Prevalence of resistant enterobacteria and ESBL *Escherichia coli* isolated from wastewater discharged into the environment by hospitals in the Abidjan district, Ivory Coast

ABSTRACT

Aims: Hospital effluent constitutes a reservoir for antibiotic-resistant bacteria. In particular, Gram-negative bacteria carrying several antibiotic resistance genes. This study was to evaluate the prevalence of antibiotic-resistant Enterobacteriaceae and ESBL Escherichia coli in hospital effluents.
Methodology: In total, 112 wastewater samples from 4 hospital sites were collected and examined during 2020 at the Pasteur Institute of Côte d'ivoire. Enumeration of total enterobacteria was carried out on McConkey agar. McConkey agar was supplemented with 4 mg/l ceftazidime to detect resistant enterobacteria. Antibiotic-resistant Escherichia coli were identified using Malditof. The production of ESBLs was investigated by synergy tests on Mueller-Hinton agar.
Results: The results indicate a prevalence of resistant enterobacteria varied between 4.6% and 2.6% with an average of 3.9%. A proportion of 69.2% of resistant enterobacteria were strains of Escherichia coli. ESBL Escherichia coli represented a rate of 32.7% among resistant enterobacteria. A rate of 47.3% of resistant Escherichia coli strains produces an ESBL. It appears from this study that the phenomenon of multi-resistance is undoubtedly present in bacteria isolated from hospital effluents, because the enterobacteria isolated from these waters are mainly strains of ESBL-producing Escherichia coli.
Conclusion: Resistant Enterobacteriaceae and particularly ESBL-producing Escherichia coli present in hospital effluents released without treatment into the environment are likely to cause public health problems. These bacteria should be considered environmental pollutants.

Keywords: Antibiotics, Enterobacteriaceae, Escherichia coli, resistance, ESBL, hospital effluent.

1. INTRODUCTION

Hospital wastewater is a highly complex effluent containing antibiotic compounds, metabolized drugs, disinfectants (Emmanuel et al., 2005), patient feces and microorganisms, potentially containing multidrug-resistant genes (Galvin et al., 2010) (Chagas et al., 2011). As such, hospital wastewater is considered a reservoir for antibiotic resistance, generating an environment conducive to the exchange of antibiotic resistance

genes. Gram-negative bacteria carrying multiple resistance genes are increasingly found in hospital wastewater (Zhang et al., 2012). Hospital effluents are discharged as common community wastewater to the wastewater treatment plant (WWTP), without any pre-treatment or cleaning process. After treatment, the water is discharged into surface waters. Some pathogenic microorganisms can remain longer in an aquatic environment, creating dissemination pathways and environmental reservoirs of antibiotic resistance genes (ARGs)(Perron et al., 2008). Over the past two decades, the presence of antibiotics in water bodies and the subsequent development of microbial resistance have attracted the attention of scientists and the public as a potential concern (Kümmerer, 2009) (Rodríguez-Gil et al., 2010). Hospital wastewater is one of the sources that contribute significantly to the environmental burden of antibiotics and, consequently, to antibiotic resistance (Zhang et al., 2009) (Davies & Davies, 2010) (Harris et al., 2012).

Enterobacteria, particularly Escherichia coli, are commensal bacteria present at approximately 10^8 colony-forming units per gram of human stool. In 2024, the WHO list of priority antibiotic-resistant pathogens specifically classifies Enterobacterales resistant to third-generation cephalosporins (3GCEs), including *Escherichia coli* and *Klebsiella pneumoniae,* as “critical priority”. The burden of these bacteria in AMR requires targeted action, especially in low-income countries (Da et al., 2023). In sub-Saharan Africa, several studies indicate Escherichia coli as the most common and most resistant enterobacteria (Conforti et al., 2024). Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL *E. coli*) are a real health problem for the populations of these countries because these strains escape medically relevant antibiotics and constitute essential supports in the propagation of resistance genes via mobile genetic elements (Traoré M., 2019). ESBL *E coli* are commonly found in wastewater worldwide (Bréchet et al., 2015). Recently, authors have established the relationship between wastewater indicators and AMR carriage in the population. This approach shows the scope of wastewater surveillance to inform effective public health interventions (Guessennd et al., 2013). As in many sub-Saharan countries, the problem of ESBL-producing *Escherichia coli* is a concern in Côte d’Ivoire (Lorcy & Dubé, 2018). Indeed, despite the increasing prevalence of ESBL-producing enterobacteria (BSE), particularly ESBL *E. coli*, their epidemiology is not yet well understood overall and the role of wastewater in their propagation is poorly documented (Laber et al., 1999). According to a study conducted at a university hospital in the country, liquid discharge from hospitals can contain most of the multi-resistant bacteria involved in nosocomial infections (Nuñez & Moretton, 2007). Reducing the spread of the most common multi-resistant bacteria (MRB) (ESBL *E. coli*) and that of the most resistant is one of the challenges facing health authorities in Côte d'Ivoire (Reinthaler et al., 2003).

The objective of this work was to estimate the prevalence of resistant Enterobacteriaceae, resistant *Escherichia coli* and BLSE *Escherichia coli* in hospital effluents in the district of Abidjan.

2. MATERIAL AND METHODS

2.1 COLLECTION OF HOSPITAL WASTEWATER

Hospital wastewater was collected from 4 hospital sites between January and December 2020, distributed as follows: 1 university hospital, 2 general hospitals and 1 specialized day hospital for tuberculosis (Figure 1).

FIG. 1. MAPPING OF HOSPITAL WASTEWATER SAMPLING SITES

2.2 SAMPLE SIZES

Twenty-eight campaigns were conducted overall. During each campaign, each of the 4 sites was collected once between 9:00 and 12:00, corresponding to a period of intense activity in the hospitals. A total of 112 samples were collected during this study. Samples were manually collected in sterile vials from the main manholes (collecting the discharge from all other manholes in the hospital) and then transported within 3 hours, in coolers containing ice packs, to the laboratory for immediate analysis.

2.3 DETERMINATION OF THE PREVALENCE OF RESISTANT ENTEROBACTERIA

A volume of 100 µl of water samples serially diluted to the 10th degreein saline was plated on McConkey agar without antibiotics to enumerate Enterobacteria and on McConkey agar supplemented with 4 mg/L ceftazidime to enumerate resistant Enterobacteria. Plates were incubated for 24 to 48 hours at 37°C. The percentage of resistant Enterobacteria was calculated by dividing the total number of colonies on McConkey medium with antibiotic by the number of colonies on McConkey medium without antibiotic.

2.4 DETERMINATION OF THE PREVALENCE OF RESISTANT ESCHERICHIA COLI

For the detection of antibiotic-resistant Escherichia coli strains, presumptive Enterobacteria colonies on McConkey agar supplemented with 4 mg/l ceftazidime were randomly selected

on the basis of their macroscopic differences. Five colonies of each morphology type were identified using Malditof (BioMérieux, Marcy-l-Étoile, France). The prevalence of resistant Escherichia coli was calculated by dividing the number of Escherichia coli identified with Malditof by the number of enterobacteria identified with Malditof.

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2.5 DETERMINATION OF THE PREVALENCE OF BLSE-PRODUCING ESCHERICHIA COLI (E. COLI BLSE):

Among the isolated Escherichia coli strains, the production of BLSE was investigated by synergy tests using the Mueller-Hinton agar diffusion technique as previously described.(Jarlier et al., 1988). The presence of an ESBL was confirmed between a third-generation cephalosporin (C3G) disc (cefotaxime, ceftazidime, cefepime) and amoxicillin + clavulanic acid separated by 3 cm from center to center. The appearance of a synergy image called "champagne cork" between these discs allowed the confirmation of the production of a broad-spectrum beta-lactamase. Subsequently, the number of ESBL E. coli was divided respectively by the number of resistant Enterobacteriaceae and by the number of resistant E. coli in order to determine the prevalence of ESBL E. coli in relation to these 2 sets

2.5 STATIC DATA ANALYSIS

Statistical analysis of the data was performed using IBM SPSS statistics 27 and GraphPad Prism 9.5.0 (730) software (San Diego, California, USA). Values were given as percentages. The significance threshold was set at $P < 0.05$ for the expression of results.

3. RESULTS

The prevalence of resistant Enterobacteriaceae in hospital effluents varied between 4.6% and 2.6% with a mean of 3.9%. However, no statistically significant differences between these prevalences from one type of hospital to another were noted (Table 1).

Table 1. P Prevalence of resistant enterobacteria in hospital effluents

Collection sites	Count (CFU/ml)		Prevalence of resistant enterobacteria (%)
	Total Enterobacteria	Resistant Enterobacteria	
Site 1	$3.8\ 10^6$	$1,75\ 10^5$	4.6
Site 2	$3.8\ 10^6$	$1,47\ 10^5$	3.9
Site 3	$3.66\ 10^6$	$1,61\ 10^5$	4.4
Site 4	$2.86\ 10^6$	$7,48\ 10^4$	2.6
Average	$3,53\ 10^6$	$1,38\ 10^5$	3.9

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Approximately 69.2% of resistant Enterobacteriaceae in hospital effluents are strains of Escherichia coli (Table 2).

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Table 2. Malditof's identification of Escherichia coli strains within the population of resistant enterobacteria

Resistant Enterobacteria	Effective Malditof Identification (N)	Prevalence (%)
Escherichia coli species	296	69.2
Non-*Escherichia coli* species	132	30.8
Total	428	100

32.7% of resistant enterobacteria in hospital effluents are strains of BLSE Escherichia coli (Table 3)

Table 3. Distribution of BLSE *Escherichia coli* strains in the population of resistant enterobacteria

Resistant Enterobacteria	Staff (N)	Prevalence (%)
Escherichia coli BLSE	140	32.7
Resistant enterobacteria other than Escherichia coli ESBL	288	67.3
Total	428	100

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: 47.3% of resistant Escherichia coli strains found in hospital effluents produce ESBL (Table 4)

Table 4. Distribution of BLSE *Escherichia coli* strains in the population of resistant enterobacteria

Resistant *Echerichia coli*	Staff (N)	Prevalence (%)
Escherichia coli ESBL	140	47.3
Non- ESBL *Escherichia coli*	156	52.7
Total	296	100

4. DISCUSSION

Hospitals are places where the use of antibiotics and the density of individuals favor the dissemination of antibiotic resistance (Russell, 2001). The operation of these structures is accompanied by the discharge of a high quantity of wastewater containing pathogens mainly from patients (Wellington et al., 2013) (LaPara et al., 2011). These germs can be exposed to a wide variety of antibacterials contained for the development of resistance via cellular mutation or acquisition of genetic elements in the form of plasmids or transposons (Conforti et al., 2024). Studies indicate that even when treated by wastewater treatment plants (WWTP), hospital effluents constitute a source of release of antibiotic-resistant pathogens into the downstream environment (Wellington et al., 2013) (LaPara et al., 2011). Wastewater monitoring is a cost-effective and less burdensome way to track changes in RAM carriage within a community (Conforti et al., 2024). In countries of the South, particularly in sub-Saharan Africa where antimicrobial resistance is less well documented, this approach could be an alternative to this problem (Da et al., 2023).

Prevalence of resistant Enterobacteriaceae in discharged hospital wastewater

In wastewater, little information is available on RAM levels in other bacterial populations because most studies in this area have been carried out using coliforms as bacterial indicators (Guardabassi et al., 2002). The results obtained in this study confirm the presence of bacteria of the Enterobacteriaceae family such as *Escherichia coli*, in hospital wastewater. The results of this study show that approximately 1.47.105 CFU/ml or 3.9% of the enterobacteria contained in the wastewater continuously discharged into the environment by these structures are resistant. This prevalence of resistant enterobacteria is much lower than that of 77.41% noted by authors in samples from rivers influenced by strong anthropogenic pressure characterized by the nearby presence of a hospital, a significant concentration of population and refugees, associated or not with agricultural activity (Hobeika, 2021). Similarly, other studies have shown that approximately 15% of clinical strains of enterobacteria in hospitals could be resistant to 3rd generation cephalosporins (3GC) with high prevalences recorded in intensive care units (48%), hematology-oncology departments (27%) and the pediatric department (25%) (Mkaouar et al., 2008). Once in hospital effluents, these enterobacteria have the power to easily transfer genetic information between themselves or with other bacteria in the environment (Yala et al., 2024). The low prevalence of resistant Enterobacteria observed in this study could be related to the probable presence of residues of specific substances in the effluents as suggested by Nunez and Moretton (Nuñez & Moretton, 2007). According to some studies, the prevalence of resistant bacteria in wastewater can vary considerably depending on several factors including the physicochemical parameters and the analytical methods used (Guardabassi et al., 2002) (Gyorgy & Sandor, 2010). For others, this difference in resistance rates can be explained by the variability of epidemiological factors, antibiotic usage policies and levels of hospital hygiene from one country to another (Mkaouar et al., 2008). The application of infection limitation provisions and the determination of the mechanism underlying resistance to C3G in enterobacteria are essential to control their dissemination at the health and community level (Yala et al., 2024).

Prevalence of Escherichia coli in the population of resistant enterobacteria isolated Among the bacteria isolated from hospital wastewater, the species *Escherichia coli* occupies an important place with a proportion of 69.2%. This high proportion could be explained by the predominance of Escherichia coli commonly isolated among the enterobacteria. Indeed, studies conducted in Togo found *Escherichia coli* (63.93%) as the most frequently isolated species in the group of enterobacteria followed by *Klebsiella* (22.86%) (Salah et al., 2021). This trend is confirmed by other authors, notably by Ebongue et al. in Cameroon (48.5%) and Kalambry et al. in Mali (44.7%) (Ebongue et al., 2015) (Kalambry et al., 2019). *Escherichia coli* is an enterobacteria present in the digestive tract and involved in infections, mainly urinary. As such, the bacterium is frequently the therapeutic target of antibiotics (Sonnet, 2020) (Public Health France a, 2023). Third-generation cephalosporins (3GCs) occupy an essential place in the therapeutic arsenal in human health. Thus, the control of resistance to these molecules in enterobacteria such as *Escherichia coli* is a concern both in hospital and community settings. If in Europe, the average proportion of resistance to 3GCs in *Escherichia coli* decreased from 15.7% to 14.3% between 2018-2022, this is not yet the case in most developing countries (Public Health France a, 2023) (Tagajdid et al., 2010). The share of resistant *Escherichia coli* in this study shows the interest in determining the impact of hospital effluent discharges on the contamination of aquatic ecosystems downstream, particularly at the fauna level.

Prevalence of ESBL Escherichia coli in the population of isolated resistant Escherichia coli

The ESBL *Escherichia. coli* rate obtained in this study is 32.7%. The presence of ESBL *Escherichia coli* in hospital effluents has been reported in several studies conducted worldwide (Guessennd et al., 2013) (Cahill et al., 2019) (Fadare & Okoh, 2021). This rate could be explained, on the one hand, by a higher frequency and density of carriage among hospitalized patients (Mkaouar et al., 2008) (Ruppé et al., 2013). On the other hand, the high consumption of antibiotics in hospitals leads to their elimination in the form of compounds that are still active in wastewater. These compounds exert a selection pressure favoring resistant bacteria including ESBL *Escherichia coli*. The presence of disinfectants, widely used in hospitals, can also contribute to the selection of resistant bacteria (Coutu et al., 2013).

Analysis of Table 4 shows a rate of 47.3% of resistant *Escherichia coli* strains found in hospital effluents producing an ESBL. Indeed, in recent years, several studies have highlighted the presence of ESBL *Escherichia coli* strains in hospital effluents, both in developing and developed countries (Drieux-Rouzet & Jarlier, 2014). In their study, Galvin and colleagues investigated the impact of hospital effluent by quantifying antibiotic-sensitive and antibiotic-resistant *Escherichia coli* strains in effluent samples collected at different levels at the discharge of an Irish hospital, in the municipal network upstream and downstream of the hospital sewer connection. They showed that the proportion of antibiotic-resistant *Escherichia coli* strains found in the municipal network was higher downstream of the hospital connection than upstream of the hospital connection (Galvin et al., 2010). These data suggest the importance of organizing the monitoring of antibiotic consumption and bacterial resistance at the environmental level as well as in human and animal health (Public Health France b, 2023).

5. CONCLUSION

This study shows that the phenomenon of multiresistance is undoubtedly present in bacteria isolated from hospital effluent wastewater, because the germs isolated from these waters are mainly strains of Escherichia coli producing ESBL. These effluents discharged without treatment into the lagoon can constitute a source of dissemination of these potentially pathogenic bacteria. Controlling ESBL-producing Escherichia coli strains in water remains a public health issue in Côte d'Ivoire. These bacterial strains are the cause of many therapeutic failures. More rigorous environmental management is more than necessary. On the one hand, improving wastewater treatment processes and on the other hand decontaminating hospital effluents by physicochemical and microbiological processes is therefore essential. Also, quantification and genotypic characterization by searching for resistance genes would make it possible to assess the risks associated with possible transfers of resistance genes and infections

COMPETING INTERESTS

The authors declare that they have no conflict of interest

. ETHICAL APPROVAL

Access to the various hospital sites for sampling and analysis of discharged effluents was subject to written authorizations from the managers of these structures. These authorizations are kept with the authors.

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