None of the AME's tested was found to display an ideal enzymic behaviour over the whole concentration range of all substrates tested; all AME's suffered more or less from substrate inhibition. APH(3′)-I and APH(3′)-II could not be distinguished from each other since kanamycin and neomycin were phosphorylated very well, amikacin only very badly, and the other aminoglycosides not. According to the literature only butirosin should discriminate between these types. For clinical practice such a discrimination is thought irrelevant. Since APH(2″) modifies all the kanamycin and gentamicin derivatives tested, but not neomycin (which lacks an identical 2″-hydroxy group), APH(3′) will be recognized easily. Discrimination between ANT(2″) and ANT(4′) should be easy since ANT(4′) can not modify gentamicin derivatives in contrast to ANT(2″). Until recently, subtyping of ANT(2″) seemed unnecessary, but recently Coombe and George (1981) claimed the existence of an ANT(2″)-II. Their conclusions are incomplete, since the reference ANT(2″)-I had not been included in their tests. A clear distinction between the reference AME's AAC(3)-I, -II and -IV, AAC(6′)-I and AAc(2′) could be made now by comparing the sets of eight activity curves. This was demonstrated by the fact, that an AAC present in a *Proteus mirabilis* strain and received as an AAC(2′), was unmistakably typed as an AAC(3)-I. Also the AAC(6′)-I differed strongly from the staphylococcal AAC(6′).

As expected these sets of activity curves offer better characteristics with regard to the substrates than the conventional substrate profiles do. Probably these sets will also be very helpful in the elucidation of the relation between enzymatic activity and MIC-increase.

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Cell surface hydrophobicity of *Staphylococcus* species and adhesion onto biomaterials

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Infections associated with biomaterials (prosthetic heart valves, vascular grafts, indwelling catheters, etc.) in the early post-operative phase are mostly caused by coagulase-negative staphylococci (Garvey, 1980). Adhesion of bacteria onto these materials might be the first step in these infections (Moore et al., 1980; Sugerman, 1982).

Table 1. Bacterial affinity towards xylene and adhesion onto FEP-teflon and cellulose acetate (CA).

Species	Treatment	Xylene affinity	Number of adhering bacteria ($\times 10^3$/mm^2 ± S. D.) onto	
			FEP	CA
S. epidermidis	none	high	52 ± 17	0.6 ± 0.5
(strain 1)	trypsin	high	68 ± 8	0.4 ± 0.3
	chymotrypsin	high	47 ± 6	0.5 ± 0.4
	pepsin	low	1.4 ± 0.3	0.2 ± 0.2
S. epidermidis	none	high	24 ± 6	0.6 ± 0.6
(strain 2)	SIC penicillin	high	23 ± 6	1.5 ± 0.8
	aqueous phenol	moderate	12 ± 1	ND
S. saprophyticus	none	low	0.3 ± 0.2	0.2 ± 0.2

ND, not determined; bacterial cell concentration 10^9 cells/ml; exposure time 2.5 h.

In the present study adhesion of three strains of coagulase-negative staphylococci onto different materials was investigated and related to the cell surface hydrophobicity of the bacteria. The bacteria were characterized according to Kloos and Schleifer (1975). Two strains identified as *Staphylococcus epidermidis* were non-encapsulated and one strain identified as *Staphylococcus saprophyticus* was encapsulated. Late logarithmic-phase bacteria grown in Trypticase soy broth (TSB) were suspended in phosphate-buffered saline (0.113 M, pH 7.2; PBS) to a final bacterial cell concentration of approximately 10^9 cells per ml. In some experiments the bacteria were pretreated with proteolytic enzymes for 1.5 h at 37°C (0.1% w/v trypsin or chymotrypsin in PBS, pH 8.0, or 0.1% w/v pepsin in citrate-buffered saline, 0.2 M, pH 3).

To obtain lipoteichoic acid (LTA)-deficient bacteria, the penicillin-sensitive *S. epidermidis* (strain 2; minimal inhibitory concentration (MIC) of penicillin of 0.02 U/ml broth) was grown in TSB containing a sub-MIC (SIC) of penicillin of 0.005 U/ml, or grown in TSB overnight and extracted with hot aqueous phenol following the method of Westphal et al. (1952).

The cell surface hydrophobicity of the bacteria was determined by measurement of the affinity of the bacteria towards xylene in a water–xylene two-phase system according to the method described by Rosenberg et al. (1980).

In the adhesion experiments two different polymeric films were used: hydrophobic FEP-teflon films (Du Pont de Nemours, Geneva, Switzerland) and non-hydrophobic cellulose acetate films (CA; Fabelta S.A., Tubize, Belgium; $\overline{M}_n$ = 53000, degree of substitution 2.49, 40.1% acetyl; cast from a 10% w/v solution in acetone). Specimens (7 cm^2) of the films were exposed to the bacterial suspensions in PBS (3 ml) for 2.5 h at 37°C, washed eight times with PBS (3 ml) and the adhering bacteria were fixed with 4% (w/v) glutaraldehyde in PBS and counted microscopically (six areas of 0.05 mm^2 in duplicate experiments).

The affinities towards xylene of the non-treated and treated bacteria are given in Table 1. A high affinity implies a hydrophobic bacterial cell surface. The adhesion of *S. epidermidis* (strain 1) onto CA was much less than onto FEP (Table 1). Repeated rinsing of the CA films with PBS significantly reduced the numbers of adhering bacteria, while after repeated rinsings of the FEP films the numbers of the adhering bacteria remained stable. The encapsulated *S. saprophyticus* adhered only slightly onto both films. Stationary-phase, heat-killed or formaldehyde-killed *S. epidermidis* and *S. saprophyticus* showed comparable adhesion behaviour as viable log-phase bacteria onto FEP and CA, indicating that growth phase or viability of the bacteria are not important in the adhesion process. Treatment of *S. epidermidis* (strain 1) with pepsin strongly reduced the adhesion of the bacteria onto FEP as compared to that of trypsin- or chymotrypsin-treated and non-treated bacteria. *S. epidermidis* (strain 2) treated with aqueous phenol showed lower adhesion numbers onto FEP

as compared to non-treated bacteria, while adhesion of *S. epidermidis* (strain 2) grown in TSB with SIC of penicillin onto both FEP and CA was unchanged. Separately, it was found that EDTA did not interfere with the adhesion of *S. epidermidis* onto FEP, which indicates that divalent cations are not essential in the adhesion process.

Comparison of both *S. epidermidis* strains and *S. saprophyticus* in the xylene-affinity test showed that *S. saprophyticus* has a more hydrophilic cell surface due to the presence of a capsule. Proteinaceous material present at the surface of *S. epidermidis* is probably digested by pepsin treatment leading to an increased hydrophilicity of the bacterial cell surface. Trypsin or chymotrypsin treatment of the bacteria had no effect. Extraction of LTA and other cell wall components of *S. epidermidis* by aqueous phenol resulted also in a more hydrophilic bacterial cell surface. Reduced adhesion of bacteria onto FEP is paralleled by an increased cell surface hydrophilicity of the bacteria.

The adhesion of bacteria onto polymeric substrates is related to the surface tensions of the bacteria involved and the substrates used. In the present system the adhesion of bacteria onto polymeric films can be described by the thermodynamic approach of Absolom et al. (1979) for the adhesion of cells in a liquid medium to various substrates with various surface tensions.

The surface tension of *S. epidermidis* is less than that of the suspending liquid (PBS), and an increased adhesion onto FEP is predicted in comparison to that onto less hydrophobic CA. The surface tensions of the more hydrophilic *S. saprophyticus* and pepsin-treated or aqueous phenol-treated *S. epidermidis* might be close to the surface tension of the suspending liquid, which explains the low adhesion of these bacteria onto both substrates.

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Haemolytic activity of *Proteus mirabilis* strains

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Young broth cultures of all *Proteus mirabilis* strains tested exhibited haemolytic activity. This activity seemed to be strongly cell-associated as only a very small fraction of this activity was found in the cell-free supernatant fluid.

The haemolysin was only produced by actively growing cells. Inhibition of the haemolytic activity by chloramphenicol and trypsin suggested that the haemolysin is of protein nature. Lecithin and