ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 12, pp.76061-76063, December, 2018

DOI: https://doi.org/10.24941/ijcr.33325.12.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

"NOSOCOMIAL INFECTIONS CAUSED BY BIOFILM-PRODUCING STAPHYLOCOCCI"

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ARTICLE INFO

Article History:
Received 24th September, 2018
Received in revised form
16th October, 2018
Accepted 17th November, 2018
Published online 29th December, 2018

Key Words:
Staphylococcus spp, Biofilm, Methicillin-Resistance.

ABSTRACT

Introduction: In Mexico, one of the main causal agents of nosocomial infections belong to the genus *Staphylococcus*, bacteria that in many cases have multiresistance to antibiotics and also produce biofilm. **Objective:** The purpose of the study was to determine the frequency of *Staphylococcus spp* isolated from various biological samples from patients hospitalized methicillin resistant by detecting the *mec* A gene and biofilm production by phenotypic detection Agar Congo Red. **Materials and Methods:** This work was carried out in the Laboratory of Microbiology and Molecular Biology of the Center for Biomedical Research of the UAC. We studied 143 strains of staphylococci donated by the Clinical Analysis Laboratory of a Hospital of the City of San Francisco de Campeche from January 2015 to December 2015. Staphylococcal strains included in this study were isolated from biological samples from patients hospitalized. To phenotypic characterization, strains were cultured on CRA plates in duplicate; while to genotypic detection of gene *mec*A was done by PCR reaction. **Results and conclusions:** Our results show that the staphylococcal strains included in this study from infectious processes hospitalized patients have a high frequency of methicillin resistance by detecting the *mec*A (80.4%) gene and more than half are able of expressing biofilm (55.2%). This gives them not only a resistance to beta-lactam antibiotics but also the ability to produce biofilm, increasing their chances of survival in the face of various adverse events and the use of antibiotics.

Citation: Luis Núñez Oreza, Betty Sarabia Alcocer, Rosita Tovar León, Paulino Tamay Segovia, Selene Blum Domínguez et al. **2018.** ""Nosocomial infections caused by biofilm-producing staphylococci"", *International Journal of Current Research*, 10, (12), 76061-76063.

INTRODUCTION

Intra-hospital infections are a health problem with a high cost in health services. In Mexico, one of the main bacteria causing these infections belong to the genus *Staphylococcus*, with numerous species whose natural habitat is the skin and mucous membranes of the human being that facilitates its transmission between medical personnel and hospitalized patients (Baz, 2018). Several reports in the world show an increase in the multi-resistance of these microorganisms, thus the resistance to methicillin (oxacillin) is an important marker in nosocomial infections. The resistance is acquired by horizontal transfer of the gene *mec*A between the staphylococcal species. Gene *mec*A codes for a transpeptidase with a low affinity for the beta-lactam ring, making the carrier strains of *mec* A resistant to the cephalosporins and semi-synthetic penicillins such as oxacillin and nafcillin (Velázquez, 2005). In addition, several of their species have the ability to produce microstructures called biofilm, which allows them to survive in the host due to the protection it gives against the immune system and the action of antibiotics. When small structures of the biofilm are released into the bloodstream, staphylococci can spread causing a systemic infection and cause sepsis in immunocompromised patients. The biofilm is an important factor of virulence of staphylococci in intra-hospital infections related to the implantation of catheters, prostheses and valves between and

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even tissues (Ziebuhr, 1999). Therefore, the objective of this work was to identify methicillin-resistant staphylococcal strains able of producing biofilm from nosocomial infections.

MATERIALS AND METHODS

This work was carried out in the Laboratory of Microbiology and Molecular Biology of the Center for Biomedical Research of the UAC.

Staphylococcal strains: We studied 143 strains of staphylococci donated by the Clinical Analysis Laboratory of a Hospital of the City of San Francisco de Campeche from January 2015 to December 2015. Staphylococcal strains included in this study were isolated from biological samples from patients hospitalized. The samples were urine cultures, purulent secretions, abscesses, wounds, blood cultures and catheter tips. Each strain was re-isolated and identified in our laboratory. For re-isolation, the strains were seeded in soy broth and trypticasein (CST) and incubated for 24 h at 35 ° C. They were then striated on salt and mannitol agar (ASM), and incubated for 18 h at 35 ° C. In the identification stage, every of the strains gram positive and with catalase-positive and coagulase-positive were identified by automated Vitek II (Biomerux) equipment.

Phenotypic characterization: The strains were cultured on CRA plates, prepared by adding 0.8 g of Congo Red, 12 g bacto agar and 36 g of glucoseforone liter of brain heart infusion. The plates were subsequently incubated for 24 h at 37°C and additionally overnight at room temperature(Arciola, 2001 and Castro, 2013). The CRA plates assays weremadeby duplicate, and consistent results were obtained. For each strain, this experiment was repeated three times. The presence of black colonies were interpreted as positive biofilm strainwhile red colonies were interpreted as negative biofilm.

Genotypic detection of *mec* A: From each one of the staphylococcal strains evaluated, the DNA was isolated. Subsequently, by means of the PCR reaction and using the corresponding forward and reverse oligonucleotides, a fragment of gene *mec*A was amplified. The product obtained from the PCR reaction was subjected to agarose gel electrophoresis, which was then stained with ethidium bromide. Detection of fragment of gene *mec*A was made by the visualization of a band of size of 397 bp using a UV trasnsilluminator.

RESULTS

Identification of positive and negative coagulase staphylococcal strains: All strains included in this study tested positive for gram stain and catalase test, being identified as strains belonging to the genus Staphylococcus using the automated Vitek II equipment. The coagulase test was positive for 47 strains (32.7%) and negative for the remaining 96 (67.1%). The strains were isolated from urine cultures in 24.5%, from purulent secretions, abscesses or wounds in 56.6% and from blood culture and catheter tip in 18.9%. The number of isolated positive (CoP) or negative (CoN) strains to the coagulase test was more predominant in the CoN from urocultures with 94.3%, followed by the blood cultures and tip of catheter with 85.2%. In the case of staphylococcal strains from purulent secretions, abscesses or wounds, the isolations were similar for both the CoP strains (50.6%) and the CoN strains (49.4%) (Figure 1).

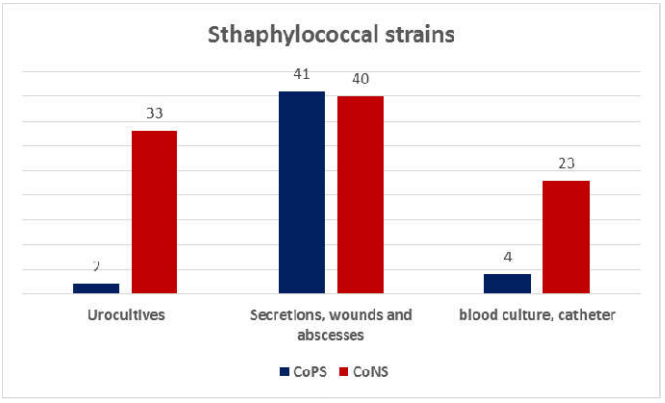

Figure 1. Coagulase positive and negative staphylococcal strains isolated from various samples from hospitalized patients.

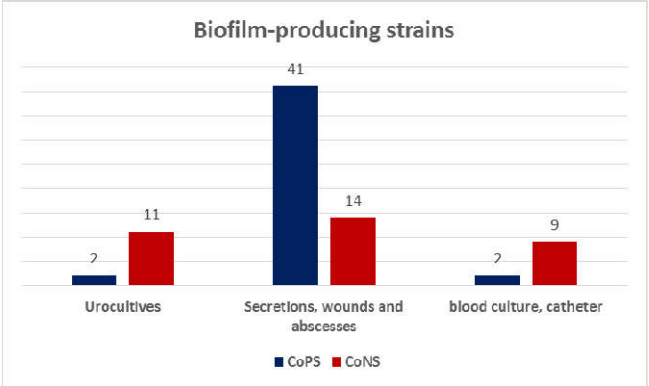

Figure 2. Staphylococcal positive and negative coagulase strains producing biofilm isolated from various samples from hospitalized patients

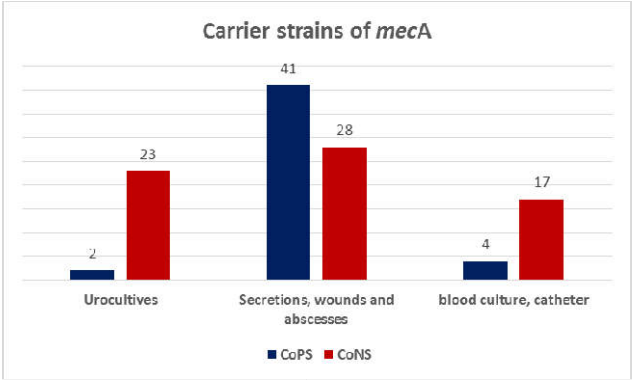

Figure 3. Coagulase positive and negative staphylococcal strains carrying the gene*mec*A that confers resistance to methicillin isolated from various samples from hospitalized patients.

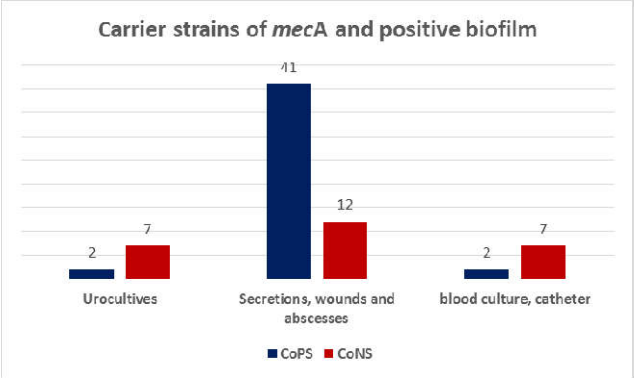

Figure 4. Strains of coagulase positive and negative staphylococci isolated from several samples of hospitalized patients. Strains were carriers of the *mec*A, *ica*A and *ica*D genes, which when expressed confer resistance to methicillin and become producers of biofilms.

Detection of biofilm in Congo Red Agar: Phenotypically, the biofilm production was positive in 55.2% of the isolated strains. All strains of coagulase-positive staphylococci, except for two isolates from blood cultures, were biofilm-producing (95.7%). In the case of CoN, 34 biofilm producing strains were detected, equivalent to 35.4%, which represents that almost two thirds of the coagulase-negative staphylococci do not produce this virulence factor. Strains isolated from urine cultures formed 33.3%, of purulent secretions, abscesses or wounds 35% and of blood culture and catheter tip 39.1% (Figure 2).

Strains carrier of gene *mec* A: The genotypic detection of the *mec*A gene determined that of all staphylococcal strains included in the study, 80.4% carried the gene. The positive coagulase strains that carried *mec*A were the 47 strains (100%). In the case of negative coagulase strains, the gene was found in 68 of them, representing 70.8%. The foregoing indicates that the resistance to methicillin is very widespread in the strains studied (See graphic 3). Thus, the negative coagulase strains resistant to methicillin from urine cultures were found in 69.7%, purulent secretions, abscesses or wounds corresponded to 70% and blood culture and catheter tip 70.8% (Figure 3). The analysis of strains of positive coagulase or negative staphylococci carriers of the *mec*A gene indicated that 49.7% are phenotypically resistant to methicillin and phenotypically biofilm producers (Velazquez, 2005 and Nuryastuti, 2008). Of the total positive coagulase strains, 95.7% (45 strains) carried the *mec*A gene and were able to phenotypically produce biofilm, while of the total negative coagulase they reached 27.1% (26 strains). The frequency of CoNS strains carrying the genes of interest isolated from urocultures were found in 7 strains (21.2%), purulent secretions, abscesses or wounds in 12 (30%) and from blood culture and catheter tip corresponded to 7 strains (30.4%) (Figure 4).

DISCUSSION

In the last two decades several studies have described that the pathogenesis of *Staphylococcus* spp seems to be attributed to the production of biofilm. In addition, it has been reported that regardless of whether they are colonizing a catheter or causing sepsis, the biofilm gives the bacteria the characteristic of surviving any environmental condition, making it difficult to eradicate them through antibiotics (Martín, 2004 and Otto, 2008). In this study we found that coagulase negative strains have a higher frequency of resistance to methicillin compared to the production of the biofilm, which suggests that the biofilm is not an indispensable factor for its survival in the host.

In contrast, staphylococcal coagulase positive strains tested are resistant to methicillin and almost all have the ability to produce biofilm, suggesting that for these bacteria the biofilm is a much more important virulence factor.

Conclusion

Almost half of staphylococcal strains included in this study, which were isolated from infectious processes in hospitalized patients, were both carriers of gene *mec*A and biofilm producers, which confers to the bacteria resistance to beta-lactam antibiotics and also gives them the ability to produce biofilm, increasing their chance of surviving various adverse events such as antibiotic therapy. In addition, as the positive coagulase staphylococci included in this study were in its totality resistant to methicillin and almost all strains were producers of biofilm, it allows us to suggest that in these strains of *Staphylococcus aureus*, survival in the human host to be seems associated with the biofilm production with multiresistance to beta-lactam drugs. Finally, due that in coagulase-negative staphylococcal strains, resistance to methicillin was found at a higher frequency than biofilm production, probably for coagulase-negative strains during host colonization, is more important the resistance to beta-lactam antibiotics than biofilm production.

Acknowledgment

We acknowledge Center for Biomedical Research of the Autonomous University of Campeche for the permission to carry out the study.

Funding Source: This study was financially supported by Center for Biomedical Research of the Autonomous University of Campeche.

Declaration of conflicts of interest: The authors declare no have conflicts of interest

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