Report of unusual clinical appearance in bacteraemia with non-haemolytic M-type 58 \textit{Streptococcus pyogenes}

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\textbf{Background & objectives:} The occurrence of haemolytic colonies on blood agar often provides the starting point for the laboratory diagnosis of pyogenic streptococci, while non-haemolytic variants could pass unrecognised, leading to a failure of diagnosis. We report the details of two epidemiologically unrelated patients with bacteraemia featuring M-type 58 \textit{Streptococcus pyogenes}, a seemingly rare cause of human infection in the UK, and briefly review previous reports of infection with non-haemolytic strains of this species.

\textbf{Methods:} Case notes of the two patients were reviewed. Isolates obtained from clinical specimens were recovered and identified and cultured on horse blood agar to observe pattern of haemolysis.

\textbf{Results:} In the first case, of a 75 year-old man with leukaemia and a retropharyngeal abscess, the isolate was consistently non-haemolytic, probably due to a failure to produce streptolysin S as has been described before in a small number of reports involving various M-types. In the second case, of an 84 year-old woman with dermatitis and septicaemia, the organism was principally beta-haemolytic but with no haemolysis on aerobic culture where the colonies were well spaced, a phenomenon thought to be associated with abundant production of serum opacity factor (OF).

\textbf{Interpretation & conclusion:} These cases are a reminder that misleading cultural appearances can occur with \textit{S. pyogenes} and that OF positive strains can produce poor haemolysis on aerobic culture, or fail even to do so at all.

\textbf{Key words} M-type 58 - non-haemolytic variants - \textit{Streptococcus pyogenes}

The occurrence of haemolytic colonies in cultures on blood agar of clinical specimens from non-sterile sites provides a common starting point for the laboratory diagnosis of \textit{Streptococcus pyogenes}. The presence of non-haemolytic variants would most likely pass unrecognised in routine laboratory practice, leading to a failure of diagnosis. There are occasional earlier reports\textsuperscript{1-5} of non-haemolytic variants of this species isolated from patients with clinical infection. These have been found where the organism has been sought with particular diligence (as in outbreaks of rheumatic fever\textsuperscript{1} and sepsis on a plastic surgery unit\textsuperscript{1}), where unusual colonial appearances have led to its recognition, or where it has been isolated in pure culture from otherwise sterile sites\textsuperscript{1,2,4-6}. To date there has been little systematic work done to establish how prevalent non-haemolytic strains of \textit{S. pyogenes} might be in their human host. A three-month study involving 361 throat swabs from patients with pharyngitis in the Dunedin district of New Zealand in 1999\textsuperscript{7} yielded 64 isolates of \textit{S. pyogenes} of which two (3\%) were non-haemolytic. Non-haemolytic variants of Lancefield group B, C, G streptococci and \textit{S. milleri} were also sometimes encountered in clinical specimens\textsuperscript{7} and thought to occur more frequently in organisms from
animal sources. We report the details of two patients with bacteraemia featuring M-type 58 \textit{S. pyogenes}, a seemingly rare cause of human infection in the UK.

Material & Methods

Initial isolates from clinical specimens were recovered from commercially available or in-house prepared culture media and diagnosed by standard laboratory techniques, and saved without delay at -80°C in vials with beads (Protect: Technical Service Consultants, Heywood, UK). Identity was confirmed and T/M serotyping performed at the Streptococcus Reference Unit, Laboratory of Respiratory and Systemic Infection, Central Public Health Laboratory, Colindale. The streptococcal isolates were studied fresh from the -80°C saves using cultures on 5 per cent defibrinated horse blood in Columbia Blood Agar base (Biosoconnections, Bardsey, Leeds code BC 2001) in petri dishes incubated aerobically and anaerobically for up to 72 h to observe the patterns of haemolysis around the colonies. A recent isolate of M-type 1 \textit{S. pyogenes}, OF negative from a patient with bacteraemia was grown in parallel for comparison.

Study subjects: Clinical specimens were collected from two patients. The first patient was a 75 yr old man admitted with a 12 h history of sore throat, dysphagia and fever. His medical history included the development of hairy cell leukaemia 10 months before this admission, and his medication included aspirin. On examination he had a fever of 38°C, pharyngeal erythema, swelling on the left side of the neck and enlarged cervical lymph nodes. Flexible laryngoscopy revealed a paralysed left vocal cord. CT scan of the neck showed a thickening of the retropharyngeal tissue over a distance of 6 cm craniocaudally anterior to the 3rd and 4th cervical vertebrae indicating a retropharyngeal abscess. Investigations showed haemoglobin 9.5 g/dl, circulating white cell count 18.7 x 10^9/l (neutrophils 13.6 x 10^9/l, lymphocytes 1.4 x 10^9/l) and creatine phosphokinase 739 IU/l (normal range 24-195 IU/l). Blood culture and a peri-urethral swab yielded \textit{S. pyogenes} which was confirmed by the Streptococcus Reference Unit, Colindale and shown to be of T-type 8/25/Imp 19, M-type 58, OF positive. She was treated initially with flucloxacillin, and intravenous penicillin G was added when the culture results were known. For a brief period she became hypotensive (blood pressure 68/31 mm Hg) but fever resolved, her dermatitis improved and she made a good recovery. The systemic infection was thought to have arisen from infected dermatitis, either from the recent excorations or from the inflamed area around the urinary catheter.

Results

In aerobic culture, colonies from the first patient were umbonate with an entire edge and colony diameters up to 1 mm by 48 h; from the second patient they were at first convex, becoming umbonate and of a similar size; the M-type 1 isolate was mucoid with some collapse to produce a heaped edge and colony size up to 2.8 mm
diameter by 48 h incubation. In the first patient there was a very narrow zone of greening around the colonies but no haemolysis in either aerobic or anaerobic cultures; failure to lyse red blood cells around the colonies in any part of the culture plate was confirmed by low power light microscopy. In the second patient the aerobic cultures showed haemolysis over most of the blood agar plate by 48 h incubation, with zones of haemolysis up to 2.8 mm diameter around the colonies located more closely together. However, zones of haemolysis were smaller where the colonies were more widely separated and, in the most thinly spread parts of the plate, the colonies showed small haloes of greening but no haemolysis (confirmed by microscopy), resembling those of the first patient. Colonial morphology was no different whether the colonies were haemolytic or non-haemolytic. Separate subcultures from haemolytic and from non-haemolytic colonies of the second patient produced the same mixed picture with haemolysis predominant but some isolated non-haemolytic colonies by 48 h incubation. In contrast to the results with the first patient, all colonies from this patient showed haemolysis on anaerobic incubation. The M-type 1 cultures showed clear zones of haemolysis around all colonies in both aerobic and anaerobic conditions, with diameters up to 5.8 mm by 48 h incubation in air.

Discussion

A review of previous studies involving streptococcal typing in these districts showed that there were no patients other than these with detected bacteraemia featuring M-type 58 S. pyogenes during 22 yr of observation. We detected two persons with asymptomatic pharyngeal colonisation with haemolytic M-type 58 S. pyogenes in a screening programme of 1640 young adult applicants for poultry processing work in North Yorkshire during 1980-88 (i.e., two of 20 isolates saved for serotyping from 25 positive applicants). Haemolytic M-type 58 S. pyogenes was not found in a study of 263 typed isolates of this species from the wounds of infected meat handlers in North Yorkshire, 1978-86, nor in a separate collection of 64 typed isolates from patients with sore throat studied here in 1981-82.

Non-haemolytic variants of S. pyogenes are thought to occur rarely but they are hard to recognise in mixed cultures and there have been few studies to determine their prevalence in clinical specimens. Streptolysin S is the product responsible for haemolysis around colonies in aerobic culture and occasional non-producing strains have been reported. To date these have included isolates of S. pyogenes of T-type 1, M-type 4, T-type 11, T-type 12 in a ward-based outbreak involving infants and nurses, the same serotype causing acute post-streptococcal glomerulonephritis (PSGN) in patients in Japan, mucoid M-type 18 in the throats of a series of military personnel in Colorado during an outbreak of rheumatic fever, T-type 25, M-type 49 from skin infection, T-type 12 M-type 66 from an outbreak of sepsis in a plastic surgery unit and mucoid M-type 68 from an infection of the eye. Non-haemolytic isolates such as these are variable in their production of a greening effect (alpha haemolysis) beneath colonies on aerobic incubation. The organism from the first patient in this study was most probably streptolysin S-deficient, although this was not studied at molecular level. Such strains usually produce streptolysin O but, nonetheless, may fail to produce haemolysis on anaerobic incubation. This effect has not been fully explained but might relate to the reported inactivity of streptolysin O in strictly anaerobic conditions. In the Dunedin pharyngitis study the use of a special streptolysin O-enhancing medium revealed group A, C and G streptococci, non-haemolytic on conventional laboratory media, in cultures from 2 per cent of the patients. Mutant strains with an inability to produce streptolysin O are rarely encountered. The first patient in present study showed a high ASO titre in convalescence, suggesting that the infecting organism produced streptolysin O.

The genetics behind the production of streptolysins O and S is under study, and the role of these products as virulence factors in streptococcal infection, as judged by in vitro laboratory and animal model research, appears to be substantial. However, the clinical significance of infection in man with organisms that fail to produce these substances is not known. The limited data published to date suggest that various strains with this failure have been able, nonetheless, to produce local and systemic infection, PSGN and acute rheumatic fever.

The serum opacity factor (OF) of S. pyogenes is closely related to M protein and occurs as a diffusable substance, described in earlier years as having
lipoproteinase activity but now thought to have the ability to produce agglomeration with lipoproteins. Members of some 40 M-types of *S. pyogenes* are producers of OF, including the more frequent M-types 4, 49, 73, 81 and R28 as well as M-type 58 and others; these are described as OF+ strains and many are particularly associated with streptococcal infections of the skin. Pinney and colleagues in 1977 showed that OF+ strains tend to produce narrow, fuzzy zones of haemolysis in comparison with the larger, clear-cut zones of OF negative strains. Under some cultural conditions isolates can produce greening but no haemolysis, as noted by other workers. The effect is thought to be due to disturbance of streptolysin S by OF. Streptolysin S consists of a small, active polypeptide moiety induced and carried by a range of larger molecules, such as RNA, polyribonucleotides, alpha-lipoprotein and albumin.

The isolate from our second patient which was of the same serotype as that in the first patient, is an example of an OF+ streptococcus; in this case the isolate showed non-haemolytic colonies only where they were well-separated on the aerobic culture plate. Colony separation provides less competition for nutrients from the underlying agar and presumably allows the vigorously growing organisms to produce high levels of diffusible OF. In anaerobic culture such strains usually produce good zones of haemolysis.

The index strain of M-type 58 *S. pyogenes* came from Trinidad, an island where streptococcal infection has been intensively studied. It was isolated in 1967 from a patient with pyoderma of the foot and PSGN (A. Efstratiou, personal communication). In subsequent times this serotype has been encountered only infrequently and, in a summary of isolates referred to the Streptococcus Reference Unit, Colindale, UK over 11 yr, it accounted for less than 1 per cent of strains. Of the 139 cultures of this type received, 80 were from the throat, 37 from skin and wounds, 12 from the vagina and only two isolated from the blood; single isolates were from patients with suspected PSGN and rheumatic fever. M-type 58 streptococci have a wide geographical distribution with further reports including Czechoslovakia, India and the USA. Our local isolates were widely separated in time and place. M-type 58 has been generally regarded as a ‘skin-infecting type’ of *S. pyogenes*; bacteraemia with M-type 58 streptococci appears to be rare and it is possible that the NSAIDs taken by our two patients might have predisposed to serious, invasive forms of infection, as has been suggested earlier. Whether M-type 58 streptococci have a special propensity to produce non-haemolytic forms, and how prevalent such strains might be, remain unknown. The detection of non-haemolytic M type 58 streptococci resulted from the serendipity of isolating the organism from blood, when further tests are routinely applied to identify organisms. The recognition of such strains in mixed cultures from body sites with a normal flora would be unlikely, unless special techniques were applied. It is important that microbiologists recognise this shortcoming in their reliance on haemolysis for the detection of *S. pyogenes* in clinical specimens. These cases also act as a reminder that OF+ strains of *S. pyogenes* may produce poor haemolysis on aerobic culture, or fail to do so at all. With the general resurgence of serious forms of streptococcal infection and their sequelae in many parts of the western world in recent years, the possibility of unusual cultural appearances in diagnostic tests, such as we describe here, should be borne in mind.

### References


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