PLASMID PROFILE OF Staphylococcus hyicus ISOLATED FROM SWINE EXUDATIVE EPIDERMITIS IN BRAZIL

PERFIL PLASMIDIAL DE Staphylococcus hyicus ISOLADOS DE SUINOS COM EPIDERMITE EXSUDATIVA NO BRASIL

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SUMMARY

Staphylococcus hyicus cultures, isolated from piglets with skin lesions, were investigated for their plasmid profile and their resistance to antimicrobial agents. Plasmid of different sizes could be detected in six Staphylococcus hyicus isolates. All that samples showed tetracycline resistance and presented small plasmid of 4.0 Kb. Curing and transformation experiments showed that the 4.0 Kb plasmid harboured the genetic determinant of tetracycline resistance in this strain.

UNITERSMS: Staphylococcus hyicus; Plasmid; Swine

INTRODUCTION

A typical swine disease, the exudative epidermitis is caused by Staphylococcus hyicus and occurs more frequently in suckling piglets. This affection has been described with relative frequency in Brazil. This microorganism in pigs was still isolated from polyarthritis and also from the healthy animals skin. In bovines it was observed in skin with lesions and causing mastitis.

The knowledge about the susceptibilities of this bacterium to the different antimicrobial agents is very important to the treatment Staphylococcus hyicus infections.

The reason of Staphylococcus aureus resistance to several antimicrobial drugs has been imputed to the plasmids presence. Plasmid occurrence in Staphylococcus hyicus was reported by Kloos et al. (1981) and correlated posteriorly to the antimicrobial drugs resistance. However, nothing is known about plasmids from coagulase negative staphylococci isolated from animals in Brazil.

MATERIAL AND METHOD

Samples

Six samples of Staphylococcus hyicus isolated from skin pigs with exudative epidermitis were used. The samples were collected from different places located in Londrina region, Paraná State, Brazil.

The identification of species level was done as described by Devriese et al. (1978, 1985) and by the API Staph (API System, Montalieu, Vercieu, France).

Antimicrobials Sensitivity

The sensitivity to different antimicrobials was determined according to Bauer et al. (1966) in agar Muller-Hinton using DIFCO discs with: Ampicillin (Ap) 10 ug; Chloramphenicol (Cm) 30 ug; Erythromycin (Em) 15 ug; Gentamicin (Gm) 10 ug; Tetracycline (Tc) 30 ug; Sulfazotrin (St) 25 ug; Neomycin (Nm) 30 ug; Novobiocin (Nv) 30 ug; Cephalothin (Ct) 30 ug; Amikacin (Ak) 30 ug; Nitrofurantoin (Nf) 30 ug; Streptomycin (Sm) 10 ug; Oxacillin (Ox) 5 ug; Trimethoprin (Tp) 5 ug; Sulfonamide (Su) 300 ug; Nalidixic Acid (Nal) 30 ug; Penicillin (Pc) 10 U; Lincomycin (Lm) 5 U; Kanamycin (Km) 30 ug.

Plasmids Extraction

A modification of a method described by Birnboin; Doly; (1978), and adapted for Staphylococci by Schwarz; Blobel (1989), was done in our laboratory and used for the extraction of plasmid DNA.

The samples were grown in 3 ml of Brain Heart Infusion (BHI) for 18h at 37°C bath shaking. Then 1.5 ml of the cultures was sedimented by microcentrifugation. Each pellet was resuspended in 50 mM glucose, 25 mM EDTA, 25 mM Tris-HCl at pH 8.0. The cell walls of each culture were lysed by subsequent incubation during 30 min. at 37°C in the presence of 40 ug/ml lysostaphyn (SIGMA, St. Louis, USA). The alkaline lysis of the protoplasts was reached by adding 1% sodium dodecylsulfate and 0.2N NaOH at 4° for 5 min. Then 3 M sodium acetate at pH 4.8 was added to neutralize the mixture. After microcentrifugation, the supernatant was precipitated with 1 volume of ammonium acetate and 1.5 volume of absolute ethanol (PA Grade), that was kept at -20°C for 2 h. The mixture was centrifuged and the precipitate washed with 70% ethanol being resuspended in 20 mM Tris-HCL and 1 mM EDTA at pH 7.5.

Agarose Gel Electrophoresis

Gel of 0.8% agarose was used. Electrophoresis was carried out for 90 min. at 80 V. The gel was stained with 10 ug/ml
Tab. 1 shows the resistance to different antimicrobial drugs, the six staphylococcal strains were identified as *Staphylococcus hyicus* showed resistance to only seven. All the strains were resistant to *Staphylococcus hyicus* was chosen as the recipient. The transformed protoplasts were selected to tetracycline resistance and also screened for plasmid DNA.

**Plasmid Transformation**

Protoplast transformation experiments were performed according to the method of Chang; Cohen (1979), modified by Gotz et al. (1981). *Staphylococcus aureus* ATCC 25923 was chosen as the recipient. The transformed protoplasts were selected to tetracycline resistance and also screened for plasmid DNA.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Drugs resistance pattern</th>
<th>Plasmid profile</th>
<th>Plasmid Molecular Weight (Kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Tc Su Tp</td>
<td>1</td>
<td>4.00</td>
</tr>
<tr>
<td>B</td>
<td>Tc Su Tc</td>
<td>2</td>
<td>4.00; 95.38</td>
</tr>
<tr>
<td>C</td>
<td>Tc Su Tp Em Lm</td>
<td>2</td>
<td>5.08; 95.38</td>
</tr>
<tr>
<td>D</td>
<td>Tc Su Tp Em Lm Cm</td>
<td>1</td>
<td>4.00</td>
</tr>
<tr>
<td>E</td>
<td>Tc Su Tp Em Lm Cm</td>
<td>2</td>
<td>4.00; 95.38</td>
</tr>
<tr>
<td>F</td>
<td>Tc Su Tp Em Lm Cm</td>
<td>3</td>
<td>4.00; 60.00; 95.38</td>
</tr>
</tbody>
</table>

Tab. 1 shows the resistance to different antimicrobial drugs, the number of plasmids found and their respective MW.

From the 19 antimicrobial drugs tested, *Staphylococcus hyicus* showed resistance to only seven. All the strains were resistant to the TC, Su and Tp. Three strains (D, E and F) were also resistant to the Em, Lm, Cm and the sample C to the Lm and Em. The resulting clones were then replicated on selective TSA plates containing 15 ug/ml tetracycline. Apparently cured clones were screened for plasmid DNA.

**RESULTS AND DISCUSSION**

The six staphylococcal strains were identified as *Staphylococcus hyicus* as described by Devriese et al. (1978, 1985).

Finally, the use of the same antimicrobial drugs for treating human and animal infections, can play a role on selecting staphylococci strains with a similar pattern of drugs resistance and plasmids profile.

**REFERENCES**


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