Infection with *Streptococcus agalactiae* in a London hospital

F. S. MHALU

From the Department of Bacteriology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12

SYNOPSIS *Streptococcus agalactiae* is presently the commonest β -haemolytic streptococcus isolated from clinical material in this hospital. Between October 1974 and March 1975, 81 patients with such infections were seen. Seventeen had urinary tract infections, six had septicaemia, and one neonate had meningitis. Three of those with septicaemia were neonates and two died. The organism was also found to be a cause of pyogenic skin conditions in five patients. Isolates from throat swabs in 12 patients, from sputum in four, and from the female genital tract in 18 were considered part of the normal flora. Human strains of *Str. agalactiae* were found to be biochemically different from animal strains.

Streptococcus agalactiae (β -haemolytic streptococcus Lancefield group B) is classically known as a cause of mastitis in cattle. Only after the development by Lancefield (1933) of her method for grouping streptococci was human colonization and infection by this organism recognized. Although not yet universally accepted, workers in the United States of America and the Netherlands recognize that the varieties of *Str. agalactiae* found in man are biochemically, physiologically, and serologically different from those causing bovine mastitis (El Ghoroury, 1950; Brown, 1953; Butter and De Moor, 1967). Human infection, unless due to direct exposure to infected animals or their products, is acquired from other human sources. However, cross infection between man, except in cases of neonates acquiring the organism in passage through the birth canal, has not been demonstrated, and most cases arise from autoinfection.

As a result of observing that *Str. agalactiae* was currently the commonest β -haemolytic streptococcus isolated from clinical material in this hospital, a prospective study was undertaken to establish the type of patients involved. It was also decided to test whether the strains isolated from patients in the United Kingdom conformed to the biochemical criteria characteristic of *Str. agalactiae* from human material as described in other countries.

Material and Methods

In the six months from October 1974 to March 1975

a record was kept of all specimens from which Lancefield group B streptococcus was isolated. All such strains isolated during the period were kept for biochemical and antibiotic sensitivity tests. Thirteen strains of *Str. agalactiae* of animal origin, kindly made available by Weybridge Central Veterinary Laboratories, were studied in parallel with the human strains for comparison. The following tests were carried out on all the strains:

LACTOSE AND SALICIN FERMENTATION

1% peptone water sugars with Andrade's indicator were inoculated with each of the strains and incubated for up to seven days.

HIPPURATE HYDROLYSIS

The method of Hare and Colebrook (1934) was used.

CAMP TEST (Munch-Petersen and Christie, 1947)

4% sheep blood agar plates were inoculated in a line along the middle with a β -haemolysin producing strain of *Staphylococcus aureus*. Up to 10 strains per plate of the *Str. agalactiae* were each streaked in a line on either side and at right angles to the *Staph. aureus*; a gap of about 2 mm was left between the medial ends of the streptococcus streaks and the staphylococcal inoculum. Plates were then incubated aerobically overnight and inspected for the presence of 'arrowheads' of complete haemolysis, as shown in the figure. Such haemolysis did not occur when the plates were incubated anaerobically.

PIGMENT PRODUCTION

Strains were streaked on plates of Columbia agar

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Figure Camp test: (1) 'Arrowhead' shaped zone of complete haemolysis; (2) Camp positive *Str. agalactiae* strains; (3) Camp negative strain; (4) β -haemolysin producing *Staph. aureus*.

base (Oxoid), which contains 0.1% starch, and incubated overnight using an anaerobic system employing the Gas-Pak, as described by Fallon (1974).

HAEMOLYSIS

Since *Str. agalactiae* does not produce streptolysin O, the ability to produce haemolysis was investigated using 4% horse blood agar plates incubated aerobically overnight.

ANTIBIOTIC SENSITIVITY TESTS

Sensitivity tests were done using tetracycline/penicillin ditch plates containing 5 μ g/ml ampicillin and 25 μ g/ml of tetracycline in the ditches. The Oxford strain of *Staph. aureus* was used on each plate as a control. Disc diffusion tests to bacitracin were done on 4% lysed horse blood agar plates by the method of Stokes, using Oxoid discs containing 0.05 unit of bacitracin. A standard bacitracin-sensitive *Streptococcus pyogenes* was used as a positive control.

ASSESSMENT OF SIGNIFICANCE OF ISOLATION

Whenever *Str. agalactiae* was isolated from a specimen which is normally sterile, for example from blood or cerebrospinal fluid, its significance is not in doubt. Isolation from other sites not normally sterile was assessed as significant on finding the streptococcus in pure culture and associated with the presence of pus cells, thus providing evidence of

inflammation. Urinary tract infection due to *Str. agalactiae* was diagnosed when a pure growth of more than 10^5 colony-forming units of *Str. agalactiae* per millilitre of mid-stream urine was found in association with pyuria. This was defined as the presence of more than 5 pus cells per high-power field of microscopy of deposit obtained by centrifuging 10 ml of well-mixed urine.

Results

In six months, 99 isolates of *Str. agalactiae* were obtained from 81 patients. The patients' sex, type of clinical specimen, and whether or not the isolates were considered significant are shown in table I. Three of the five patients from whose pus a pure growth of *Str. agalactiae* was isolated were adults, a male and a female with paronychia and a male with pyogenic arthritis. The others were male children, one with burns and the other with a pilonidal sinus wound infection. The patients with sputa from which *Str. agalactiae* was isolated had other recognized respiratory pathogens in the specimens, hence the streptococcus was not considered significant. Although the streptococcus was commonly isolated from patients with sore throats, no significance could be laid on the isolates because so far no conclusive proof has been produced to incriminate *Str. agalactiae* in the causation of sore throat.

Six patients had *Str. agalactiae* isolated from blood cultures. Three were adults and the others were neonates. The three adults with septicaemia included one man with endocarditis and one woman with puerperal pyrexia. The third was a woman with septicaemia complicating carcinoma of the breast with widespread metastases. Two of the three neonates with septicaemia died. In another neonate the organism was isolated from cerebrospinal fluid. She also died in spite of chemotherapy.

No significance was attached to group B strepto-

Type of Specimen	Sex		Significance of Isolate	
	Male	Female	Significant	Insignificant
Pus (wound, abscess, and joint fluid)	14	8	5	17
Sputum	2	2	nil	4
Throat swab	4	9	1 (neonate)	12
Blood	1	5	6	nil
Cerebrospinal fluid	1	nil	1	nil
Vaginal swab	nil	18	nil	18
Urine	nil	17	17	nil
Total	22	59	30	51
	81			

Table I Type of patient specimen from which *Str. agalactiae* was isolated

coccus isolation from the female genital tract except that such isolates were thought to indicate the source of infection of the babies. Seventeen patients, all adult females and mostly with abnormal urinary tracts, were found to have urinary tract infection due to *Str. agalactiae*.

All the 99 human and all the 13 animal strains of *Str. agalactiae* were sensitive to penicillin and the animal strains were also sensitive to tetracycline. However, only 38% of the human strains were sensitive to tetracycline. One per cent of the human strains and 61% (8 out of 13) of the animal strains were sensitive to bacitracin.

Results of the biochemical tests on the 99 human and 13 animal strains are shown in table II. The biotypes of *Str. agalactiae* based on source, and biochemical and haemolytic properties proposed by Brown (1953) and reviewed by Butter and De Moor (1967) are:

Human *Str. agalactiae*

var. *opportunus*, haemolytic, lactose negative, salicin positive

var. *mastitidis*, haemolytic, lactose positive, salicin positive.

Bovine *Str. agalactiae*

var. *mastitidis*, haemolytic, lactose positive, salicin positive

var. *asalignus*, haemolytic, lactose positive, salicin negative

var. *agalactiae*, non-haemolytic, lactose positive, salicin positive.

Discussion

In addition to being one of the common bacterial causes of bovine mastitis, *Str. agalactiae* is now recognized to be part of the normal bacterial flora in the human throat, genitourinary tract, and rectum. Butter and De Moor (1967) found that 10% of normal individuals harbour the streptococcus in the throat, while Baker and Barrett (1973) found up to 30% of normal adult females carried it in the vagina. Christensen *et al.* (1974) found *Str. agalactiae* to be the commonest streptococcus in the male urethra and the female lower genitourinary tract in patients under investigation for gonorrhoea. They concluded that *Str. agalactiae* in women is spread by sexual intercourse. During the past decade most interest in *Str. agalactiae* has centred on infections of the neonate; in some areas this has constituted the commonest cause of neonatal meningitis with a prevalence rate of 65% in 1972 (Yow, 1974). In this country an epidemiological study (*British Medical Journal*, 1973) recorded 14 cases of this condition from the whole of Britain in 1972. However, Reid (1975) found overall rates for neonatal morbidity and mortality of 2.7 and 1.0 per thousand live births respectively. The results of this study, which reports seven serious cases (septicaemia and meningitis) seen in a single hospital over a period of six months, indicate that infections by the organism are more frequent than was previously believed.

The female vagina is known to be the source of infections of the neonate and of auto-infection leading to puerperal pyrexia. Other sources of the organism must be considered in septicaemia and wound infections in the adult male. The throat is a likely source of such infections but this has not yet been shown to be the case. A possible source of septicaemia, especially in the adult female, is the urinary tract. In this study, urinary tract infection was found to be the commonest type of systemic infection with *Str. agalactiae* in the selected group of hospital patients studied.

Biochemical test results in this survey give support to the widely held view that human strains of *Str. agalactiae* differ from animal strains. Whereas the human strains fermented salicin but not lactose and uncommonly fermented both lactose and salicin, the animal strains mostly fermented both sugars and uncommonly fermented lactose but not salicin. Only one of the animal strains was found to give the opposite pattern. Six of the human strains did not ferment either salicin or lactose. Such strains have been recognized before by Butter and De Moor (1967) but have been neglected. It is time that such strains were recognized as a specific biotype. Ninety-three per cent of the human strains produced

Biochemical Test	Positive Reactions	
	Human strains	Animal strains
Hippurate hydrolysis	99	13
CAMP test	99	13
β -haemolysis (small zone)	97	11
α -haemolysis	1	2
Non-haemolytic	1	0
Pigment production	93	3
Salicin fermented, lactose not fermented	81	1
Salicin and lactose fermented	12	10
Lactose fermented, salicin not fermented	nil	2
Salicin and lactose not fermented	6	nil
Total number of strains	99	13

Table II Biochemical reactions of human and animal *Str. agalactiae* strains

Using the above biotyping scheme, 82% of the 99 human strains in this study were *Str. agalactiae* var. *opportunus*, 12% were var. *mastitidis*, and 6% could not be placed in this scheme because they did not ferment either salicin or lactose or they were non-haemolytic. Of the 13 animal strains studied, 10 (77%) were var. *mastitidis*, two were var. *asalignus*, and one could not be biotyped.

pigment overnight; only 23% of the animal strains did so. Pigment production is therefore a potential differential test between animal and human strains. No significant difference in the haemolytic properties of the animal and human strains could be found in this study; whereas 97% of the human strains produced β -haemolysis on horse blood agar, 84% of the animal strains were also β -haemolytic. It can be inferred that *Str. agalactiae*, whether of human or animal origin, is more often haemolytic than not on horse blood agar.

Most of the human *Str. agalactiae* strains in this study were resistant to bacitracin while more than half of the animal strains were sensitive. Thus the test for sensitivity to bacitracin of β -haemolytic streptococci from throat swabs in man maintains its usefulness as a screening method for differentiating *Str. pyogenes* from the commonly found 'non-pathogenic' *Str. agalactiae*. Sixty-two per cent of the human strains were shown to be resistant to tetracycline. This indicates that, like *Str. pyogenes*, *Str. agalactiae* infections should not be treated with tetracyclines unless sensitivity results are available.

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