CHARACTERIZING METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

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Background. Antibiotics inhibit the growth of bacterial agents through the inhibition of cell wall synthesis, cytoplasmic membrane function, protein synthesis, and nucleic acid synthesis. The ideal drug of choice has low toxicity to human cells. However, new antibiotics are continuously needed because of the ability of microorganisms to develop resistance to these drugs, which could lead to several medical problems.

Objective. In this study, we tested the resistance of *Staphylococcus aureus* to the antibiotics SXT 25, GM 10, OX 1, and Va 30 using the Kirby Bauer Sensitivity Test.

Methods. To perform this test, we inoculated several bacterial samples onto Petri dishes containing Mueller Hinton agar. Discs containing the antibiotics were placed on the plates, which were incubated overnight. On the following day, the zone of inhibition was measured. The susceptibility of the organisms to each antibiotic was determined. Eight samples with various resistance patterns were chosen for molecular characterization.

Results. The results of the Kirby Bauer Test showed that all bacterial specimens were resistant to OX 1, most of them had no zone of inhibition. Approximately 81.8% of the specimens were susceptible to Va 30, which had only 6 out of 33 specimens with zones of inhibitions small enough to be considered resistant. The SXT 25 and GM 10 had approximately an equal number of resistant and susceptible bacteria. After antibiotic sensitivity was determined, eight samples with similar patterns of resistance were chosen for molecular characterization. The DNA of the bacteria was isolated by centrifuging the bacterial samples and adding solutions to them.

Conclusion. We concluded that OX 1 has no effect on *Staphylococcus aureus* and Va 30 has the greatest ability to inhibit the growth of the bacteria.

INTRODUCTION

Antibiotics have several different methods of inhibiting bacterial growth. The method with lowest toxicity to human cells is the inhibition of cell wall synthesis because humans lack a cell wall. Many *Staphylococcus aureus* bacteria have gained resistance to several antibiotics that inhibit cell wall synthesis. If left untreated, *Staphylococcus aureus* has the ability to cause toxic shock or endocarditis. Resistance in bacteria arises from indiscriminate antibiotic use by physicians, inappropriate use of antibiotics by nonphysicians in some countries where they can be purchased without a prescription, or patient apathy toward the regimen prescribed by a physician.

Drug-resistant bacteria develop because of mechanisms of gene transfer between them. Transformation occurs when cell-free DNA causes changes in another strain, transduction is when bacteriophages act as vectors of genetic material, and conjugation is direct transfer of DNA from one cell to another. Most antibiotic resistance genes are located on transposons, which contain information on them that allows them to move copies of themselves to other sites on the bacterial chromosome or to bacterial plasmids. The transposon allows drug resistance genes to be spread from one generation to another within a strain and to other bacterial species. The genetic exchange between bacterial species allows the development of multiple drug resistant strains which have caused several hospital-associated epidemics.

In this study, we tested antibiotic sensitivity of several *Staphylococcus aureus* using the Kirby Bauer Sensitivity Test and after resistance was determined, certain samples were chosen for DNA isolation to see if there were differences in the patterns of organisms with similar antibiograms.

MATERIALS AND METHODS

To determine bacterial susceptibility to antibiotics, we conducted the Kirby Bauer Sensitivity Test. The first step was to make agar plates for the *Staphylococcus aureus* to grow on. To make the plates, 19 grams of Mueller Hinton agar was mixed with 500 mL of water and heated until the agar was completely in solution. The agar was poured into sterile Petri dishes and allowed to cool. Next, 33 existing samples of bacterial plates were labeled 129–161 and the newly made agar plates were also labeled 129–161. Also, several tubes containing nutrient broth were labeled 129–161. A colony of the existing samples of bacteria were taken using a sterile swab and diluted in the nutrient broth with the same corresponding number label to a dilution of 10^8 cells per mL. The diluted bacteria were swabbed onto the newly made agar plate that also contained the same corresponding number label. The plate was swabbed to produce a lawn type growth. To produce the lawn, half of the plate was swabbed with the broth inoculum, then the plate was rotated 90° and the next half was swabbed. Then the plate was once again rotated 90° and the next half was swabbed and finally it was rotated another 90° and the last half was swabbed.

Discs containing the antibiotics were added to the agar plate. The antibiotics used were SXT 25, GM 10, OX 1, and Va 30. One disc of each antibiotic was added to each plate using sterile tech-

niques. After the discs were placed, they were gently pressed to make sure they adhered to the agar surface. Then the plates were inverted and incubated overnight (18 hours) at 37°C. The next day the zone of inhibition was measured and resistance or susceptibility was determined for each bacteria and antibiotic. Eight samples with similar patterns of resistance were chosen for DNA isolation. Samples 134, 141, 145, 146,147, 154, 155, and 158 were chosen. To isolate the DNA, it was first necessary to take a bacterial colony from each agar plate and let them grow in a tube containing LB broth. The tubes were incubated at 37°C overnight. The next day, 1.8 mL of the bacterial culture was added to a 2 mL collection tube and they were centrifuged at 10,000× g for 30 seconds at room temperature. The resulting supernatant was decanted and the tubes were centrifuged again at 10,000× g for 30 seconds at room temperature and the remaining supernatant was removed with a pipet tip. Next, 300 µL of microbead solution were added and the pellet was resuspended. Then 50 µL of solution MD1 were added to the tube. The tubes were centrifuged at 10,000× g for 30 seconds at room temperature. Then the supernatant was transferred to a clean 2 mL collection tube. Then 100 µL of solution MD2 were added to the tube and the tube was incubated at 4°C for five minutes. After incubation, the tubes were centrifuged at room temperature for 1 minute at 10,000× g. We transferred the supernatant to a clean 2 mL collection tube and added 900 µL of solution MD3 to the tube. After loading about 700 µL into the spin filter and centrifuging it at 10,000× g for 30 seconds at room temperature, we discarded the flow through and added the remaining supernatant to the spin filter. After a series of centrifuge steps, adding solutions, and discarding flow through liquid, the DNA in the tube is ready for any downstream application.

Results and Discussion

The antibiotic sensitivity patterns of the methicillin-resistant *Staphylococcus aureus* (MRSA) differed from sample to sample. Only three specimens out of 33 were resistant to all four antibiotics. Six specimens were resistant to three antibiotics, being susceptible to only vancomycin. This shows that there were not a large number of multiresistant strains. Also, because of the differences in antibiotic sensitivity, it is more probable that the samples came from different sources. Also, since vancomycin and oxacillin are both cell wall inhibitors, yet 27 samples were susceptible to vancomycin and 32 samples were resistant to oxacillin, it demonstrates that the two antibiotics must have different methods of working. In the future, gel electrophoresis will be conducted on the eight isolated DNA samples to determine if the MRSA have similar genetic patterns.

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