Braz. J. vet. Res. anim. Sci.,
São Paulo, v. 30, n. 1, p. 39-41, 1993.

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

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

Vaneril BELOTI[1]; Amauri Alcindo ALFIERI[1]; Marilda Carlos VIDOTTO[2]; Ernst Eckehardt MULLER[2]; Julio Cesar de FREITAS[2]

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 strains.

UNITERMS: ***Staphylococcus hyicus***; Plasmid; Swine

INTRODUCTION

A typical swine disease, the exudative epidermitis is caused by ***Staphylococcus hyicus*** and occurs more frequently in suckling piglets[4]. This affection has been described with relative frequency in Brazil[7]. 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[5].

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[11]. Plasmid occurence in ***Staphylococcus hyicus*** was reported by KLOOS et al.[9] (1981) and correlated posteriorly to the antimicrobial drugs resistance [11, 12, 13, 14]. 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.[5, 6] (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.[1] (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 (Sft) 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[2]; (1978), and adapted for Staphylococci by SCHWARZ; BLOBEL[12] (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 preciptate 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

1-Mestre em Microbiologia - Centro de Ciências Agrárias da Universidade Estadual de Londrina
2-Doutor em Microbiologia - Centro de Ciências Agrárias da Universidade Estadual de Londrina

ethidium bromide (SIGMA, St. Louis, USA). The molecular weight (MW) standards used were ***Escherichia coli*** plasmids provided by Dr. E. Chartoni (Universidade Federal de Minas Gerais): p307 (83.076 Kb), pSa (35.384 Kb), pRK290 (20.307) and pBR322 (4.0 Kb).

Curing Experiments

The tetracicline resistant ***Staphylococcus hyicus*** culture, was incubated in Trypticase-soy-broth (TSB - DIFCO, Detroit, U.S.A.) for 48 h at 42°C with 10 ug/ml ethidium bromide, plated on Tryp-ticase-soy-agar (TSA - DIFCO, Detroit, U.S.A.) and incubated for 24 h at 37°C. The resulting colones were then replicaplated on selective TSA plates containing 15 ug/ml tetracycline. Apparently cured clones were screened for plasmid DNA.

Plasmid Transformation

Protoplast transformation experiments were performed according to the method of CHANG; COHEN[3] (1979), modified by GOTZ et al.[8] (1981). ***Staphylococcus aureus*** ATCC 25923 was chosen as the recipient. The transformed protoplasts were selected to tetracycline resistance and also screened for plasmid DNA.

RESULTS AND DISCUSSION

The six staphylococcal strains were identified as ***Staphylococcus hyicus*** as described by DEVRIESE et al.[5,6] (1978, 1985).

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 strains A and E presented intermediate resistance to Nal.

It was observed more than one plasmid in four strains. The strains A, B, D, E and F have a plasmid weighting approximately 4.0 Kb while strain C harboured a plasmid of 5.1 Kb; B, C, E and F also present a large plasmid of 95.38 Kb and F carried a third plasmid of 60 Kb.

Thus, all those strain showing tetracycline resistance had a small plasmid of 4.0 Kb. LYON; SKURRAY[10] (1987) report in ***Staphylococcus aureus*** and SCHWARZ; BLOBEL[13] (1990) described in ***Staphylococcus hyicus*** a little plasmid of 4.5 Kb responsible by tetracycline resistance. The plasmid found in all the strains with 4.0 Kb did not appear only on strain C. In this strain another plasmid with 5.1 Kb was observed.

For curing experiments the strain A culture was used which contained only one plasmid. The resulting clones appeared cured, were screened for plasmid DNA. They lost their plasmid and became tetracycline sensitive showing a large zone of growth inhibition in the antibiogram. However, curing experiments alone may result in misleading information. Therefore, in addition to curing experiments, we conducted the more specific protoplast transformations. This led to clones which carried only the 4.0 Kb plasmid and became resistant to tetracycline. Thus, the extrachromossomal nature of tetracycline resistance ***Staphylococcus hyicus*** was demonstrated.

The high MW plasmids present in the strains B, C, E and F (Tab. 1) are not associated with tested drugs resistance, since strain D does not carry the high MW plasmids (95.38 Kb and 60 Kb) but shows resistance to the same drugs as F, which harbour this plasmids.

TABLE 1
Resistance and plasmid profile presented by six samples of ***Staphylococcus hyicus*** isolated from swine exudative epidermitis. Londrina, 1992.

Strains	Drugs resistance pattern	Plasmid profile	Plasmid Molecular Weight (Kb)
A	Tc Su Tp	1	4.00
B	Tc Su Tc	2	4.00; 95.38
C	Tc Su Tp Em Lm	2	5.08; 95.38
D	Tc Su Tp Em Lm Cm	1	4.00
E	Tc Su Tp Em Lm Cm	2	4.00; 95.38
F	Tc Su Tp Em Lm Cm	3	4.00; 60.00; 95.38

Tc= tetracycline; Su= sulfonamide; Tp= trimethoprin; Em= erythromicin; Lm= lincomycin; Cm= chloranphenicol.

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.

RESUMO

Staphylococcus hyicus isolados de leitões com lesões de pele foram estudados no que se refere aos seus plasmídios e resistência a agentes antimicrobianos. Plasmídios de diferentes pesos moleculares foram detectados nas seis amostras de *Staphylococcus hyicus*. Todas as amostras mostraram resistência à tetraciclina e um pequeno plasmídio de 4.0 Kb, com uma exceção. Experimentos de cura e transformação demonstraram que este plasmídio abriga o determinante genético para resistência à tetraciclina nas amostras estudadas.

UNITERMOS: *Staphylococcus hyicus;* Plasmídios; Suínos

REFERENCES

01-BAUER, A.W.; KIRBY, W.M.M.; SHERRIS, J.G.; TURK, M. Antibiotic susceptibility testing by standardized single disc method. **Amer. J. clin. Path.**, v. 45, p. 493-6, 1966.

02-BIRNBOIN, H.C.; DOLY, J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. **Nucl. Ac. Res.**, v.7, p.1513-23, 1978.

03-CHANG, S.; COHEN, S.N. High frequency transformation of **Bacillus subtilis** protoplasts by plasmid DNA. **Molec. Gen. Genet.**, v. 168, p. 111-9, 1979.

04-DEVRIESE, L.A.; DERYCKE, J. **Staphylococcus hyicus** in cattle. **Res. Vet. Scl.**, v. 26, p.356-8, 1979.

05-DEVRIESE, L.A.; HAJEK, V.; OEDING, P.; MEYER, S.A.;

SCHLEIFER, K.H. **Staphylococcus hyicus** (Sompolinsky 1953) comb. nov. and **Staphylococcus hyicus** subsp. **chromogenes** subsp. nov. **Int. J. system. Bacteriol.**, v. 28, p. 482-90, 1978.

06-DEVRIESE, L.A.; SCHLEIFER, K.H.; ADEGOQUE, G.O. Identification of coagulase-negative staphylococci from farm animals. **J. appl. Bact.**, v. 58, p.54-5, 1985.

07-FREITAS, J.C.; ALFIERI, A.A.; MULLER, E.E.; BELOTI, V. Identificação de **Staphylococcus hyicus** subsp. **hyicus** em suínos com dermatite exudativa na região de Londrina, Pr. In: CONGRESSO LATINO DE VETERINARIOS ESPECIALISTAS EM SUÍNOS, 1., *Anais*. 1985. p.111.

08-GOTZ, F.; AHRNE, S.; LINDBERG, M. Plasmid, transfer and genetic recombination by protoplast fusion in staphylococci. **J. Bact.**, v. 145, p. 74-81, 1981.

09-KLOOS, W.E.; ORBAN, B.S.; WALKER, D.D. Plasmid composition of **Staphylococcus** species. **Canad. J. Microbiol.**, v.27, p. 271-8, 1981.

10-LYON, B.R.; SKURRAY, R. Antimicrobial resistance of **S. aureus**: Genetic Basis. **Microbiol. Rev.**, v.51, p.88-134, 1987.

11-NOBLE, W.C.; RAHMANN, M.; LLOYD, D.H. Plasmid in **Staphylococcus hyicus**. **J. appl. Bact.**, v. 64, p.145-9, 1988.

12-SCHWARZ, St.; BLOBEL, H. Plasmids and resistance to antimicrobial agents and heavy metal in **Staphylococcus hyicus** from pigs and cattle. **J. Vet. Med. B.**, v. 36, p.669-73, 1989.

13-SCHWARZ, St.; BLOBEL, H. Isolation and restriction endonuclease analysis of a tetracycline resistence plasmid from **Staphylococcus hyicus**. **Vet. Microbiol.**, v.24, p.113-22, 1990.

14-SCHWARZ, St.; CARDOSO, M.; BLOBEL, H. Plasmid mediated chloranphenicol resistance in **Staphylococcus hyicus**. **J. gen. Microbiol.**, v.135, p. 1-8, 1989.

Recebido para publicação em 16/01/92
Aprovado para publicação em 15/12/92