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L-forms of *Staphylococcus epidermidis* Induced by Penicillin

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A b s t r a c t

L-forms of *S. epidermidis* were induced at 35°C with the use of an L-form medium with penicillin. The aim of this study was to evaluate the frequency of L-form induction and demonstrate whether the origin of the clinical strains affects the frequency of L-forms induction, as well as to study whether the time of action of the antibiotic has an influence on frequency of L-form induction.

K e y w o r d s: L-forms, *Staphylococcus epidermidis*

Cell wall deficient forms of *Staphylococcus epidermidis* were isolated directly from clinical material of patients with jaw muscle infections (McGregor, 2000), urinary tract infections (Dominugue *et al*., 1993; Świerczewski and Reyes, 1970), secretory otitis media (Ataoglu *et al.*, 1994). It is supposed, that L-forms of bacteria might be a cause of infections in patients with chronic idiopathic prostatitis (Dominigue and Hellstrom, 1998), and can be a cause of malignant tumors located on sun-exposed areas such as head, arms and legs (Cantwell, 2003). Some studies in the 1960s and 1970s showed that L-forms of *Staphylococcus aureus* may be produced *in vitro* by the action of penicillin (Banville 1964; Rosdahl and Vejlsgaard, 1970; Simon and Yin, 1970). However, there are no reports available concerning the studies of the evaluation of frequency of L-form induction of *S. epidermidis*, the demonstration whether the origin of the clinical strains affects the frequency of L-form induction and examination if the time of action of the antibiotic has an effect on L-form induction.

36 strains of penicillin resistant *S. epidermidis* were included in this study to determine *in vitro* L-form induction. Strains were identified with the API Staph biochemical tests (bioMérieux). The *S. epidermidis* strains, were isolated from blood (12), urine (12) and biomaterials (catheters, drains) (12) from patients of A. Jurasz University Hospital in Bydgoszcz. The *S. epidermidis* strains were tested for induction to L-forms on Tryptic Soy Agar, TSA (BBL). Next, the bacteria were suspended in Tryptic Soy Broth, TSB (BBL) to a turbidity approximating 0.5° on the McFarland scale and were incubated for 2 hours. In order to induce L-forms, 0.05 ml of TSB suspension was added to 4.95 ml of Brain Heart Infusion, BHI (Difco) containing 100 U/ml penicillin G (Biochemie GmbH). The samples (0.1ml) were taken after 10 minutes of incubation and again after 24 hours. The samples were plated on BHI agar supplemented with 5% NaCl, 5% sucrose (Polskie Odczynniki Chemiczne, Gliwice), 0.5% yeast extract (Difco), 10% fresh horse serum and 100 U/ml penicillin G. All cultures were incubated at 35ºC. The plates were checked for presence of L-form colonies for 8 days (Jakubczak *et al*., 2002; Owens, 1988). Homogenous growth of colonies which had irregular areas, “fried egg” shapes with centrally located core growing above the colony surface was taken as positive results.

In comparison with the parental strains colonies, the colonies of *S. epidermidis* L-forms were larger than the vegetative cells they had been derived from. In this study only cultures of L-forms colonies with “fried egg” shapes, irregular areas, centrally located core growing above the surface of the colony were taken into consideration. After 10 minutes and 24 hours of incubation in BHI with penicillin 11.1% and 25.0% strains of *S. epidermidis* transformed into L-forms, respectively. The same strains of L-forms grew after 10 minutes

Table I
The number of *S. epidermidis* strains of inducing L-forms

Source of isolation	Number of strains producing L-forms	
	isolated after 10 min of exposure to penicillin	isolated after 24 h of exposure to penicillin
Urine (n = 12)	1	4
Blood (n = 12)	2	2
Biomaterials (n = 12)	1	3
In general	4	9

n – number of strain

and 24 hours of incubation in BHI with penicillin. Table I shows the frequency of the induction of *S. epidermidis* L-forms from the strains of blood, urine and biomaterial.

The L-forms of bacteria have not been carefully studied yet, because it is difficult to characterize them by light microscopy. They do not grow on common media, but they can be cultured on hypertonic medium containing fresh horse serum and penicillin (Jakubczak *et al*., 2002; Owens, 1988). Dominique *et al*. (1993) isolated L-forms of *Staphylococcus haemolyticus* and *Streptococcus agalactiae* from a 22-year old woman with haematuria and routine culture-negative urine. They drew the conclusion that L-forms of bacteria were present in the genitourinary tract of the patient and caused idiopathic haematuria after antibiotic treatment. Ataoglu *et al*. (1994) cultured L-forms of coagulase negative staphylococci, for example *S. epidermidis*, from patients with secretory otitis media who had been treated by cefaclor or a combination of ampicillin and sulbactam. However, there are no reports available about of the intentional *in vitro* induction of L-forms of *S. epidermidis*. It appears that the cause of phenomenon is the fact that *S. epidermidis* has been considered as pathogen in clinical cases in the 1980s and most of the studies of L-forms induction took place in 1960s and 1970s (Banville, 1964; Rosdahl and Vejlsgaard, 1970 and Simon and Yin, 1970). These studies showed that the strains of *S. epidermidis* which are penicillin resistant were capable to induce L-forms. Similar studies with the use of strains of *S. aureus* producing penicillinase were carried out by Rosdahl and Vejlsgaard (1970); Simon and Yin (1970). They concluded that it is possible that strains of *S. aureus* producing penicillinase induced L-forms. So it was supposed that the mechanism of bacterial resistance does not have an influence on the bacteria changing into L-forms. Jakubczak *et al*. (2002) and Owens (1988) proved that most of strains of *S. aureus* changed into L-forms after 10 minutes incubation in BHI with penicillin. Our studies suggest that the period of 24 hours is more optimal to induce L-forms of *S. epidermidis* strains. The origin of strains of *S. epidermidis* did not play a significant role in changing bacteria into L-forms. It was also proved by *Jakubczak et al*. (2002) in their studies of *S. aureus*. However, this problem requires further studies with the strains of *S. epidermidis* that cause clinically proved urinary tract infections, bacteriemia and shunt and catether-related infections.

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