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Listeria Species Isolated from the Wastewater Samples of a Private and Tertiary Hospital in Ibadan and their Antibiotics Resistance Patterns

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Authors' contributions

This work was carried out in collaboration between both authors. Author OIF designed the study the protocol. Authors OIF and MJA managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: In hospitals, a significant amount of water is consumed and equally, significant amounts of wastewater is disposed with high levels of contaminants, including disease-causing bacteria such as *Listeria* spp. have been found in wastewater effluent and surrounding freshwater bodies. Recent studies suggest that *Listeria* species readily survive conventional wastewater treatment processes even after tertiary treatment. This study was carried out to determine the antibiotic resistance pattern of *Listeria* spp. isolated from hospital wastewater (treated and untreated) from private and tertiary hospital samples in Ibadan and comment on the public health significance.

Materials and Methods: Hospital wastewater samples were collected between April and July, 2016. Listeria Selective Agar Base with Listeria Selective Supplement (Oxoid, UK) was used for the isolation of the *Listeria* species and the isolates were identified using standard conventional methods. Antimicrobial susceptibility testing was done against ampicillin (10 µg), cloxacillin (5 µg), amoxicillin (5 µg), streptomycin (10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), sulfamethoxazole-trimethoprim (25 µg) and tetracycline (30 µg) by the

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Kirby-Bauer disk diffusion method.
Results: A total of 96 *Listeria* spp. were isolated comprising *L. monocytogenes* 23 (24%), *L. innocua* 13 (13.5%), *L. ivanovii* 14 (14.6%) and other *Listeria* spp. 46 (47.9%). Furthermore, all the 96 (100%) isolates were resistant to ampicillin while all (100%) the *L. monocytogenes* and *L. ivanovii* showed resistance to both ceftriaxone and cloxacillin. In addition, all the L. *ivanovii* exhibited complete resistance to ciprofloxacin. Also, three (3.1%) isolates (*L. monocytogenes*, *L. ivanovii* and *Listeria* spp.) were resistant to a combination of eight antibiotics (sulfamethoxazole-trimethoprim, chloramphenicol, tetracycline, ampicillin, ceftriaxone, cloxacillin, amoxicillin and ciprofloxacin).
Conclusion: The observation from this study showed that the wastewater from both the private and tertiary hospitals could be a source of transmission of multi-drug resistant bacteria to human and animals. More so, the wastewater treatment processes did not reduce the load of the *Listeria* species.

Keywords: Listeria species; antibiotics resistance; hospital; wastewater.

1. INTRODUCTION

Water is one of the most abundant compounds on earth covering approximately three-quarters of the earth's surface. Large part of water available on earth is saline in nature and only a small quantity exists as fresh water. Water resources are among the most critical resources and the importance of water, particularly surface water (rivers), in meeting the need of humans, animals and industries underscore the need to protect them against contaminations [1]. However, fresh water has become a scarce commodity due to over exploitation and pollution [2]. Hospitals consume a significant amount of water daily and these ranges from 400 to 1200L per bed and equally generate significant amounts of wastewater loaded with microorganisms, heavy metals, toxic chemicals, disinfectants, specific detergents as a result of routine diagnosis, laboratory, research activities and medicine excretion by patients and radioactive elements [3]. There is increasing attention on the presence of pollutants in surface and groundwater such as surfactants and pharmaceuticals [4]. Hospital wastewater has similar quality to municipal wastewater and is important sources of pharmaceuticals residues in all wastewater treatment plant effluents due to their inefficient removal with the conventional systems [5,6]. Indeed hospital wastewater may have an adverse impact on environmental and human health. High levels of contaminants, including disease-causing bacteria such as *Listeria* spp. have been found in wastewater effluent and surrounding freshwater bodies such as rivers and estuaries [7].

Modern knowledge of the need for sanitation and treatment of polluted water however, started with the frequently cited case of John Snow [8], in which he proved that a cholera outbreak in London was due to sewage contaminated water obtained from the Thames River [9]. Wastewater treatment practices vary from country to country across the globe. Even in areas with a high degree of wastewater treatment, pathogens and some chemicals, many with unknown ecological consequences, may still be released into the environment [10]. The ability of these organisms to survive conventional wastewater treatment processes could lead to major environmental and human health problems, resulting from the highly contaminated surface waters receiving the wastewater [7].

Listeria spp. which was thought to be only associated with food related infections and diseases has now been discovered and reported in water and are widely spread in natural environment and animals [11]. *Listeria. monocytogenes* is the principal pathogen in humans and animals; *L. ivanovii* is a pathogen of animals but is implicated in human disease [12]. Ingestion of *L. monocytogenes* through food and water is generally the main mode of transmission of infection [13]. *Listeria monocytogenes* has an unusual characteristic of surviving in temperatures from -7°C to 45°C while its optimum growing temperature is around 37°C and it is able to grow minimally at a temperature as low as 0°C [14].

Some studies suggest that *Listeria* species readily survive conventional wastewater treatment processes even after tertiary treatment [10,15]. With reports of inadequate removal of *Listeria* pathogens from wastewater from the developed world [10], it can be presumed that wastewater treatment plants in developing countries may be inefficient for the removal of these pathogens from wastewater effluents prior to discharge into the receiving water bodies. Several studies have revealed the preponderance of *Listeria* species to exist as biofilms attached to surfaces such as stainless steel, glass, propylene and food [16,17]. In a recent study in Ibadan, *Listeria spp*. was isolated from abattoir wastewater [18]. Antibiotic resistant strains of *Listeria* species from different environmental samples have been reported including poultry droppings [13], abattoir wastewater [18], and fish pond water [19]. This study was therefore carried out to determine the antibiotic resistance pattern of *Listeria* spp. isolated from hospital wastewater of both private and tertiary hospital in Ibadan and comment on the public health significance.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The study was carried out in a private and tertiary hospital in Ibadan, Nigeria between April and July, 2016. Ibadan is the largest city in West Africa and located at 70 24'N; 30 54'E; 234 m above sea level [20]. Samples of untreated and treated wastewater were aseptically collected into sterile universal bottles at the point of discharge into the receiving water body. The samples were transported immediately to the laboratory in ice packs for microbiological analyses.

2.2 Isolation and Identification of *Listeria* species

Isolation of *Listeria* species was carried out according to the method that was previously described [21]. Analysis of the wastewater was carried out using the Listeria Selective Agar Base (Oxoid, UK). The media was prepared according to manufacturers' instruction. A vial of Listeria Selective Supplement (SR0140) was added to 500 ml of Listeria selective agar (CM0856) at 50°C and mix well. Briefly, 1 ml of different wastewater samples were serially diluted and the standard pour plate technique was used by plating out 1 ml of the appropriate dilutions on Listeria selective agar. The plates were inverted and incubated at 35°C for 24-48 hours. Suspected colonies with gray colour and dark background were sub-cultured on Listeria Selective Agar media to obtain pure isolates. The isolates were identified using microscopy, Gram staining and biochemical tests (oxidase, catalase, citrate, methyl red, Voges-Proskauer, motility test glucose, sucrose, lactose, maltose, galactose, D-xylose, mannitol and β- hemolytic activity).

2.3 Antibiotics Susceptibility Test of the Isolates

Using the standard disk diffusion technique on Mueller-Hinton agar, antibiotics susceptibility test of the isolates was carried out based on the recommendation of Clinical Laboratory Standards Institute [22]. A total of ten antibiotics obtained from Oxoid, U.K. were used and they include: tetracycline (30 µg), streptomycin (10 µg), cloxacillin (5 µg), ofloxacin (5 µg), amoxillin (5 µg), ceftriaxone (30 µg), sulfamethoxazole-trimethroprim (25 µg), chloramphenicol (30 µg), ampicillin (10 µg) and ciprofloxacin (5 µg). The isolates were sub-cultured and colonies of 18-24 hours old culture were picked and suspended in a saline solution and adjusted to 0.5 McFarland standard. The suspension of the isolates was inoculated in Mueller Hinton agar plates using a sterile swab stick. Sterile forceps was used to aseptically place the antibiotics disc on the inoculated plates and incubated at 35°C for 18-24 hours. A positive control of *Listeria monocytogenes* was used for each set of analyzed samples. The zones of inhibition were measured (mm) after 24 hours, recorded and interpreted according to the CLSI guidelines.

3. RESULTS

A total of 96 (100%) *Listeria* spp. were isolated from the wastewater samples comprising *Listeria monocytogenes* 23 (24%), *L. innocua* 13 (13.5%), *L. ivanovii* 14 (14.6%) and other *Listeria* spp. 46 (47.9%). The isolates obtained from the wastewater samples of the private hospital was 58 (60.4%) while it was 38 (39.6%) from the tertiary hospital. In addition, 14 (14.6%) and 24 (25.0%) isolates were obtained from untreated and treated wastewater of the tertiary hospital respectively (Table 1).

The result of the susceptibility test showed that all 96 (100%) the isolates were resistant to ampicillin, 88 (91.7%) were resistant to ciprofloxacin, while all the *L. ivanovii* and *L. monocytogenes* showed resistance to ceftriaxone and cloxacillin. Furthermore, while all *L. ivanovii* isolated exhibited total resistance to ciprofloxacin, 46 (47.9%) of the isolates were resistant to sulfamethoxazole-trimethoprim (Table 2). In addition, 54 (93.1%), 20 (34.5%) and 12 (20.7%) of the isolates obtained from the untreated wastewater samples of the private hospital showed resistance to cloxacillin, sulfamethoxazole-trimethoprim and streptomycin respectively, while 55 (94.8%) of the isolates showed resistance to ceftriaxone and ciprofloxacin. It was further observed that 15 (25.9%) and 30 (51.7%) *L. monocytogenes* and the other *Listeria* spp. from the untreated wastewater of the private hospital showed resistance to ciprofloxacin respectively (Table 3).

Furthermore, the susceptibility tests results of the isolates obtained from the untreated wastewater of the tertiary hospital showed that all 24 (100%) were resistant to ceftriaxone and cloxacillin while, 10 (71.4%) showed resistance to ciprofloxacin and sulfamethoxazole-trimethroprim. Similarly, all the *L. ivanovii* exhibited resistance to ciprofloxacin while, two (14.3%) *L. monocytogenes* and six (42.9%) other *Listeria* spp. were resistant to ciprofloxacin. More so, all the *L. monocytogenes* and *L. ivanovii* were completely resistant to sulfamethoxazole-trimethroprim, however, none of the isolates showed resistance to chloramphenicol (Table 4). From the isolates obtained from the treated wastewater samples collected from the tertiary hospital, all 14 (100%) were absolutely resistant to ceftriaxone and cloxacillin while, 23 (95.8%) and 16 (66.7%) were resistant to ciprofloxacin and sulfamethoxazole-trimethroprim respectively. Moreover, three (12.5%) *L. monocytogenes,* and six (25.0%) other *Listeria* spp. that were resistant to ciprofloxacin was low (Table 5). Additionally, the phenotype of resistance of the isolates showed that 25 (26.0%) of the isolates were resistant to a combination of five antibiotics that included sulfamethoxazole-trimethroprim, cloxacillin, ampicillin, ceftriaxone and ciprofloxacin while three (3.1%) isolates (*L. monocytogenes*, *L. ivanovii* and *Listeria* spp.) showed resistance to the combination of the following eight antibiotics: chloramphenicol, tetracycline, ampicillin, ceftriaxone, cloxacillin amoxicillin and ciprofloxacin sulfamethoxazole-trimethoprim, (Table 6).

4. DISCUSSION

Information on listeriosis in the environment, especially in the developing countries of the world, is limited. However, listeriosis was previously believed to be a foodborne disease, studies have shown that the environment can also serve as reservoir for this pathogen. In the present study, isolation of *L. monocytogenes* and *L. ivanovii* from the studied hospital wastewater (treated and untreated) is an indication that hospital wastewater may serve as reservoir of the pathogens to both humans and animals. Different species of *Listeria* were isolated from the hospital wastewater samples which corroborate other studies that had previously reported the isolation of the bacteria from different environmental samples such as abattoir wastewater, freshwater samples, poultry wastes, fishpond water and municipal waste effluents [7,13,18,19]. The observation from this study that showed *L. monocytogenes* 23 (24.0%) having the highest rate of occurrence followed by *L. ivanovii* 14 (14.6%) and *L. innocua* 13 (13.5%) is in agreement with a recent report on a study on poultry waste and fishpond water in which *L. monocytogenes* had the highest rate of occurrence compared to the other species [19]. However, from the samples collected from the treated hospital wastewater, *L. ivanovii* had the highest rate of occurrence (11.5%) compared to *L. monocytogenes* (4.2%) and *L. innocua* (3.1%). Among the wastewater from the tertiary hospital, the treated sample has the highest number 24 (25.0%) of isolates as compared to that from the untreated source 14 (14.6%). Also, the number of isolates from untreated private sample 58 (60.4%) is higher compared to that of the tertiary hospital 14 (14.6%). In addition, the observation from this present study that showed the isolates obtained from the treated wastewater samples being higher than that from the untreated wastewater of the tertiary hospital may be due to the previous reason given that convectional wastewater treatment may not be effective in the removal of bacterial pathogens [23].

In the present study, all 96 (100%) the isolates were found to be resistant to ampicillin, this is in line with total resistance of similar isolates to the same antibiotic in previous studies on raw milk and processed meat from cattle herds within Sokoto Metropolis [24]. However, the 100% resistance observed in this study is slightly higher than the 92.9% resistance reported on salad vegetables and vegetable salads sold in Zaria [25]. The observed 88 (91.7%) resistance of the isolates to ciprofloxacin in this study is in contrast to the 0% resistance of *Listeria* spp. that were reported from previous studies on clinical and food samples in India [26], ready to eat foods in South Africa [27], treated wastewater effluent and receiving surface water also in South Africa [15]. The reason for the disparity may be due to the studied samples. However, resistance rate of the isolates to ciprofloxacin 88 (91.7%) in this study is comparably similar to the 91.0% resistance of *Listeria* isolates to the drug in a report of a study on municipal wastewater from South Africa [7].

Table 1. The percentage of occurrence of *Listeria* species isolated from hospital wastewater n (%)

Source	*L. monocytogenes*	*L. ivanovii*	*L. innocua*	*Listeria* spp.	Total
Untreated tertiary	*3.00±1.00[a] (3.1)	2.00±0.00[a] (2.1)	0.00±0.00[a] (0.0)	9.00±2.00[a] (9.4)	14(14.6)
Treated tertiary	4.00±1.00[a] (4.2)	11.00±2.00[b] (11.5)	3.00±0.00[b] (3.1)	6.00±0.00[a] (6.2)	24(25.0)
Untreated private	16.00±2.00[b] (16.7)	1.00±0.00[a] (1.0)	10.00±2.00[c] (10.4)	31.00±3.00[b] (32.3)	58(60.4)
Total	23 (24)	14 (14.6)	13 (13.5)	46 (47.9)	96(100.0)

**Values are means ± Standard Deviation of duplicate observations (Means with same alphabets down each column are not statistically significant at p<0.05)*

Table 2. Antibiotic resistant pattern of *Listeria* species isolated from all the hospital wastewater n (%)

Antibiotics	*L. monocytogenes* n=23	*L. ivanovii* n=14	*L. innocua* n=13	*Listeria* spp. n=46	Total n=96
Ampicillin (10 µg)	23(24.0)	14(14.6)	13(13.5)	46(47.9)	96(100.0)
Streptomycin (10 µg)	2(2.1)	0(0.0)	4(4.2)	8(8.3)	14(14.6)
Ceftriazone (30 µg)	23(24.0)	14(14.6)	12(12.5)	44(45.8)	93(96.9)
Chloramphenicol (30 µg)	4(4.2)	0(0.0)	3(3.1)	3(3.1)	10(10.4)
Sulfamethoxazole-trimethroprim (25 µg)	9(9.4)	9(9.4)	6(6.3)	22(22.9)	46(47.9)
Cloxacillin (5 µg)	23(24.0)	14(14.6)	11(11.5)	44(45.8)	92(95.8)
Tetracycline (30 µg)	2(2.1)	0(0.0)	2(2.1)	5(5.2)	9(9.4)
Ciprofloxacin (5 µg)	20(20.8)	14(14.6)	12(12.5)	42(43.8)	88(91.7)
Ofloxacin (30 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Amoxicillin (25 µg)	3(3.1)	1(1.0)	2(2.1)	5(5.2)	11(11.5)

Table 3. Antibiotic resistant pattern of *Listeria* species isolated from untreated private hospital wastewater n (%)

Antibiotics	*L. monocytogenes* n=16	*L. ivanovii* n=1	*L. innocua* n=10	*Listeria* spp. n=31	Total n=58
Ampicillin (10 µg)	16(27.6)	1(1.7)	10(17.2)	31(53.5)	58(100.0)
Streptomycin (10 µg)	1(1.7)	0(0.0)	3(5.2)	8(13.8)	12(20.7)
Ceftriazone (30 µg)	16(27.6)	1(1.7)	9(15.5)	29(50.0)	55(94.8)
Chloramphenicol (30 µg)	4(6.9)	0(0.0)	2(3.4)	3(5.2)	9(15.5)
Sulfamethoxazole-Trimethroprim (25 µg)	4(6.9)	0(0.0)	3(5.2)	13(22.4)	20(34.5)
Cloxacillin (5 µg)	16(27.6)	1(1.7)	8(13.8)	29(50.0)	54(93.1)
Tetracycline (30 µg)	2(3.4)	0(0.0)	1(1.7)	5(8.6)	8(13.8)
Ciprofloxacin (5 µg)	15(25.9)	1(1.7)	9(15.5)	30(51.7)	55(94.8)
Ofloxacin (30 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Amoxicillin (25 µg)	3(5.2)	0(0.0)	2(3.4)	4(6.9)	9(15.5)

Table 4. Antibiotic resistant pattern of *Listeria* species isolated from untreated tertiary hospital wastewater n (%)

Antibiotics	*L. monocytogenes* n=4	*L. ivanovii* n=11	*L. innocua* n=0	*Listeria* spp. n=9	Total n=24
Ampicillin (10 µg)	3(21.4)	2(14.3)	0(0.0)	9(64.3)	14(100.0)
Streptomycin (10 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Ceftriazone (30 µg)	3(21.4)	2(14.3)	0(0.0)	9(64.3)	14(100.0)
Chloramphenicol (30 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Sulfamethoxazole-trimethroprim (25 µg)	3(21.4)	2(14.3)	0(0.0)	5(35.7)	10(71.4)
Cloxacillin (5 µg)	3(21.4)	2(14.3)	0(0.0)	9(64.3)	14(100.0)
Tetracycline (30 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Ciprofloxacin (5 µg)	2(14.3)	2(14.3)	0(0.0)	6(42.9)	10(71.4)
Ofloxacin (30 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Amoxicillin (25 µg)	0(0.0)	0(0.0)	0(0.0)	1(7.1)	1(7.1)

Table 5. Antibiotic resistant pattern of *Listeria* species isolated from treated tertiary hospital n (%)

Antibiotics	*L. monocytogenes* n=4	*L. ivanovii* n=11	*L. innocua* n=3	*Listeria* spp. n=6	Total n=24
Ampicillin (10 µg)	4(16.7)	11(14.3)	3(12.5)	6(25.0)	24(100.0)
Streptomycin (10 µg)	1(4.2)	0(0.0)	1(4.2)	0(0.0)	2(8.3)
Ceftriazone (30 µg)	4(16.7)	11(14.3)	3(12.5)	6(25.0)	24(100.0)
Chloramphenicol (30 µg)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	1(4.2)
Sulfamethoxazole-trimethroprim (25 µg)	2(8.3)	7(29.1)	3(12.5)	4(16.7)	16(66.7)
Cloxacillin (5 µg)	4(16.7)	11(14.3)	3(12.5)	6(25.0)	24(100.0)
Tetracycline (30 µg)	0(0.0)	0(0.0)	1(4.2)	0(0.0)	1(4.2)
Ciprofloxacin (5 µg)	3(12.5)	11(45.8)	3(12.5)	6(25.0)	23(95.8)
Ofloxacin (30 µg)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Amoxicillin (25 µg)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	1(4.2)

Table 6. Multiple antibiotic resistance patterns of *Listeria* species isolated from the hospital wastewater samples

Antibiotypes	***L. monocytogenes* n= 23**	***L. ivanovii* n=14 N (%)**	***L. innocua* n=13**	***Listeria* spp. n=46**	**TOTAL n =96**
OB AMP	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.0)	2 (2.1)
OB CRO AMP	2 (2.1)	0 (0.0)	0 (0.0)	2 (2.1)	4 (4.2)
AMP-CRO-CIP	0 (0.0)	1 (0.0)	1 (1.0)	1(1.0)	3 (3.1)
OB-AMP-CRO-CIP	8 (8.4)	4 (4.2)	3 (3.1)	16 (16.8)	31(32.3)
SXT-OB-AMP-CRO	1 (1.0)	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.1)
SXT-AMP-CRO-CIP	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.0)
STR-OB-AMP-CRO-CIP	1 (1.0)	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.1)
OB-AMP-CRO-CIP C	2 (2.1)	0 (0.0)	1 (1.0)	0 (0.0)	3 (3.2)
STR-SXT-AMP-CRO-CIP	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)
C-OB-AMP-CRO-CIP	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)
SXT-OB-AMP-CRO-CIP	5 (5.2)	9 (9.5)	2 (1.0)	9 (9.5)	25 (26.0)
OB-AML-AMP-CRO-CIP	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
SXT-STR-AMP-CIP-CRO	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)
TE-OB-AMP-CIP-CRO	1 (1.0)	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.1)
OB-STR-SXT-AMP-CRO-CIP	1 (1.0)	0 (0.0)	0 (0.0)	4 (4.2)	5 (5.2)
OB-TE-SXT-AMP-CRO-CIP	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.1)	2 (2.1)
OB-SXT-AML-AMP-CRO-CIP	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.0)
OB-TE-SXT-C-AMP-CIP	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.1)	2 (2.1)
OB-STR-AML-AMP-CRO-CIP	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	1 (1.0)
OB-STR-SXT-AML-AMP-CRO-CIP	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.1)	2 (2.1)
OB-TE-AML-SXT-C-AMP-CIP	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.0)
OB-SXT-C-TE--AML-AMP-CRO-CIP	1(1.0)	0 (0.0)	1 (1.0)	1 (1.0)	3 (3.1)

Keys: TET- Tetracycline, STR- Streptomycin, C- Chloramphenicol, CRO- Ceftriaxone, CIP- Ciprofloxacin, OB- Cloxacillin, SXT- Sulfamethoxazole-trimethoprim, AMP- Ampicillin, AMX- Amoxicillin

The high 93 (96.9%) resistance of all the listerial isolates to ceftriaxone in this study is higher compared to the resistance (85.2%) reported in a study on abattoir effluent in Lagos [21]. Comparing the resistance of the isolates obtained from the samples of the tertiary hospital with that of the private hospital, the 100% resistance of the isolates from the tertiary hospital to ceftriaxone is a bit higher than the 55 (94.8%) resistance observed among the isolates obtained from the untreated wastewater samples of the private hospital. These were however much higher than the 24.36% resistance to ceftriaxone reported from a previous study in South Africa on treated municipal wastewater and receiving surface water [15]. The observed difference may as a result of exposure of the isolates obtained from the hospital wastewater to the antibiotics compared to the later study which could have led to selection of antibiotic resistance. Conversely, resistance of the *Listeria* isolates from the treated wastewater of the tertiary hospital in this study to sulfamethoxazole which was 16 (67.67%) is lower compared to the 10 (71.4%) resistance of the isolates from the untreated wastewater samples of the tertiary hospital to the same antibiotic but is similar to 67.95% reported by Olaniran *et al*. [15]. However, this is much higher than the 20 (34.5%) resistance of the isolates from untreated wastewater samples collected from the private hospital. The reason for this difference could be due to the fact that there is a higher usage of antimicrobials in the tertiary hospital compared to the private hospital, as a result of larger population of patients patronizing the tertiary hospital.

In this study, four (6.9%) of the *L. monocytogenes* isolated from the untreated wastewater samples of the private hospital wastewater showed resistance to chloramphenicol while it was observed that none of the *L. monocytogenes* isolated from both treated and untreated wastewater of the tertiary hospital showed resistance to chloramphenicol. This observation is not in agreement with a higher (36.4%) resistance to the antibiotic as recently reported from a study on *L. monocytogenes* isolated from the wastewater samples collected from an abattoir in Ibadan [18]. This variation could be as a result of different samples studied and the possibility of abuse of this antibiotic in animal husbandry in the studied area. Furthermore, the 1(4.2%) of the *L. monocytogenes* isolated from the treated wastewater samples of the tertiary hospital that was resistant to tetracycline is lower compared to the 47.4% resistance reported from a treated effluent in Durban, South Africa to the same drug [15].

Comparing the resistance of the *Listeria* isolates from the studied samples (treated wastewater from tertiary hospital, untreated wastewater from tertiary hospital and the untreated wastewater from private hospital) in this study, resistance of the isolates obtained from the private hospital to tetracycline (13.8%), streptomycin (20.7%) and chloramphenicol (15.5%) were higher than the ones observed from the isolates from the treated wastewater of the tertiary hospital which were 4.2% (tetracycline), 8.3% (streptomycin) and 4.2% (chloramphenicol) whereas, none of the isolates obtained from the untreated wastewater of the tertiary hospital exhibited any resistance to the three antibiotics. The lower resistant rate observed in the wastewater samples collected from the tertiary hospital could be as a result of better management of the usage of the antibiotics in the tertiary hospital compared to the private hospital. Furthermore, the observation that 25 (26.0%) of the isolates were resistant to a combination of five antibiotics that included sulfamethoxazole-trimethroprim, cloxacillin, ampicillin, ceftriaxone and ciprofloxacin is high. More so, three (3.1%) isolates also showed resistance to a combination of eight antibiotics (sulfamethoxazole-trimethoprim, hloramphenicol, tetracycline, ampicillin, ceftriaxone, cloxacillin amoxicillin and ciprofloxacin) which is higher than resistance by some *Listeria* spp. to combination of six antibiotics that was reported on abattoir wastewater in Ibadan [18].

5. CONCLUSION

This study revealed that both the treated and untreated wastewater from the selected hospitals could be a potential source for the transmission of multi-drug resistant bacteria directly or indirectly. In addition, the water treatment processes do not reduce the load of *Listeria* spp. from the treated wastewater and the water treatment tank may serve as pool of several antibiotics thus being a potential source of transfer of multi-drug resistance genes. Hence, there is a need for devising a more efficient method for hospital wastewater treatment before discharge into the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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