform Count

Eosin methylene blue agar used for study of enterobacteria. The use of eosin methylene blue agar enable differentiation between lactose fermentation and non-lactose fermentation organisms. It has been used widely for detection and enumeration of coliforms bacteria in water and food. Eosin methylene blue agar was prepared by suspending 36g of the medium in 1liter distilled water in a conical flask. This was then sterilized by autoclaving at 121^0C for 15minutes under 15lbs pressures and allowed to cool. Each culture sample was inoculated by an aseptic transferred. The medium was then incubated at 37^{0C} for 24hrs. the viable bacterial isolates which grew in to visible colorless in nutrients agar eosin methylene blue agar medium were counted one after the other on the flat form of a Quebec colony counter and calculated as follows,

$$\frac{Number\ of\ colonies\ counted\ \times Dilution\ factor}{Volume\ of\ Sample}$$

Gram Staining

Gram staining techniques is an important analysis and classification for microbial characteristics (Bartholomew and Mitter, 1952). The gram staining test has being used in water testing (Adeyomi *et al*. 2007) and is useful in identification of groups such as *Salmonella species, Shigella species, Escherichia coli, Klebsilla species, Proteus species and Pseudomona species among others.* Specimens were observed under 100X oil immersion objective lenses of microscope.

Biochemical Characterization

Preliminary *Escherichia coli, Shigella species* and *Staphylococcus aureus* isolate were identified and confirmed after conducting some conventional biochemical tests. The following biochemical tests were carried out.

Catalase test

A suspension of 24hours old culture of the test organism was made with sterilized distilled water on a clean glass microscope slide a few drop of hydrogen peroxide were added using a dropping pipette. The evolution of gas bubbles caused by the liberation of free oxygen indicated the presence of catalase enzyme.

Oxidase test

This was carried out for the detection of cytochrome oxidase in the microorganisms. The overnight broth culture of isolates was inserted bactident oxidase test strips. The strips were withdrawn at once and left for 10 minutes for color change. Color change from yellow to dark purple confirms the presence oxidase. The oxidase test strips were impacted with 1% tetramethyl-p-phenyldiamine solution.

Citrates test

Simmon's citrate medium is a nutrient substrate that offers ammonium salt as the only source of nitrogen and citrate as the only carbon source. The degradation of the citrate lead to alkalinization of the medium which is indicate by the pH bromothymol blue changing color from green to deep

blue. Salt of Simmon's citrates agar were inoculated with light inoculums of the isolates incubated at 37^0C for 5 days. Color changes from green to blue indicate a positive result (Garrity *et.,al,* 2005)

Urease test

The slants of Christensen's urea agar medium were inoculated with the isolates and incubated at 37^0C for 5days watching daily for any color change. The development of change in color from yellow to pink shows positive urease activities.

Coagulase test

The coagulase test can be performed using two different method: slide and test tube test. The slide test is simple, giving result within 10second, but it can give false negatives, the tube test is the definitive test, however, it can take up to 24hrs to complete. For both test, clumping, or clots of any size indicate a positive response. While *S. aureus*is the most commonly isolated coagulase-positive organism. There are several other species of *Staphylococcus* which are positive for coagulase activity. *S. schleiferi* and *S. lugdunesis* may give positive result in the slide test for bound coagulase and *S. schleiferi and S. intermedius* may give positive result in the tube coagulase test.

The slide test were performed by preparing a suspension of bacterial cells mixed into a drop of rabbit plasma on a microscope slide, coagulase were present on the bacterial cells. The presence of plasma caused the bacterial cells to clump. The clumping would occur because of the clumping factor in an adhesion as a result of the binding of the cells with the fibrinogen in the plasma. The visible clumping of the bacterial cell was observed under microscope.

Antibiotic test

Muller Hinton agar of 38 gram was weight into 1000ml of distilled water and sterilized at 121^0C for 15minutes under 15lbs pressure in an autoclave. The cool molten Muller Hinton agar was poured into sterile Petri-dishes. Petri-dishes were allowed to set a surface dried in an oven at 45^0C. the Mueller Hinton agar plate were seeded with freshly test strains of about 24hrs by swabbing using sterile swab-sticks to make cell suspensions of the organisms to give a concentration of about 10^{-5}cfu/ml, ready preferred sensitivity discs were used for the sensitivity test which contain various antibiotics such as *Streptomycin, Septrin, Chloramphenicol, Sparfloaxin, Cipofloaxin, Amoxcilin, Augumentin, Gentymycin, Pefloaxacin*. The sensitivity disc were aseptically placed on to the media (Muller Hinton agar) containing the streaked organisms. The plate were incubated at 37^oC for 24-48hours in which zone of inhibitions were determined.

Antibiotics, such as, *Ciproflaxacin, Tetracycline, Septrin* and *Amoxicillin* were used for well diffusion method. 1000g of *Ciprofloxacin* was dissolved in 100ml distilled water, 1000g of *Tetracyclin* dissolved in 100ml distilled water, 1000g of *Amoxicillin* dissolve in 100ml distilled water, like wise *Septrin* respectively.

Well diffusion was used in the application of these antibiotics against the listed organisms on Mueller Hinton Agar. Zone of inhibition and Minimal Inhibitory Concentration (MIC) were determined.

Results

Table 1. Sample collection

S/N	Number of sample	Place of Collection
1	2	Damaturu Modern Abattoir, Yobe State, Nigeria

Table 2. Bacterial load (total viable counts) cfu/ml

S/N	Sample	Dilution factor	Number of colonies
1	A	10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5}	$7.28x10^{-1}$cfu/ml $6.46x10^{-3}$cfu/ml $4.4x10^{-4}$cfu/ml $3.8x10^{-6}$cfu/ml $2.9x\ 10^{-4}$cfu/ml
2	B	10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5}	$8.34x10^{-1}$cfu/ml $5.96x10^{-1}$cfu/ml $5.56x10^{-1}$cfu/ml $4.16x10^{-2}$cfu/ml $4.11x10^{-4}$cfu/ml

Table 3. Gram's reactions for bacterial isolates

S/N	Sample	Dilution Factor	Gram's Reaction	Microscopic Appearance
1	A	10^{-1}	+ve cocci in clusters	*Staphylococci*
2		10^{-2}	-ve thin rod	*Bacilli*
3		10^{-3}	-ve thin rods	*Bacilli*
4		10^{-4}	+ve cocci in cluster	*Staphylococci*
5		10^{-5}	-ve rods	*Bacilli*
1	B	10^{-1}	-ve thin rods	*Bacilli*
2		10^{-2}	-ve rods	*Bacilli*
3		10^{-3}	+ve cocci in cluster	*Staphylococci*
4		10^{-4}	-ve thin rods	*Bacilli*
5		10^{-5}	-ve rods	*Bacilli*

Table 4. Biochemical Characterization of Bacterial isolates

S/N	Sample	Suspected organisms
1	A	*Escherichia coli, Staphylococcus aureus, and Shigella species.*
	B	*Escherichia coli, Staphylococcus aureus, and Shigella species*

Table 5. Bacterial Colony Characterization on Differential Media

S/N	Sample	N.A	MAC	EMB	XLD	Suspected Organism
1	A	Yellowish, opaque, cocci in shape with rough surface				*Staphylococcus aureus*
			Pink mucoid smooth surface and rough surface	Green metallic sheen with opaque, smooth surface and rough edge		*Escherichia Coli*
					Pinkish colonies with black spots at the centre, rough surface and rough edge	*Shigella Species*
				Translucent colourless colonies, mucoid with rough surface and rough edge		*Shigella species*
2	B	Yellowish, opaque cocci in shape with rough surface				*Staphylococcus aureus*
			Pink muccoid smooth surface and rough surface	Green metallic sheen with opaque, smooth surface and rough edge		*Escherichia coli*
					Pinkish colonies with black spot at the centre, rough surface and rough edge.	*Shigella species*
				Translucent colourless colonies, mucoid with rough surface and rough edge		*Shigella species*

KEY: N.A = Nutrient Agar; MAC= Mac'conkey Agar; EMB= Eosin Methylene Blue Agar; XLD = Xylose Lysine Deoxychocolate Agar

Table 6. Biochemical Characterization of bacterial isolates

S/n	Sample	Catalase	Oxidase	Citrate	Urease	Coagulase	organisms presence
1	A	ND ND +	- - ND	- ND ND	- ND ND	ND ND +	*Escherichia coli* *Shigella species* *Staphylococcus aureus*
2	B		- - ND	- ND ND	- ND ND	ND ND +	*Escherichia coli* *Shigella species* *Staphylococcus aureus*

Positive: (+) indicated the presence of organism in the sample.
Negative: (-) indicate the absence of organism in the sample
ND: Not detected

Table 7. Sensitivity test for bacterial isolates by antibiotic disc method

S/N	Organism	Antibiotic drugs	Zone of inhibition (mm/dm)	Sensitivity
1	*Escherichia coli*	*Ciprofloxacin*	3.5	S
		Septrin		R
		Amoxicillin		R
		Tetracycline	1.5	S
2	*Shigella species*	*Ciprofloxacin*	4.0	S
		Septrin	-	R
		Amoxicillin	-	R
		Tetracycline	2.5	S
3	*Staphylococcus aureus*	*Ciprofloxacin*	5.5	S
		Septrin	-	R
		Amoxicillin	-	R
		Tetracycline	1.8	S

Table 8. Sensitivity test for bacterial isolates by Well diffusion method

S/N	Organism	Antibiotic	Zone of inhibition dm/mm	Sensitivity
1	*Escherichia coli*	Septrin 30mg	21	S
		Chlorophenicol 30mg	-	R
		Sporfloaxzcin 10mg	-	R
		Ciprofloxacin 10mg	-	R
		Amoxicillin 30mg	-	R
		Augumintin 30mg	-	R
		Gentamycin 10mg	-	R
		Petflexacin 30mg	-	R
		Tarivin10mg	2	S
		Streptomycin 30mg	2	S
		Streptomycin 30mg	2	S
2	*Shigella species*	Septrin 30mg	2.2	S
		Chlorophenicol 30mg	2.2	S
		Sporfloaxzcin 10mg	-	R
		Ciprofloaxacin 10mg	-	R
		Amoxicillin 30mg	-	R

		Augumintin 30mg	-	R
		Gentamycin10mg	-	R
		Pefloexacin 30mg	1.7	S
		Tarivin10mg	2	S
		Streptomycin 30mg		R
3	*Staphylocucus aureus*	Pefloaxacin 10mg	2.1	S
		Gentamycin10mg	1.9	S
		Ampillox 30mg	-	R
		Zinacef 20mg	-	R
		Amoxicillin 30mg	-	S
		Ciprofloxacin 10mg	2.8	S
		Streptomycin 30mg	2.3	S
		Septrin 3mg	1.5	S
		Erythromycin 10mg	-	R

Key: S= Sensitivity, R= Resistance

Discussion

The study of the abattoir waste water showed that the water was contaminated with high levels of *Escherichia coli, Shigella sp.* and *Staphylococcus* aureus indicating faecal contamination. The source of the contamination is most likely the abattoir waste that is being washed out into the drainage channel during and after slaughtering. Such contamination conducts polluted water unfit for human consumption and even transmits diseases. This is a potential hazard in the case of the people residing in nearby areas or localities.

Salmonella, common pathogen of North-eastern Nigeria, were not found in the untreated waste water; nevertheless the conclusion that the abattoir waste water does not contain *Salmonella* cannot be drawn since the study was limited which may be due to random selection of samples. Another possible reason may be, waste from slaughtered animal disposed to effluent on the day of sample collection was not contaminated with *Salmonella species*.

Bacterial isolates detected were *Shigella sp., Escherichia coli* and *Staphylococcus aureus.* It is reasonable to suggest that the slaughter waste may not be the source of the *Shigella sp.* since *Shigella spp.* are bacteria with humans and primates as hosts (Strockbine & Maurelli 2005). More likely the source is faecal contamination from humans indicating that abattoir workers or other people resident at the abattoir area defecate into the drainage channel. From the coliform count, number of colonies investigated was ranging between $2.9x10^{-4}$ – $7.28x10^{-1}$ cfu/ml for A and $4.11x10^{-4}$- $8.34x10^{-1}$ for B, the maximum count of $7.28x10^{-1}$ and $8.34x10^{-1}$ were recorded in 10^{-1} for A and 10^{-1} for B, whereas minimum of $2.9x10^{-4}$ and 4.11×10^{-4}cfu/ml for A and B were recorded at 10^{-5} for both the samples. *Escherichia coli* were found as gram negative bacteria and showed negative results for oxidase, citrate, and urease test. *Staphylococcus aureus* was grams positive bacteria on microscopy after gram staining and positive to catalase and coagulase test. *Shigella sp.* was found to be gram negative bacteria which were pinkish colonies with black spot at the center, rough surface and rough edge.

Test for antibiotics susceptibility indicates that the *Escherichia* coli from the abattoir waste water were more sensitive to the tested antibiotics, supporting reports form Magiorakos and Srinivasan (2012). Antibiotics were sensitive to some microorganisms while others were resistance to some microorganisms for synthetic disc and Well diffusion methods. Septrin, Tarivid, Streptomycin were sensitive to *Escherichia* coli while others antibiotics were resistance to *Escherichia* coli in which the *Septrin* was more effective than the *Tarivid* and *Streptomycin*. For,

Shigella species, Septrin, Chlorophenicol, Pefleoxacin and Tarivid were sensitive. For *Staphylococcus aureus*, Pefleoxacin, Gentamycin, Ciprofloxacin, Streptomycin and Septrin were sensitive whereas, Amphiclox, Zinacef, Amoxacillin and *Erythromycin* were resistance. Ciprofloxacin were more effective in above study. For Well diffusion method, Ciprofloxacin and Tetracycline were sensitive to *Escherichia coli,* whereas Septrine and Amoxacillin were resistance. Ciprofloxacin was more effective than Amoxicillin in above test*. Shigella sp.* were resistance to Septrin and Amoxicillin sensitive to *Ciprofloxacin* and *Tetracycline* in which *Ciprofloxacin* were more effective than tetracycline. Likewise, for *Staphylococcus aureus,* Ciprofloxacin and Tetracycline were effective, while Septrin and Amoxacilin were resistance. Here, Ciprofloxacin was found to be more effective than Tetracycline. From our present study, resistant development capacity was detected in bacterial isolates against commercially available antibiotics. In some countries of African Continent overuse of antibiotics and supply of non-prescribed antibiotics by the local pharmacy has been reported. Over use of antibiotics without proper medical supervision and prescription may lead to risk of resistance to intestinal pathogens, such as *Salmonella sp., Shigella* sp. and *Staphylococcus aureus* (Mukonzo *et al.,* 2013).

Conclusion

Present research study showed a high level of Coliform and other pathogens in the effluent sample such as, *Escherichia coli, Shigella sp. and Staphylococcus aureus*. It is evident that this effluent could pose a risk of infection to the people in nearby area or locality. The disposal of abattoir wastewater containing *Shigella sp.* and high levels of *Escherichia coli* and *Staphylococcus sp*. into the drainage channel, are considered to be a risk for human health. To reduce the risks and to minimize the possible transmission to human and animals in the environment, it is suggested that the following preventive measures are introduced at the abattoir:

1. Faeces and other abattoir waste be collected and destroyed or made non-hazardous instead of being excreted into the drainage channel.
2. It is also advisable to have a continuously running treatment facility that minimizes the amount of bacteria in the effluent water before discharge into the channels.
3. There is need to create awareness about presents situation of the sources and the necessity for further sanitation.

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References

Abiade-Paul, C., Kene, I. & Chah, K. (2005). Occurrence and antibiogram of Salmonellae in effluent from Nsukka Municipal abattoir. *Nigerian Veterinary Journal*, 27(1), pp.48–53.

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

Adeyemo, O. (2002). Unhygienic operation of a city abattoir in south western Nigeria: environmental implication. *African Journal of Environmental Assessment and Management*, 4(1), pp.23–28.

Adeyemo, O., Ayodeji, I. & Aika-Raji, C. (2002). The water quality and sanitary conditions in a major abattoir (Bodija) in Ibadan, Nigeria. *African Journal of Biomedical Research*, 5, pp.51–55.

APHA. (1980). Standard Methods for the Examination of water and Waste Water (19th Ed.) *American Public Health Association and water pollution control federation.* Washington D. C. pp 1134.

APHA. (1985). Standard methods for the examination of water and wastewater, 16th edition. *American Public Health Association*, Washington DC.

Bartholomew J. W and Mitter T. (1952).The gram stain. *Microbiology and Molecular biology Review*. 16, pp.1-29.

Benka-Coker, M. & Ojior, O.(1995). Effect of slaughterhouse wastes on the water quality of Ikpoba River, Nigeria. *Bioresource Technology*, 52, pp.5–12.

Berger, P.S. & R.K. Oshiro. (2002). Source water protection: Microbiology of source water, pp. 2967-2978, *In: Encyclopedia of Environmental Microbiology*, G. Bitton, editor in chief, Wiley Inter science, N.Y.

Black, R. E., Cousens, S., Johnson, H. L., Lawn, J. E., Rudan, I., Bassani, D. G.,Jha, P., Campbell, H., Fischer Walker, C., Cibulskis, R., Eisele, T., Liu, L. & Mathers, C. (2010). Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*, 375(9730), pp.1969–87.

Blake, D. P., Hillman, K., Fenlon, D. R., & Low, J. C. (2003). Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. *Journal of Applied Microbiology*, 95(3), pp.428–436.

Dufour, A., Bartram, J., Bos R & Gannon V. (2012). *Animal Waste, WaterQuality and Human Health*. IWA Publishing, London.

Garrity G, Brenner DJ, Krieg NR, Staley JR. (2005). *Bergey's manual of systematic bacteriology, vol 2: the proteobacteria, part B: the gammaproteobacteria*. Springer, New York, NY, USA.

Krishnaveni, R., Devi, P. Y. and Rao, S. R. (2013). Bioremediation of steel industrial effluents using soil microorganisms.*International Journal of Advance Biotechnology and Research*. 4(1), pp.51-56.

Magiorakos, A. P., Srinivasan, A., Carey, R. B, Carmeli, Y., Falagas, M. E., Giske, Harbarth C. G., Hindler S., J. F., Kahlmeter, G, Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Webber, J. T. & Monnet, D. L. (2012). Multidrug -resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*. 8(3), pp.268–281.

Mukonzo, J. K., Namuwenge, P. M., Okure, G., Mwesige, B., Namusisi, O. K., & Mukanga, D. (2013). Over-the-counter suboptimal dispensing of antibiotics in Uganda. *Journal of Multidisciplinary Healthcare*, 6, pp.303–310.

Nwanta, J.A., Onunkwo, J. & Ezenduka, E. (2010). Analysis of Nsukka metropolitan abattoir solid waste and its bacterial contents in south eastern Nigeria: public health implication. *Archives of environmental & occupational health*, 65(1), pp.21–6.

Selvi, A. T., Anjugam, E., Devi, R. A.,Madhan, B.,Kannappan, S. and Chandrasekaran, B. (2012).Isolation and Characterization of Bacteria fromTannery Effluent Treatment Plant and their Tolerance to Heavy Metals and Antibiotics.*AJEBS*, 3(1), pp. 34-41.

Swai, E.S. and Schoonman, L. (2012). A survey of zoonotic diseases in trade cattle slaughtered atTanga city abattoir: a cause of public health concern. Asian *Pacific Journal of Tropical Biomedicine,* 2(1), pp. 55-60.

United States Environmental Protection Agency. (2006). "Terms of Environment Beginning With"E"". Assessed June 9, 2010.

WHO 2013: World Health Organization. (2013). *Guidelines for Drinking-water Quality,* 4th ed. (2013), pp. 296-299.