Case report

A 67 year old woman presented with a two week history of fever, weight loss, malaise and increasing shortness of breath. There were no predisposing factors for endocarditis; she was pale with severe dyspnoea. Her pulse was 120 and temperature 38°C. Splinter haemorrhages were evident but no other peripheral stigmata of endocarditis were present. She had the murmurs of mixed mitral valve disease. Staphylococci were isolated after 24 hours' incubation from six sets of blood cultures. All the isolates were penicillin sensitive and all showed positive clumping factor in the slide test, were Staphaurex positive, but tube coagulase was negative. Biochemical tests were carried out.³ The closest identification was as *S hominis* or SV in the Baird Parker 1963 scheme, but unusual results were seen with very strong DNase activity, positive phosphatase, and ornithine decarboxylase. Acetoin was positive but acid production from mannitol and ribose was absent.

The table⁵ compares the standard biotyping profile of the isolate with those of closely related staphylococcal species: *S hominis*, *S intermedius*, and *S aureus*.

Echocardiography showed a thickened aortic valve compatible with endocarditis. Initial treatment consisted of intravenous benzyl penicillin 1.2 g two hourly and gentamicin 80 mg twice a day. Both a deterioration in cardiac and renal function and multiple emboli necessitated emergency mitral valve replacement.

At operation there was gross mitral valve destruction and vegetations, and a Carpentier Edwards prosthesis was inserted. The valve was sterile.

Postoperative treatment was changed to benzyl penicillin 1.2 g five times a day and rifampicin 600 mg twice a day (in vitro synergy was demonstrable). She made a good recovery and after two weeks was changed to oral amoxycillin 1 g three times a day for four further weeks. Peak and trough serum bactericidal titre were adequate (1/64, 1/16, respectively). She was discharged and remained well on follow up at five months.

Comment:

The anomalous results of tube coagulase, clumping factor and Staphaurex latex agglutination and the clinical course strongly suggest an association with *S aureus*. The strong DNAse production also supports this, but the lack of acidification of mannitol by two methods, combined with ribose negativity and the positive maltose and Voges Proskaver reaction, prevent identification as either *S aureus* or *S intermedius*. The carbohydrate reactions suggest *S hominis*.

Overall, the results would be compatible with an anomalous variety of the *S aureus*/*S intermedius*/*S hyicus* species group.

This case raises the question of which tests should be used in the routine laboratory to identify staphylococci. Tube coagulase testing alone may misidentify strains that share characteristics of different staphylococci. We therefore feel that clinically important staphylococcal isolates should also be identified

by clumping factor tests, irrespective of their coagulase reaction. The Staphaurex latex agglutination method is more sensitive than the slide technique⁴ and in this case would have highlighted the anomaly. We believe that isolates of coagulase negative staphylococci from patients with serious sepsis deserve detailed identification so that the epidemiology and natural history can be further elucidated.

One case is merely interesting, two form an anecdote, and three a series, so we ask for contributions.

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Glycocalyx in virulent and avirulent strains of *Shigella flexneri*

External polysaccharidic coats (glycocalyx) in bacteria have been largely overlooked

Table Biotyping profile⁵ of blood culture isolate compared with those of closely related staphylococci

Test	Blood culture isolate	<i>S hominis</i>	<i>S intermedius</i>	<i>S aureus</i>
Acid (aerobically) from:				
Arabinose	-	-	-	-
Lactose	+	v	+	+
Maltose	+	+	-	+
Mannitol	-	-	+	+
Fructose	+	+	+	+
Ribose	-	-	+	+
Sucrose	+	+	+	+
Trehalose	+	+	+	+
Xylitol	-	-	-	-
Phosphatase	+	-	+	+
Acetoin (VP)	+	+	-	+
Thermolabile DNAase	+	-	+	+
Nitrate reduction	+	v	+	+
Ornithine decarboxylase	+	-	-	-
Coagulase human plasma	-	-	v	+
Coagulase rabbit plasma	-	-	+	v
Clumping factor	+	-	+	+
Staphaurex	+	-	+	+

Key: + positive, - negative, v variable

Letters to the Editor

they are not demonstrable by conventional electron microscopy methods and because they are lost *in vitro* by repeated subcultures. They can be stabilised before being prepared for transmission electron microscopy examination, however, and with this procedure the glycocalyx of several bacterial species has been shown. Their presence has been related to high virulence, resistance to the bacterial action of serum,¹ and anti-phagocytic activity² as well as to invasiveness of bacteria.³ In this study we looked for the presence of glycocalyx in an avirulent strain of *Shigella flexneri*, using the virulent strain as a control.

A virulent strain of *S flexneri* was isolated from a patient with dysentery and identified biochemically and serologically. The avirulent derivative was obtained from the original isolate throughout the Witkowska and Mulczyk procedure.⁴ Virulence was repeatedly assayed by both the kerato-conjunctivitis test in guinea pigs and rabbit small intestine, isolated by ligature. A battery of frozen preparations of both strains was made after the initial growth was obtained in modified Luria broth and stored at -20°C . For electron microscopy analysis both bacterial strains were cultured in modified Luria broth and further processed according to the procedure originally described by Lam *et al* and modified by Mora Galindo *et al*.⁵ Sections were photographed without contrast with a Zeiss EM-10 electron microscope and the glycocalyx thickness of virulent and avirulent strains was measured. The significance of data was determined by an analysis of variance.

The exopolysaccharidic coat stained with ruthenium red was recognised as a heavy electron dense layer outside the outer membrane; there were no morphological differences when the glycocalyx of virulent strains was compared with their avirulent derivatives (figure); both bacterial strains showed a homogeneous and continuous electron-dense layer adhered to the outer membrane, as well as fibrillar projections arranged in a patchy pattern. There were no significant differences in relation to glycocalyx thickness between virulent and avirulent bacteria (table).

Figure Outer membrane of virulent *S flexneri* is covered by electron dense layer: two zones are observed: a continuous basal one (white arrow) and patched peripheral fibrillar projections (black arrow). Bar = 200 nm. Insert. Glycocalyx of avirulent variant.

In *Vibrio vulnificus* a clear relation between virulence and the presence of glycocalyx has been found; avirulent strains are morphologically devoid of glycocalyx, or it was decreased in thickness; but virulent strains possess a prominent one.¹ In this study the external polysaccharidic coat in a recently isolated virulent strain of *S flexneri* was similar in morphological appearance as well as in thickness to that of the avirulent derivative, so it would seem that this structure was not related to virulence. In both strains of *S flexneri* (virulent and avirulent) several kinds of modifications in chemical composition of the external polysaccharidic coat can occur, but the nature and extension of such changes cannot be determined simply by electron microscopic observation of bacterial cells stabilised with anti-O antibodies and stained with ruthenium red. Despite this we have shown that the presence of glycocalyx does not seem to be involved in virulence as is the case with *Vibrio vulnificus*.

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Table Glycocalyx dimension in *Shigella flexneri* stabilised with anti-O antibodies

		<i>S flexneri</i> cells	
		virulent (n = 15)	avirulent (n = 15)
Glycocalyx zone (region)	Mean (SD)	14.74 (3.93)	14.60 (2.95)
Amorphous (nm) basal		30.18 (8.28)	31.86 (8.14)
Projections (nm) (peripheral)			