Research Article

Hospital effluents as sources of antibiotics residues, resistant bacteria and heavy metals in Benin

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Received: 25 January 2022 / Accepted: 22 June 2022
Published online: 08 July 2022

Abstract

This study aims to evaluate the bacteriological, chemical, and toxicological quality of hospital effluents in Benin. Eighteen (18) samples were collected from the south and north of Benin, and poles were set up. Bacteriological analysis was carried out according to the French National Organization for Standardization (AFNOR) standard. Identification of the bacterial species isolated was performed using the API 20E gallery and specific biochemical tests. Antibiotic residues were searched by following ELISA kit instructions. 109 bacterial isolates were obtained, with a predominance of non-enterobacteria (37.7%) and *Acinetobacter* spp. (24.6%), followed by *Klebsiella* spp. (11.48%); *S. aureus* and *Coagulase-negative staphylococci* (*CoNS*) were isolated at the same frequency (6.5%). Physico-chemical and toxicological parameter analyses showed that they were in conformity with the standards of discharge into the environment. The pH, temperature, electrical conductivity, total solids below, P-redox and suspended solids measured were, on average, 6.83, 27.21 °C, 693.68 µS/cm, 693.68 mg/l, 1.68 mV and 0.15 mg/l, respectively. For heavy metals, cadmium was most prevalent (0.22 mg/l), followed by nickel (0.18 mg/l) and lead (0.03 mg/l). The average antibiotic residue concentrations ranged from 0.043 to 7.65 µg/l. Ciprofloxacin, metronidazole and sulfamethoxazole had the highest residue concentrations of 7.65 ± 2.272 µg/l, 6.61 ± 0.051 µg/l and 3.88 ± 3.088 µg/l, respectively. The heavy metal concentrations obtained were below those required by Benin standards. These effluents therefore present health and environmental risks. It is then more than necessary to develop treatment methods for them before their rejection into the natural environment.

Article Highlights

- The study carried out made it possible to rule on the nature and quality of hospital effluents generated in the health structures in Benin. Physico-chemical parameters such as pH, temperature, electrical conductivity, total solids below, P-redox and suspended solids measured has been determined. The research of pathogenic bacteria as well as the research of heavy metals and antibiotic residues was done.
- Data were provided on the nature of the bacteria present in hospital effluents and their degree of patho-

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genicity. The types of heavy metals contained in the samples were reported and compared to the standards in force at the national and international level. The presence of antibiotic residues was evaluated and the most common ones were documented. The presence of residues of some specific antibiotics has confirmed their wide use in human therapeutics.

- The study raised awareness of the fact that untreated hospital effluent can enter the water table, end up in natural waterways and be the source of many environmental and health problems. The study highlighted the urgency of installing wastewater treatment plants for health facilities in Benin.

Keywords Hospital effluents · Antibiotic residue · Antibioresistance · Heavy metals · Health and environmental risks · Benin

1 Introduction

Water is an essential resource for life. It plays an important role in daily activities and needs (domestic, agricultural, industrial, artisanal, etc.). As a result of these uses, it can be contaminated by micropollutants that present potential environmental and health risks. Health care activities especially generate different types of waste, including effluents that are complex mixtures of chemical and biological substances that are continuously released. Hospital effluents generally come from all sections of health care facilities. In many developing countries, they represent a significant source of emerging contaminants. These contaminants include heavy metals, antibiotic resistance genes, antibiotic resistant bacteria and antibiotic residues [1, 2]. contaminants can be discharged into various environments, such as rivers, lakes and seas, without prior treatment and can then accumulate [3]. The health risk of the discharge of hospital effluents can thus be well perceived since humans are in constant interaction with the environment. For many years, antibiotics have made it possible to fight effectively against pathogenic bacteria, which have today developed mechanisms of resistance to them. Excessive and abusive use has very quickly led to a dramatic increase in antibiotic resistance and the selection of multiresistant bacteria throughout the world [4]. The adverse effects of pharmaceuticals such as antibiotics in the environment have been reported in some studies [5]. Of most concern is the development of bacteria and antibiotic resistance genes, as this would lead to the transfer of these genes to microbial species normally sensitive to antibiotics. Infections due to antibiotic-resistant bacteria have higher mortality and morbidity rates and directly result in longer hospitalizations [6]. It is estimated that these infections could cause 10 million deaths worldwide by 2050 [7]. The situation has worsened due to the presence of heavy metals in the environment. It has been demonstrated that the concentrations of heavy metals in the environment are associated with the levels of antimicrobial resistance genes [8]. Heavy metals, which must be remembered, are metallic elements that have a high density compared to water. They are very soluble and can therefore be easily absorbed by living organisms [9]. It is easy to understand why the emergence of toxic heavy metals in the environment is associated with selective pressure on bacterial species [10].

The heavy metals generally found in hospital effluents are copper (Cu), iron (Fe), cadmium (Cd), lead (Pb) and others [11]. Several studies in Benin have reported the presence of resistant bacteria and resistance genes in the environment due to the untreated discharge of hospital effluents [12]. Very few studies, on the other hand, have revealed data on antibiotic residues and the presence of heavy metals in hospital effluents. The present study was then initiated to assess not only the presence of antibiotic-resistant bacteria but also heavy metals and antibiotic residues in hospital wastewater in Benin. The results generated by this study will allow us to reveal updated and exploitable scientific data to initiate a sustainable treatment plan for effluents in Benin's hospitals before their release into the environment.

Besides the introduction, the manuscript contains three other parts. Part two comes right after the introduction and highlights the methodology followed, part 3 presents the results obtained and part 4 is devoted to the discussion.

2 Materials and methods

2.1 Study area and sample collection

The study was carried out in the southern and northern parts of the Benin Republic in the main public health centers where health care activities are carried out on a continuous basis.

The collection concerned a total of eighteen (18) samples at a rate of at least three per site. In southern Benin, samples were collected from Abomey-Calavi/So-Ava hospitals, which are zone hospitals covering two large areas. DHC Zou is the main hospital that covers the whole area of Abomey and Bohicon. The hospitals of Aplahoué, Lokossa and Grand-Popo cover the Mono department. Zone Hospital of Mènontin is located in the Atlantic department and especially covers the cities of Calavi and Cotonou. Pobè

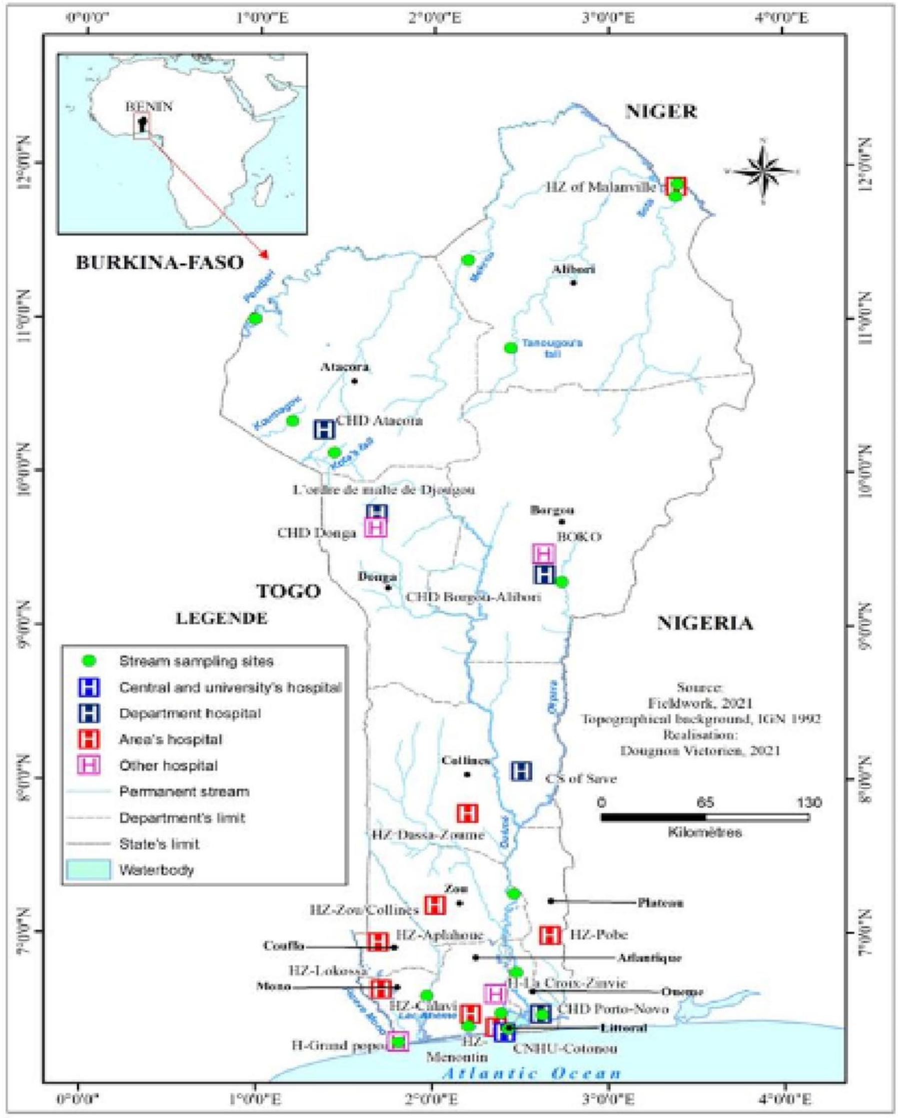

Fig. 1 Hospital effluents collection sites

Hospital is located in the plateau department at the border with Nigeria. Zinvié Hospital is located 40 km from Cotonou in the Department of the Atlantic, commune of Abomey Calavi more precisely in the District of Zinvié. The National University Hospital is the reference hospital in Benin and is also located in the Altlantique Department. Departmental Hospital Center (DHC) is located in the plateau department and is the second largest hospital in Benin. In northern Benin, the samples were collected at zone hospitals of Boko which is located in the department of Borgou and share the sanitary zone of DHC Borgou-Alibori. Malanville is located in the department of Alibori. Dassa and Savè hospitals are located in the Department of Collines. The order of Malte Hospital is located in the Donga department and shares the sanitary area with the DHC Donga. The samples were also collected in the DHC Atacora. Figure 1 shows the geographical distribution of the water sample collection sites, while Table 1 gives the specifications for each site (number of beds, levels in the health pyramid and others).

All hospitals identified for sample collection are general hospitals with the following main services: medicine, paediatrics, surgery, gynaecology, laboratory, medical imaging, ENT, Anaesthesia, Stomatology, operating theatre, physiotherapy and a nursing service. We made point sampling in sterile 400 ml bottle. In order to avoid the bias associated with paralleling the results from each hospital, the samples collected were of a composite type from the wastewater storage points of the following departments: intensive care, resuscitation, emergency, laboratory, pediatrics and maternity (departments with high antibiotic). The samples were collected in May 2021 (rainy season). The time of collection was the same (between 8 and 9 am), to avoid any bias.

Table 1 Specification of sampling sites (hospital)

N	Name of the hospital	Type	Number of beds	Level in the health pyramid
1	Atacora	General and public hospital	83	Departmental level
2	Departmental Hospital Center of Donga	General and public hospital	88	Departmental level
3	Departmental Hospital Center of Borgou-Alibori	General and public hospital	272	Departmental level
4	Area Hospital of Boko	General and public hospital	93	Peripheral level
5	Area Hospital of Dassa-zoumè	General and public hospital	67	Peripheral level
6	Area Hospital of Ordre de Malte	General and public hospital	106	Peripheral level
7	Area Hospital of Malanville	General and public hospital	90	Peripheral level
8	Area Hospital of Savè	General and public hospital	48	Peripheral level
9	Hubert Koutoukou Maga National University Hospital Center	General and public hospital	679	Central level
10	Departmental Hospital Center of Oueme	General and public hospital	327	Departmental level
11	Area Hospital of Aplahoué	General and public hospital	100	Peripheral level
12	Area Hospital of Abomey-Calavi So-Ava	General and public Hospital	110	Peripheral level
13	Departmental Hospital Center of Zou	General and public hospital	512	Departmental level
14	Area Hospital of Grand-Popo	General and public hospital	50	Peripheral level
15	Area Hospital of Pobè	General and public hospital	84	Peripheral level
16	Area Hospital of Lokossa	General and public hospital	70	Peripheral level
17	Area Hospital of Mènontin	General and public hospital	115	Peripheral level
18	Zinvié Confessional Center	General and public hospital	112	Peripheral level

They were transported in a cooler equipped with a cold accumulator to the laboratory where a pole was formed from the different samples collected per site. Figure 1 show the samples collection sites.

2.2 Bacteriological analysis of samples

All the samples collected were spread on agar plates incorporated to search for bacteria resistant to four (4) antibiotics, namely, ampicillin (AP), ciprofloxacin (CPFX), metronidazole (MET), and doxycycline (DOX), at different concentrations and combinations in nutrient agar [13]. Agar was prepared by incorporating an initial concentration of antibiotic into Mueller Hinton agar. The antibiotic concentration of each type of medium is as follows: Metronidazol 0.02 g/ml; Ampicillin 0.1 g/ml; doxyciclin 0.5 g/ml and ciprofloxacin 0.0015 g/ml. The antibiotics were obtained from Sigma Chemical Company, St. Louis, MO. Double the concentrations were also tested for each antibiotic. Then, the plates were incubated at 37 °C for 24 h. Biochemical identification was carried out to further identify resistant bacteria (those grown on embedded agars). These colonies were streaked on Mueller–Hinton agar plates for purification and then Gram staining, biochemical tests (catalase and oxidase), seeding of the API 20^E gallery (Biomerieux, France) (for only Gram-negative bacilli) and free staphylocoagulase and DNA tests (for only Gram-positive cocci).

2.3 Physico-chemical analysis of samples

Physicochemical characterization was performed to determine the following parameters: temperature, hydrogen potential (pH), salinity, electrical conductivity (EC), resistivity, redox potential (P-redox), and total dissolved solids (TDS). They were analyzed according to the protocols recommended by Rodier et al. [14] by direct measurement with a multimeter Multi 3630 IDS SET KS2 (Xylem—WTW, France).

2.4 Determination of antibiotics residues

Seven antibiotics have been searched in this study. These are β-lactam amoxicillin (AMO) and ampicillin (AP), chloramphenicol (CAP) a phenicol, ciprofloxacin (CPFX) a fluoroquinolone, metronidazole (MNZ) an imidazole, sulfamethoxazole (SMZ) a sulfonylurea and neomycin (NEO) an aminoglycoside. They were chosen in view of their frequency of use in hospitals in West African countries [15]. Both detection and quantification were carried out according to the instructions of the MyBioSource® ELISA kit (MyBioSource, San Diego, California, USA) specific to each antibiotic sought. The plate in each kit was read at 450 nm on a microplate reader, and then the calibration curve of the standards was plotted with the percentages of absorbances (A/A0 × 100 with A: average absorbance of the standard or sample and A_0 the average absorbance of the 0 ppb standard) as a function of logC of the standards contained in each kit. Determination of the concentrations of the samples from the straight-line equation.

$$Y = ax + b$$

obtained for each antibiotic was done.

2.5 Determination of heavy metal concentration

2.5.1 Mercury concentration determination

Samples were collected in prewashed 100 ml borosilicate glass bottles. The water samples were acidified with HCl (5%), stored and transported at 2–8 °C and protected from light. Prior to analysis, the samples were brought to room temperature. The quartz boats were sterilized (30 min at 600 °C) and allowed to cool to room temperature. The balance was tarred with one of the sterilized and empty boats before introducing 100 µl of the homogenized sample. For each sample, three boats were loaded and introduced into the spectrophotometer (DMA-80). When the analysis error displayed by the instrument exceeds 50%, the analysis is repeated. Otherwise, the mercury concentration considered is the average displayed by the apparatus.

2.5.2 Lead, cadmium and nickel concentration determination

For lead (Pb), cadmium (Cd) and nickel (Ni), their respective concentrations were determined by the MERCK test kit method using a photo Lab 6600UV–Vis molecular absorption spectrophotometer (WTW, USA). The test kits are specific to each metal, and preliminary mineralization of the sample is necessary. Before starting the analyses, it is also important to check the pH of the samples and to adjust if necessary with 65% nitric acid or sodium hydroxide. The principle is that in the presence of (pyridyl-2′-azo)-4-resorcinol (PAR), the metal forms a complex that is determined photometrically. In the case of lead, the procedure also depends on the purity of the water and requires a total hardness test. It should be noted that with the determination of hardness, the analysis of lead is done in two steps. In the first step, reagent 1 added to the sample in the test tube was read by a molecular absorption spectrophotometer and gave a lead concentration of A. Then, the addition of reagent 2 results in a lead concentration result B. The final lead concentration in the sample is determined from the following formula:

$$\text{Lead content in mg/l} = \text{Result A} - \text{Result B}.$$

For the other metals (Cd and Ni), the concentration of the metal was determined directly with a photo Lab 6600UV–Vis molecular absorption spectrophotometer (WTW, USA).

2.6 Data processing

The data were recorded in an Excel 2016 spreadsheet, and graphs were produced with GraphPad software version 8. A comparison mean test was carried out with the software R. The average of parameters was compared to World Health Organization (WHO) and Benin standards.

3 Results

3.1 Bacterial species identified

109 bacterial isolates were obtained. The bacteria identified were mostly nonenterobacteria (37.7%) and *Acinetobacter spp*. (24.6%), followed by *Klebsiella spp*. (11.48%). *S. aureus*- and *coagulase-negative staphylococcus* were isolated at the same percentages (6.5%). *Yersiniae enterolitica* was the less isolated (1.64%). Figure 2 presents the different bacterial species isolated from samples.

3.2 Physico-chemicals characterization

The values of the different parameters vary according to the sites without much difference. In general, these values comply with WHO standards for wastewater discharge into the environment. The temperature varied between 25.8 °C and 28.8 °C, with averages of 26.56 °C in the north and 27.86 °C in the south. The hydrogen potential (pH) expresses the concentration of H+ ions and measures the acidity or alkalinity on a scale from 0 to 14. There was very little variation in pH, which varied between 5.85 and 7.11, with averages expressing the global acidity of the samples. The electrical conductivity (EC) and resistivity indicate the

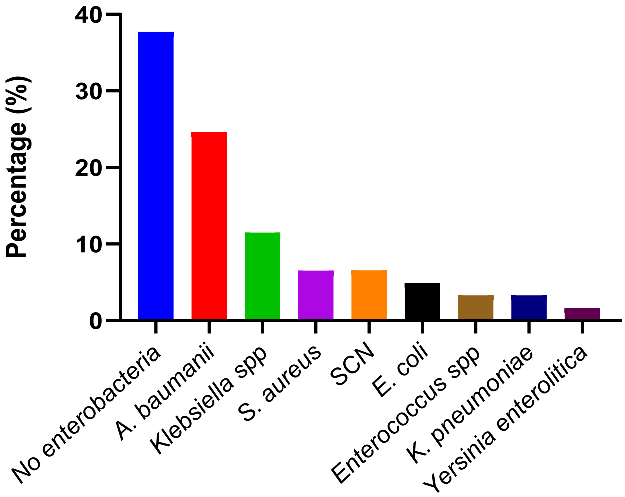

Fig. 2 Bacterial species isolated from hospital effluent samples

ability of a water to conduct power, which depends on the content of mineral salts in the water. The resistivity is the inverse of the electrical conductivity, and they provide information on the mineralization of water. All values were in accordance with the standard (˂ 2000 µS/cm), except Area Hospital of Dassa-Zoumè and CNHU, who recorded the highest values for conductivity (1557 and 1887 µS/cm). The average values of these parameters indicate that the effluents have a high average mineralization. The total dissolved solids (TDS) provides information on the salt content of water samples. The highest values were obtained at the Area Hospital of Dassa-Zoumè and Hubert Koutoukou Maga National University Hospital Center sites and were correlated with the conductivity measured at these sites. Additionally, the salinity values at these sites agree since the highest salinity values were recorded there (0.7 and 0.8, respectively). The redox potential (P-redox) indicates the oxidizing or reducing power of a substance relative to another. Average values of 19.96 mV in the north and 7.54 mV in the south were recorded. In general, this reflects the strong oxidizing power of the samples. Table 2 summarizes the results of the physicochemical analysis.

3.3 Antibiotics residues detection

Chloramphenicol, metronidazole, ciprofloxacin, and sulfamethoxazole were the most preponderant residues, with frequencies ranging from 100% to 87.5%. Ampicillin was detected at a lower frequency (33.75%). Although neomycin was found in the samples, none of its concentrations reached the detection limit for this antibiotic (Fig. 3).

The mean comparison test between the northern and southern samples reveals that the residue concentrations recorded in the north are higher than those in the south for amoxicillin (AMO), ampicillin (AP), metronidazole (MNZ), sulfamethoxazole (SMZ) and neomycin (NEO). In contrast, for chloramphenicol (CAP) and ciprofloxacin (CPFX), concentrations in the south are higher than those in the north. The average residue concentrations ranged from 0.043 to 7.65 µg/l, with the maximum value recorded for ciprofloxacin (Table 3).

3.4 Determination of heavy metal concentration

The results reveal the presence of heavy metals (lead, cadmium, nickel) in variable concentrations, all of which comply with Beninese standards for discharge into the environment (35 mg/l). Cadmium (Cd) is the most prevalent, with an average concentration of 0.21 mg/l, followed by nickel (Ni) and lead (Pb), with average concentrations of 0.17 mg/l and 0.029 mg/l, respectively. Table 4 presents the results of the analysis of heavy metals in the hospital effluents collected.

4 Discussion

Contamination of water resources by anthropogenic pollutants is a public health concern because good-quality water is important for drinking, domestic use, etc. [16]. The pollution of water resources by various contaminants, including heavy metals, antibiotic-resistant bacteria, antibiotic resistance genes, and antibiotic residues, remains a real problem in many parts of the world [2]. The situation is particularly alarming in developing regions such as sub-Saharan Africa, where many rivers and lakes often receive untreated hospital wastewater containing various types of anthropogenic pollutants [17]. Hospital effluents present characteristics of great variability that are a function of elements such as the size of hospitals, the number of beds, inpatients and outpatients, and the number and type of sections [18, 19]. The present study aims to assess the presence of antibiotiresistant bacteria, heavy metals, and antibiotic residues in hospital wastewaters in Benin. The bacteriological and chemical characterization of the effluents revealed the presence of micropollutants such as antibiotic residues but also a variety of bacterial species. These results are close to works in the literature that reported that liquid discharges from the hospital environment are highly loaded with chemical pollutants and pathogens and thus constitute a threat to the environment and health [20–24]. The physicochemical characterization of the effluents revealed that the values were in accordance with the standards for discharge to the environment. Our results are different from those of Iké et al. [24], who reported that apart from temperature, all values are above Nigerian regulatory standards for wastewater discharge to

Table 2 Physico-chemicals analysis characterization

Parameters	Electrical Conductivity (EC) (µS/cm) (OMS ˂2000)	Temperature (°C) (OMS˂30)	pH (OMS: 6–9)	Salinity (%)	Total Dissolved Solid (TDS) (mg/l)	P-redox (mV)	Resistivity (KΩ/cm)
Departmental Hospital Center Atacora	647	26.8	6.84	0.2	647	7.5	1.546
Departmental Hospital Center Donga	290	26.4	6.82	0	290	8.5	3.46
Departmental Hospital Center of Borgou-Alibori	685	26.8	6.49	0.3	685	86.9	1.46
Area Hospital of Boko	544	25.8	6.92	0.2	544	2.9	1.837
Area Hospital of Dassa-zoumè	1557	26.8	7.07	0.7	1557	− 6.2	0.642
Area Hospital of Ordre de Malte	675	26.9	6.93	0.3	675	2.4	1.482
Area Hospital of Malanville	492	26.3	5.85	0.2	492	65.9	2.04
Area Hospital of Savè	1296	26.7	7.11	0.6	1296	− 8.2	0.772
Average ± sd	773.25 ± 428.63	26.56 ± 0.37	6.75 ± 0.41	0.31 ± 0.23	773.25 ± 428.53	19.96 ± 35.77	1.69 ± 0.87
Hubert Koutoukou Maga National University Hospital Center	1687	26.5	7.06	0.8	1687	− 5.5	0.582
Departmental Hospital Center of Porto-Novo	691	28	7.7	0.3	691	− 38.1	1.448
Area Hospital of Aplahoué	708	28.2	7.02	0.3	708	2.4	1.413
Area Hospital of Abomey-Calavi So-Ava	376	26.6	6	0.1	376	57.3	2.66
Departmental Hospital Center of Zou	531	27.9	6.67	0.2	531	23.6	1.882
Area Hospital of Grand-Popo	206	28.5	7.6	1	206	− 32.4	0.485
Area Hospital of Pobè	376	27.7	6.5	0.1	376	34.1	2.66
Area Hospital of Lokossa	244	28.8	6.83	0	244	14.1	4.09
Area Hospital of Mènontin	154	28.6	6.92	0.7	154	8.7	0.649
Zinvié Confessional Center	1168	27.8	6.78	0.5	1168	11.2	0.857
Average ± sd	614.1 ± 483.96	27.86 ± 0.78	6.91 ± 0.5	0.4 ± 0.34	614.1 ± 483.96	7.54 ± 28.62	1.67 ± 1.17

the environment. The average temperature and pH values of the effluents are in line with Beninese standards for liquid waste discharge, which call for a temperature of 30 °C and a pH between 6 and 9 for domestic wastewater. Indeed, in Benin, no standards exist for parameters related to hospital effluents. Temperature plays an important role in the solubility of salts and gases and the determination of pH. It also acts as a physiological factor in the metabolism of microorganisms living in water. In this study, the temperature ranged from 25.8 to 28.8 °C with an average of 27.21 °C, which is in line with the international standard established by the WHO, [25] that set the maximum value

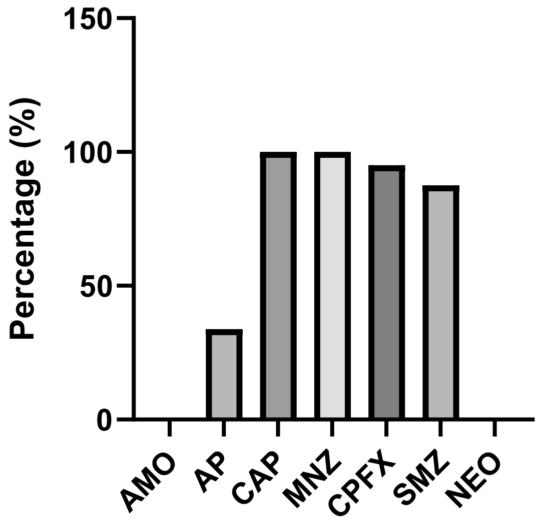

Fig. 3 Frequencies of detection of antibiotic residues

Table 3 Average concentration of antibiotic residues

Antibiotics	Northern (μg/l)	Southern (μg/l)	Average
AMO	0.05	0.03	0.043 ± 0.026
AP	1.96	1.84	1.90 ± 1.042
CAP	0.1054	0.1063	0.105 ± 0.001
MNZ	6.62	6.61	6.61 ± 0.051
CPFX	7.41	7.84	7.65 ± 2.272
SMZ	4.89	3.06	3.88 ± 3.088
NEO	1.00	0.99	0.99 ± 0.027

Table 4 Heavy metals concentation

Metallic Trace Elements	Lead 1 mg/l*	Cadmium 1 mg/l*	Nickel 2,5 mg/l*
HZ Pobè	IND	0.23	0.29
Departmental Hospital Center of Porto	0.33	0.13	0.13
Departmental Hospital Center of Zou	0.01	0.15	0.27
Area Hospital of Aplahoué	IND	0.07	0.09
Area Hospital of Lokossa	0.06	0.10	IND
Area Hospital of Grand popo	0	0.31	IND
Area Hospital of Mènontin	0.02	0.12	0.52
Zinvié Confessional Center	IND	0.36	0.35
Area Hospital of Abomey-Calavi	IND	0,29	IND
Hubert Koutoukou Maga National University Hospital Center	IND	0.16	0.18
Average ± sd	0.04 ± 0.10	0.2 ± 0.1	0.18 ± 0.20
Area Hospital of Boko	0.01	0.09	0.11
Area Hospital of Malanville	IND	0,18	IND
Area Hospital of Ordre de Malte	IND	0,20	IND
Departmental Hospital Center of Donga	0.08	0,07	IND
Area Hospital of Dassa	IND	0,36	IND
Departmental Hospital Center of Atacora	IND	0,21	IND
Departmental Hospital Center of Borgou-Alibori	0.02	0.15	1.15
Area Hospital of Savè	IND	0.64	IND
Average ± sd	0.02 ± 0.29	0.24 ± 0.18	0.18 ± 0.04

at 30 °C. Todedji et al. [26] obtained temperatures ranging from 18.6 to 30.2 °C with an average of 24.52 °C in a study on effluents from a university hospital in Benin. The temperature found by Sadek et al. [20] in the effluent of Sidi Kacen Provincial Hospital was 19.8 °C, and that found by Tahiri et al. [27] was 17.11 °C.

The study conducted by Bouzid et al. [21] at Mohamed V Hospital in Meknes, Morocco reported a temperature between 19.4 and 20.4 °C. The pH values of the effluent ranged from 5.85 to 7.11. This is consistent with WHO [25] standards between 6.5 and 8.5 and the results obtained in several studies [20, 21, 26, 28]. Electrical conductivity, salinity and total dissolved solids are indicators of mineral pollution in wastewater. Electrical conductivity estimates overall mineralization and total soluble salts in water and is one of the simplest and most important indicators for monitoring hospital effluent quality [14]. Electrical conductivity values ranged from 154 to 1687 µS/cm. The values obtained in most samples remain below the WHO. [25] lower limit of 2000 µS/cm. Values higher than ours have been reported [20, 26]. Since water with an electrical conductivity between 600 and 1000 µS/cm is considered highly mineralized [14], the analyzed effluents in this study are highly mineralized. Salinity follows the same trend as electrical conductivity. Indeed, salinity values vary between 0 and 0.8 with an average of 0.35. This was further verified by the fact that the sites where the highest values were recorded for conductivity were the same for salinity (HZ Dassa-Zoumè and CNHU). A positive value of the redox potential of water reflects its oxidizing power. The average value of P-redox is 1.68 mV, and it can be concluded that in general, the effluents of this study have oxidizing power. Hospital effluents contain a great variability of pathogens. A high percentage of nonenterobacteria (22.05%) was recorded. According to morphology, gram-negative bacilli were also found with the presence of *Acinetobacter baumanii,* followed by *Klebsiella* spp., *E. coli, K. pneumoniae,* and *Yersinia enterolica*. This preponderance of gram-negative bacilli was reported by Guessennd et al. [29]. *S. aureus* and *CoNS* were isolated at a frequency of 6.5%. This frequency is much lower than the result of Guessennd et al. [30], which was 100%. Our results are related to those of several studies carried out in Africa (Benin, Morocco) and in Europe (Spain, France), in which the authors have also highlighted a bacterial flora of hospital effluents with the presence of gram-negative bacteria belonging to the Enterobacteria family [30–32] and, to a lesser extent, Staphylococci [33].

Various antibiotic residues were detected in the effluent samples. Aquatic organisms are therefore exposed to cocktails of residues if these effluents reach the water table. Even if their individual concentrations measured in the environment are low, when combined, they can be significant and cause significant toxicity to aquatic organisms by interacting with each other and having either a significant effect (synergistic effect) [34]. In this study, different antibiotic residues were sought in effluent samples. This may be due to the wider use of antibiotic molecules in hospitals than elsewhere. Metronidazole, ciprofloxacin, chloramphenicol and sulfamethoxazole were the most frequently detected in effluents at high concentrations. The average residue concentrations ranged from 0.043 to 7.65 µg/l, with the maximum value recorded for metronidazole. This could be explained by the frequent use of these molecules but also by their low biodegradation in the environment. The concentrations found are highly variable in relation to variations in consumption patterns (type of antibiotic and quantity) and to the persistence of the different compounds disseminated [35]. On the other hand, the conditions of the receiving environments and the intrinsic chemical characteristics of the molecules condition their persistence [36]. Therefore, the study of processes governing persistence, such as sorption, mobility and degradation of antibiotics, is an essential step. The most important mechanisms conditioning sorption and thus mobility of antibiotic compounds are interactions with organic matter and mineral constituents in the soil, as well as ion exchange, hydrogen bonding, and complex formation with metal ions [37]. In addition, the lack of sanitation and wastewater treatment facilities in low-income countries means that the average concentration of antibiotic residues is generally significantly higher. Metronidazole is an example of a widely empirically used first-time treatment in many African countries [38]. Against various protozoan parasites and anaerobic bacteria, it is an effective antimicrobial drug [39]. The mean concentration of metronidazole was 6.62 µg/l in hospital effluents. Indeed, this antibiotic is wrongly considered more as an antiparasitic than an antibiotic by the population. The metronidazole concentrations found in this study are much higher than those obtained in the effluents of the G-point CHU by Levy et al. [40] (0.15 µg/l) and Maiga et al. [41] in Bamako (0.02 µg/l). Ciprofloxacin, one of the most potent antibiotics at low concentrations, was also concentrated in effluents (7.63 µg/l). These results are similar to those obtained in France by Andreozzi et al. [42], in Italy by Miao et al. [43]. in Canada, TamTam et al. [44] and Kouadio et al. [45] in Côte-d'Ivoire. Our results are different from those reported by Maiga et al. [41] in Mali, which makes the case of the nondetection of this molecule in hospital effluents. Chloramphenicol, despite its detection limit of approximately 0.05 µg/l, was detected and quantified in hospital effluents at concentrations of approximately 0.1 µg/l in the present study. These results are not corroborated by those of Maiga et al. [41], who reported the nondetection of chloramphenicol in hospital effluents. Indeed, this antibiotic has been withdrawn from the Malian market for a few years because of its toxicity to blood cells and bone marrow. Amoxicillin was not detected in hospital effluents. This result is different from that of Maiga et al. [41], who reported the detection of amoxicillin in hospital effluents at concentrations on the order of 0.066 µg/l. The northern samples recorded the highest concentrations of ampicillin at an average concentration of 1.90 µg/l. These results are different from those of Christian et al. [46] who obtained concentrations on the order of ng/l. Sulfamethozaxole is a widely used antibiotic for the treatment of diseases in humans and animals and is one of the antibiotics commonly detected in wastewater treatment plant processes (WWTPs) [47, 48]. In the present study, sulfamethozaxole was also detected in hospital effluents. It has been shown that low-cost antibiotics, such as sulfamethozaxole and trimetoprim, seem to be more frequently observed and at higher concentrations in developing countries [49]. This can also be due to their persistence and poor elimination in the effluents [50, 51]. The results in the present study are superior to the 0.06 µg/l obtained by Maiga et al. [40] in effluents in Mali. Values ranging from 0.04 to 0.162 µg/l were found by Kouadio et al. [45], TamTam et al. [44], and Andreozzi et al. [41] for cotrimoxazole, a combination molecule of sulfamethoxazole and trimethoprim. The three heavy metals, namely, lead, cadmium and nickel, investigated in this study were all present in varying concentrations. The presence of heavy metals in hospital effluents is related to the presence of iodinated contrast agents used for radiography, certain drugs and their metabolites that may contain organohalogen elements, the use of disinfectants, detergents, and chlorinated solvents, as well as other substances from laboratories [26]. A predominance of cadmium (0.22 mg/l) followed by nickel (0.18 mg/l) and lead (0.03 mg/l) was observed. This predominance of cadmium could be due to cleaning activities that involve the use of large volumes of detergents, the use of dye products when refurbishing treatment rooms, and the lack of pretreatment of effluents at the department level before their discharge into septic tanks [26]. Metal concentrations in hospital effluents vary at each hospital. While the different concentrations obtained for these metals all complied with Beninese standards for discharge into the environment (1 mg/l for cadmium and lead and 2.5 mg/l for nickel), this was not the case in comparison with WHO standards. Indeed, for cadmium, all the concentrations obtained were higher than the WHO standard of 0.01 mg/l, with a maximum value recorded at the HZ of Savè (0.64 mg/l). For lead, all the concentrations obtained conformed to the WHO standard, which is 5 mg/l.

It should be noted that although data exist on the presence of resistant bacteria and heavy metals, this is the first time that a study has provided national data on the presence of these pollutants in Benin. In addition, this is one of the first studies in Benin to show the presence of antibiotic residues in hospital effluents. As a pioneering study, this work opens up many research perspectives to investigate the link between the most commonly used antibiotics in Benin, the presence of antibiotic residues and resistant bacteria in hospital effluents and the resultant role of these chemical and biological pollutants in the spread of antimicrobial resistance. This study is supported by the One Health approach, which highlights the link between environmental and human health, through the massive use of antibiotics in human health and their diffusion in the environment.

5 Conclusion

Effluents generated by hospital activities can present a potential danger for humans and the environment. The physico-chemical parameters of the effluents in this study conformed with the accepted standards in Benin. Various bacterial strains were isolated with a wide range of gram-negative bacilli. Antibiotic residues were also detected in variable concentrations in these effluents. These results can be explained by hospital practices and the lack of adequate treatment of these effluents. The concentrations of heavy metals obtained in the hospital effluents were below those required by Benin standards. The lack of systematic treatment of this wastewater before disposal exposes real health and environmental risks, given by the nature and content of the pollutants that can be found in effluents. It is therefore imperative and necessary to develop treatment methods for these effluents before their discharge into the natural environment.

Acknowledgements The authors are very grateful to The World Academy of Sciences (TWAS) and the Islamic Development Bank (ISDB), which funded one of them for this study. They also thank the L'OREAL FOUNDATION for giving the Young Talents Prize to the first author. They are finally grateful to the Minister of the Living Environment and Sustainable Development in Benin, his Excellency his Excellency M. José TONATO and all his staff.

Author contributions ED, VD, VMCH, JRK, WM, CL and MB wrote the protocol. ED, VMCH, WM got the funding. ED, VD, JRK, VMCH, EG, RAA, CL and KF processed the samples. ED, VD, JRK, JA and FT performed the statistical analyses. ED, VD, EG, JRK wrote the draft of the manuscript. VD, JRK, MB and HB reviewed the manuscript. All authors read and approved the final manuscript.

Funding The authors are very grateful to The World Academy of Sciences (TWAS) and the Islamic Development Bank (ISDB). These two institutions have made this research possible through research funding allocated to the research team under the IsDB-TWAS Grants for Research Collaboration in Sustainability Sciences (Grant No. 506808). They reviewed the research protocol and validated the design of the study and collection, analysis, and interpretation of data. The authors are also grateful to The L'OREAL FOUNDATION, which provided the YOUNG TALENTS 2021 Prize to one of the authors (Dr Esther DEGUENON).

Data availability All data generated or analyzed during this study are included in this published article and Additional file.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate Not applicable. The authors received written approval from the hospital managers prior to collecting effluent samples. The study protocol was approved by the Ministry of the Living Environment and Sustainable Development in Benin.

Consent for publication Not applicable.

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