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Group C streptococci in human infection: a study of 308 isolates with clinical correlations

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SUMMARY

A collection of 308 clinical isolates of β-haemolytic Lancefield group C streptococci was assembled from laboratories in England, Nigeria and New Zealand. Of these, 276 isolates were *Streptococcus equisimilis*, 23 *S. milleri* and nine *S. zooepidemicus*. Isolates of *S. equisimilis* in the African collection, though few, gave higher rates of lactose and raffinose fermentation, aesculin hydrolysis and positive α-galactosidase reactions than those from elsewhere. Erythromycin resistance was found in 1·9% of the English isolates of *S. equisimilis*. Strains from superficial infections accounted for 88% of the collection and were most commonly isolated from the upper respiratory tract, skin or wounds. Amongst the 36 patients yielding isolates from deep sites *S. equisimilis* was found in septicaemia, cellulitis, abscess, peritonitis, septic arthritis, pneumonia, mycotic aneurysm and acute epiglottitis. *S. milleri* was found in abdominal abscesses, peritonitis, pleural empyema and osteomyelitis and *S. zooepidemicus* was found in septicaemia, pneumonia, meningitis and septic arthritis. Within the collection an unselected general catchment of 214 isolates of group C streptococci from the laboratories in Yorkshire showed the following species: from 199 superficial infections 94% *S. equisimilis*, 5% *S. milleri* and 1% *S. zooepidemicus* and 15 patients with deeper, more agressive infections 67, 27 and 6·7% of these species respectively.

INTRODUCTION

Group C streptococci have accounted for 9–17% of β-haemolytic isolates from all clinical sites in recent reports from diagnostic microbiology laboratories in England (Millar, 1984; Morris & Berry, 1985; Barnham, 1987). Reports of bacteraemia to the PHLS Communicable Disease Surveillance Centre indicate that these organisms have been found less commonly in serious infection than organisms of groups A, B and G (Young, 1982; Barnham, 1983).

The Lancefield group C antigen is carried by four particular species of streptococci – *Streptococcus equisimilis*, *S. zooepidemicus*, *S. equi* and *S. dysgalactiae* – but only the first two of these have been certainly found in man; the group C antigen is also carried by some strains of *S. milleri* and occasionally by other α-haemolytic streptococci that may cause human infections. Recent DNA homology

studies have shown close relationships within the cluster of *S. equisimilis*, *S. dysgalactiae*, large colony group G and group L streptococci and, separately, between the pair *S. zooepidemicus* and *S. equi* (Kilpper-Bälz & Schleifer, 1984; Farrow & Collins, 1984).

Significant superficial or invasive infection with *S. equisimilis* is known to occur in man (Portnoy & Reitler, 1944; Benjamin & Perriello, 1976; Colman, Efstratiou & Gaworzewska, 1988; Teare *et al.* 1989). Recent outbreaks of serious infection with the animal-associated *S. zooepidemicus* (Ghoneim & Cooke, 1980; Edwards, Roulson & Ironside, 1988), sometimes complicated by acute post-streptococcal glomerulonephritis (Barnham, Thornton & Lange, 1983) have renewed interest in this group of organisms. However, many reports have described group C streptococcal disease without distinguishing the species involved: this is unfortunate, as the epidemiology, clinical spectrum of disease and immunological responses to infection vary according to the organism responsible.

We assembled an international collection of β-haemolytic group C streptococci from human infection with the following aims: (1) to determine the species and decide the proportion of each in the normal laboratory catchment, (2) to assess the variability of isolates from three continents in laboratory identification tests and (3) to compile the clinical information on patients yielding the various species of group C streptococci. The results are given and discussed in this report.

MATERIALS AND METHODS

Five diagnostic microbiology laboratories in Yorkshire and one each in Nigeria and New Zealand were invited to contribute Lancefield group C β-haemolytic streptococci to a study collection. We also placed a request in the PHLS Communicable Disease Report for isolates of group C streptococci from serious infection and as a result received organisms from a further 13 centres in England. The organisms were isolated from clinical specimens according to the normal diagnostic procedures of the individual laboratories and submitted to us together with brief notes of the clinical details. We obtained more detailed clinical information on selected patients with serious infection by discussion with doctors and a review of the casenotes where necessary.

Candidate cultures of streptococci for the study were received in the Harrogate laboratory in a freeze-dried state or on blood agar slopes in bijou bottles; they were tested without delay for β-haemolysis by overnight aerobic culture at 37 °C in Petri dishes containing Blood Agar Base (Gibco Ltd, Paisley, Scotland) with 5% defibrinated horse blood and were tested for a Lancefield group C reaction with the Streptex grouping Kit (Wellcome Diagnostics, Dartford, Kent); only cultures giving positive results in these tests were included in the study.

Overnight anaerobic cultures of the streptococci at 37 °C on Columbia Agar (Oxoid Ltd, Basingstoke, Hants) containing 5% horse blood were harvested and tested for identification using the API 20 STREP kit (API Laboratory Products Ltd, Basingstoke, Hants). Disk diffusion antibiotic susceptibility tests were peformed in Petri dishes using penicillin (2 units) and erythromycin (5 μg) disks on overnight aerobic cultures of the streptococci at 37 °C on 5% horse blood agar, using the Oxford staphylococcus (NCTC 6571) as a susceptible control.

Twenty-five selected isolates, including 13 from blood cultures, were tested for T-antigens at the Division of Hospital Infection, Central Public Health Laboratory, Colindale according to the system described by Efstratiou (1983).

Certain isolates of *S. zooepidemicus* and *S. equisimilis* from the collection were characterized further using bacteriocin typing, bacteriophage typing, DNA fingerprinting and more detailed antibiotic susceptibility tests; the methods employed and results are given elsewhere (Barnham *et al.* 1987; Skjold *et al.* 1987; Millar, Langdale & Barnham, 1988).

RESULTS

During the period November 1985 to January 1988 a total of 308 assembled streptococcal cultures passed the entry criteria of the study of β-haemolysis on horse blood agar and a positive reaction in tests for the Lancefield group C antigen. These included a collection of 214 unselected, sporadic isolates of β-haemolytic group C streptococci saved from the range of routine diagnostic microbiology specimens tested at Harrogate (72 isolates), Northallerton (52), York (23), Leeds and Wakefield (67). Similar smaller, general collections were contributed during 1986 and 1987 from Wellington, New Zealand (48 isolates) and from Lagos, Nigeria (23 isolates). Twenty-three isolates from a further 13 laboratories in England, including 13 isolates from systemic sites, were received following the request in the Communicable Disease Report.

Identification of the 308 isolates with the API 20 STREP test showed three species: 276 isolates of *S. equisimilis* (90% of the collection), 23 isolates of *S. milleri* (7%) and 9 isolates of *S. zooepidemicus* (3%). All 23 isolates received from Nigeria were *S. equisimilis*; in the collection of strains from New Zealand 40 were *S. equisimilis*, 7 *S. milleri* and 1 *S. zooepidemicus*. In the general catchment of 214 strains from the Yorkshire laboratories 197 were *S. equisimilis* (92·1%), 14 *S. milleri* (6·5%) and 3 *S. zooepidemicus* (1·4%). Of the 23 isolates sent from other centres in England, largely from serious clinical infections, 16 were *S. equisimilis*, 5 *S. zooepidemicus* and 2 *S. milleri*.

The reactions of the 276 isolates of *S. equisimilis* in the API 20 STREP test are shown in Table 1. All isolates fermented trehalose but not sorbitol. In comparison with those from England and New Zealand the isolates from Nigeria, though small in number, appeared more commonly to ferment lactose and raffinose, to give a positive α-galactosidase reaction and to hydrolyse aesculin. The collection of *S. equisimilis* gave 21 different patterns of reactions; most commonly seen were: (*a*) β-glucuronidase, phosphatase and leucine aminopeptidase formation, arginine hydrolysis, ribose and starch fermentation (57% of isolates); (*b*) as (*a*) but with lactose fermented (19%); (*c*) as (*a*) but aesculin hydrolysed (4·3%); (*d*) as (*a*) but hippurate and aesculin hydrolysed (2·9%); (*e*) as (*a*) but β-glucuronidase not formed (2·5%). In the Nigerian collection the above 5 patterns accounted for 15 (65%) of the isolates and 3 patterns were found that were not seen from elsewhere: (*f*) α-galactosidase, β-glucuronidase, phosphatase and leucine aminopeptidase formation, arginine hydrolysis, ribose, starch and lactose fermentation (3 isolates); (*g*) as (*f*) but α-galactosidase and β-glucuronidase not formed (2 isolates); (*h*) as (*f*) but glycogen fermented (1 isolate). In the New Zealand collection patterns (*a*)–(*c*)

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Table 1. *Reactions of 276 isolates of β-haemolytic* Streptococcus equisimilis *in the AP 20 Strep test*

Test	Positive reactions in isolates (number tested) from (%) England (213)	New Zealand (40)	Nigeria (23)	All sources (276)
Alkaline phosphatase	100	100	100	100
Leucine aminopeptidase	100	100	100	100
Arginine dihydrolase	100	100	100	100
Trehalose fermentation	100	100	100	100
Starch fermentation	100	100	100	100
Ribose fermentation	97·7	95	100	97·5
β-Glucuronidase	94	100	87	95
Lactose fermentation	28	18	52	28
Aesculin hydrolysis	10	15	30	12
Glycogen fermentation	3·8	2·5	4·3	3·6
Raffinose fermentation	1·4	0	30	3·6
Hippurate hydrolysis	3·8	0	4·3	3·2
α-Galactosidase	0	2·5	17	1·8
β-Galactosidase	0·5	5	0	1·1
Voges-Proskauer	0	0	0	0
Pyrrolidonyl arylamidase	0	0	0	0
Arabinose fermentation	0	0	0	0
Mannitol fermentation	0	0	0	0
Sorbitol fermentation	0	0	0	0
Inulin fermentation	0	0	0	0

accounted for 35 (88%) of the isolates and 2 patterns were found that were not seen from elsewhere: (*i*) as (*a*) but lactose fermented and ribose not fermented (2 isolates); (*j*) as (*a*) but α-galactosidase and β-glucuronidase not formed, and glycogen fermented (1 isolate). The API 20 STREP system identified the organisms with these various profiles as *S. equisimilis*, group G, group L or as combinations of these with low discrimination and advised that identification should be confirmed by serological tests.

With one exception the nine isolates of *S. zooepidemicus* showed the following pattern of reactions: β-glucuronidase, phosphatase, leucine aminopeptidase and arginine dihydrolase formed, aesculin hydrolysed, ribose, sorbitol, lactose, starch and glycogen fermented; one strain showed trehalose fermentation but no fermentation of ribose. The 23 strains of *S. milleri* gave ten different patterns of reactions; most commonly seen were: (1) Voges-Proskauer test positive, phosphatase, leucine aminopeptidase and arginine dihydrolase formed, aesculin hydrolysed, lactose, trehalose and starch fermented (26% of isolates); (2) as (1) but starch not fermented (17%); (3) as (1) but arginine dihydrolase not formed (13%).

In T-antigen testing of 25 selected strains at Colindale all 9 isolates of *S. zooepidemicus* were non-typable. Typing of 16 isolates of *S. equisimilis* showed 4 of type T301, 4 of T305, 3 of T7, 2 of T202 and 1 each of types T4, T204 and T301/305; the strain of T-type 4 was from New Zealand, the others all from

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Table 2. *Species of group C streptococci from 36 deep clinical infections*

(Percentage of the total shown in parentheses.)

Condition	Principal diagnostic specimen	Number of patients yielding		
		S. equisimilis	*S. zooepidemicus*	*S. milleri*
Abscess	Pus	6	—	4
	Blood	1	—	—
Bursitis	Pus	1	—	—
Epiglottitis	Blood	1	—	—
Meningitis	Csf, blood	—	1	—
Mycotic aneurysm	Blood	1	—	—
Osteomyelitis	Pus	—	—	1
Peritonitis	Pus	1	—	1
Pleural effusion	Aspirate	—	—	1
Pneumonia	Blood	1	1	—
	Lung	1	—	—
Septicaemia	Blood	7	2	1
Septic arthritis	Aspirate	1	1	—
	Blood	1	1	—
Totals		22 (61%)	6 (17%)	8 (22%)

England. Eight of these isolates, displaying five different T antigens, were from blood cultures. There appeared to be no special relationship between T-types and particular API 20 STREP profiles.

Antibiotic disk tests showed that all isolates in the collection were susceptible to penicillin but 4 isolates of *S. equisimilis*, all from England, were resistant to erythromycin (1·9 % of the isolates of that species from that country).

The clinical sources of the collected streptococci are shown in Tables 2 and 3. Isolates from Nigeria included 19 from the upper respiratory tract, 3 from skin and wounds and 1 from the vagina; the collection from New Zealand included 5 from blood cultures, 3 from abscesses and 40 from various superficial sites.

Table 2 shows the sources in 36 patients with deep clinical infection. Of the 22 (61 %) with *S. equisimilis* infection the organisms was present in the blood cultures of 12; septicaemia was associated with cellulitis of the legs (2 patients), acute sinusitis, conjunctival infection, cot death, and neutropenia in a patient with lymphoma (1 each); abscesses yielding this organism were in the brain, lumbar vertebrae, axilla, appendix and in ischio-rectal and subcutaneous positions. Of the 6 patients (17 %) infected with *S. zooepidemicus* the organism was found in the blood cultures of 5; septicaemia was associated with pregnancy in 1 patient and with cellulitis of the groin in another who was immunosuppressed after receiving a renal transplant; septic arthritis of the knee occurred in 2 patients, acute meningitis in 1 and fatal pneumonia, septicaemia and post-streptococcal glomerulonephritis in another. Of the 8 patients (22 %) infected with *S. milleri* the organism was found in the blood culture of 1 with abdominal pains and fever; abscesses yielding this organism were in the peritoneum, buttock, perineal and perianal positions.

Table 3 shows the sources of group C streptococci in 272 patients with superficial infections. *S. equisimilis* was found in 254 (93·4 %), most commonly in infections of the upper respiratory tract, skin and wounds; 25 patients with clinical infection

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Table 3. *Species of group C streptococci from 272 superficial infections*

(Percentage of the total shown in parentheses.)

Site of infection	No. of patients yielding		
	S equisimilis	*S. zooepidemicus*	*S. milleri*
Conjunctiva	4	—	—
External ear	5	1	—
Intact skin and wounds	65	1	1
Paronychia	5	—	—
Skin ulceration	31	—	—
Upper respiratory tract	115	1	12
Urethral/genital	25	—	1
Urine	4	—	1
Totals	254 (93·4%)	3 (1·1%)	15 (5·5%)

yielded the organism from genital swabs. *S. milleri* was found in 15 patients (5·5%), most commonly in the upper respiratory tract. *S. zooepidemicus* was found in only 3 patients (1·1%): 1 with otitis externa, 1 with infection in a finger after a crush injury to the nail and 1 with pharyngitis and cervical lymphadenopathy.

There appeared to be no predominant API 20 STREP profiles of *S. equisimilis* and *S. milleri* in the isolates from deep as compared with superficial infection. The general catchment of 214 isolates of group C streptococci from the Yorkshire laboratories gave the following species in 15 deep infections: 10 isolates (67%) *S. equisimilis*, 4 (27%) *S. milleri* and 1 (6·7%) *S. zooepidemicus*, and in 199 superficial infections: 187 isolates (94%), 10 (5%) and 2 (1%) respectively.

DISCUSSION

The general collection of 214 group C isolates from the Yorkshire laboratories was thought to be representative of the normal laboratory catchment from the examination of all types of clinical specimens. Identification of the isolates revealed 92·1% *S. equisimilis*, 6·5% *S. milleri*, and 1·4% *S. zooepidemicus*. These findings are similar to those of an earlier study of a year's general catchment of 130 group C streptococcal isolates in the Harrogate and Northallerton laboratories where we found a distribution of the above species of 84·5, 13·9% and 1·5% respectively (Barnham, 1987). The organism called *S. milleri* in this report is likely to be known in future as *S. anginosus* (Ruoff, 1988).

S. equisimilis was the predominant organism collected in England and New Zealand and the only species found in the small collection from Nigeria. *S. milleri* accounted for 7% of the collected streptococci in England and 15% in those from New Zealand. All but one isolate of *S. zooepidemicus* came from England; the organisms was found in the blood culture of a renal transplant recipient with cellulitis in New Zealand, the first recorded case of the infection from that country.

The species composition in collections such as this depends to some extent upon technical and procedural influences. The decision to include only those organisms β-haemolytic on horse blood agar excluded certain α- or non-haemolytic group C streptococci that may cause infection in man. We have encountered group C

antigen-carrying α-haemolytic strains of *S. equisimilis*, *S. salivarius* and *S. mitior* in clinical specimens and they have been reported by others (Plummer, 1941). Beta-haemolytic strains of *S. milleri* may be found more commonly on sheep than on horse blood agar cultures (Bucher & von Gravenitz, 1984) and the agar base employed may promote β-haemolysis in otherwise α-haemolytic organisms (Saunders & Ball, 1980; Spencer & Pease, 1985). Technical decisions as to what colonial appearances merit picking from culture plates and whether to consider the number of colonies present on the plate may have some influence on the isolates selected for a collection. The cross reactions between group C streptococcal latex reagents and *S. pneumoniae* and *Klebsiella pneumoniae* may further lead to misidentification of isolates if they are not tested pure (Lee & Wetherall, 1987; Gordon, Damm & Anderson, 1987). We did not specify the procedures to be used for primary isolation or identification of group C streptococci in the laboratories contributing to this study.

The *S. equisimilis* isolates in the collection gave more than 20 patterns of reactions when tested with the API 20 STREP system but just two common patterns accounted for more than three quarters of the isolates. Certain patterns were seen only in isolates from Nigeria or New Zealand and the African isolates, though few, seemed to show a higher frequency of lactose and raffinose fermentation and aesculin hydrolysis than those from elsewhere. There was no special relationship between API 20 STREP pattern and T antigen type in the 16 strains tested. For identification of *S. equisimilis* the API 20 STREP test needs to be supplemented by an accurate Lancefield grouping test (Tillotson, 1982; Colman & Ball, 1984) and as an epidemiological biotyping tool its use is limited by the occurrence of certain very common reaction patterns. In further studies on *S. zooepidemicus* (Barnham *et al.* 1987) we found that the API 20 STREP test had similar limitations as an epidemiological tool, giving only a few common patterns of reactions.

The three species of group C streptococci we found were easily distinguished by the use of the API 20 STREP test. A simpler and cheaper technique for quickly distinguishing between those organisms in clinical cultures from man would be useful. *S. milleri* may often be recognized by its small colonies and caramel-like smell; measurement of the haemolytic zone on anaerobic incubation, a rapid Voges-Proskauer test and a rapid test to show the absence of Fc(γ) receptors on the cell surface have been suggested for its identification (Bucher & von Graevenitz, 1984; Lebrun *et al.* 1986). Primary cultures of *S. zooepidemicus* on agar usually show mucoid colonies that can easily be distinguished from the relatively dry colonies of *S. equisimilis*; in nutrient broth cultures with 10% horse serum the capsulated *S. zooepidemicus* gives a diffuse cloudy growth compared with the discrete, granular growth of *S. equisimilis*. A small range of further tests may be helpful to distinguish the two provisionally, including fermentation tests for sorbitol and trehalose. Study of a newly-developed enzyme kit gave good and rapid discrimination between the species in Lancefield group C, including a distinction between human and animal strains of *S. equisimilis* (Efstratiou & Colman, 1989).

We found erythromycin-resistance in 1·9% of the English isolates of *S. equisimilis*, a figure similar to the 1·6% previously reported by us in group C

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streptococci isolated from clinical specimens in North Yorkshire (Barnham & Cole, 1986). All isolated in the collection were susceptible to penicillin by disk testing but in a more detailed study of 15 of these isolates we found that *S. zooepidemicus* was killed by penicillin more slowly than *S. equisimilis*, even in the presence of gentamicin (Millar, Langdale & Barnham, 1989).

This study confirms that most isolates of β-haemolytic group C streptococci from infection and carriage in man are either *S. equisimilis* or C antigen-bearing *S. milleri*, while *S. zooepidemicus* is only occasionally found. In the medical literature we are aware of reports of only two human infections with group C streptococci showing the characteristics of *S. equi* (Duma *et al.* 1969; Downing & Spirazza, 1986) and one with those of *S. dysgalactiae* (Quinn *et al.* 1978); moreover, the organisms from these patients were not sent to a national reference laboratory for confirmation of identity.

Group C streptococci have been reported to cause a spectrum of disease in man including upper respiratory tract, skin, wound and puerperal infections, septicaemia, pneumonia, endocarditis, meningitis and septic arthritis (Benjamin & Perriello, 1976; Lancefield & Hare, 1935; Armstrong *et al.* 1970; Mohr *et al.* 1979; Stamm & Cobbs, 1980; Stein & Panwalker, 1985; Stewardson-Krieger & Gotoff, 1977; Kuskie, 1987). Studies of naso-pharyngeal carriage rates of group C streptococci in normal populations have shown rates between 1·9 and 3·6% (Hare, 1940; Ogunbi *et al.* 1978; Barnham, 1983) but there has been little assessment of the carriage rates in other anatomical sites. The species of group C streptococci were often not determined in these studies of infection and carriage.

The proportions of the total group C streptococci isolated from various clinical sites in the study (Table 3) broadly agree with those reported by others (Morris & Berry, 1985). The isolates were from patients with clinical infection but it is likely that, in some cases, they were commensal organisms picked up incidentally from sites of carriage; however, it is reasonable to assume that all the isolates from deep infections (Table 2) contributed to illness in the patients.

S. equisimilis was found most commonly in the upper respiratory tract, ulcerations and other wounds of the skin and in the genital tract. Nineteen of the 23 isolates we examined from Africa, all *S. equisimilis*, were from the upper respiratory tract. Group C streptococci are particularly common amongst throat carriers and patients with tonsillitis in that part of the world (Ogunbi *et al.* 1978). The clinical features of 22 patients yielding *S. equisimilis* from deep sites (Table 2) showed a spectrum of serious disease including epiglottitis, pneumonia, septicaemia, abscess and septic arthritis; this accords with published case reports of group C streptococcal infection in which the organism was determined as *S. equisimilis*. Reports of the typing of strains of *S. equisimilis* from human infection suggest that these are mainly human-derived organisms, distinct from those isolated from animals (Efstratiou, 1983; Colman, Efstratiou & Gaworzewska, 1988); occasional zoonotic infections do probably also occur (Barnham, 1988). Infection with *S. equisimilis* often leads to a rise in anti-streptolysin O antibody in contrast to infection with *S. zooepidemicus*, when this does not occur (Barnham, Cooper & Lange, 1989).

In the general collection of 214 group C streptococcal isolates from the Yorkshire laboratories *S. zooepidemicus* was found in one deep and two superficial infections, i.e. 6·7% of the 15 isolates from deep infection and 1% of the 199

isolates from superficial sites. In the whole study collection six of the nine isolates of *S. zooepidemicus* (67%) came from deep infections. Although the numbers are small and the catchment of isolates from some contributing laboratories highly selected, the data suggest that this organism causes a higher proportion of aggressive group C infections than would be expected from its rare occurrence at superficial sites. In all, the species accounted for 17% of the isolates from deep infection in the study. In an *in vitro* bactericidal test for virulence in man Facklam & Rutledge (1985) found that *S. zooepidemicus* was potentially very virulent, *S. equisimilis* variable and *S. milleri* much less so. *S. zooepidemicus* has a very high capacity to bind to fibronectin, which may enhance its ability to adhere and colonize at superficial anatomical sites and perhaps also in tissue lesions in certain deeper sites (Mamo *et al.* 1987).

Genetic analysis suggests that *S. zooepidemicus* is closely related to *S. equi* and should perhaps be classified as a subspecies of it (Farrow & Collins, 1984). For reasons that remain unclear the organism has a much wider host range among animals than *S. equi* but it seems not to be a regular, natural commensal in man. Human infection with *S. zooepidemicus* appears to be a zoonosis, principally seen in patients consuming unpasteurized milk or dairy products or those in close contact with horses; a variety of local and systemic infections has been described (Barnham, Thornton & Lange, 1983). The range of serious infections seen with the organism in the patients in this study included septicaemia, pneumonia, meningitis, septic arthritis and post-streptococcal glomerulonephritis. The species was also found in a few superficial cultures from patients with mild, uncomplicated infection. In other studies occasional respiratory carriers of *S. zooepidemicus* have been detected amongst healthy farm hands (Barnham, Thornton & Lange, 1933). Further clinical details of many of the patients in this study infected with *S. zooepidemicus* are given and discussed elsewhere (Barnham, Ljunggren & McIntyre, 1987; Barnham & Edwards, 1989); isolates from the study were also included in a collection used to develop epidemiological typing systems for *S. zooepidemicus*, including bacteriocin, bacteriophage and DNA fingerprinting techniques (Barnham *et al.* 1987; Skjold *et al.* 1987). All isolates of the species tested so far have been T antigen-untypable in the scheme described by Efstratiou (1983).

C antigen-bearing β-haemolytic *S. milleri* made up 7% of the total isolates in this study; in the unselected catchment from the Yorkshire laboratories it accounted for 5% of isolates from superficial sites and 27% of the isolates from deep infection. In the patients with deep infection the organism was found in suppurative abdominal conditions and pleural empyema; these are typical of infection with *S. milleri*, as described by Parker (1978). The clinical significance of *S. milleri* in cultures from superficial sites is still uncertain (Ruoff, 1988). In our study *S. milleri* accounted for only 9% of β-haemolytic group C streptococci isolated from the upper respiratory tract, contrasting with the 67% *S. milleri* reported by Bucher & von Graevenitz (1984) in their study of throat cultures on sheep blood agar; this difference may be largely due to the cultural conditions employed, as discussed above. There is no evidence that strains of *S. milleri* carrying the Lancefield group C antigen have different biological behaviour or pathogenicity compared with those that do not.

In conclusion, this study shows that *S. equisimilis* and *S. milleri* are predominant

amongst the β-haemolytic group C streptococci isolated from human infection while the animal-associated *S. zooepidemicus* accounts for only a small percentage of isolates. Each of these three species may, on occasion, cause serious disease in man. We recommend that the species of group C streptococcal isolates should be determined for case reports, carrier studies, in outbreaks, in serious disease or when complications such as nephritis occur, so that the natural history and epidemiology of these different infections can be clearly distinguished.

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