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Identification of *Listeria* spp. with Antibiotic Resistance in Wastewater from Hospitals in Libreville, Gabon

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ABSTRACT: This study focuses on the use of Gram staining in the identification of *Listeria monocytogenes* in hospital wastewater in Libreville. The study aims to highlight the presence of this bacterium, which can cause illness and mortality in these wastewater systems. The study was conducted on 27 samples, of which 14 were identified as green colonies and 13 as white colonies after culture and isolation. Gram staining revealed that these were Gram-positive bacteria, with respective prevalence rates of 51.85% and 48.15%. Phenotypic identification of these colonies confirmed that they were *Listeria monocytogenes*. Antibiotic susceptibility testing showed that these bacteria exhibited an overall resistance rate of 36.4% (59/162) and an overall sensitivity rate of 63.6% (103/162) to the tested antibiotics. Resistance to amoxicillin was the highest at 63.6% (103/162), followed by cefotaxime (37%, 10/27), gentamicin (29.6%, 8/27), kanamycin (25.9%, 7/27), ofloxacin (14.8%, 4/27), and tetracycline (11.11%, 3/27). These results demonstrate that this bacterium, widespread in our environment, requires particular attention due to the risk of epidemics associated with multidrug-resistant *Listeria monocytogenes*. Hospital wastewater could be a source of human listeriosis resistant to antibiotics. This poses a significant risk to human health, as hospital wastewater can infiltrate the soil and contaminate groundwater. Additionally, it may pollute nearby effluents, thereby exposing human and animal populations that use these waters to potential contamination.

KEYWORDS: Antibiotic resistance, Hospitals, *Listeria monocytogenes*, Libreville, Wastewater.

INTRODUCTION

Hospitals are potential hubs for the proliferation of various microorganisms due to the presence of individuals suffering from a range of diseases and infections. Numerous procedures are carried out in these environments, often requiring the use of water, making hospital wastewater carriers of diverse pathogenic agents such as *Listeria monocytogenes* [1]. In Libreville, hospital wastewater systems often discharge into nearby rivers or are directly released into the environment. Populations affected by water shortages and scarcity may resort to using this runoff for activities such as farming, livestock rearing, cooking, and other domestic uses. Consequently, humans consuming products from these activities are at risk of infection by *Listeria monocytogenes*, which can cause a potentially fatal illness known as listeriosis.

Listeriosis is a bacterial infection that can manifest in both invasive and non-invasive forms. At-risk groups include pregnant women and their newborns, the elderly, and immunocompromised individuals. Clinical symptoms of blood infections caused by *Listeria monocytogenes* are similar to those caused by other etiological agents of bacteraemia, including fever, myalgia, headaches, abdominal pain, vomiting, and diarrhoea [2].

The danger posed by listeriosis is exacerbated by the emergence of multidrug-resistant strains of *Listeria monocytogenes* against antibiotics and antimicrobials commonly used to treat this condition and more severe infections [3]. This underscores the importance of our study, which aims to determine the prevalence of *Listeria monocytogenes* in hospital wastewater in Libreville.

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MATERIALS AND METHODS

Sample Collection Sites

For this study, sampling authorisations were obtained from the Directorate General of Healthcare Institutions. Samples were collected from four different sites, namely:

- Centre National de Santé de Nzeng-Ayong (CNSA),
- L'Hôpital de Coopération Sino-Gabonaise (HCSG),
- Centre Hospitalier Universitaire de Libreville (CHUL), and
- Centre Hospitalier Universitaire Mère et Enfants Jeanne Ebory (CHUJE).

Biological Material

The biological material consisted of hospital wastewater samples collected from septic tanks and hospital discharge zones.

Sampling Process

The sampling process began with site reconnaissance. Following the identification of sampling locations and equipped with personal protective equipment, two samples were collected per site.

- The first sample was collected by suspending a bottle attached to a rope into the wastewater to ensure adequate exposure to environmental bacteria.
- The second one was collected under the same conditions.

Samples Analysis

Bacterial Culture, Isolation, and Colony Identification

The culturing of *Listeria* was conducted by inoculating the wastewater samples onto *Listeria* Mono Differential Agar ACC. The cultures were incubated at 37°C for 24 hours. The resulting colonies exhibited green or white pigmentation. Isolation was carried out by subculturing each colony type on the same medium under identical conditions. Colony identification was performed using the Listeria 18R system [4].

Antibiotic Susceptibility Testing

Antibiotic susceptibility was assessed using the disc diffusion method on Mueller-Hinton (MH) agar (BioMérieux) [5], following Clinical Laboratory Standards Institute (CLSI) recommendations. The antibiotics tested were the following: Amoxicillin (AML, 25 µg), Gentamicin (GEN, 10 µg), Cefotaxime (CTX, 30 µg), Kanamycin (KAN, 30 µg), Ofloxacin (OFX, 5 µg), and Tetracycline (TET, 30 µg).

RESULTS

Microscopic Observation of Gram-Stained Slides

After Gram staining and microscopic observation, both green and white colonies displayed an elongated (bacillary) shape and violet staining, confirming their classification as Gram-positive bacteria. These bacteria were arranged in small chains and occasionally isolated.

Prevalence of *Listeria*

A total of 27 samples were analysed, of which 14 green colonies and 13 white colonies were identified. Using the *Listeria* System 18R, green colonies were confirmed as *Listeria monocytogenes* (51.85%, 14/27), while white colonies were identified as *Listeria seeligeri* (48.15%, 13/27).

Antibiotic Susceptibility Testing

Antibiotic susceptibility tests revealed an overall resistance rate of 36.9% (59/160) and an overall sensitivity rate of 63.1% (101/160).

Two *Listeria* species were isolated from hospital wastewater: *Listeria monocytogenes* and *Listeria seeligeri*.

For both species, resistance was highest against amoxicillin, followed by cefotaxime, gentamicin, kanamycin, ofloxacin, and tetracycline. Amoxicillin demonstrated 100% resistance in both cases. However, all other antibiotics were more effective, exhibiting lower resistance rates compared to their sensitivity rates (Figure 1).

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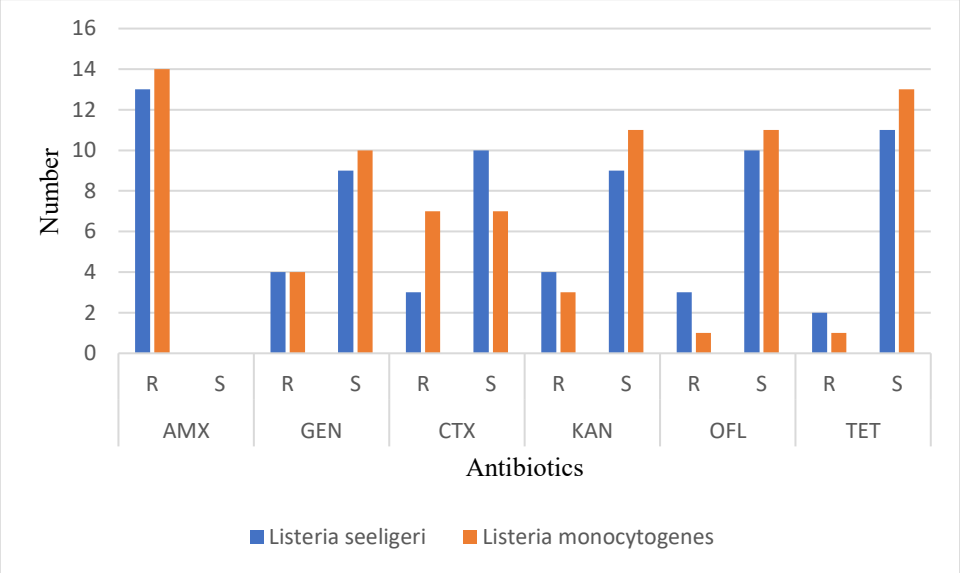

Figure 1. Susceptibility to Antibiotics

DISCUSSION

Hospital wastewater and the surrounding freshwater bodies, such as rivers, often serve as reservoirs for significant levels of contaminants, including a variety of pathogenic bacteria. Among these pathogens, *Listeria* spp. has been identified as important environmental and public health threats [6]. Such effluents typically contain biological waste and residual antibiotics, which can contribute to the survival, proliferation, and selection of resistant bacterial strains, further exacerbating the potential risks to both environmental and human health.

In this study, a total of 27 pure colonies of *Listeria* spp. were successfully isolated following bacterial culture. Detailed identification of these colonies revealed that *Listeria monocytogenes* accounted for 51.85% of isolates, while *Listeria seeligeri* made up the remaining 48.15%. These findings align closely with those of Gartley et al. (2022) [1], who examined *Listeria monocytogenes* in watersheds globally, reporting a prevalence that varied between 0% and 67%, depending on the geographical location and sample size, which ranged from 26 to 1405 water specimens.

Despite this general consistency, our study found a comparatively higher prevalence of *Listeria* spp., particularly *Listeria monocytogenes*, when compared with the work of Falodum et al. (2018). Their study reported prevalence rates of 4.2% for *Listeria monocytogenes* and 6.2% for other *Listeria* spp. [7]. The elevated prevalence observed in the current research is likely attributed to the concentration of hospital-derived contaminants in the sampled effluents. Hospitals are known to release significant biological loads, including bacteria and residual antibiotics, into wastewater systems, thereby creating hotspots for microbial proliferation and potential resistance development [8].

Listeria spp. are ubiquitous microorganisms that can thrive in diverse environments, including soil [9], food processing facilities, and wastewater [10]. While they are widely distributed, their concentrations in natural environments are typically low, generally below 20% [11]. This low baseline prevalence underscores the heightened concern associated with hospital wastewater contamination. The direct discharge of untreated or poorly treated effluents into freshwater systems not only elevates *Listeria* levels but also increases the risk of human exposure and the spread of antimicrobial resistance. This highlights the urgent need for stringent wastewater management practices, particularly in healthcare settings, to mitigate environmental contamination and associated health risks.

About antimicrobial resistance, *Listeria monocytogenes* is typically susceptible to aminopenicillins such as penicillin and ampicillin, with resistance being a rare occurrence [12]. However, our findings reveal an unusually high resistance to amoxicillin, which is likely linked to the widespread and frequent use of this antibiotic in hospital settings [13]. Overuse or improper disposal of antibiotics can exert selective pressure on bacterial populations, leading to the emergence and persistence of resistant strains.

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Resistance to gentamicin, an aminoglycoside, is extremely rare or almost non-existent in *Listeria monocytogenes*, as confirmed by well-documented studies [12]. In contrast, tetracycline resistance is more frequently reported, particularly in human isolates of *Listeria monocytogenes*. This aligns with our observations, which show a notable, though not predominant, occurrence of tetracycline resistance among the isolates studied. These findings emphasise the complexity of resistance patterns in *Listeria* spp. and the role of hospital effluents in driving the emergence of resistant strains.

Overall, this study underscores the critical role of hospital wastewater as a source of both *Listeria* contamination and antibiotic resistance, thereby highlighting the importance of targeted interventions to mitigate these risks.

The findings of our study indicate low resistance to aminoglycosides (gentamicin and kanamycin), consistent with the observations of Baquero et al. Nevertheless, while the prevalence of tetracycline resistance in this study is low, this resistance phenotype is indeed present in the isolates analysed.

CONCLUSION

Gram staining is a bacterial pre-identification technique that enables the determination of the Gram type of bacteria. This pre-identification step facilitates the selection of an appropriate system for the final phenotypic identification of bacterial colonies.

In the present study, this system identified *Listeria monocytogenes* as the predominant *Listeria* species in hospital wastewater. *L. monocytogenes* is a bacterium that can be found in various ecosystems and is pathogenic, particularly to the elderly (due to weakened immune systems) and children (due to immature immune systems).

The risk posed by this bacterium to human health is significant, especially when antibiotic-resistant strains come into contact with humans. This contact could occur through contamination of hospital effluents. Our study demonstrates that hospital wastewater contamination could be a contributing factor to the emergence of antibiotic-resistant human listeriosis.

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REFERENCES

1. Gartley, S., Anderson-Coughlin, B., Sharma, M. and Kniel, K.E. *Listeria monocytogenes* in irrigation water: an assessment of outbreaks, sources, prevalence, and persistence, Microorganisms, 10 (7), 2022, pp. 1319.
2. Wiśniewski, P., Zakrzewski, A.J., Zadernowska, A. and Chajęcka-Wierzchowska, W. Antimicrobial resistance and virulence characterization of *Listeria monocytogenes* strains isolated from food and food processing environments, Pathogens, 11(10), 2022, pp. 1099.
3. Vallim, D.C., Hofer, C.B., Lisbôa, R.C., Barbosa, A.V., Rusak, L.A., Reis, C.M.F., Hofer, E. Twenty years of *Listeria* in Brazil: occurrence of *Listeria species and Listeria monocytogenes* serovars in food samples in Brazil between 1990 and 2012, Biomed Res. Int, 2015 (1), 2015, pp. 540204.
4. Faria, A.S., Rodrigues, G., Miranda, R.B., Bonilla-Luque O.M., Carvalho L., Fernandes N., Prieto, M.A., Gonzales-Barron, U. Preliminary, Assessment of Microbiological and Physicochemical Properties of Artisanal Chouriço de Carne Fermented Sausages, Manufactured in Northern Portugal, Biol. life sci. forum, 26 (1), 2023, pp. 56.
5. Bauer, A., Kirby, W., Sherris, J.C. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Pathol, 45 (4),1996, pp. 493-496.
6. Odjadjare, E. and Okoh, A. Prevalence and distribution of *Listeria* pathogens in the final effluents of a rural wastewater treatment facility in the Eastern Cape Province of South Africa, J. Microbiol. Biotechnol, 26, 2010, pp. 297-307.
7. Falodun, O. and Amusan M. *Listeria* Species Isolated from the Wastewater Samples of a Private and Tertiary Hospital in Ibadan and their Antibiotics Resistance Patterns, J. adv. microbiol, 10 (3), 2018, pp. 1-10.
8. Bai, W. Li, L., Fu, P., Han, H., Liu J. and Guo, Y. The epidemiology of *Listeria monocytogenes* in China, Foodborne Pathog. Dis, 15 (8), 2018, pp. 459-466.
9. Dowe, M.J., Jackson, E.D., Mori J.G. and Bell C.R. *Listeria monocytogenes* survival in soil and incidence in agricultural soils, J. Food Prot, 60 (10), 1997, pp. 1201-1207.

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10. Moreno, Y., Ballesteros, L., Garcia-Hernàndez, J., Santiago, P., Gonzàlez, A., Ferrú, A. Specific detection of viable *Listeria monocytogenes* in Spanish wastewater treatment plants by Fluorescent In Situ Hybridization and PCR, Water research, 45 (15), 2011, pp. 4634-4640.
11. Ryser, E.T. and Marth, E.H. *Listeria,* listeriosis, and food safety, CRC press, 2007.
12. Baquero, F., Lanza, V.F., Duval M. and Coque T.M. Ecogenetics of antibiotic resistance in *Listeria monocytogenes,* Mol. Microbiol, 11(3), 2020, pp. 570-579.
13. Mascarello, M., Simonetti, O., Knezevich, A., Carniel, L. I., Monticelli, J., Busetti, M., Schincariol, P., Torelli, L., Luzzati, R., Correlation between antibiotic consumption and resistance of bloodstream bacteria in a University Hospital in North Eastern Italy, 2008–2014, Infection, 45, 2017, p. 459-467.

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