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Identification, bacterial load and antibiotic resistance profile of Enterobacteriaceae isolated from effluent from the three abattoirs in the city of Lubumbashi (DR Congo)

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ABSTRACT

Introduction. Slaughterhouses represent a strategic sector of the food chain in the Democratic Republic of the Congo. By supplying the meat consumed on a daily basis, they contribute to the food security of urban populations. However, slaughtering activities produce large volumes of liquid effluent containing blood, fats, organic debris and intestinal microorganisms. When discharged untreated into the environment, this effluent becomes a major source of biological pollution.

Objectives. The aim of this study was to identify the enterobacteria present in the effluents from three abattoirs, to quantify their load, to describe their distribution across the sites and to assess their susceptibility to antibiotics.

Materials and methods. This was a descriptive cross-sectional study conducted from 14 July 2025 to 14 September 2025. Eighteen samples were collected, at a rate of six samples per abattoir: the Lubumbashi Public Abattoir, the Mzée Grand Market Abattoir and the Kibwe Farm Abattoir. The analyses were carried out at the bacteriology laboratory of the Lubumbashi University Clinics. Isolation was carried out on MacConkey agar and CLED agar, whilst identification was performed using the Le Minor panel of five media: Kligler, urea-indole, Simmons' citrate, lysine-iron and SIM. Antibigram testing was performed by agar diffusion in accordance with the CLSI/EUCAST 2024 recommendations. The data were analysed using SPSS version 23. Qualitative variables were compared using the chi-squared test and quantitative variables using analysis of variance (ANOVA). The threshold for statistical significance was set at $p < 0.05$.

Results. Our results show that, out of 18 samples, six species were isolated: *Salmonella typhi* 12/18, *Citrobacter freundii* 12/18, *Klebsiella pneumoniae* 5/18, *Escherichia coli* 2/18, *Klebsiella oxytoca* 1/18 and *Edwardsiella tarda* 1/18. *S. typhi* and *C. freundii* were the most common, each accounting for 36.36 per cent. They were found exclusively at the public abattoir and the Mzée market abattoir, with six strains at each site. The Kibwe farm, meanwhile, had four different species, with *K. pneumoniae* being the most prevalent at 15.15 per cent. Bacterial counts revealed very high loads, particularly at the Kibwe farm: ranging from 130.10^2 to 290.10^5 CFU/ml, with a mean of $1.97 \times 10^7 \pm 1.06 \times 10^7$ CFU/ml, $p = 0.006$. Mzée and Public had lower averages: 2.70×10^4 and 2.81×10^4 CFU/ml, $p = 0.000$. The ANOVA confirmed that the difference was significant. The resistance profile shows very high rates of resistance to beta-lactams. *S. typhi*: 100 per cent resistant to aztreonam and ampicillin, 92 per cent to cefotaxime. *C. freundii*: 100% resistant to aztreonam,

piperacillin/tazobactam, cefotaxime and cefixime. *K. pneumoniae*: 100% resistant to amikacin, cefotaxime, piperacillin/tazobactam, aztreonam and ampicillin. The MAR index is >0.2 for *S. typhi* (0.69), *C. freundii* (0.69) and *K. pneumoniae* (0.63). The main limitation of this study lies in the small number of samples analysed and the lack of molecular confirmation of the bacterial strains.

Conclusion. We therefore conclude that the effluents from the three slaughterhouses in Lubumbashi are heavily contaminated with multidrug-resistant Enterobacteriaceae. These results enable the risk of contamination to be assessed and should inform public health policy.

Keywords: Identification, enterobacteria.

1. Introduction

According to the World Health Organisation, antibiotic resistance is now one of the top ten threats to global public health. Slaughterhouse effluent represents a key link in the environmental spread of multidrug-resistant bacteria within the framework of the 'One Health' approach, which considers the interactions between human, animal and environmental health.

Slaughterhouses are a strategic sector of the food chain in the Democratic Republic of the Congo. By supplying the meat consumed on a daily basis, they contribute to the food security of urban populations. However, slaughtering activities produce large volumes of liquid effluent containing blood, fats, organic debris and intestinal microorganisms. When discharged untreated into the environment, this effluent becomes a major source of biological pollution.

Among the microorganisms present, enterobacteria occupy a special place. This group of Gram-negative, facultative anaerobic bacilli normally colonises the digestive tract of animals. However, several species are implicated in severe human diseases. *Escherichia coli* is the classic indicator of faecal contamination; *Salmonella typhi* causes typhoid fever; whilst *Klebsiella pneumoniae*, *Citrobacter freundii*, *Klebsiella oxytoca* and *Edwardsiella tarda* are associated with nosocomial, urinary and gastrointestinal infections that are becoming increasingly difficult to treat. SOKARI and OGHENETEGA (2008), in their study in Port Harcourt, Nigeria, had already demonstrated that slaughterhouse effluent carries a high load of enterobacteria. More recently, Ojo et al. (2019) reported a marked increase in antibiotic resistance among bacteria isolated from slaughterhouse effluent in south-western Nigeria.

In the Democratic Republic of the Congo, the situation is particularly worrying. MISCHLER et al. (2018) highlighted that the lack of suitable infrastructure for liquid waste management contributes to the contamination of aquatic ecosystems and drinking water sources. In Lubumbashi, the country's second-largest city, three sites account for the bulk of slaughtering: the Lubumbashi public abattoir; none of the abattoirs has a functioning wastewater treatment plant. Effluent flows directly into drains and watercourses, exposing market gardeners and riverside communities to a high health risk.

This situation is exacerbated by the phenomenon of multi-drug resistance. The MAR index, developed by KRUMPERMAN (1983), is used to assess the degree of exposure of an environment to antibiotics. A value greater than 0.2 indicates a high-risk environment, where antibiotics are used frequently. Research by AWUGCHEW et al. (2020) in Ethiopia and by GUESSENND et al. (2013) in Côte d'Ivoire have shown that urban and hospital wastewater are reservoirs of multidrug-resistant bacteria. KATELE et al. (2025) confirm that wastewater in Africa now constitutes a major reservoir of antibiotic-resistant bacteria.

To our knowledge, no study to date has simultaneously assessed the diversity of Enterobacteriaceae, their bacterial load, their antibiotic susceptibility profile and the multi-resistance index in effluents from the main abattoirs in the city of Lubumbashi.

It is against this background that the present study was conducted. The aim was to identify the Enterobacteriaceae present in the effluent from three abattoirs, to quantify their load, to describe their distribution across the sites, and to assess their antibiotic susceptibility. Beyond the descriptive aspect, the aim was to provide evidence to alert health and environmental authorities to the urgent need for action.

2. Materials and methods

2.1. Study setting and type

A descriptive cross-sectional study involving prospective data collection on the identification of Enterobacteriaceae isolated from slaughterhouse effluents in the city of Lubumbashi from 14 July 2025 to 14 September 2025. The slaughterhouses targeted were: the Lubumbashi public slaughterhouse, the Mzée Grand Market slaughterhouse and the Kibwe Farm slaughterhouse. This study was carried out at the biomedical analysis laboratory of the Lubumbashi University Clinics within the bacteriology department.

2.2. Sample collection and transport

Aseptic sampling of effluent from three abattoirs in the city of Lubumbashi (the Lubumbashi Public Abattoir: 1, the Mzée Grand Market Abattoir: 1 and the Kibwe Farm Abattoir: 1) were taken manually from drainage areas where water was actively flowing, into a sterile 30 ml bottle, then placed in a cool box with a cooling pack and transported directly to the laboratory at the Lubumbashi University Clinics, taking the necessary precautions (storage temperature: 4°C). Care must be taken to avoid the risk of contamination and degradation of the sample during transport. A total of 18 samples were collected, at a rate of one sample per day over six days, amounting to six samples per site. The relatively small number of samples constitutes a limitation of the study.



Figure 1. Vial of slaughterhouse effluent.

2.3. Counting, isolation and identification of enterobacteria

2.3.1. Counting of colonies

Homogenisation of the sample using a shaker. Due to the high concentration of microorganisms in the abattoir effluent, the sample is subjected to serial decimal dilutions (10^{-1} à 10^{-5}) using a sterile solution (distilled water). This produces Petri dishes containing a countable number of colonies, generally between 30 and 300. The spread plate method: A small volume (usually 0.1 ml) of each dilution is dispensed onto the surface of a Petri dish containing a gelled culture medium. The sample is then spread evenly across the surface using a sterile spreader.

Inoculation and incubation: The Petri dishes are incubated under specific conditions (temperature and time) depending on the type of bacteria being tested for. For the total count of mesophilic aerobic flora, incubation is usually carried out at 30 °C for 72 hours. For indicators of faecal contamination

such as *E. coli* and coliforms, selective media are used and incubation takes place at 37 °C or 44 °C.

Counting and calculation: After incubation, the visible colonies are counted. The result is expressed as Colony-Forming Units per millilitre (CFU/ml) of sample. The calculation takes into account the number of colonies counted and the dilution factor used for that plate. (ISO 8199: 2018).

2.3.2. Isolation and identification of enterobacteria

Bacterial Culture:

- **Culture Media:** We used MacConkey agar selective medium and CLED (Cystine Lysine Electrolyte Deficient) agar non-selective medium for the culture of enterobacteria.
- **Incubation:** Incubate the plates at 37°C for 24 hours (MacFADDIN, J. F., 2000)



Figure 2. CLED agar and MacConkey agar.

2.3.3. Identification of enterobacteria

The identification of enterobacteria was carried out using the Le Minor test on Kligler-Hajna medium, SIM, Simmons' citrate, lysine-iron and urea-indole media to characterise enzymatic and metabolic activity, thereby differentiating them from other bacterial species through a series of specific reactions, leading to reliable identification of the strain or contamination.



Figure 3. The five media in the Le Minor panel.

2.3.4. Antibiotic sensitivity test

Diffusion Method:

We used the disc diffusion method (Kirby-Bauer) to assess antibiotic susceptibility (JORGENSEN J. et al., 2015).

A bacterial suspension equivalent to 0.5 McFarland was prepared to ensure accurate and reproducible results when performing an antibiogram. To prepare the standard 0.5 McFarland suspension, 0.5 ml of barium chloride (1.175 per cent) was mixed with 99.5 ml of sulphuric acid (1 per cent). The resulting solution has an optical density (OD) of 0.08 to 0.1, corresponding to a bacterial concentration of approximately 1.5×10^8 CFU/ml. A total of seventeen antibiotic discs were used for the susceptibility test.

Incubate upside down at 37°C for 24 hours.



Figure 4. Incubator.

The inhibition zones were interpreted in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, version

2025). The reference strain *Escherichia coli* ATCC 25922 was used as a quality control for the susceptibility testing.



Figure 5. Antibiogram.

Table 1. Antibiotics selected for the susceptibility test.

No.	Antibiotics	Abbreviation	Sensitivity	Intermediate	Resistant	
1	Amikacin 30	AK	17	15–16	14	Aminoglycoside
2	Gentamicin 10	CN	15	13 – 14	12	
3	Piperacillin/Tazobactam 110	TPZ	25	21–24	20	Penicillin
4	Ampicillin 2	AP	17	14–16	13	
5	Amoxycylav 30	AMC	18	14–17	13	
6	Aztreonam 30	ATM	21	18–20	17	Monobactam
7	Cefixime 5	CFM	19	16–18	15	Cephalosporin
8	Cefotaxime 5	CTX	26	23–25	22	
9	Cefuroxime 30	CXM	18	15–17	14	

10	Imipenem 10	IPM	23	20–22	19	Carbapenem
11	Ciprofloxacin 5	CIP	26	22–25	21	Quinolone
12	Levofloxacin 5	LE	21	17–20	16	
13	Norfloxacin 10	NX	17	13–16	12	
14	Chloramphenicol 30	C	18	13–17	12	Phenicolé
15	Doxycycline 30	DXT	14	11–13	10	Cycline
16	Tetracycline 30	T	15	12–14	11	

2.3.5. MAR (Multiple Antibiotic Resistance) Index

MAR index per isolate:

The MAR index (MarI) is a quantitative measure of a bacterium's resistance to multiple antibiotics, reflecting the degree of multidrug resistance in bacterial isolates.

The MAR index is determined for each isolate using the formula:

$$MARI = \frac{a}{b}$$

Where:

a: the number of antibiotics to which the test isolate was resistant.

b: the total number of antibiotics to which the test isolate was found to be susceptible.

A MARI value greater than 0.2 indicates a high-risk environment in which antibiotics are commonly used (AWUGCHEW T., et al., 2020).

MARI index per site (PH KRUMPERMAN., 1983):

$$MARI \text{ for a site} = \frac{\text{Nombre d'antibiotiques pour lequel tous les isolats sont résistants}}{\text{Nombre des isolats présents dans le site} \times \text{Nombre des antibiotiques testés}}$$

Table II. Interpretation of the MAR index per site.

	MAR index > 0.4	MAR index < 0.4
Risk of contamination	High	None or very low
Source of contamination	Human	Non-human

2.4. data analysis

Statistical analyses were carried out using SPSS version 23. Quantitative variables were expressed as mean $\pm$ standard deviation or median, depending on their distribution. The normality of the distributions was tested using the Shapiro-Wilk test. Comparisons between groups were performed using ANOVA or the Kruskal-Wallis test, depending on the conditions of application. Qualitative variables were compared using Pearson's chi-squared test or Fisher's exact test. Effect sizes (Cramer's V) and 95% confidence intervals were reported. A p-value of < 0.05 was considered statistically significant.

2.5. Results

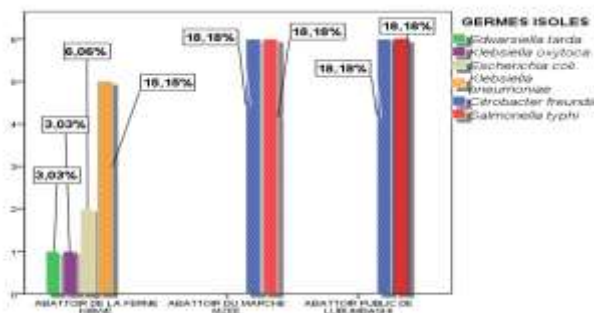


Figure 6. Distribution of isolated bacteria by slaughterhouse.

Two bacteria were isolated in significant numbers: *Salmonella typhi* and *Citrobacter freundii*, followed by *Klebsiella pneumoniae*. The Kibwe Farm abattoir isolated four different bacteria, with *Klebsiella pneumoniae* accounting for the highest proportion at 15.15 per cent. In contrast, the Mzee Market abattoir and the Lubumbashi public abattoir each isolated two bacteria equally: *Citrobacter freundii* and *Salmonella typhi*, at 18.18 per cent each. The chi-squared statistical test shows that the isolation of these bacteria in these abattoirs was strongly and significantly associated ($p = 0.000$) (V-Cramér = 0.7). The detection of *Salmonella typhi* in slaughterhouse effluent is an unusual finding, as this bacterium is primarily found in humans. Confirmation by

serotyping or molecular biology (PCR or sequencing) would be necessary to rule out any possible confusion with non-typhoid serovars of *Salmonella*.

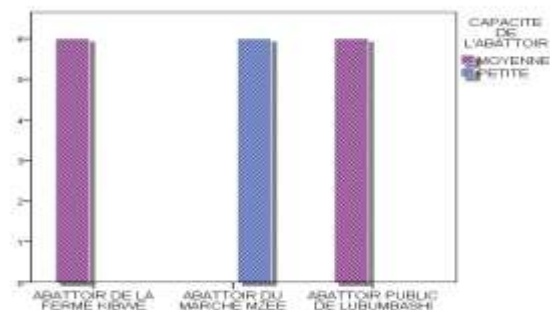


Figure 7. Distribution by slaughterhouse capacity.

The Mzee market abattoir has a small capacity compared with the other two, which have an average capacity. The chi-squared statistical test shows that the capacity of these abattoirs was statistically significant, with a strong association ($p = 0.000$) (V-Cramér = 1.0).

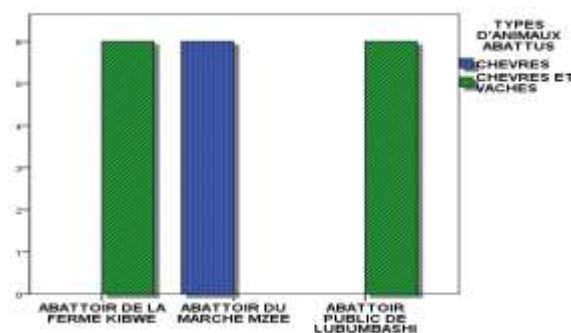


Figure 8. Breakdown by type of animal slaughtered in abattoirs.

Figure 8 shows that the Mzee market abattoir slaughters only goats, whereas the other two abattoirs, in addition to goats, also slaughter cows. The chi-squared statistical test shows that the types of animals slaughtered in these abattoirs were statistically significant, with a strong association ($p = 0.000$) (V-Cramér = 1.0).

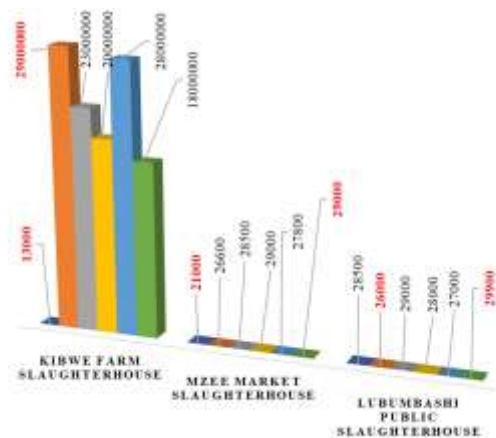


Figure 9. Distribution according to bacterial count (in CFU/ml) in the abattoirs.

Figure 9 shows that, following bacterial counting, the effluents from the Kibwe Farm slaughterhouse had very high concentrations ranging from 130×10^2 to 290×10^5 CFU/ml; with a mean of $1.966883333 \times 10^7 \pm 1.055293458 \times 10^7$ CFU/ml ($p = 0.006$). The Mzee Market abattoir: concentrations ranging from 210×10^2 to 290×10^2 CFU/ml; with a mean of $2.69833 \times 10^4 \pm 3.675 \times 10^4$ CFU/ml ($p = 0.000$). And finally, the Lubumbashi public abattoir: concentrations ranging from 260×10^2 to 299×10^2 CFU/ml; with a mean of $2.80667 \times 10^4 \pm 14.024 \times 10^4$ CFU/ml ($p = 0.000$). The ANOVA test showed that the bacterial counts from these three abattoirs were statistically significant.

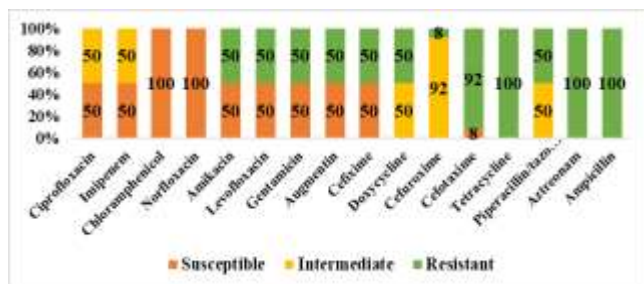


Figure 10. Antibiotic resistance profile of *Salmonella typhi*.

Figure 10 shows that *Salmonella typhi* isolated from abattoir effluent exhibited resistance to aminoglycosides (50 per cent for amikacin and 50 per cent for gentamicin), to cyclines (50 per cent for doxycycline and 100 per cent for tetracycline), to fluoroquinolones (50 per cent for levofloxacin) and to beta-lactams (100 per cent for aztreonam and ampicillin; 92 per cent for cefotaxime; 50 per cent for piperacillin/tazobactam, cefixime and Augmentin; and 8 per cent for cefuroxime).

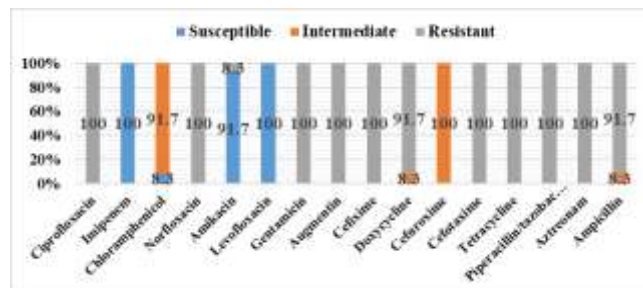


Figure 11. Antibiotic resistance profile of *Citrobacter freundii*.

Figure 11 shows that *Citrobacter freundii* isolated from slaughterhouse effluent exhibited resistance to aminoglycosides (100 per cent for gentamicin and 8.3 per cent for amikacin), to cyclines (100 per cent for tetracycline and 91.7 per cent for doxycycline), to fluoroquinolones (100 per cent for ciprofloxacin and norfloxacin) and to beta-lactams (100 per cent for aztreonam, piperacillin/tazobactam, cefotaxime, cefixime, Augmentin and 91.7 per cent for ampicillin).

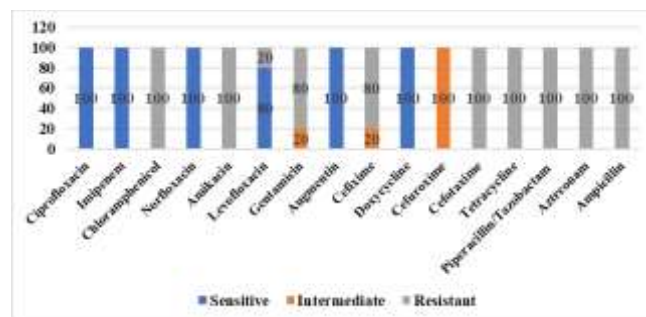


Figure 12. Antibiotic resistance profile of *Klebsiella pneumoniae*.

Figure 12 shows that *Klebsiella pneumoniae* isolated from slaughterhouse effluent exhibited resistance to aminoglycosides (100 per cent for amikacin and 80 per cent for gentamicin), to cyclines (100% for tetracycline), phenicols (100% for chloramphenicol) and beta-lactams (100% for cefotaxime, piperacillin/tazobactam, aztreonam and ampicillin; 80% for cefixime).

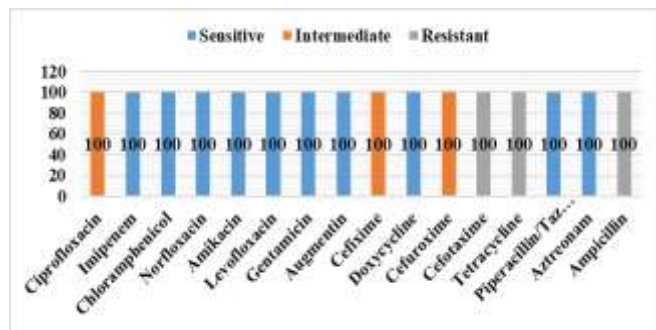


Figure 13. Antibiotic resistance profile of *Klebsiella oxytoca*.

Figure 13 shows that *Klebsiella oxytoca* isolated from slaughterhouse effluent exhibited resistance to cyclines (100% for tetracycline) and beta-lactams (100% for cefotaxime and ampicillin).

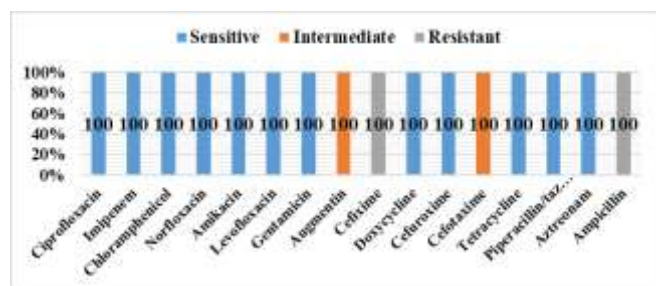


Figure 14. Antibiotic resistance profile of *Edwardsiella tarda*.

Figure 14 shows that *Edwardsiella tarda* isolated from slaughterhouse effluent exhibited resistance to beta-lactams (100% for ampicillin and cefixime).

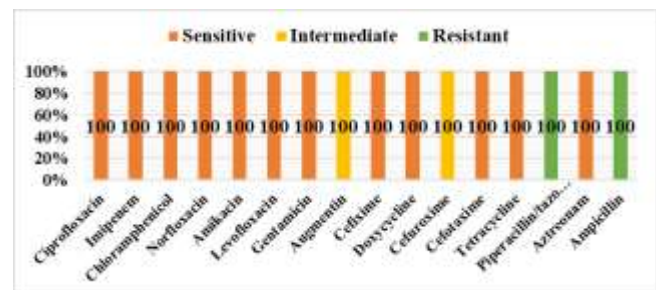


Figure 15. Antibiotic resistance profile of *Escherichia coli*.

Figure 15 shows that *Escherichia coli* isolated from slaughterhouse effluent exhibited resistance to beta-lactams (100% for ampicillin and piperacillin/tazobactam).

Table III: Multiple antibiotic resistance index.

	A	B	IMRA
<i>Salmonella typhi</i>	132	192	0.69
<i>Citrobacter freundii</i>	132	192	0.69
<i>Klebsiella pneumoniae</i>	50	80	0.63
<i>Klebsiella oxytoca</i>	3	16	0.19
<i>Edwardsiella tarda</i>	2	16	0.13
<i>Escherichia coli</i>	4	32	0.13

This table shows that the multiple antibiotic resistance index is greater than 0.2 for *Salmonella typhi* (0.69), *Citrobacter freundii* (0.69) and *Klebsiella pneumoniae* (0.63); these values indicate a high-risk source of contamination.

2.6. Discussion

The aim of this study was to analyse effluent from three abattoirs in Lubumbashi, focusing on the identification of Enterobacteriaceae, their diversity and their antibiotic resistance.

• Diversity and prevalence of enterobacteria

Bacterial strains were identified using conventional biochemical methods. Although widely used in resource-limited laboratories, these techniques are less accurate than modern methods such as the API 20E, MALDI-TOF mass spectrometry or 16S rRNA gene sequencing.

Out of 18 samples, 6 species were isolated: *Salmonella typhi* 12/18, *Citrobacter freundii* 12/18, *Klebsiella pneumoniae* 5/18, *Escherichia coli* 2/18, *Klebsiella oxytoca* 1/18 and *Edwardsiella tarda* 1/18. *S. typhi* and *C. freundii* were the most common, each accounting for 36.36 per cent. They were found exclusively at the public abattoir and the Mzée market abattoir, with six strains per site. The Kibwe farm, meanwhile, yielded four different species, with *K. pneumoniae* being the most prevalent at 15.15 per cent.

The chi-squared test shows a statistically significant association between the slaughterhouse and the isolated

bacteria, $p = 0.000$; V-Cramér = 0.7. This difference can also be explained by the animals slaughtered: Mzée slaughters only goats, whilst the other two slaughter both goats and cows, $p = 0.000$; V-Cramér = 1.0.

These results confirm those of SOKARI and OGHENETEGA (2008), who reported microbial contamination of slaughterhouse effluent in Nigeria with enterobacteria such as *E. coli*, *Salmonella*, *Klebsiella* and *Enterobacter*.

- **High microbial load in effluents**

Bacterial counts revealed very high loads, particularly at the Kibwe farm: ranging from 130×10^2 to 290×10^5 CFU/ml, with a mean of $1.97 \times 10^7 \pm 1.06 \times 10^7$ CFU/ml, $p = 0.006$. Mzée and Public had lower averages: 2.70×10^4 and 2.81×10^4 CFU/ml, $p = 0.000$. The ANOVA confirms that the difference is significant.

These high levels are consistent with the context in Lubumbashi, where the lack of adequate infrastructure for waste management and effluent treatment contributes to the contamination of aquatic ecosystems. MISCHLER et al. (2018) also characterised slaughterhouse effluent as a major environmental risk factor.

- **Multidrug resistance of concern**

The resistance profile shows very high rates of resistance to beta-lactams. *S. typhi*: 100 per cent resistant to aztreonam and ampicillin, 92 per cent to cefotaxime. *C. freundii*: 100 per cent resistant to aztreonam, piperacillin/tazobactam, cefotaxime and cefixime. *K. pneumoniae*: 100 per cent resistant to amikacin, cefotaxime, piperacillin/tazobactam, aztreonam and ampicillin.

The MAR index is >0.2 for *S. typhi* (0.69), *C. freundii* (0.69) and *K. pneumoniae* (0.63). According to P.H. Krumperman (1983), a MAR >0.4 indicates high-risk human-derived contamination. AWUGCHEW et al. (2020) consider that a MAR > 0.2 reflects an environment where antibiotics are commonly used.

These findings are consistent with those of OJO et al. (2019), who described a sharp increase in antibiotic resistance among bacteria isolated from slaughterhouse effluent.

Identification was based on the Le Minor panel, and susceptibility testing on the Kirby-Bauer method according to EUCAST. Sampling over a two-month period does not allow for an assessment of seasonality. The absence of PCR means that resistance genes cannot be identified.

2.7. Conclusion

Following this descriptive cross-sectional study, conducted from 14 July to 14 September 2025 in three slaughterhouses in Lubumbashi, it is evident that slaughterhouse effluent constitutes a significant reservoir of enterobacteria.

Firstly, the analysis identified six species: *Salmonella typhi*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *E. coli*, *K. oxytoca* and *E. tarda*. *S. typhi* and *C. freundii* were the most prevalent, each accounting for 36.36 per cent. The Kibwe farm had the highest bacterial load: an average of 1.97×10^7 CFU/ml.

Secondly, the isolated strains exhibited marked multidrug resistance. The MAR indices of 0.69 for *S. typhi* and *C. freundii*, and 0.63 for *K. pneumoniae*, exceed the threshold of 0.4, indicating a high risk of human-derived contamination. Resistance is particularly evident against beta-lactams, aminoglycosides, cyclines and fluoroquinolones.

Thirdly, the presence of *S. typhi*, the causative agent of typhoid fever, in untreated effluent poses a threat to public health. In the DRC, the lack of effluent treatment infrastructure puts populations reliant on local water sources at risk. Slaughterhouses are key sources of foodborne pathogens, with resistance levels rising sharply.

We therefore conclude that effluent from the three slaughterhouses in Lubumbashi is heavily contaminated with multidrug-resistant enterobacteria. These findings enable the risk of contamination to be assessed and should inform public health policy.

2.8. Recommendations and outlook

The results of this study support the establishment of a microbiological monitoring system for slaughterhouse effluent, the construction of wastewater treatment plants, the strengthening of regulations on the use of antibiotics in veterinary medicine, and the integration of slaughterhouses into national programmes to combat antibiotic resistance in line with the One Health approach. In light of the results obtained, several avenues for further research emerge:

- Molecular characterisation of the isolates;
- Screening for resistance genes (*bla*CTX-M, *bla*TEM, *bla*SHV, *bla*NDM, *bla*KPC, *mcr*-1, etc.);
- Whole-genome sequencing of the most resistant strains;

- A multicentre study covering several provinces of the DRC;
- Longitudinal surveillance to assess seasonal variations;
- A quantitative assessment of the microbiological risk to exposed populations.

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