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Antibiotic Resistance of *Staphylococcus* spp. Isolated from Sewage in Manaus, Amazonas

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ABSTRACT

***Staphylococcus* spp. have become important human pathogens in recent decades due to the selection of resistant bacteria and the spread of their resistance genes in the environment. This study aimed to evaluate the resistance of *Staphylococcus* spp. obtained from sewage in the city of Manaus, Amazonas state, Brazil. The isolates were tested for susceptibility to antimicrobials using the Kirby-Bauer method for ampicillin, azithromycin, ciprofloxacin, clindamycin, chloramphenicol, erythromycin, gentamicin, oxacillin, cefoxitin, linezolid, penicillin, rifampicin, sulfazotrim, tetracycline and vancomycin. Among the strains isolated from sewage, the greatest resistance was observed for penicillin and oxacillin, with 100% of isolates resistant to these antibiotics. Some antibiotics had resistant and sensitive strains (ampicillin, clindamycin, erythromycin, cefoxitin, azithromycin). *Staphylococcus* spp. were identified as sensitive to ciprofloxacin chloramphenicol gentamicin linezolid sulfazotrim tetracycline, vancomycin, with no strain resistant to these antibiotics.**

Keywords: Antibiotic, Resistance, Sewage, *Staphylococcus*.

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I. Introduction

Staphylococcus aureus is one of the main human pathogens, which can trigger a variety of infectious diseases, such as skin and soft tissue infections, endocarditis, osteomyelitis, bacteremia and lethal pneumonia. Infectious diseases are the second leading cause of human deaths worldwide [1]. In recent decades, due to the adaptation of bacteria and the use of large amounts of antibiotics, resistant *S. aureus* strains have been selected. The ability of *Staphylococcus* to acquire and/or develop resistance to antibiotics is well known, mainly due to the presence of mobile genetic elements (plasmids, transposons and bacteriophages), which play a fundamental role in the ability of *S. aureus* to adapt to environmental stresses, including exposure to antibiotics [2]. In addition to the traditional mechanisms of resistance, a special feature of the pathogenesis of *Staphylococcus* spp. is their ability to survive on biotic and abiotic surfaces by forming biofilms. The formation of biofilm has the function of protecting bacteria in hostile environments. The bacteria inside the matrix are more resistant to the host's immune response and to antimicrobial agents than free bacteria, requiring a much higher concentration of antibiotics to eliminate them [3]. The ability of bacteria to develop and acquire antimicrobial resistance has quickly led to the emergence of multidrug-resistant strains, such as methicillin-resistant *S. aureus* (MRSA). These strains have become endemic in most hospitals worldwide. Strains have also been reported to be resistant to vancomycin (VRSA) [4]. The importance of *Staphylococcus* as a pathogen lies not only in human populations, but also in the environment. The frequent use of antibiotics has contributed to the spread of resistant bacteria or their resistance genes in the environment. In addition, most of the antibiotics consumed are not absorbed and can end up in wastewater, which can infect people with resistant bacteria [5]. Hence, there is a need to monitor *Staphylococcus* resistance, as well as the appropriate use of antibiotics, to minimize the prevalence of antimicrobial resistance.

II. Materials and Methods

A. *Bacterial Samples*

Five milliliters of sewage water was collected in Manaus, Amazonas (3 ° 08'10.3 "S 60 ° 00'33.7" W - Google). Strains of *Staphylococcus* spp. were cultured in Baird-Parker Agar medium (Merck) for 24 hours at a temperature of 35 °C, according to [6]. Colonies with typical morphology on Baird-Parker Agar supplemented with egg yolk tellurite emulsion were selected and subjected to Gram staining. The strains were grown at the ESA / UEA Microbiology Laboratory at

35 °C in soybean triptych broth (TSB) for antibiotic resistance tests.

B. *In vitro Susceptibility to Antimicrobials*

For the susceptibility test, the Kirby-Bauer disk diffusion method (Polisensidisc – DME), one of the most widely used antimicrobial sensitivity tests, was employed, with the following antibiotics and concentrations: ampicillin-AMP 10 µg; azithromycin-AZI 15 µg; ciprofloxacin-CIP 05 µg; clindamycin-CLI 02 µg; chloramphenicol-CLO 30 µg; erythromycin-ERI 15 µg; gentamicin-GEN 10 µg; oxacillin-OXA 01 µg; cefoxitin-CFO 30 µg; linezolid-LNZ 30 µg; penicillin G-PEN 10 µg; rifampicin-RIF 05 µg; *sulfazotrim*-SUT 25 µg; tetracycline-TET 30 µg; and vancomycin-VAN 30 µg. The medium used was Mueller-Hinton agar (Himedia), which is the recommended standard for antimicrobial susceptibility testing.

The disk-diffusion method was carried out according to [7]. With the aid of a bacteriological loop, the *Staphylococcus* isolates were inoculated in 5 ml of TSB broth (Himedia), and grown at 35 °C and 150 rpm for 24 hours. After growth, a sterile swab was introduced into the tube. The inoculation was done in the form of streaks on the surface of the Mueller-Hinton agar in three directions. Then the discs were applied to the surface of the culture medium and incubated for 24 hours at 37 °C.

C. *Reading the Plates and Interpreting the Results*

After 24 hours of incubation, the plates were evaluated to read the inhibition halo, measured with a ruler. The diameters of the inhibition halos were interpreted according to the criteria recommended by [8].

III. Results

It was possible to isolate several strains with dark coloring in the Baird-Parker medium, characteristic of *Staphylococcus* spp. Nine isolates of *Staphylococcus* spp. were selected and evaluated. The pattern of antibiotic resistance among the isolates varied widely, as shown in Table 1. The highest resistance was observed for penicillin and oxacillin, with 100% of isolates resistant to these antibiotics. Some antibiotics had resistant and sensitive strains (ampicillin, *clindamycin*, erythromycin, cefoxitin and azithromycin). The UEA-07 isolate showed resistance to the largest number of antibiotics (40%), being resistant to six of the fifteen tested antibiotics (clindamycin, azithromycin, ampicillin, erythromycin, oxacillin and penicillin).

According to the results of the sensitivity tests, all *Staphylococcus* isolates were identified as sensitive to ciprofloxacin, chloramphenicol, gentamicin, linezolid, sulfazotrim, tetracycline and vancomycin, with no strain resistant to these antibiotics (Fig. 1). For azithromycin and erythromycin, there was large variation between the isolates, with some sensitive and others resistant. For gentamicin and *rifampicin*, although there were no resistant strains, some strains were intermediate, as can be seen in Fig. 1.

The UEA-03 isolate was able to grow in the presence of erythromycin, despite forming an inhibition halo (Fig. 2), showing that *Staphylococcus* spp. are capable of acquiring *resistance* to certain antibiotics easily. For clindamycin, the inhibition halo was not as well defined as in the other antibiotics, as can be seen in Fig. 2.

TABLE I: Patterns of Antimicrobial Resistance of Selected *Staphylococcus* spp

Antibiotics *Isolates*	*AMP* 10µg	*AZI* 15µg	*CIP* 5µg	*CLI* 2µg	*CLO* 30µg	*ERI* 15µg	*GEN* 10µg	*OXA* 1µg	*CFO* 30µg	*LNZ* 30µg	*PEN* 10µg	*RIF* 5µg	*SUT* 25µg	*TET* 30µg	*VAN* 30µg
UEA-01	18	14	30	R	28	17	14	R	17	35	13	17	36	28	25
UEA-02	15	10	40	R	40	18	30	R	34	40	R	40	25	40	26
UEA-03	10	22	25	17	25	30	26	R	27	30	R	22	25	30	18
UEA-04	10	14	44	10	40	12	31	R	36	45	R	32	26	40	30
UEA-05	R	16	36	14	38	15	38	R	38	40	20	36	27	40	30
UEA-06	19	14	24	R	24	17	14	R	20	38	14	20	33	18	24
UEA-07	*R*	*10*	*34*	*R*	*42*	*12*	*30*	*R*	*30*	*40*	*R*	36	24	44	24
UEA-08	R	14	36	R	30	R	30	R	30	44	R	34	26	40	25
UEA-09	R	15	36	R	38	15	34	R	32	44	R	34	24	36	30
Resistant *Sensitive*	R≤17 S≥24	R≤13 S≥18	R≤15 S≥21	R≤14 S≥21	R≤12 S≥18	R≤13 S≥23	R≤12 S≥15	R≤17 S≥18	R≤21 S≥22	R≤20 S≥21	R≤28 S≥29	R≤16 S≥20	R≤10 S≥16	R≤14 S≥19	R≤ S≥

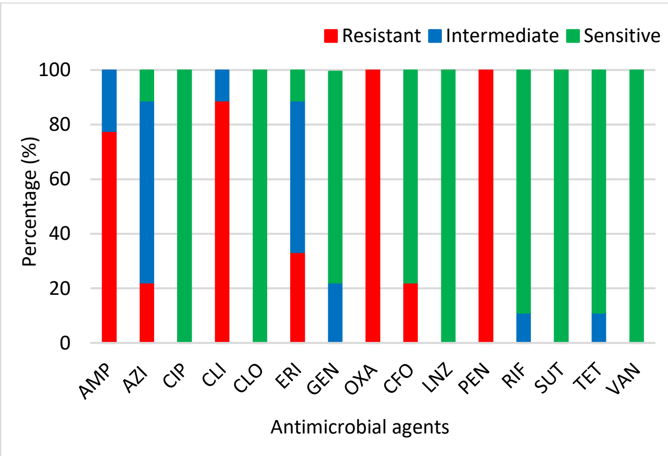

Fig. 1. Antibiotic susceptibility and resistance of *Staphylococcus* isolates.

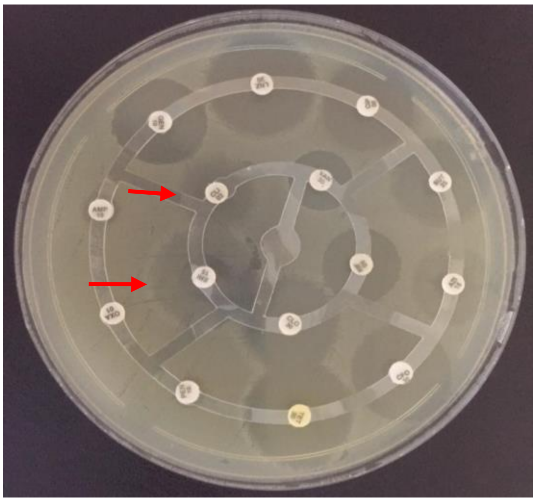

Fig. 2. Sensitivity of the UEA-03 isolate to the tested antibiotics.

IV. Discussion

The spread of antibiotic resistance of the Staphylococcus genus has become worrying because of its presence in in wastewater, mainly in the sewers of large cities. The use of antibiotics is one of the main resistance *inducers*, because part of the antibiotic used reaches the environment with activity intact, promoting the selection of resistant microorganisms [9].

Similar to the results found in the present study, other authors have also reported a high rate of resistance to β-lactams. Assessing the prevalence of *Staphylococcus* spp. and *S. aureus* in a dental clinic, [10] observed that 79.5% of *S. aureus* isolates showed β-lactamase production, conferring resistance to ampicillin and erythromycin. [11], evaluating the microbiological quality of lettuce salads *in* restaurants, found 30 *Staphylococcus* spp. isolates, with respective resistances to penicillin (56.7%), oxacillin (46.7%) and erythromycin (26.7%). [12] evaluating the patterns of antibiotic resistance of *S. aureus* in dairy herds in South Africa, observed a higher prevalence of resistance to penicillin G and ampicillin, probably due to the fact that these antibiotics of the beta-lactam class are the most used as intramammary remedies in that country. Although a high level of clindamycin-resistant isolates has been observed, few studies have reported this resistance. [13] evaluated the profile of antibiotic resistance in *Staphylococcus* isolated from a stream in Southern Brazil. Erythromycin resistance was found in 37.50% of the strains, followed by 27.27% to *penicillin*, 12.50% to clindamycin, 6.81% to trimethoprim-sulfamethoxazole, 5.68% to chloramphenicol and 2.27% to norfloxacin, while none of the strains investigated showed resistance to gentamicin and ciprofloxacin. These findings are similar to ours in Manaus.

Bacterial infections remain a challenging public health problem due to the emergence and spread of multidrug-resistant strains, such as *S. aureus* resistant to vancomycin, which is the main antibiotic used to treat resistant strains [14]. In this study, no vancomycin-resistant strain was observed. This can be attributed to its relatively restricted use in Brazil. Knowledge and monitoring of local resistance patterns are essential for the formulation of effective strategies for the treatment of bacterial infections. In addition, overuse of antibiotics causes the emergence of bacterial resistance and increases health costs and sepsis-related deaths.

Some *Staphylococcus* strains are considered inhabitants of the normal microbiota, and do not have all the virulence factors found in *S. aureus*. Their emerging threat comes from the fact that these bacteria can carry a large number of antimicrobial resistance *genes* [15], [16]. Due to the increase in antibiotic resistance in the recent years as a result of the use of these antibiotics, the World Health Organization announced a "global action plan on antimicrobial resistance" [17]. Today, the persistent, indiscriminate and inappropriate use of antibiotics and the growing spectrum of antibiotic resistance is a challenge to public health.

V. Conclusion

This study showed the incidence of *Staphylococcus* spp. in sewage from Manaus, Amazonas, and their susceptibility patterns to different antibiotics. May species showed sensitive and resistant strains, and the main resistance was to antibiotics of the class of β-lactamics. The indiscriminate use of antibiotics can turn commensal bacteria into multidrug-resistant pathogens.

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