Coagulase-negative strains of *Staphylococcus pyogenes*

R. BAYSTON

From the Department of Bacteriology, Children's Hospital, Sheffield

SYNOPSIS The isolation of two coagulase-negative strains of *Staphylococcus pyogenes* is reported, and their biochemical characteristics are given. The reliability of coagulase tests alone in the identification of *Staph. pyogenes* is discussed, and the deoxyribonuclease test is recommended as a useful confirmatory test.

Two strains of staphylococci have recently been isolated in this laboratory, which had the biochemical characteristics of *Staphylococcus pyogenes* (Baird-Parker, 1963) but failed to produce either free or bound coagulase. Both strains were phage-typable using routine phages.

The first strain (A) was isolated from a hydrocephalic child of 3 months with a ventriculo-atrial shunt. It was suspected that she had septicaemia secondary to colonization of the shunt system, and blood cultures grew a staphylococcus which was coagulase-negative by both slide and tube methods. Sensitivity tests by the disc method (Multidisc, Oxoid) showed that the organism was sensitive to chloramphenicol and gentamycin, and resistant to penicillin, tetracycline, streptomycin, erythromycin, sulphonamides, and trimethoprim-sulphamethoxazol (Septrin). Further tests on salt agar showed that it was also resistant to methicillin. The organism was

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again isolated from later blood cultures, from fluid aspirated from the Spitz-Holter valve, from the ventricular fluid, and from all the components of the shunt system after it had been removed.

The second strain (B) was isolated from the urine of a male patient aged 4 years who had had a small sacral meningocele repaired. He was incontinent of urine, but otherwise normal. The organism was isolated from two consecutive specimens of urine. In each case there were between 20 and 25 white blood cells per high-power field in the centrifuged deposit and the organism was present as a heavy pure growth, the viable count being 10^7 orgs/ml. This strain was resistant to penicillin only.

Investigation of the Staphylococci

All strains of coagulase-negative staphylococci isolated from lesions in this hospital are examined biochemically as part of a study of the problem of

Test	Staph. pyogenes Subgroup SI	Strain A	Strain B
Catalase	+	+	+
Acid from glucose anaerobically	+	+	+
Pigment	Variable, typically golden	Golden	Grey
Coagulase (see text)	+	-	-
Phosphatase production	+	+	+
Acetoin production	+	+	+
Acid from arabinose	-	-	-
Acid from galactose	Variable	+	+
Acid from inositol	-	-	-
Acid from lactose	+	+	+
Acid from maltose	+	+	+
Acid from mannose	+	+	+
Acid from salicin	-	-	-
Acid from mannitol anaerobically	+	+	+
Gelatin liquefaction	Variable	-	-
DNA-se production	+	+	+
Fibrinolysin production	+	+	+
Phage type	+	77	52/52A/80/81

Table Comparison of characteristics of strains A and B with *Staph. pyogenes*

shunt colonization, and the above organisms were included in this study because of the coagulase results. Biochemical tests indicated that both organisms belonged to subgroup SI (*Staph. pyogenes*) (Baird-Parker, 1963), and both organisms produced deoxyribonuclease (DNA-se), so attempts were then made to detect coagulase. First, the plasma was checked and found to give typical coagulation with control strains. Strains A and B were then inoculated into a series of 10% plasma broths kept at 37°C, 30°C, room temperature, and +4°C for five days, the broths being examined daily. No clot was formed. The organisms were then inoculated onto plasma-agar plates (Lack, 1956) and incubated at 37°C for two days. A reduced zone about half the size of that of the control was produced. Neither strain produced more fibrinolysin than control organisms.

A culture of strain A was sent to Dr M. T. Parker at the Cross-Infection Reference Laboratory, Colindale, who kindly agreed to investigate the organism. Dr Parker's results indicated that the organism was *Staph. pyogenes* and confirmed that it was coagulase-negative. The results of biochemical and other investigations are summarized in Table I.

Discussion

Mutants of *Staph. pyogenes* resistant to one or more common antibiotics and which were coagulase-negative have been described previously (Klimek, Cavallito, and Bailey, 1948; McVeigh and Hobdy, 1952; Massieu, 1960). However, Brown and Evans (1963) found that their antibiotic-resistant strains were coagulase-positive and suggested that their strains arose as spontaneous mutants with genetic change affecting only loci pertaining to resistance, whereas the mutants described by other workers were produced as a result of exposure to toxic levels of antibiotics, ultraviolet irradiation, or chemical mutagens and probably suffered multiple genetic damage. One of the two strains described here, strain A, showed multiple resistance, but was capable of producing pigment, and generally had all the characteristics of *Staph. pyogenes* except, of course, production of coagulase. Other strains of methicillin-resistant *Staph. pyogenes* isolated in this hospital have been coagulase-negative when tested by the slide test, but have produced free coagulase rapidly in the tube test. They have all belonged to phage type 77. Strain A is not known to have been exposed to mutagens of any sort, and was isolated before treatment was begun.

Until recently, coagulase production was the only criterion used in this laboratory for the identification of staphylococci as *Staph. pyogenes*. However, with the isolation of these two coagulase-negative strains,

it became obvious that a confirmatory test was required.

Mannitol fermentation has been used by some workers to indicate pathogenicity (Mossel, 1962; Branson, 1968), but some coagulase-positive strains fail to attack this substrate (Mossel, 1962; Person, Yü, and Washington, 1969) and a few strains of the non-pathogenic subgroup SVI were found by Baird-Parker (1963) to ferment it. Members of this subgroup, and some micrococci, usually attack mannitol oxidatively, and anaerobic conditions are essential if media containing mannitol are to be used.

Tests for phosphatase production were recommended by Baird-Parker (1965) to be used in conjunction with coagulase tests, but his subgroups SII, SIII, and M6 produce phosphatase and thus a coagulase-negative strain of *Staph. pyogenes* could be wrongly identified as *Staph. albus*.

As both organisms gave small zones of opacity on plasma-agar plates, the use of these was considered. However, several anomalous results were obtained when the method was tested with various staphylococci, and it was decided that the plate-coagulase test was unsatisfactory for routine use.

The test for DNA-se production was found to be both more specific and more convenient in use. Brandish and Willis (1970) found that all coagulase-positive staphylococci isolated by them were DNA-se positive, and only two out of 596 coagulase-negative strains were positive. One of these was investigated in this laboratory and was found to belong to subgroup SIII (Baird-Parker, 1963). Similar results were obtained by other workers (Jacobs, Willis, and Goodburn, 1964; Jarvis and Wynne, 1968; Stickler, 1970).

All staphylococci isolated in this laboratory are now tested for DNA-se production as well as for coagulase production. A few strains of *Staph. albus* produce zones of clearing on the plates, but these zones are much smaller than those produced by *Staph. pyogenes* and are easily distinguished. It is suggested that this system provides a reliable safeguard against failure to recognise *Staph. pyogenes* and that this may apply particularly when multiple resistant strains are being examined.

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