Mannose Inhibition as a Significant Marker for Differentiating among Novobiocin-resistant Staphylococci of Relevance in Clinical Microbiology

By JOSÉ A. G. M. CRISTINO, ARTUR T. PEREIRA, MARIA L. MIRA AND CARLOS MANSO

1Instituto Bacteriológico Câmara Pestana, University of Lisbon, 1100 Lisbon, Portugal
2Instituto de Química Fisiológica, Faculty of Medicine of Lisbon, Av. Prof. Egas Moniz, 1600 Lisbon, Portugal

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A specific method for the identification of Staphylococcus saprophyticus among novobiocin-resistant species isolated from man is described. The test is based on novobiocin resistance, non-fermentation of D-mannose and early inhibition with late secondary growth on glucose/mannose + novobiocin agar plates. On this medium novobiocin-resistant Staphylococcus cohnii showed a confluent, continuous and homogeneous growth after 24 h which remained unchanged at 48 h whether or not it fermented D-mannose, whereas novobiocin-resistant Staphylococcus xylosus fermented D-mannose. These results are discussed in relation to a possible causal role of PTS enzymes and phosphomannose isomerase deficiency in mannose inhibition.

INTRODUCTION

Staphylococcus saprophyticus is a coagulase-negative novobiocin-resistant species responsible for a common urinary tract infection in young females. Current identification is based on the simplified Kloos and Schleifer scheme containing 13 key characters (Kloos & Schleifer, 1975). Mannose inhibition of S. saprophyticus has been previously described (Pereira & Cristino, 1985). Here we report a specific method of identification of S. saprophyticus among novobiocin-resistant species based on mannose inhibition, and also the demonstration of antigen 51 on strains responsible for urinary infection.

METHODS

Staphylococcus strains. The novobiocin-resistant species S. cohnii (119 strains), S. xylosus (14 strains) and S. saprophyticus (27 strains) were isolated from human skin and mucosal membranes. In addition, 50 strains of S. saprophyticus isolated from urine, 5 strains of S. aureus and 10 strains of S. epidermidis were included in the tests for comparison. A total of 225 strains was tested.

Identification of species. Susceptibility to lysostaphin (100 µg ml⁻¹) was tested on plates. Purple agar plates with different substrates were used as described by Kloos & Schleifer (1975). Gelatinase production tubes (Lab-Lemco, peptone, nutrient gelatine) were incubated at 22 °C and observed for 15 d. Most strains were also studied with API system galleries containing 19 substrates.

Glucose/mannose + novobiocin agar medium. Plates were prepared with Columbia Agar Base (Oxoid), glucose (1%, w/v) and bromocresol purple (0-002%, w/v). One hour later half the solidified medium was removed and replaced by the same base with bromocresol purple (0-002%, w/v), D-mannose (1%, w/v) and novobiocin (5 µg ml⁻¹). Glucose and D-mannose stock solutions were filter-sterilized.

Inoculation of the medium. A saline suspension of each strain was prepared with an opacity equivalent to tube 4 of the McFarland scale. A loopful of the suspension was lightly streaked perpendicularly to the separation line of the two carbohydrates, starting on the half of the plate containing D-mannose and novobiocin. Four strains were inoculated on each plate. Cultures were incubated at 37 °C and examined after 24 and 48 h.

Slide agglutination. Slide agglutinations were performed as previously described (Pereira, 1962). Rabbits were inoculated with the S. saprophyticus reference strain no. 51877 (Pereira, 1962), and sera were absorbed with standard strain 18 from overnight plates, for 4 h in a water-bath at 50 °C.

Phosphomannose isomerase (EC 5.3.1.8) determination. The assays were done as described by Bergmeyer et al. (1974).

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RESULTS

Identification of *S. saprophyticus* in glucose/mannose + novobiocin agar medium. On this medium the *S. aureus* and *S. epidermidis* strains could be excluded because they did not grow on the half of the plate containing novobiocin. Novobiocin-resistant strains grew on both halves of the plate (Fig. 1).

*S. xylosus* and mannose-positive *S. cohnii* (subsp. 2) could be excluded because D-mannose fermentation produced a colour change. This reaction and glucose fermentation were responsible for the colour change extending from the culture streak in the surrounding medium along the whole streak.

*S. saprophyticus* and mannose-negative *S. cohnii* (subsp. 1) produced a colour change only on the glucose half of the plate. On the D-mannose half there was a striking difference between the two species: *S. cohnii* showed confluent, homogeneous growth after 24 h which remained unchanged after 48 h, while *S. saprophyticus* showed inhibition after 24 h and after 48 h an irregular secondary growth with colonies of many different sizes (Fig. 1).
Mannose inhibition was shown by all 77 strains of *S. saprophyticus*, by one out of 14 strains of *S. xylosus* and by none of the 119 *S. cohnii* strains.

**Identification of antigen 51 on *S. saprophyticus* strains.** Of the strains isolated from urine and identified as *S. saprophyticus* by the above method, 94% revealed antigen 51, whereas it was present only on 38% of the strains isolated from skin and mucosal membranes.

**Phosphomannose isomerase assays.** Phosphomannose isomerase activity was detected in all 10 strains of *S. cohnii* subsp. 2 and in both strains of *S. xylosus* that were tested, whereas the 14 strains of *S. saprophyticus* and 5 of *S. cohnii* subsp. 1 tested revealed no enzyme activity.

**Discussion**

Among novobiocin-resistant coagulase-negative *Staphylococcus* species, only *S. saprophyticus* is responsible for urinary infections in young females. The non-fermentation of D-mannose and the carbohydrate inhibition in *S. saprophyticus* seem to provide a specific method for its identification. On glucose/mannose + novobiocin agar medium, novobiocin selected the three species that occur in man: *S. saprophyticus*, *S. cohnii* and *S. xylosus*. Only *S. saprophyticus* and *S. cohnii* subsp. 1 do not ferment mannose, but only the former showed early inhibition with late secondary growth in the presence of D-mannose.

The lethal influence of D-mannose in honeybees was explained by a phosphomannose isomerase deficiency (Saunders et al., 1969; Sols et al., 1960). A teratogenic effect of mannose in early rat embryos has also been reported (Freinkel et al., 1984), where decreasing levels of gluolysis were not yet counterbalanced by the oxidative Krebs-cycle metabolism. Mannose inhibition in *S. saprophyticus* may be due to a direct effect of D-mannose or to an effect of some metabolite(s) of mannose. A phosphotransferase system (PTS), which has been demonstrated in various species of bacteria (Postna & Lengeler, 1985) may be expressed in *S. saprophyticus* with enzymes II^Glc^ and II^Man^.

When the whole system of three or four PTS enzymes functions, phosphorylated mannose may be formed. The absence of phosphomannose isomerase would lead to accumulation of phosphorylated mannose with a toxic effect as seen in the mannose inhibition phenomenon. The absence of enzyme activity in all 14 *S. saprophyticus* strains studied would support this hypothesis.

The presence of antigen 51 appears to be significant, since almost all the *S. saprophyticus* strains isolated from urine specimens, but only 38% of the strains from the skin or mucosal membranes, possessed the antigen. Antigen 51 appears to behave as a virulence factor, since evidently only strains with this antigen are able to colonize bladder mucosal membrane and give rise to disease.

**References**


