



Scientific Journals

ISSN 2052-0751

Journal of  
Biology

Research Paper

# Antibiotic Resistance of Faecal Bacteria Indicators and Pathogens Isolated from Sludge and Wastewaters of Abattoirs in Nairobi, Kenya

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## Abstract

Bacterial antibiotic resistance has become a serious problem among pathogenic bacteria and has led to increased concern surrounding environmental risks and potential spread of resistance in microbial species. Besides having clinical consequences, resistant bacteria of animal origin may be the source of determinants of resistance for the possible transfer to human strains. The objectives of the present study were to determine the abundance and distribution of antibiotic and multiple drug resistance among faecal bacteria indicators and pathogens found in wastewaters of animal abattoirs in Nairobi, Kenya. Standard microbiological methods were used to isolate and identify faecal streptococci, faecal coliforms, *Vibrio* and *Salmonella* species. Sensitivity to antibiotics was determined by agar diffusion method. The mean intermediary sensitive case (8.1% ( $\pm 5.6$ )) was significantly lower ( $p=0.00$ ) compared to the mean sensitive (41.3% ( $\pm 23.1$ )) and mean resistant (50.6% ( $\pm 22.3$ )) cases at  $p<0.05$ . Isolates showed high resistance to lincomycin (90%), ampicillin (80%), and methicillin (72.5%) and low resistance to chloramphenicol (22.5%). The results provided dynamics of resistance development in warm blooded animals usually consumed by humans. Multiple antibiotic resistance index was  $>0.2$  indicating high risk of exposure to the various antibiotics.

**Keywords:** Antibiotic Resistance, Bacterial Indicators, Pathogens, Sewage and Abattoirs

## 1. Introduction

During the past sixty years, antimicrobials were extensively used as growth promoters in breeding practices as well as in human and veterinary medicine. Nevertheless concerns about the use and disposal of these pharmaceuticals have been rising during the past decade (Garcia-Armisen et al, 2011) because of their impact on both human health and the environment. Bacterial resistance to antibiotics has become a serious problem among pathogenic bacteria, which has led to an increased concern surrounding environmental risks and potential spread of antibiotic resistance among microorganisms. In Kenya, a bacterium, *Neisseria gonorrhoea*, which was previously sensitive to penicillin, has now developed resistance to this drug (John, 2012). Senior ministry of health officials and doctors in Kenya have

warned that misuse of drugs is making them ineffective as treatments for various diseases and ailments. Antibiotics are easily available over the counter with buyers not having to submit a doctor's prescription. This easy availability means many people are either overdosing or under dosing or simply buying antibiotics when they don't need them. Resistance is typically common where antibiotics are heavily used (hospitals, long term care centres and large livestock operations), although antibiotic resistant bacteria are also shown to be present in wastewater, surface water, ground water, sediments and soils and increasingly in surface aquatic environments (Zbigniew, 2005; Baquero et al, 2008 and Zhang et al, 2009). The rapid increase in multiple antibiotic resistant in aquatic bacteria is partially due to

the ability of those bacteria to transfer antibiotic resistance markers among the bacterial population by cell to cell contact. The prolonged exposure to low doses of antibiotics leads to the selective proliferation of resistant bacteria, which could horizontally transfer genes to other bacterial species (Aminov & Mackie, 2007 and Zbigniew et al, 2010).

In the livestock sector, different types of farm animals are capable of carrying a wide range of zoonotic pathogens (Swai & Schoonman, 2012). Livestock often act as non-symptomatic carriers of human pathogens such as *E. coli* 0157, *Salmonella* species and *Campylobacter*, which are rarely detected during routine ante-mortem examination and their wastes may contain high concentrations of the organisms. Animal waste can therefore contaminate human and animal drinking water sources and even soil when used as manure (Christina et al, 2012).

In relation to the health of animals and food safety concern for human consumption it is crucial to control the susceptibility of bacteria in animal wastewaters to antibiotics. The contribution of abattoirs and associated wastewaters is rarely considered and yet abattoirs are potential sources of enteric bacteria that could possess antibiotic resistance genes. Due to the limited scientific documentation regarding these aspects, this study was conducted to evaluate the prevalence of Antibiotic Resistance (AR) and Multiple Drug Resistance (MDR) among two groups of faecal pollution indicators; Faecal Streptococci (FS) and faecal coliforms and two pathogenic bacteria i.e. *Vibrio* and *salmonella* species.

## 2. Materials and Methods

### 2.1. Sample Collection and Preparation

The wastewater and sludge samples were collected from cattle abattoir in Kayole, sheep and goat abattoir in Kiamai, Nairobi, Kenya. The samples were collected in clean sterile 200 mL plastic bottles and transported to Kenyatta University laboratory in an ice cooler box for analysis. Wastewater samples that were not analyzed within four hours were stored at a temperature of 4 °C. All samples were analyzed within 24 h.

### 2.2. Isolation and Identification of Bacterial Isolates

Standard microbiological methods (Mariita & Okemo, 2009) were used to isolate Faecal Coliforms (FC), Faecal Streptococci (FS), *Vibrio* and *Salmonella* species in the samples of wastewaters and sludge. Pigmentation of the colonies and Gram's staining followed by standard biochemical characterization {such as mortality, urease, TSI (triose sugar iron), glucose fermentation, indole, citrate utilization, and the cytochrome oxidase tests} were used to confirm the bacterial isolates.

### 2.3. Antibiotic Sensitivity Test

Sensitivity to antibiotics was determined by the agar diffusion technique recommended by the NCCLS (National Committee for Clinical Laboratory Standards) (NCCLS, 2003) on Mueller-Hinton agar (Oxoid) using the following antibiotic impregnated disks: ampicillin (25 µg); cotrimoxazole (25 µg); streptomycin (10 µg); chloramphenicol (30 µg); kanamycin (30 µg); gentamicin (10 µg); penicillin G (1 unit); methicillin (5 µg); minocycline (30 µg); lincomycin (2 µg); erythromycin (15 µg); tetracycline (25 µg) and sulfamethoxazole (200 µg). Interpretation of the results namely sensitive (S), Intermediary Resistant (IR) and resistant (R) was made in accordance to the standard measurement of inhibitory zones in millimetre (mm). Multiple Drug Resistance (MDR) index values were calculated using the formula by Lee et al (2009) as follows:

$$\text{MDR index} = X / (Y \times Z)$$

*X*=Total of antibiotic resistant case

*Y*=Total of antibiotic used in the study

*Z*=Total number of isolates

### 2.4. Data Analysis

SPSS computer software version 16.0 was used for data entry and statistical analysis. Groups significance tests were performed using one way ANOVA at 5% significance level and P value of <0.05 was considered as significant. The means were separated using Tukey's Honest Significance Difference (HSD) test at 5% level.

## 3. Results

### 3.1. Antibiotic Resistance Patterns

High percentages of bacterial isolates were resistant to lincomycin (90.0%), ampicillin (80.0%) and methicillin (72.5%) (Table 1). Some isolates were sensitive to gentamicin (72.5%) and chloramphenicol (75%), hence the latter two were considered to be the most effective antibiotics. The study shows that in Kenya the drugs of choice for treatment of the isolates are chloramphenicol (75.0%), gentamicin (72.5%) and minocycline (60.0%). Lincomycin (5.0%), ampicillin (12.5%), tetracycline (22.5%) and sulfamethoxazole (25%) are ineffective according to the study. The mean intermediary sensitive case {8.1% (±5.6)} was significantly lower ( $p=0.00$ ) compared to the mean sensitive {41.3% (±23.1)} and mean resistant {50.6% (±22.3)} cases at  $p<0.05$ .

When sources of isolates are considered (Table 2), a high percentage of cattle wastewater isolates showed resistance to ampicillin (100%), lincomycin and tetracycline (87.5%) and methicillin (75%). None of the cattle wastewater isolates was resistant to minocycline.

**Table 1. Antibiotic Sensitivity of Randomly Selected Bacterial Isolates (n=40) to 13 Types of Antibiotics**

Antibiotic (ng)	Resistance (%)	Inter. Sensitive (%)	Sensitive (%)
Ampicillin (25)	80.0	7.5	12.5
Chloramphenicol (30)	22.5	2.5	75.0
Cotrimoxazole (25)	30.0	2.5	67.5
Erythromycin (15)	50.0	7.5	42.5
Gentamicin (10)	27.5	0.0	72.5
Kanamycin (30)	37.5	17.5	45.0
Lincomycin (2)	90.0	5.0	5.0
Methicillin	72.5	7.5	20.0
Minocycline (30)	32.5	7.5	60.0
Penicillin G (1 unit)	50.0	7.5	42.5
Streptomycin (10)	32.5	20.0	47.5
Sulfamethoxazole (200)	62.5	12.5	25.0
Tetracycline (25)	70.0	7.5	22.5
Mean	50.6 <sup>a</sup> (±5.6)	8.1 <sup>b</sup> (±5.6)	41.3 <sup>a</sup> (±23.1)

n= total number of isolates tested; Inter.= Intermediary  
Means having the same letters within the row are not significantly different according to Tukey's HSD at 5% level

omycin and ampicillin (100%) and penicillin (75.0%) and only 12.5% were resistant to chloramphenicol. Bacterial isolates from goat and sheep sludge mostly showed resistance to lincomycin (100%), ampicillin (87.5%) and none of the isolates was resistant to chloramphenicol. High percentages of the sheep wastewater isolates were resistant to lincomycin (87.5%) but none of the isolates was resistant to cotrimoxazole. Goat wastewater isolates were mostly resistant to methicillin (100.0%), lincomycin and tetracycline (75%) while only 12.5% were resistant to kanamycin and gentamicin. There was no significant difference ( $p=0.971$ ) in resistance to antibiotics of bacterial isolates collected across the waste-waters and sludge samples.

All bacteria isolated from cattle sludge were resistant to lincosamides (100%) (Table 3). Overall, a high percentage of bacterial strains isolated from all sites were more resistant to lincosamides, and  $\beta$ -lactam classes of antibiotics compared to aminoglycosides, macrolides, sulfonamides, phenolics and tetracyclines. Only 22.5% ( $\pm 18.5$ ) of the studied isolates showed resistance to phenicols. There was significant difference ( $p=0.00$ ) in resistance to  $\beta$ -lactams, lincosamides and phenicols at  $p<0.05$ .

### 3.2. Multiple Antibiotic Resistance

All the studied bacterial strains exhibited resistance to mo-

**Table 2. Resistance of Bacterial Isolates Collected From Wastewaters and Sludge**

Antibiotic (ng)	Percentage of AR Among Isolates in Wastewaters and Sludge				
	1	2	3	4	5
Ampicillin (25)	100.0	100.0	62.5	50.0	87.5
Chloramphenicol (30)	50.0	12.5	25.0	25.0	0.0
Cotrimoxazole (25)	37.5	37.5	37.5	0.0	37.5
Erythromycin (15)	50.0	50.0	50.0	50.0	50.0
Gentamicin (10)	12.5	25.0	12.5	37.5	50.0
Kanamycin (30)	50.0	25.0	12.5	62.5	37.5
Lincomycin (2)	87.5	100.0	75.0	87.5	100.0
Methicillin	75.0	62.5	100.0	62.5	62.5
Minocycline (30)	0.0	37.5	50.0	62.5	12.5
Penicillin G (1 unit)	62.5	75.0	37.5	37.5	37.5
Streptomycin (10)	25.0	37.5	50.0	25.0	25.0
Sulfamethoxazole (200)	62.5	62.5	62.5	62.5	62.5
Tetracycline (25)	87.5	62.5	75.0	62.5	62.5
Mean	53.8 <sup>a</sup>	53.4 <sup>a</sup>	50.3 <sup>a</sup>	48.1 <sup>a</sup>	48.1 <sup>a</sup>

AR-antibiotic resistance, 1-cattle wastewater, 2-cattle sludge, 3-goat wastewater, 4-sheep wastewater and 5-goat and sheep sludge. Means followed by the same letters within the row are not significantly different according to Tukey's HSD at 5% level.

Most cattle sludge isolates also showed resistance to linc- re than 3 antibiotics (Table 4). However, the patterns of

resistance among the 40 cultures varied as represented in Table 4. Of all the studied bacterial isolates, 52.5% (21) showed 6 Multiple Antibiotic Resistance (MAR) pattern (i.e. resistance to 6 of the 13 antibiotics tested). Only a small percentage of the isolates (2.5%) showed a 4 MAR pattern. None of the studied bacteria showed a 0-3 and 9-13 MAR pattern.

**Table 3. Resistance of Bacteria to Antibiotics With Respect to the Nature of the Antibiotics (in %)**

Antibiotic	Wastewaters and Sludge					Mean ( $\pm$ SD)
	1	2	3	4	5	
AM	29.2	43.8	37.5	41.7	37.5	37.9cd ( $\pm$ 5.6)
LA	79.2	79.2	66.7	50.0	62.5	67.5b ( $\pm$ 12.3)
LI	87.5	100.0	75.0	87.5	100.0	90.0a ( $\pm$ 10.5)
MA	50.0	50.0	50.0	50.0	50.0	50.0bc ( $\pm$ 0.0)
PH	50.0	12.5	25.0	25.0	0.0	22.5d ( $\pm$ 18.5)
SUL	50.0	50.0	50.0	31.3	50.0	46.3bc ( $\pm$ 8.3)
TET	43.8	50.0	62.5	62.5	37.5	51.3bc ( $\pm$ 11.2)

AM-aminoglycosides (kanamycin, gentamicin, streptomycin), SUL-sulfonamides (sulfamethoxazole, cotrimoxazole), LA- $\beta$ -lactams (ampicillin, penicillin, methicillin), LI-lincosamides (lincomycin), MA-macrolides (erythromycin), PH-phenicols (chloramphenicol) and TET-tetracycline (tetracycline, minocycline). 1-cattle wastewater, 2-cattle sludge, 3-goat wastewater, 4-sheep wastewater and 5-mixture of goat and sheep sludge. Means followed with same letters within the column are not significantly different according to Tukey's HSD at 5% level

**Table 4. Patterns of MDR of Faecal Coliforms, Faecal Streptococci and *Vibrio* and *Salmonella* Species (n=40) to 13 Antibiotics**

No. of Antibiotics	Types of Antibiotics	No. (%) of Strains
8	Tet, sulf, pen, ery, kan, gen, cot, chlo	1 (2.5)
7	Linc, amp, met, tet, ery, sulf, mino	8 (20)
	Linc, amp, met, tet, ery, pen, gen	9 (22.5)
6	Linc, amp, kan, met, sulf, pen	10 (25)
	Linc, amp, kan, tet, strep, cot	3 (7.5)
	Linc, sulf, strep, cot, mino, chlo,	6 (15.0)
	Amp, met, ery, chlo, strep, cot	2 (5)
4	Tet, kan, gen, strep	1 (2.5)

Amp:ampicillin, Linc:lincomycin, Pen:penicillin, Met:methicillin, Ery:erythromycin, Tet:tetracycline, Cot:cotrimoxazole, Strep:streptomycin, Kan:kanamycin, Gen:gentamicin, Sulf:sulfamethoxazole, Chlo-chloramphenicol and Mino:minocycline

All MAR index values were found to be more than 0.20, where the highest MAR index was exhibited by FC (0.40), followed by *Salmonella* spp. (0.35), Faecal *Streptococci* (0.30) and *Vibrio* spp. (0.225) (Table 5).

### 3. Discussion

Bacterial resistance to antibiotics is a growing medical and ecological problem worldwide generated by the selection process following the indiscriminate use of antibiotics (Meirelles-Pereira et al, 2002). Environmental bacteria may play an important role as reservoirs of antibiotics; resist-

ance genes which are exchanged by bacteria from different ecosystems (Dang et al, 2008). The five sources of bacterial contamination characterized in this study were investigated because they differ in terms of the origin of the bacteria strain they release in the environment (cattle, goat and sheep) and in the expected exposure of these bacteria to antimicrobial selective pressure.

**Table 5. Multiple Antibiotic Resistance (MAR) Value of Bacterial Isolates**

Bacterial Isolate	MDR Value
Faecal coliform	0.400
<i>Salmonella</i> species	0.350
Faecal streptococci	0.300
<i>Vibrio</i> species	0.225

Bacterial isolates were most resistant to lincomycin, ampicillin and methicillin and most sensitive to chloramphenicol, gentamicin and cotrimoxazole. These results, therefore, show that ampicillin, lincomycin and methicillin are not effective in the control of these bacteria since high percentages of the isolates (i.e. 80%, 90% and 72.5%, respectively) were resistant to these antibiotics. Bacterial resistance to erythromycin, streptomycin, penicillin, ampicillin, amoxicillin, kanamycin, tetracycline, oxytetracycline and chloramphenicol has been reported (Roberts, 2011).

The present study demonstrated that bacterial isolates were resistant to antibiotics commonly used as feed additives (tetracycline, streptomycin and sulfonamides) or therapeutics (penicillin and tetracycline). According to Zbigniew et al (2005) bacteria resistance to antibiotics depends on the chemical structure of the antibiotics; this is confirmed by results of the present study.

All the bacteria isolated from cattle sludge were resistant

to lincosamide antibiotics and therefore studied bacteria are capable of detoxifying these antimicrobial agents. Lincosamides are one of the commonly used antibiotic classes in human and veterinary clinical practice (Andreozzi et al, 2006), thus the resistance of bacteria isolated from the studied wastewater and sludge samples to this class of antibiotics is not surprising. Besides lincosamides, 67.5% of bacterial strains were resistant to  $\beta$ -lactam antibiotics. This study supports the idea that  $\beta$ -lactam resistance is now widespread in pathogenic bacteria isolated from a variety of location and sources (Maciedo et al, 2010). High resistance to antibiotics may be attributed to several factors; first it may be that the source of drinking water for the animal had been polluted by antibiotic agents; secondly introduction of antibiotic supplemented commercial feeds may have initiated the resistance as previously suggested (Le et al, 2005). High resistance may also be attributed to the history, frequency and dose of antibiotic utilization by self-medication or prolonged use as previously mentioned (Aminov & Mackie, 2007).

MAR index value beyond 0.20 in every species of bacteria in the present study indicates that there has been high risk in exposure to antibiotics such as lincomycin, ampicillin, penicillin, methicillin and tetracycline. Microorganisms in this study resisted more than 3 antibiotics with one bacterial isolates resisting up to eight out of the 13 antibiotics used. Antibiotics which are commonly used in human and veterinary medicine as well as in agriculture lead to the selection of antibiotic resistant microorganisms (Monika et al, 2011). Resistance to one antibiotic could contribute to selection for resistance to antibiotics of other groups. High multi-resistance incidence, similar to or higher than those found in this study, has been reported in aquatic environments (Matyar et al, 2007).

#### 4. Conclusion

Though there may be differences in growth media, bacteria species and incubation conditions, results from the present study provide an early warning on antibiotic resistance in isolated FC, FS, *Vibrio* and *Salmonella* species from wastewaters and sludge. Additionally, data is also provided on potential effective antibiotics aimed at farmers and veterinarians towards setting a more efficient farm management and thus farmers could improve their practices with appropriate use of antibiotics.

#### Acknowledgement

We thank Kenyatta University, Kenya, for giving us laboratory space and Mr. D. Ng'ang'a, a technician in the Plant and Microbial Science Laboratory for technical assistance. Our special thanks also go to the workers of Kayole and Kiamako Abbottoirs in Nairobi, Kenya who participated in the collection of samples. Dr. C. Makori of the department

of Veterinary Services of Kenya is also acknowledged for giving us ethical clearance.

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