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Antibiotic Sensitivity Profile of Enteric Bacteria Isolated from Beach Waters and Sewage from the Municipality of Vila Velha-ES, Brazil

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Abstract

Since 1928 when antibiotics were discovered they have promoted the control of infectious diseases of bacterial origin. However, the chemical characteristics of these medications may represent a potential risk to public health and the environment because their residues have some components which are resistant and difficult to decompose and, once administered to the general population or in large pharmacotherapeutic centers such as hospitals, may cause the contamination of soil, water sources and wastewater becomes imminent and worrysome. This research quantified the thermotolerant fecal coliforms *E. coli* and *Enterococcus* and also determined the antibiotic sensitivity profile of *E. coli* and *Enterococcus* spp. isolated from sewer waters and beach waters in the city of Vila Velha, Espírito Santo. The quantified microorganism indicators from beach waters demonstrated scores within the standards for balneability. The sewer water collection points allowed the isolation of enteric bacteria with scores between <3à >2, 4 × 10^5. All the isolated *E. coli* showed (100%) sensitivity to aztreonam, ciprofloxacin, chloramphenicol, ceftriaxone, gentamicin, imipenem and nitrofurantoin, while for amoxicillin, sulphazotrim and tetracycline the sensitivity profile was varied, showing lower percentages for samples from sewer waters. The isolated *Enterococcus* spp. showed (100%) sensitivity only to the antibiotics bacitracin, chloramphenicol and vancomycin. This lower sensitivity profile of isolated bacteria to some antibiotics may be related to the presence of these drugs in the environment.

Keywords: *Escherichia coli*; *Enterococcus* spp.; Water; Sewer; Antibiotics

Introduction

The drugs have an unquestionable relevance in our society, from its importance in the fight against diseases, to the function of promoting human longevity. Among these substances are highlights the antibiotics, discovered in 1928, resulting in a significant change in the control of infectious diseases of bacterial origin with impacts on public health and quality of life [1].

In Brazil, during urgent or routine treatment to solve health problems, people get drugs that often are not consumed completely and end up being stored for possible future use. Many of these products are left over after treatment and end up being disposed of as household waste or sewage common [2]. Furthermore, studies has shown that after administration of drug, significant portions thereof are secreted by human domestic sewage and for keeping its chemical properties long enough to meet a therapeutic purpose, about 50% to 90% a drug administered is excreted unchanged [3]. Therefore, the chemical characteristics of drugs pose a potential risk to public health and the environment. Their waste has some resistant components, difficult to decompose, which can contaminate soil and water and some groups of residual drugs, deserving of special attention, like the antibiotics [2-4].

Between 30% and 90% of the dose of antibiotic administered to humans and animals are excreted in the urine as active substances enabling such remain in the environment [5]. These factors are aggravated because it is estimated that each year, 11.2 million kg of antimicrobials are used in animals for non-therapeutic purposes and 900,000 kg are administered for therapy; and that for humans are spent annually 1.3 million kg of antibiotics [6], and the annual consumption of antibiotics, globally, has been estimated between 100,000 to 200,000 tons [4].

Based on these parameters It is no surprise that worldwide, analysis of domestic-, waste-, surface- and underground-water detects the presence of drugs such as antibiotics and anesthetics, hormones, anti-inflammatory and other hazardous molecules [2,7,8].

Several authors have reported in several countries, including Brazil, the occurrence of residues of different groups of drugs along water bodies [8,9]. In water of 139 rivers of the United States of America (USA) were identified about a hundred organic contaminants, including pharmaceuticals [8,10]. Antibiotics such as tetracyclines (oxytetracycline, tetracycline and chlortetracycline), sulfonamides (sulfadimethoxine, sulfamethazine and sulfamethoxazole), macrolides (roxitrmicina, clarithromycin), fluoroquinolones (ciprofloxacin, norfloxacin), lincomycin, trimethoprim and tylosin were detected in surface water samples in the USA [3,10]. A study in effluents of five boroughs of Canada was found ciprofloxacin, ofloxacin, clarithromycin, erythromycin-H_2O, tetracycline, sulfamethoxazole and sulfapyridine, where the frequent occurrence of erythromycin-H_2O, roxithromycin and sulfametoxazol was observed in concentrations over 6 μg/L^{-1} [8]. The dispersion of fluoroquinolones was investigated in Valley lakes of Glatt River, Sweden [11].

The suspended solids provide ideal surfaces on which the various components are concentrated as bacteriophages, DNA and bacteria free. The high concentration of bacteria in sewage increases the possibility of horizontal transfer, because the probability of a donor bacteria of resistance genes find other receiver bacteria is greater [12].

Sewage causes exposure of bacteria to antibiotics, giving them an ecological advantage to resistant strains when compared to susceptible strains, allowing them predominate in the bacterial population. This is commonly known as the antibiotic selective pressure and can happen in the host (human or animal body) as a result of chemotherapy or the environment, for example, antibiotic residues that are thrown in the sewer [13]. In this sense the hospital sewage is composed of human waste, which by being in an environment conducive to the development of diseases has a large number of pathogenic microorganisms such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, among others, besides the presence multi-drug [14]. *Enterococcus* plays an important role in the improvement of taste and ripening cheese and sausage, besides being used as probiotics to improve the microbial balance of the intestinal tract in humans and animals [15]. These microorganisms, in that present as primary habitat the intestine, have been widely used as indicators of fecal contamination, revealing the microbiological quality of water and food due to its presence and number [16,17]. However, in recent years it has gained great prominence because it has become one of the most relevant actors when it comes to hospital infections, having acquired resistance to most antibiotics. Sensitivity tests showed phenotypes of resistance to a range of antibiotics widely administered to humans as gentamicin, streptomycin, ampicillin and vancomycin [15]. However, despite being part of the normal microflora of the intestinal tract and are present in the mucous membranes, have very subtle virulence traits that are not easily identified and that infections occur when the bacterium is translocated to organ or local more likely [18].

Another important genre, *Escherichia coli*, as *Enterococcus*, has a primary habitat the intestinal tract of humans and other warm blooded animals. But there are certain serotypes pathogenic to man and other animals. The pathogenic strains survive generally to refrigeration temperatures, although there was a slight reduction after five weeks of storage, which allows concluding that frozen foods such as meats can serve this type of bacteria [19]. The place of origin of these bacteria also allows use them as indicators of fecal contamination, ensuring a qualifying water and food for human consumption [20]. Therefore, the objective of this study was to evaluate the profile of sensitivity to antibiotics of enteric bacteria isolated from beach water and sewer of Vila Velha, Brazil.

Methodology

Samples of beach and sewage were obtained between August and October 2012, at different points of the city of Vila Velha. Points 1 and 4 relate to beach waters, where the point 1 is a non-urbanized area and point 4 a location that receives water from Vitoria bay, capital of the state of the Espírito Santo. Points 2 and 3 refer to sewage, where the point 2 is located behind on a big city hospital and the point 3, is located parallel to the avenue of this hospital, also next to it.

Analyses were performed in the Microbiology Laboratory of the University Vila Velha - UVV. Each collection bottle was sterilized with 5 ml of 15% EDTA for neutralize the purposes of the action of heavy metals present in sewage water. The collection and analysis procedures were performed according to the methodology set out in the Company's collection guide of Technology and Environmental Sanitation [21], Standard Methods of recommendations [22] and Resolution No. 430 of CONAMA [23], in depth of 15 to 30 cm below the surface, facing the mouth of the bottle against the sense of the direction of flow and the count determined by the most probable per 100 milliliters of sample number (MPN/100 ml).

A 25 ml aliquot sample was removed aseptically and homogenized in 225 ml of peptone water (0.1%) following serial dilution in assay tubes containing 9 ml of the same diluent from which it was inoculated into tubes containing Azide-Dextrose broth (Acumedia) for *Enterococcus* spp., and Lauryl Sulfate Tryptose broth (HIMEDIA) for coliform bacteria. Both placed at 37°C/24 to 48 hours. The positive tubes with azide dextrose broth were inoculated on agar Bile Esculin (MERCK) (37°C/24 hours). The positive tubes for coliform bacteria, were plated on Brilliant Green Broth and Bile (HIMEDIA) (37°C/24 to 48 hours) and *Escherichia coli* broth (HIMEDIA) (45°C/24 hours), and MacConkey Agar (OXOID). *Enterococcus* spp. was identified by Gram staining, catalase, esculin hydrolysis in the presence of bile salts and growth in BHI/6.5% NaCl (Brain-Heart Infusion HIMEDIA). *Escherichia coli* was identified by performing the gram from MacConkey, catalase, oxidase and evidence of Indole.

The determination of antimicrobial susceptibility profile was performed using the agar diffusion test [24]. The evaluation was made in Mueller Hinton agar (HIMEDIA) and, for *E. coli* we tested amoxicillin (AMO-10 mg), aztreonam (TMJ-30 µg), ceftriaxone (CRO-30 µg), ciprofloxacin (CIP-5 µg), chloramphenicol (CLO-30 µg), gentamicin (GEN-10 mg), imipenem (IPM-10 mg), nitrofurantoin (NIT-300 µg), sulfazotrim (SUT-25 mcg) and tetracycline (TET-30 µg) and, for *Enterococcus* spp., we tested bacitracin (BAC-10 mg) ciprofloxacin (CIP-5 µg), clindamycin (CLI-2 mg), chloramphenicol (CLO-30 µg), erythromycin (ERI-15 µg), gentamicin (GEN-10 mg), oxacillin (OXA-1 µg), sulfazotrim (SUT-25 mcg), tetracycline (TET-30 µg) and vancomycin (VAN-30 µg).

The antibiotics discs used in antibiotic susceptibility testing were produced by the laboratory CECON (Brazil). The reading of antibiogram was performed by measuring inhibition zones [24]. The standard strains of *E. coli* (ATCC 8739) and *Enterococcus faecalis* (ATCC 29212) were also subjected to the same tests as a form of quality control. The results regarding the sensitivity feature values in percentage referring to the 12 isolates of *E. coli* and 12 of *Enterococcus* spp.

Results and Discussion

The collection points for beach water (1 and 4) demonstrated indicator microorganisms counts within the bathing standards and were classified respectively as excellent and satisfactory, since the resolution in the 274 CONAMA [25] establishes that prevails the most restrictive indicator to 80% or more of the samples (Tables 1 and 2). The results did not allow for isolating enteric bacteria these collection points, thus not being performed the antibiotic susceptibility testing.

Regarding points 2 and 3, water from sewage, the counts of enteric bacteria (MPN/100 ml) reached values for *Fecal coliforms* between 1.1×10^3 and 1.1×10^5, *E. coli* 7×10^2 and 1.1×10^5 and *Enterococcus* <3 à 4.3×10^3 (Table 1 and Figure 1).

The raw sewage generally presents counts of *E. coli* around 10^6 (MPN/100 ml), while treated sewage may have lower scores, with values ranging 10^4-10^5 (MPN/100 ml) [1,26]. These results are close to those found by Silva et al. [27], in a study of hospital wastewater in municipality of Natal (Rio Grande do Norte, Brazil), made for

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physical-chemical and microbiological characterization, which determined high levels of fecal coliform, characteristic of sewage in natura, with fecal coliforms in the order of 2.1 × 10^5 MPN/100 ml, reflecting contamination by fecal material that is disposed of without proper treatment, resulting in biological risks to the environment and population.

Place of collect	N° of collect	Coliforms 45°C	*E. coli*	*Enterococcus* spp.
Point 1	1	0.4	0.4	0.9
	2	≤ 0.3	≤ 0.3	≤ 0.3
	3	0.4	0.4	0.4
	4	≤ 0.3	≤ 0.3	≤ 0.3
Point 2	1	1.1 x 10^3	7 x 10^2	2.3 x 10^3
	2	1.1 x 10^5	1.1 x 10^5	≤ 0.3
	3	≥ 2.4 x 10^5	1.2 x 10^4	7 x 10^2
	4	9.3 x 10^3	2.1 x 10^3	4.3 x 10^3
Point 3	1	4.6 x 10^4	1.1 x 10^3	4.3 x 10^3
	2	1.1 x 10^5	2.4 x 10^4	4.3 x 10^3
	3	2.1 x 10^4	2.1 x 10^4	<0,3
	4	4.6 x 10^4	9 x 10^2	4 x 10^2
Point 4	1	≤ 0.3	≤ 0.3	≤ 0.3
	2	≤ 0.3	≤ 0.3	≤ 0.3
	3	≤ 0.3	≤ 0.3	≤ 0.3
	4	40	40	≤ 0.3

Table 1: Counts (MPN/100 ml) of thermotolerant coliforms (45°C), *Escherichia coli* and *Enterococcus* sp in water samples and sewage in the municipality of Vila Velha-ES. Points 1 and 4 (beach water) and points 2 and 3 (sewage).

Pattern	Excellent	Very good	Satisfactory	Unfit
Coliforms 45°C	≤ 200/100 ml	≤ 500/100 ml	≤ 1000/100 ml	≥ 2500/100 ml
E. coli	≤ 200/100 ml	≤ 400/100 ml	≤ 800/100 ml	≥ 2000/100 ml
Enterococcus	≤ 25/100 ml	≤ 50/100 ml	≤ 100/100 ml	≥ 400/100 ml

Table 2: CONAMA [25] Resolution No. 274 of 29 November 2000, with values (MPN/100 ml) of reference based on 80% or more of a set of samples from each of the previous five weeks and harvested in the same place.

The Southeast stands out for more coverage in relation to water supply treated by municipalities, usually in regions most populous and, also shows 95% of cities with sewage disposal system. However, for the treatment of sewage, only 48% of the municipalities of this region do it. The state of the Holy Spirit performs a little better than average for the region, since 97% of cities have sewage system and 69% of them have sewage treatment. Several problems are related to lack of treated sewage and water collection network, highlighting pollution of rivers and seas, disease, frequent floods, and consequently, death in the population [28].

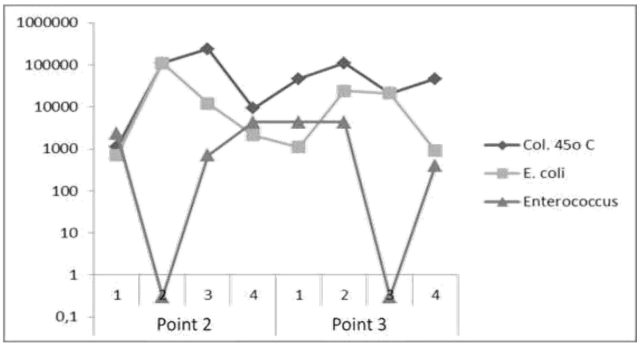

Figure 1: Count (log base 10) of enteric bacteria (MPN/100 ml) in wastewater.

Studies of Tebaldi and colleagues [29] reported that *E. coli* and *Enterococcus*, from the digestive tract has its presence correlated with pathogenic organisms, and because this are often used as indicators of fecal contamination and potential risk of the presence of patogens. Thus, quantification of these microorganisms provides a monitoring already naturally contaminated environments in relation to whether exist or not exists these treatment areas. The sewage samples were collected from raw sewage, the canal that bisects the city of Vila Velha, ES. It is a region without treatment, all of these wastes, launched in Vitória/ES bay. According to the hydrological bulletin of INCAPER [30] of 30/10/2012, there was a period of rains in August this year with an accumulated volume of about 180 mm, with a reduction to less 30 mm from the month of September. The first collections coincided with this rainy season, which may explain in part some lower scores in the first collections in the sampled sewage points. Enteric isolated from these sampling points were subjected to test sensitivity to antibiotics.

All *E. coli* strains proved 100% sensitive to aztreonam, ciprofloxacin, chloramphenicol, ceftriaxone, gentamicin, nitrofurantoin, and imipenem, and for amoxicillin (25% to 50%), sulfazotrim (75% to 100%) and tetracycline (25% to 50%), the sensitivity profile is varied (Figure 2).

Bacteria isolated from water supply sources in the city of Cascavel, Brazil, also showed resistance to tetracycline, which also corresponds to the assessment for *E. coli* isolated from hospital and sewage, and a high rate resistance to the antibiotic amoxicillin [1]. However, results described by Vasconcelos et al. [31], the sensitivity profile of *E. coli* isolated from a water of weir in Ceará, Brazil, are different. They revealed a percentage of resistance to antibiotics ciprofloxacin and nitrofurantoin, which in our results was (100%) sensitive. However still on the results of this research, the antibiotics tetracycline and sulfazotrim, are shown with very similar profile to sensitivity found in our evaluation.

Enterococcus isolates proved to be 100% sensitive only to bacitracin, chloramphenicol and vancomycin demonstrating to the other antimicrobials tested, a varied profile (Figure 3). The antibiotics that demonstrated a lower efficiency was clindamycin (0%), erythromycin (0% to 25%), gentamicin (0%) and oxaciclina (0% to 25%).

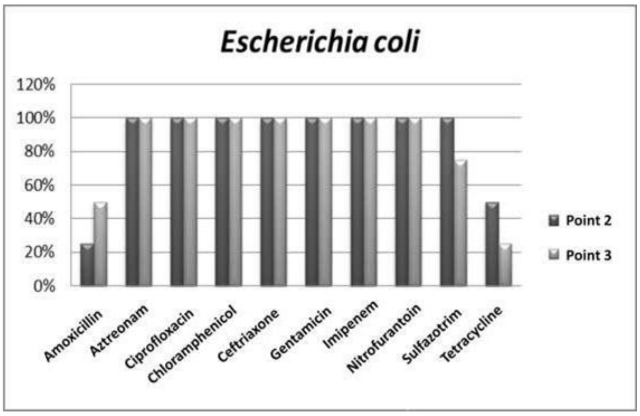

Figure 2: Profile Sensitivity antibiotic (%) of *E. coli* isolated from sewage, regarding the sampling points 2 and 3.

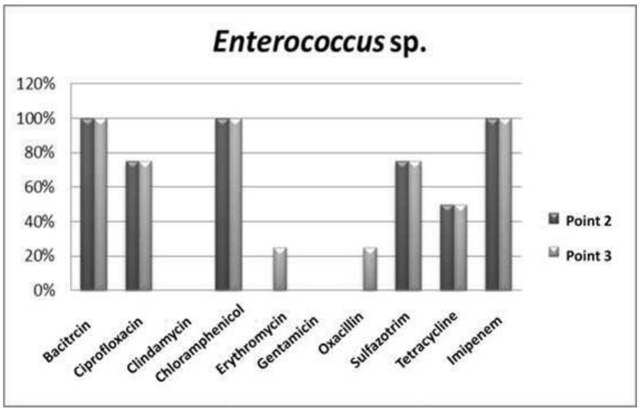

Figure 3: Sensitivity Profile (%) antibiotic of *Enterococcus* spp. isolated from sewage, referring to collection points 2 and 3.

Araújo [32] evaluating the plasmid DNA profile of bacteria resistant to antibiotics isolated from a stream in Pará de Minas Gerais, Brazil, also found a low sensitivity to the antibiotic erythromycin.

Silva Júnior et al. [2] found similar sensitivity values for ciprofloxacin and gentamicin bacterias from hospital sewage treatment system in Vitória, Brazil, as well as for aztreonam. Also according to the results, the highest sensitivity indices were obtained for vancomycin and gentamicin both with 68%, which partly differ from the results obtained in this study.

The result of great sensitivity to vancomycin draws attention because of the importance of this antibiotic against many strains resistant to other existing antibiotics. The importance of this antibiotic shown in countless research for the bacteria sensitivity profile. Remonatto et al. [33] describes that resistance to vancomycin by *Enterococcus* spp. had its explosion in 1988 in Europe and the United States and in Brazil, this resistance emerged in the second half of the 1990s.

In recent years there was concern that *Enterococcus* spp. convey resistance to vancomycin to *Staphylococcus aureus*, a much more prevalent pathogen, since this fact has been demonstrated in vitro [34], a fact that was fullfiled in 2002 with the isolation *S. aureus* resistant to vancomycin in two patients who also had Enterococcus resistant to vancomycin [35].

Making a comparative evaluation on the sensitivity profile of enteric bacteria to antibiotics tested in common to both groups, again the isolates of Enterococcus are more resistant (Figure 4). Only *E. coli* was less sensitive than *Enterococcus* spp. to tetracycline. The lower sensitivity of Enterococcus compared to *E. coli* is recognized, and may be being intensely disseminated in the environment through the discharge of sewage into waterways, where it was possible to evaluate and show the relationship between the spread of resistance genes and their ecological implications and environmental [1].

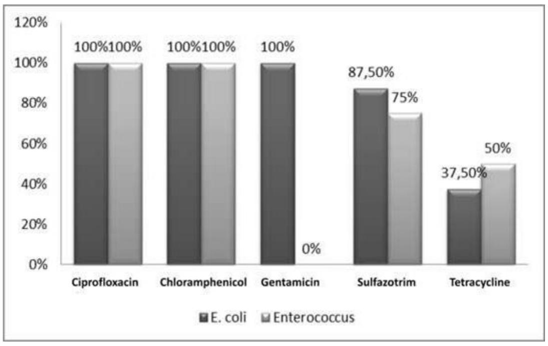

Figure 4: Comparison of the sensitivity profile (%) tested for antibiotic isolated from *E. coli* and *Enterococcus* spp.

The low sensitivity of certain strains demonstrated, reflect the actual presence of antibiotics in the environment, and in this specific case sewage where, according to Gil & Mathias [7], it is estimated that among the main classes of drugs present in the environment, the antibiotics are among the most impressive, with about 76.6%, since 55% of all microorganisms develop resistance to at least one antibiotic.

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