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Study of Amoxicillin and Cloxacillin Resistance in *Micrococcus sciuri* Strain JS 3 Isolated from a Nosocomial Infection

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (*Micrococcus aureus*) or better known as MRSA is well known. The organism has become a common name in most of the nosocomial infections. This organism is also known to be resistant to related antibiotics like cloxacillin and amoxicillin. The reverse is also true that those strains resistant to cloxacillin and amoxicillin are also resistant to methicillin. It has been hypothesized that this organism carries an extra chromosomal DNA which is responsible for the methicillin resistant. This investigation attempts to find whether such antibiotic resistance is also prevalent amongst related species of *Micrococcus*. Using the above mentioned reports, experiments were designed to find out *in vitro* resistance of *Micrococcus sciuri* strain JSG-1 which was isolated in our lab from a nosocomial infection case, to cloxacillin and amoxicillin. It was found that this strain of *Micrococcus sciuri* showed resistance to 5000 ppm of both cloxacillin and amoxicillin individually. The importance of this finding is that hospitals must step up their sanitation practices to minimize nosocomial infections by such resistant strains.

Key words: Methicillin, Cloxacillin, Amoxicillin, Micrococcus, Nosocomial

INTRODUCTION

Staphylococcus aureus better known as *Micrococcus aureus* is one of the most successful and adaptable human pathogen. It is mostly found as a commensal on skin and upper respiratory tract. However, the virulent strains have remarkable ability to acquire antibiotic resistance. Different patterns of antibiotic resistance, like drug inactivating enzyme such as β -lactamases and related plasmid profile among strains of *S. aureus* have been reported (Daini and Akano, 2009). One such antibiotic resistant organism is the MRSA. Hospitalized patients probably serve as the major reservoir of MRSA.

The mechanism of methicillin resistance in staphylococci is considerably different from penicillin resistance, where β -lactamase breaks the β -lactam ring of the antibiotic. MRSA strain produce a unique Penicillin Binding Protein (PBP2a) that has a much lower affinity for β -lactam antibiotics. The gene encoding PBP 2a has been named *mecA* and is incorporated into the chromosome of MRSA strain as a part of a conserved 30 kb region termed *mec*. The origin and evolution of the *mec* locus is the subject of considerable controversy, but it has been suggested that it may have resulted from a single mutation passed down through generations or in conjunction with horizontal gene transfer. The plasmid-encoded genes responsible penicillin resistance is readily transferable between staphylococcal species by both the horizontal and vertical route. In contrast, the

dissemination of *mec* gene (responsible for methicillin resistance) during bacterial replication is largely by vertical transfer rather than the horizontal transfer (Wielders *et al.*, 2002).

Cases of community acquired MRSA (CA-MRSA) have become more common than hospital acquired MRSA (HA-MRSA) and is found in locker rooms, daycare facilities, public schools, playgrounds and stores. Approximately 33% of any population carries CA-MRSA on their skin or in their nasal cavities, as commensal. It has become a disturbing reality that these aggressive, difficult-to-treat bacteria strains are present in most communities. This change in epidemiology poses a significant threat to the successful treatment of many common infections (Gorwitz *et al.*, 2006).

It was therefore, decided that if the spread of MRSA is like a wild forest fire, then there is all likelihood of finding such antibiotic resistance in other species of *Micrococcus*. This would result in appearance of many new infectious organisms equipped with such virulence and epidemiological factors. This was the primary objective of the present investigation.

MATERIALS AND METHODS

This study was carried out between December 2010 and March 2011

Isolation and screening of similar antibiotic resistant *Micrococcus* spp.: Swabs were collected from certain nosocomial infection cases in a hospital, which were put in 5 mL physiological saline solution.

Commercially available AMPOXIN-1000 consisting of sodium ampicillin equivalent to anhydrous ampicillin 500 mg and sodium cloxacillin equivalent to cloxacillin 500 mg. This was dissolved in 5 mL sterile distilled water to get a solution of 10000 ppm each.

Nutrient agar (Atlas, 2004) plates were prepared containing 1 mL of the antibiotic solution. The final concentrations of the antibiotics were 500 ppm each. These plates on solidification were spread with saline solutions containing the swabs and were incubated at 37°C for 48 h. Colonies showing good growth were selected and checked for morphology by Gram staining. Further identification was done by 16rDNA sequence analysis.

Study of tolerance to maximum concentration of antibiotics: One such colony was selected and was identified by 16rDNA sequencing. The above solution containing the antibiotics was diluted from 5×10^{-5} ppm to 5×10^2 ppm. Nutrient agar plates were prepared as mentioned above containing each of the dilutions and was inoculated with 0.1 mL suspension of the isolate containing 6×10^8 cells mL⁻¹. The plates were incubated as above.

Effect of pH on growth of the isolate: The experiment described in isolation and screening was repeated but with media having different pH ranging from 4 to 9.

Effect of temperature on growth of the isolate: Here too the above mentioned experiment was repeated but the plates were incubated at different temperatures ranging from 30, 37 to 50°C.

Finally an attempt was made to see whether this isolate like MRSA, was having an extra chromosomal DNA.

RESULTS

The suspensions obtained from the nosocomial infections in the hospital were cultured on nutrient agar (Atlas, 2004) containing 250 ppm of both amoxicillin and 250 ppm of cloxacillin. There was growth of certain organisms on these plates after incubation at 37°C for 48 h. The

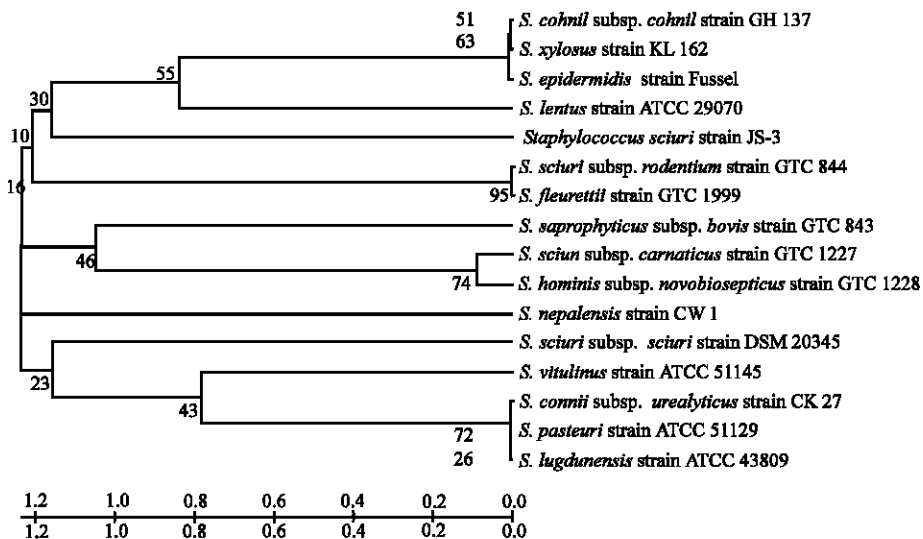


Fig. 1: Phylogenetic tree of *Staphylococcus sciuri* JS3 (*Micrococcus sciuri* JS3)

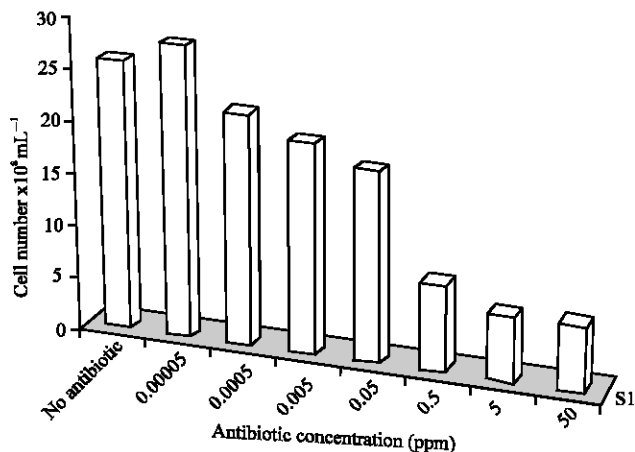


Fig. 2: Effect of different concentrations of antibiotic on the growth of the organism. The concentration ranged from 5×10^{-5} to 50 ppm

growth was not very confluent and certain Colony Forming Units (CFUs) were very pin point in size. On examining the morphology by gram staining, most of the colonies contained gram positive cocci (results not showed). One such colony of bacteria that was picked up in random, which was not showing significant pigment production.

The 16rDNA sequencing showed that the isolate was *Micrococcus sciuri*. It was deposited with Gene Bank with the accession number JN409601 and it was labeled as *Micrococcus sciuri* JS3. The culture was deposited with NCIM (NCL, Pune, India). The phylogenetic tree is as shown in Fig. 1.

It is evident from Fig. 2 that with decreasing concentration of antibiotics there was linear increase in growth of the organisms. At a very low concentration of the antibiotic (0.00005 ppm) there was slight stimulation in growth of the organism. There was complete inhibition at 5 ppm of antibiotic concentration.

Figure 3 illustrate that this organism which normally would grow at pH 7.0, is growing also at pH 6.4 in presence of 5×10^{-2} ppm of antibiotic. This is quite unusual of this organism.

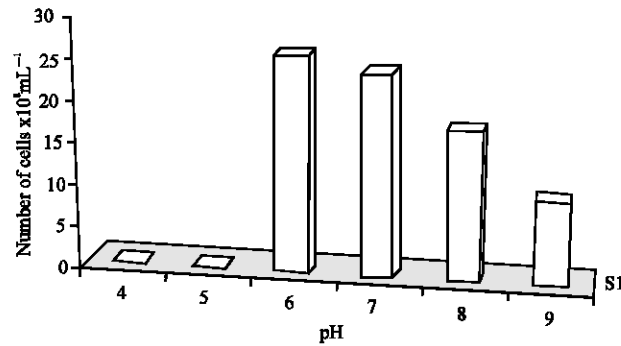


Fig. 3: The organism capable of growing at pH 6 as well as in pH 7.0

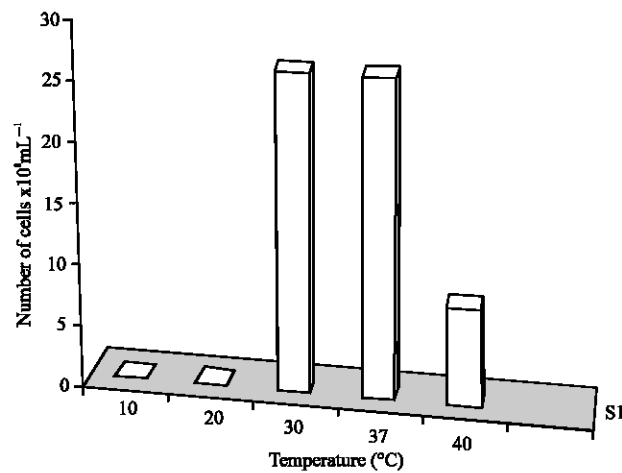


Fig. 4: The organism grows at 30 and 37°C

Figure 4 indicates that the organism is capable of growing at 37°C as well as 30°C. Again this is a unique characteristic of this strain.

Lastly an attempt was made to isolate any extrachromosomal DNA that may be present in the strain population. It was observed that 2 such extrachromosomal DNA were present having 1.5 and 2kbp size.

DISCUSSION

Micrococcus sciuri is a normal chemoorganotroph found mostly in soil. It is found on the skin of human being as it is carried by dust and wind. It has never been reported to cause any type of disease. This finding that the strain JS3 is equipped with a virulence factor like resistance to amoxicillin and cloxacillin is surprising as it may invade through either the skin or through the upper respiratory tract and can cause diseases. Since it has been isolated from nosocomial infections, it can serve to be a good indicator organism to indicate improper sterilization processes that are practiced in many hospitals, especially in developing nations. Such an alarm had been raised when widespread occurrence of methicillin resistant *Micrococcus aureus* had been detected among patients in different hospitals world wide. Even vancomycin resistant *M. aureus* is becoming very common in nosocomial infections (Hiramatsu, 2001). It seems that wound infecting gram positive cocci beside *M. aureus* is also becoming very prevalent. This could be due to the fact that

drug resistance (antibiotic resistance) is carried on extrachromosomal DNA which is getting transferred in other species, either by transduction or transformation.

The second most important factor that *Micrococcus sciuri* JS3 is capable of growing at pH 6. This acidic pH is normally the pH of certain skin secretion like sebum (Grice *et al.*, 2009) which could enhance the ability of the organism to overcome skin barriers and increase its pathogenicity.

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REFERENCES

- Atlas, R.M., 2004. Handbook of Microbiological Media. 3rd Edn., CRC Press, New York, Pages: 1282.
- Daini, O.A. and S.A. Akano, 2009. Plasmid-mediated antibiotic resistance in *Staphylococcus aureus* from patients and non patients. *Sci. Res. Essay*, 4: 346-350.
- Gorwitz, R.J., D.B. Jernigan, J.H. Powers and J.A. Jernigan, 2006. Strategies for clinical management of MRSA in the community: Summary of an experts. Proceedings of the Meeting Convened by the Centers for Disease Control and Prevention, December 5, 2006, Atlanta, GA.
- Grice, E.A., H.H. Kong, S. Conlan, C.B. Deming and J. Davis *et al.*, 2009. Topographical and temporal diversity of the human skin microbiome. *Science*, 324: 1190-1192.
- Hiramatsu, K., 2001. Vancomycin-resistant *Staphylococcus aureus*: A new model of antibiotic resistance. *Lancet Infect. Dis.*, 1: 147-155.
- Wielders, C.L.C., A.C. Fluit, S. Brisse, J. Verhoef and F.J. Schmitz, 2002. *mecA* gene is widely disseminated in *Staphylococcus aureus* population. *J. Clin. Microbiol.*, 40: 3970-3975.