

from the birth canal or the respiratory tract of those handling the baby after delivery, when colonisation would be favoured in the presence of poorly functioning nasolacrimal drainage,⁶ or both, or even overgrowth in the increased fluid formed due to any irritation—for example, excess antiseptic applied to the maternal perineum before or during delivery or inflammation caused by other well recognised pathogens.

In the spectrum of bacterial isolates the reported incidence of α haemolytic streptococci has varied between 11–62.3%,^{1 4 5 7 8} and this wide variation adds to the problem of establishing a direct pathogenic role. From our study, however, speciation does not seem to have had a direct bearing on clinical management as our isolates seem to be non-pathogenic or of a very limited pathogenicity, and treatment does not seem to be required.

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Clinical importance of production of slime by coagulase negative staphylococci in chronic ambulatory peritoneal dialysis

Coagulase negative staphylococci are an important cause of infections associated with foreign bodies, but deciding whether a particular isolate is responsible for infection or is merely a contaminant can be difficult. Unsuccessful attempts have been made to find a laboratory marker which would correlate with the clinical importance. Recently, production of slime by *Staphylococcus epidermidis* was shown to promote adherence to prosthetic devices,¹⁻³ and it has been postulated that the slime substance may protect the organism against host defences.⁴ To investigate the importance of production of the slime in vivo we examined episodes of peritonitis caused by coagulase negative staphylococci in patients undergoing chronic ambulatory peritoneal dialysis.

A retrospective analysis of the clinical records of 42 patients was made. All patients were undergoing chronic ambulatory peritoneal dialysis, and coagulase negative staphylococci were isolated from the peritoneal effluent on 115 occasions. Peritonitis was defined as pain or discomfort in the abdomen associated with cloudy peritoneal effluent (> 100 white cells/mm³). When these criteria were applied 36 patients (mean age 52 years) were found to have had 91 episodes of peritonitis. The coagulase negative staphylococci were speciated using API Staph (appareils et procédés d'identification) and further characterised by phage type, biotype, and antibiotic susceptibility pattern. Slime was detected using the method described by Christensen *et al.*¹

Species cultured from the 91 episodes of peritonitis included: *S. epidermidis* (73), *S. haemolyticus* (11), and *S. hominis* (7). Slime was detected in 37 strains (41%), all of which were *S. epidermidis*. When peritonitis recurred caused by the same bacterial strain within three to four days after stopping appropriate treatment with antibiotics the peritonitis was labelled as "recurrent." Strains were considered to be identical if they had the same species, biotype, phage type, and antibiotic susceptibility pattern. Eighteen strains were responsible for recur-

rent peritonitis (two to five episodes) and 45 for uncomplicated peritonitis. There was no increase in the length or severity of peritonitis when slime producing strains of coagulase negative staphylococci were isolated. Recurrent peritonitis, however, was more likely to occur if the strain produced slime (Table); this difference was significant (χ^2 test and Yates' correction = 6.08, $p < 0.02$).

The results of this preliminary survey, show that strains of coagulase negative staphylococci, which produce slime, are more likely to be associated with recurrent peritonitis than strains that do not produce slime. Isolating a productive strain from the peritoneal effluent was associated with a 50% chance of recurrence, compared with only a 17% chance when the isolate did not produce slime. This observation may be related to the superior adherence properties of the strains that produce slime and their ability to encase themselves in a protective matrix of slime substance on artificial surfaces.

Our results suggest that such production may be a useful prognostic marker. Laboratories concerned with the care of patients receiving chronic ambulatory peritoneal dialysis might usefully examine isolates of coagulase negative staphylococci from such patients for production of slime. Whether such advice should be extended to include isolates from infections associated with other prosthetic devices is unclear and requires further study.

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Table Correlation of peritonitis episodes with presence or absence of slime

Peritonitis	Slime	No slime	Total
Recurrent	11	7	18
Not recurrent	11	34	45
Total	22	41	63

Staphylococcus epidermidis to smooth surfaces. *Infect Immun* 1982;37:318-26.

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Commercial strip test for reduction of nitrate by bacteria

The ability of an organism to reduce nitrate is commonly tested by the Griess-Ilosvay method.¹ This entails two reagent mixtures, one of which contains naphthylamine, a potentially harmful chemical. An alternative is the Cook plate method, in which the organism is stab inoculated on a blood agar plate containing nitrate.² The reaction in the plate test is irreversible and is unaffected by any subsequent reduction of the nitrite. The zones of discolouration caused by the formation of methaemoglobin are, however, often indistinct, and the plate may require 48 hours of incubation. As an alternative to these methods we evaluated a commercial strip designed to screen for clinically important bacteriuria by detecting nitrite derived from dietary metabolites (Ames Division, Miles Laboratories). In this test the nitrite reacts with p-arsanilic acid to form a diazonium compound that is coupled with 1,2,3,4-tetrahydrobenzo(h)-quinoline-3-ol to produce a pink colour.

Material and methods

A nitrate broth was prepared containing potassium nitrate (nitrite free) 0.3% and tryptone (Oxoid L42) 0.5% w/v in distilled water. The broth was distributed in 3 ml volumes in bijoux bottles and autoclaved at 115°C for 10 minutes.

Clinical isolates from a representative range of nitrate positive and nitrate negative genera were collected. For the test a broth was inoculated from a fresh subculture to give a density of about 10⁸ colony forming units/ml. After incubation at 37°C the broth was tested for the presence of nitrite at 4 hours and again at 24 hours using the commercial strips. A strip was immersed in the broth, immediately removed, drained on the inside of the container, and read at 40 seconds, as recommended for urine testing by the manufacturer (Technical information, Miles Laboratories). Any degree of pink colour was regarded as a positive test. At 24 hours the broth was tested for nitrate by a modified Griess-Ilosvay method. Broth (200 µl) was pipetted into a microtitre well, and 50 µl of 0.8% sulphanic acid and 0.5% Cleve's acid, dimethyl-α-naphthylamine, added. A pink colour indicated the reduction of nitrate to nitrite. If no colour change occurred the test was repeated in a well containing about 30 mg of zinc dust.³ Development of a pink colour at this stage indicated that the nitrate had not been reduced by the test organism, whereas absence of colour indicated total breakdown of nitrate and nitrite. All strains were tested by the Cook plate method using a paper strip impregnated with potassium nitrate and incubated for up to 48 hours.²

Results

Fifty eight of the 66 strains tested showed concordance of the commercial strip method, Cook plate, and modified Griess-Ilosvay method at 24 hours. The 58 strains included: Nitrate positive (1): *E coli* (5), *Klebsiella* (4), *Haemophilus* (6), *Enterobacter* (2), *Proteus* (4), *S aureus* (5), *Actinomyces* (3), and *Branhamella catarrhalis* (3); Nitrate negative (1): *Listeria* (5), *Bordetella* (2), *Flavobacterium* (2), *Lactobacillus* (5), and *Aanitratum* (12). The Table shows the results given by the other eight strains.

When tested by the strip method 63 of the 66 organisms gave the appropriate nitrate reaction¹ at four hours, and the other three

strains gave the appropriate reaction at 24 hours.

From the results so far obtained we concluded that the strip test is a reliable method for the detection of bacterial nitrate reduction, as most positive tests are readable at four hours. The test avoids the problems of chemical preparation, storage, and handling.

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Human parvovirus specific IgM in antigen positive serum

In 1984 a programme of virological studies on infection with the newly described human parvovirus,¹ now designated B19 virus,² was started in Florence. We report here our first observations.

Stored sera, collected from May to July 1983 for other epidemiological purposes from 221 healthy soldiers assigned to five different units were screened for immunoelectro-osmophoresis reactivity to B19 antibodies. Polyethylene glycol 6000 was added to the gel to increase the sensitivity of the test. The antigen was detected in two subjects stationed in different units. Serum samples of both subjects had low immunoelectro-osmophoresis reactivity. The serum of one subject with mild respiratory syndrome some days before the sampling (incomplete information), was weakly positive for B19 DHA by molecular hybridisation,³ and viral particles of the B19 size and morphology were seen by electron microscopy. Although specific antibodies had not been added, virions and empty particles appeared as aggregates, and cross linking Ig was visible. Precipitating antibodies to B19

Table Nitrate reductase: results for eight bacterial strains using three different methods

Organism	Nitrate reduction test				
	Cook plate		Commercial strip		Griess-Ilosvay test 24 hour
	24 hour	48 hour	4 hour	24 hour	
Coagulase negative <i>Staphylococcus</i>	-	+	+	+	+
Coagulase negative <i>Staphylococcus</i>	-	+	+	+	+
<i>Acinetobacter lwoffii</i>	-	-	+	+	+
<i>Acinetobacter lwoffii</i>	-	+	-	+	+
<i>Pseudomonas aeruginosa</i> (four strains)	+	+	+	-	+