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STAPHYLOCOCCUS AUREUS PRESENCE OF ASSESSMENT OXACILIN TOUGH ON EMPLOYEES OF NURSING IN SANTOS –SP HOSPITAL

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ABSTRACT

Most of health professionals have opportunistic microorganisms in their nasal mucosa that can infect patients. The strain of *Staphylococcus aureus* that is usually transmitted in hospitals can bring great evils to immunocompromised patients. Recent studies showed that these bacteria are present in the hospital environment and they are multi drugs resistant. *Staphylococcus aureus* virulence is due to its own genetic. Modern antibiotics such as Methicillin are no longer effective against some strains of *Staphylococcus sp.* Now the same seems to occur with oxacillin. Health care professionals may have multidrug-resistant strains of *Staphylococcus sp.* We collected material from the nasal cavities and hands twenty-seven health professionals (fifty-four samples) and brought culture through specific tests, showing the growth of oxacillin-resistant *Staphylococcus aureus*. Twenty-one samples showed antibiotics resistance. Other researchers found similar results. We intend to continue researching with new methods.

Keywords: Staphylococcus aureus, Resistance, Oxacillin, Nosocomial infection, Transmission.

1. INTRODUCTION

In the past few decades, new findings in nosocomial infections showed that microorganisms are responsible for various types of diseases whose etiological agents disseminate easily. In 1969, because 50 thousand of 250 thousand patients died per year in the United States, Martin proposed to create a National Bacteremia Registry [1]. Since then, research on hospital infections raised rapidly, and two pathogens have been often cited Staphylococcus aureus and Pseudomonas aeruginosa [1-2].

In hospitals, the transmission of these bacteria occur by air and direct contact [3], but the staff may be a potential asymptomatic carrier and could contribute to spread them. These microorganisms are commonly present in the nose and on the skin, especially in permanent men patients [4]. Under normal conditions, they do not develop the disease because the human immune system can combat such invaders. Nevertheless, weakened people such as patients with cancer, on a postoperative period, or with HIV, are the main targets of these bacteria [5].

Researches in several countries revealed that the resistant *Staphylococcus aureus* strain is the major responsible for infections, including those outside the hospital [6].

Staphylococcus spp. are classified as gram-positive cocci shaped curl and they are members of the *Micrococcaceae* family. Those bacteria are catalase-positive, however we can divide this species into positive or negative coagulase – the last is a distinction of *Staphylococcus aureus* [7].

We can find *Staphylococcus aureus* living in commensalism in resident or transient human flora in healthy individuals, especially in mucous membranes of the nasal passages, oropharynx, skin and intestine. However, the number of hospital infections caused by

Staphylococcus aureus has increased in many countries in recent decades [8]. According to Ratard data [9], nasal colonization of *Staphylococcus aureus* resistant to methicillin (MRSA) increased significantly among health workers in United States hospitals.

Endogenous infections caused by *Staphylococcus aureus* can occur in several ways and the most common are the trauma, abrasions of the skin and mucous membranes, or during surgical procedures [10]. The infections can manifest in a simple way causing pimples, boils, and cellulitis; or even in the most severe forms, such as pneumonia, meningitis, endocarditis, toxic shock syndrome, septicemia, and others, and it may lead to death [11].

The habit of antimicrobial use is a reflection of the strength of these microorganisms and thus it is important to know the pattern of resistance in each group of patients admitted to each hospital [12].

2. METHODOLOGY

The present research was conducted at the microbiology laboratory from Monte Serrat University Center (UNIMONTE), all with the consent and approval of the ethic boards and nursing professionals through the Free Consent Term Cleared up (Termo de Livre Consentimento e Esclarecido). The data used for the research and analyses presented were analyzed and cataloged in graphs and tables, which were then compared to recent bibliographies gathered from a variety of scientific sources data-base, such as Pubmed, Scielo, Medline, Bireme, and others. In addition, in order to support the research, some nursing employees from considered risk areas such as Intensive Care Unit (ICU) were selected – except those that used antibiotics in the previous month because of infection on the airways.

The studied samples were collected from hands and nostrils, making use of pre-moistened sterile swabs with sterile saline solution. All samples and materials were placed inside sterile dry tubes and immediately sent to the microbiology laboratory of UNIMONTE. The swabs were removed and placed in BHI (Brain-Heart Infusion) for growth in the incubator at 37 °C [98.6 º F] for 24 hours. We used Agar Mannitol for the cultivation process, in which the exact needed quantity was measured in an analytical weighting balance, being then hydrated with distilled water and autoclaved for a period of 15 minutes up to a temperature of 121 ºC [249.8 °F]. As a subsequent step, in a sterile environment it was distributed into an expendable sterile Petri disposal.

For quality control purposes, one third of the sampling plates was placed in an incubator at 37 °C [98.6 º F] for another 24 hours, in order to verify possible contamination during the experiments. After finding sterile plates, the collected samples were seeded and incubated at 37 °C [98.6 º F] for 24 hours. After this period, we verified the growing process of microorganisms and the presence of positive manita. Subsequently, colored Gram was used to confirm the presence of Gram-positive and then the identification was performed by classical methods.

After identifying the *Staphylococcus aureus*, the samples were subjected to an antibiogram according to Kirby-Bauer technique [13], recommended by the National Committee for Clinical Laboratory Standards [13]. It is based on a test idealized by Bauer et al. [14]. The principle is based on diffusion through the agar of an antimicrobial impregnated filter paper, which form a zone of inhibition of bacterial growth. Therefore, we made a suspension of the bacteria under study, at 0.5 Mac Farland scale, then it was seeded in culture plates, and containing Müller Hinton agar (oxoid), in which the impregnated discs containing oxacillin were applied. After incubating the plates at 37 °C [98.6 º F] for 24 hours, each inhibition zone was read with a millimeter ruler and the bacteria tested were considered resistant if the reading of the halos was less than 10 mm for oxacillin.

3. RESULTS

Fifty-four samples were analyzed, from which 50% are from nasal cavity and the other half is from the hands (Figure 1) of nursing professionals from the Santos Hospital (state of São Paulo, Brazil). Thirty-three of these samples became negative for manita and twenty-one were positive, showing resistance to the antibiotic.

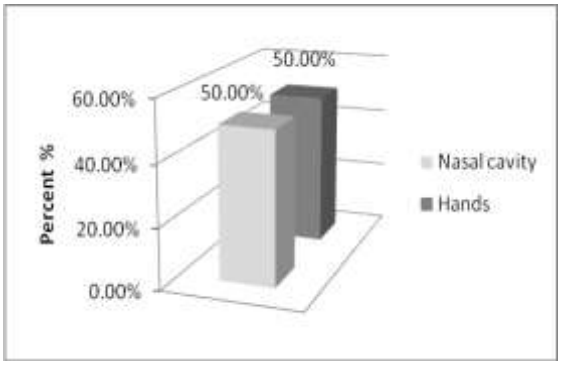

Fig 1: Level of samples collected from professionals

Table 1 shows the positivity and negativity, and the oxacillin was put into disks, after that made to identify halos measurement sensitivity. The *Staphylococcus aureus* has the ability to ferment mannitol in medium containing 7.5% sodium chloride or mannitol salt agar,

called Medium Chapman. The pH indicator is phenol red, which may indicate a positive reaction when the medium around the colonies becomes yellowish, and negative if it remains red.

Table 1. Sampling relation and presence of microorganisms.

Volunteer	Manita		Resistance to oxacillin [mm]	
	Hands	Nose	Hands	Nose
1	(-)	(-)	-	-
2	(-)	(-)	-	-
3	(+)	(+)	25	25
4	(-)	(+)	-	7
5	(-)	(-)	-	-
6	(-)	(+)	-	24
7	(+)	(+)	22	22
8	(-)	(-)	-	-
9	(-)	(+)	-	20
10	(-)	(-)	-	-
11	(+)	(+)	3	3
12	(-)	(-)	-	-
13	(-)	(+)	-	25
14	(+)	(+)	22	10
15	(+)	(-)	0	-
16	(-)	(+)	-	25
17	(-)	(+)	-	0
18	(-)	(-)	-	-
19	(+)	(+)	19	18
20	(-)	(-)	-	-
21	(-)	(-)	-	-
22	(-)	(-)	-	-
23	(+)	(+)	5	0
24	(-)	(+)	-	2
25	(-)	(-)	-	-
26	(-)	(-)	-	-
27	(-)	(+)	-	28

We found variations in determinations of halos. To evaluate the resistance, we must take into account the value, because when there halo formation greater than 11mm, the microorganism is sensitive to antimicrobial below that tough. According to Figure 1, we saw more positive incidence of *Staphylococcus aureus* manita-positive in the nostrils of the volunteers than in the hands, showing that the study agrees with the known literature.

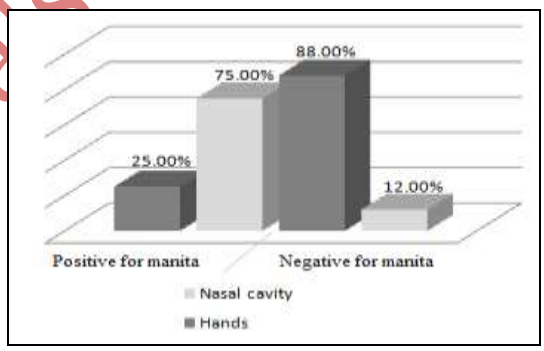

Fig 2: Analyze the reaction in mannitol for *Staphylococcus aureus*

The results also show the susceptibility of this microorganism in relation to the antimicrobial and oxacillin. In Figure 2 we can see an increased amount of resistant microorganisms in the nasal passages in comparison to the hands.

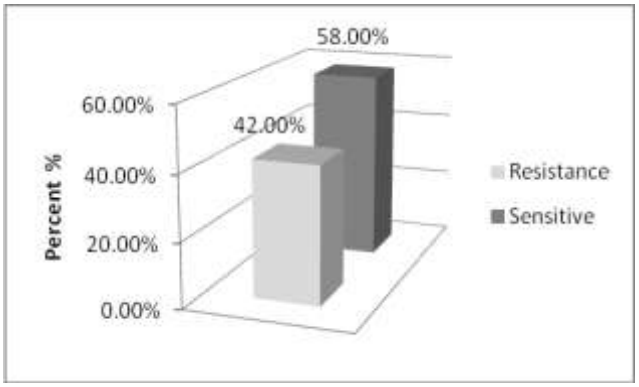

Fig 3: Antimicrobial resistance

In terms of resistance, 21 samples (36%) were positive for *Staphylococcus aureus.* By testing their resistance, 9 (42%) of these 21 were resistant and 12 (58%) were susceptible.

4. DISCUSSION

4.1 Pathogenesis

The *Staphylococcus aureus* is one of the main agents that cause infection in a large scale due to its ability to adapt quickly to different environments. These bacteria can survive even in hostile places (acid pH, humidity, osmolality, or nutrient deficiency), which enables them to colonize human beings and to create suitable cell reservoirs that can colonize other individuals [15]. Its pathogenicity involves a large amount of extracellular and cell wall components coordinately expressed during the infection's different stages [16].

The high capacity of colonization and pathogenicity of *Staphylococcus aureus* are a consequence of its virulence factors. They play a significant role "in cell adhesion, in nutrient uptake, and its evasion of the host immune response" [17]. According to Velázquez-Maze, these factors can be categorize in three categories: adhesion to the host cells, fibrinogen, fibronectin, collagen, or coagulase enzyme; evasion of host defense (staphylococcal enterotoxins, toxin of Toxic Shock Syndrome (TSS), protein A, some lipases, and capsular polysaccharides); and invasion of the host cell (tissue penetration and tissue adhesion, including proteins (toxins) α, β, δ, γe δ- hemolysins).

The focus should be on hospital infections, because the main carriers of these bacteria are health professionals [18]. The strains that acquire the multidrug resistance typically come from hospitals and have a greater clonal spread and increased virulence [19]. Some surveys proved that *Staphylococcus aureus* resistant to methicillin (MRSA) are able to survive more than one month on clothes, fabrics, and plastics commonly used in hospitals and for more than one year in disposable packaging of sterile supplies (gauze, gloves, etc.) [20]. Therefore, contamination and subsequent colonization can occur after contact with a carrier individual, such as from a contaminated object.

For Rossi, Ceccon and Krebs [21], these nosocomial infections are among the 10 leading causes of morbidity and mortality. The multidrug-resistant strains affect the blood and they are the most common nosocomial infection in this environment, which means that they concern health professionals in Brazil and worldwide.

4.2 Resistance factors of *Staphylococcus aureus* to oxacillin

The increase of resistance to most antimicrobial activity with antistaphylococcal currently available accompanied the rise of the number of infections caused by *Staphylococcus aureus* resistant to methicillin (Orsa). Therefore, glycopeptides, particularly vancomycin, have become one of the few effective therapeutic alternatives in the treatment of infections [22].

Since 2004, the Clinical and Laboratory Standards Institute (CLSI) recommends the use of cefoxitin disk (30 μg) with oxacillin disk (1μg) for oxacillin resistance's detection mediated by the mecA gene. In 2009, the CLSI excluded the oxacillin disk in predicting the strength of SCN front of β-lactam agents (CLSI, 2009).

4.3 Toxic shock syndrome (TSS) caused by staphylococcus aureus

Despite often being confused with septic shock, Toxic Shock Syndrome includes specific clinical manifestations, such as rash, peeling feet and hands, muscle weakness, pharyngeal and conjunctival hyperemia, gastrointestinal symptoms, and rapidly progressive acute renal failure [23].

The toxin acts as a superantigen, exciting the proliferation and activation of T lymphocytes, leading to a increased release of cytokines, especially tumor necrosis factor alpha and beta, interleukin-1 and interleukin-2, which causes appendage capillary permeability and hypotension, culminating in the failure of multiple organs [24].

Preliminary studies on *Staphylococcus aureus* resistance to Oxacillin presented a variety of results. Cavassini et al [25] showed with specificity of 96.7% that 61.3% of their 80 samples were resistant to oxacillin and 38.7% were sensitive. Swenson et. al [26], through its 19 samples and specificity of 89%, saw that 100% was resistant, but the amount of data collected was smaller than the previous authors. Skov. et al [27], with 99.9% specificity, studied 190 samples and 78% was resistant to oxacillin. Velasco et al [28] saw that 94.1% of their samples was resistant to oxacillin, showing that in their search the specificity was of 100%. Mimica et. al [29] came across a resistance of 96% and the specificity was 98%. Witte, Pasemann and Cuny [30] saw that 62% were resistant to oxacillin. This variation allows us to see the considerable increase in resistance of the microorganism to antimicrobial (Figure 3).

A study with Rossi [21] analyzed 40 samples with presence *Staphylococcus aureus* and saw resistance in only 3 samples, but also demonstrated that these bacteria tend to have higher resistance to β lactam. The samples of these volunteers and the high sensitivity also include factors not shown to work, perhaps by the low use of antibiotics or even the good practice of hand hygiene. Pereira [31] collected 109 samples (104 were from students). Some samples presented two different strains and 27.5% were *Staphylococcus aureus*. Samples were subjected to endurance test by the antibiotic oxacillin according to the protocol and, at the end of the tests, they noticed that 100% of the samples were sensitive to oxacillin.

Kingdom et al. [32] analyzed what was the influence in microbiota of medical college students exposed to hospitals. They saw that 29% of students in group A (students without exposure) had *Staphylococcus aureus;* 32% in group B (students with 12 months of exposure); and 32% in group C (students with 36 months of exposure). Therefore, this 3% range means a risk when it comes to the microscopic world, because it represents millions of a bacterial microbiota. This is a great risk to those exposed.

According to our data, 42% of our 54 samples proved resistant to oxacillin and 58% were sensitive. The variation is not very significant, but it proves a microorganism resistance to the antibiotic and it corroborates all the authors above.

5. CONCLUSION

We saw that Staphylococcus aureus has great potential for destruction due to its pathogenicity and that there are therapeutic options in case of contamination. Nevertheless, we showed that these drugs do not cause their expected effects, because the microorganism has acquired the means to protect itself from this medicine. Oxacillin has shown less efficacy due to the genetic medium that the microorganism adopted. This shows that Oxacillin is an invalid alternative to the desired effect.

Because of its natural habitat in our body, it is very dangerous to transport such microorganisms in our hands. Especially when it comes to healthcare professionals committed to take care of patients whose immunity system no longer responds satisfactorily.

It is possible to diminish transmission to patients by using personal protective equipment and through asepsis procedures [33]. Nevertheless, it is important to understand that we must control antibiotics use, since their indiscriminate usage is one of the principal causes of bacteria resistance.

Finally, we intend to continue researching with new methods to improve the results.

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