Non-β-haemolytic variants of *Streptococcus pyogenes*: a challenge for the microbiology laboratory

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Streptococcus pyogenes is one of the most important human pathogens isolated in the clinical diagnostic microbiology laboratory. It is carried asymptomatically, usually in the upper respiratory tract, in 5–30% of healthy individuals.[1] It also causes a wide spectrum of disease, ranging from superficial skin infection and pharyngitis to more invasive disease such as bacteraemia, necrotising fasciitis and streptococcal toxic shock syndrome (streptococcal TSS). Death occurs in 10–15% of patients with invasive *S. pyogenes* infection,[2] with mortality from streptococcal TSS reported at up to 44%.[3] *S. pyogenes* infection is also causally linked with delayed non-suppurative post-streptococcal sequelae such as acute rheumatic fever and glomerulonephritis.[4] *S. pyogenes* is classified as group A *Streptococcus* (GAS) by Lancefield grouping.[5]

The incidence of invasive group A *Streptococcus* (iGAS) disease in Ireland has been increasing since April 2012, with a further increase during 2013. From 1 January to 30 June 2013 there were 102 cases of iGAS reported in Ireland (a rate of 2.22 cases per 100,000 population). This is a significant increase from 78 cases for the same time period in 2012 (a rate of 1.70 per 100,000 population) and 48 cases for the same period in 2011 (a rate of 1.05 per 100,000 population), giving an increase of 213% since 2011.[6,7]

Group A streptococcal infections in England have also increased in 2012/2013, with higher numbers of iGAS isolates being referred to the Respiratory and Vaccine Preventable Bacteria Reference Unit between January and March 2013, compared with average referrals over the previous three years. A total of 1038 iGAS cases were referred in 2012/2013, compared to 691 for 2011/2012 and 907 for 2008/2009, the last peak season for iGAS referral.[8] It is important that patients with GAS infections are identified and treated promptly to ensure the best outcome for the patient and for the implementation of infection control precautions to minimise the risk of transmission to other patients and hospital staff.[9]

Routine isolation of GAS from clinical specimens is based on the organism's ability to produce a zone of complete haemolysis on blood agar (β-haemolysis) after incubation under aerobic or anaerobic conditions. Further identification is performed on colonies that produce this zone of haemolysis.

This study reports the identification of two unrelated isolates of *S. pyogenes* at Waterford Regional Hospital in June 2013, which lacked the ability to produce β-haemolysis under various incubation conditions. The first isolate was from a three-year-old male presenting with an abscess on the finger. The second isolate was from an 18-year-old male who also presented with an abscess. The isolates were identified after 24-h incubation at 37°C in CO_2 and 48-h in anaerobic conditions. The isolates were both slightly α-haemolytic on Columbia blood agar. Neither isolate grew on MacConkey agar.

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Both isolates were identified as *S. pyogenes* using the matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) Biotyper 3.0 (Bruker Daltonics, Germany) and Rapid ID 32 Strep system (bioMérieux, Lyon, France). The isolates reacted as Lancefield group A *Streptococcus* by Streptex (Thermo Scientific, Kent, UK). Both isolates were catalase-negative and were pyrrolidonyl arylamidase (PYR)-positive by the Rapid ID 32 Strep system (bioMérieux). Both isolates were sensitive to penicillin, erythromycin, vancomycin and teicoplanin by disk diffusion using CLSI guidelines.[10] Owing to the lack of β-haemolysis, both isolates were sent to the Epidemiology and Molecular Biology Unit, Children's University Hospital, Temple Street, Dublin, for *emm* sequence typing.

The isolation of *S. pyogenes* in the routine clinical microbiology laboratory from normally non-sterile sites (e.g., throat swabs) relies on the production of a zone of β-haemolysis on blood agar. This is used to distinguish pathogenic β-haemolytic streptococci from non-haemolytic bacteria, including non-haemolytic streptococci that are part of the normal flora of the throat. Isolates of *S. pyogenes* that do not produce β-haemolysis are indistinguishable from normal throat flora on blood agar and could therefore be disregarded, leading to the reporting of false-negative results.

The two non-β-haemolytic *S. pyogenes* isolates from the authors' laboratory were identified as they were both isolated from abscess sites. If either of these two isolates had been present in a mixed culture from a non-sterile site swab (e.g., throat swab) they would have been indistinguishable from normal flora on blood agar.

S. pyogenes is a commonly isolated pathogens from throat swabs. In the authors' laboratory, GAS were isolated from 278 out of a total 1661 throat swabs tested between 1 January and 30 June 2013 (16.7%). This compares to 301/1546 (19.5%) for the same six-month period in 2012, and 169/1299 (13%) for the same six months in 2011. These isolates were initially identified based on the presence of β-haemolysis on blood agar, with further identification to designate them as GAS.

Non-β-haemolytic variants of *S. pyogenes* have been described in the literature previously. An epidemic of pharyngitis due to non-haemolytic group A *Streptococcus* was reported in 1971 from an Air Force Base in the USA. In this report, non-haemolytic group A streptococci were found in 118 throat cultures, including six cases of rheumatic fever following the outbreak.[11] This demonstrates that infections caused by non-haemolytic strains of *S. pyogenes* have the potential to produce the same sequelae as haemolytic strains.

The prevalence of non-haemolytic strains of *S. pyogenes* is thought to be low. A three-month study of 361 throat swabs in New Zealand found that 3% of group A streptococcal isolates were non-haemolytic (two out of 64 GAS isolates).[12] In a Japanese study, nine isolates were found to be non-haemolytic among 818 group A streptococcal isolates (1.1%) from a total of 1690 throat or ear swabs tested between November 2006 and March 2009.[13] In a Canadian study of 216 throat swabs, one yielded non-haemolytic *S. pyogenes*.[14]

Non-β-haemolytic *S. pyogenes* has also been reported as a causative agent in iGAS infection. In this case, an initial throat swab was reported as *S. pyogenes* culture-negative.[14] There are also reports of bacteraemia,[15] fatal pneumonia[16] and fatal sepsis[17] due to non-haemolytic *S. pyogenes*. Cases of iGAS have a high fatality rate.[3]

There is some evidence of person-to-person transmission of non-β-haemolytic *S. pyogenes* in the literature. Yoshino *et al*.[13] suggest that *emm* type 12 variants of non-haemolytic *S. pyogenes* spread horizontally. In their study, six out of nine non-haemolytic *emm* type 12 *S. pyogenes* isolates were found between February and November 2008 in the same geographical region.[13] As previously mentioned, James *et al*. reported an epidemic of non-haemolytic GAS pharyngitis at Lowry Air Force Base, which also suggests horizontal transmission.[11]

The isolates from the authors' laboratory were typed as *emm* type 58 and *emm* type 75. Non-haemolytic *S. pyogenes emm* type 58 has previously been reported as a rare cause of bacteremia.[15] Weightman *et al*. reported on two epidemiologically unrelated patients who had septicaemia caused by non-haemolytic strains of *S. pyogenes*, both of which were *emm* type 58.[15]

The genetic mechanism responsible for lack of β-haemolysis in *S. pyogenes* variants has been examined in various studies.[13,18,19] It is likely that mutations in the *sag* operon cause a lack of production of streptolysin S, which is responsible for the haemolytic zones characteristic of *S. pyogenes*.[13] Jantsch *et al*. also looked at the role of the *sag* operon in relation to the absence of β-haemolysis. The focus of their study was an isolate that had caused a severe soft tissue infection. They found that the lack of haemolysis in this isolate was due to a premature stop mutation in the *sagC* gene. Reintroduction of the full-length version of the mutated gene using a plasmid vector restored the β-haemolytic phenotype in this isolate.[18]

Datta *et al*. have previously shown that *sagA*, *sagB*, *sagC*, *sagD*, *sagE*, *sagF* and *sagG* are each individually required for streptolysin S production.[19] Detection of non-haemolytic variants was shown to be enhanced using a selective and differential agar in a New Zealand study. CNA-P agar contains colisitin, nalidixic acid and pH 7.5-adjusted PIPES buffer. This agar enhances streptolysin activity among isolates that had been non-β-haemolytic on conventional blood agar.[12,20]

In conclusion, although the incidence of non-haemolytic variants of *S. pyogenes* is thought to be rare, clinical microbiologists should be aware that it is a possibility. The prevalence of non-β-haemolytic variants of *S. pyogenes* remains unclear. There is evidence in the literature to demonstrate that non-haemolytic variants of *S. pyogenes* can cause the same spectrum of infections clinically as typical β-haemolytic strains of *S. pyogenes*. It is likely that a low percentage (1–3%)[12,13] of *S. pyogenes* isolates are non-β-haemolytic and therefore a low percentage of GAS throat infections may be undiagnosed due to the absence of β-haemolysis on blood agar. Given the recent increase both in invasive and non–invasive GAS infections observed in Ireland, England and parts of northern Europe,[3,6–8,21] awareness of the existence of non-β-haemolytic variants is of increased importance to ensure prompt and accurate laboratory diagnosis.

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References

1 Neeman R, Keller N, Barzilai A, Korenman Z, Sela S. Prevalence of internalisation-associated gene, *prtF1*, among persisting group-A *Streptococcus* strains isolated from asymptomatic carriers. *Lancet* 1998; **352** (9145): 1974–7.

2 O'Loughlin RE, Roberson A, Cieslak PR *et al*. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. *Clin Infect Dis* 2007; **45** (7): 853–62.

3 Lamagni TL, Darenberg J, Luca-Harari B *et al*. Epidemiology of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2008 July; **46** (7): 2359–67.

4 Bisno AL. Nonsuppurative poststreptococcal sequelae: rheumatic fever and glomerulonephritis. In: Mandell GL, Bennett JE, Dolin R eds. *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases* 7th edn. Philadelphia: Churchill Livingstone, 2010: 2611–22.

5 Ruoff KL, Whiley RA, Beighton D. *Streptococcus*. In: Murray PR, Baron JB, Jorgensen JH, Pfaller MA, Yolken RH eds. *Manual of Clinical Microbiology* 8th edn. Washington: ASM Press, 2003.

6 Health Protection Surveillance Centre. Report on invasive group A streptococcal infections in Ireland, Quarters 1–2. Dublin: HPSC, 2012 (www.hpsc.ie/hpsc/A-Z/Other/GroupAStreptococcalDiseaseGAS/Publications/QuarterlyReports/File,14215,en.pdf).

7 Meehan M, Murchan S, Bergin S, O'Flanagan D, Cunney R. Increased incidence of invasive group A streptococcal disease in Ireland, 2012 to 2013. *Euro Surveill* 2013; **18** (33): 20556.

8 Public Health England. Group A streptococcal infections: seasonal activity 2012/13 (www.hpa.org.uk/hpr/archives/2013/news1713.htm#igas).

9 Steer JA, Lamagni T, Healy B *et al*. Guidelines for prevention and control of group A streptococcal infection in acute healthcare and maternity settings in the UK. *J Infect* 2012; **64** (1): 1–18.

10 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement (M100-S23). Wayne, PA: CLSI, 2013.

11 James L, McFarland RB. An epidemic of pharyngitis due to a nonhemolytic group A streptococcus at Lowry Air Force Base. *N Engl J Med* 1971; **284** (14): 750–2.

12 Dierksen KP, Tagg JR. Haemolysin-deficient variants of *Streptococcus pyogenes* and *S. dysgalactiae* subsp. *equisimilis* may be overlooked as aetiological agents of pharyngitis. *J Med Microbiol* 2000; **49** (9): 811–6.

13 Yoshino M, Murayama SY, Sunaoshi K *et al*. Nonhemolytic *Streptococcus pyogenes* isolates that lack large regions of the *sag* operon mediating streptolysin S production. *J Clin Microbiol* 2010; **48** (2): 635–8.

14 Cimolai N, Trombley C, Bhanju NM. Nonhemolytic *Streptococcus pyogenes* causing invasive infection. *Clin Pediatr (Phila)* 2002; **41** (6): 453.

15 Weightman NC, Barnham MR. Report of unusual clinical appearance in bacteraemia with nonhaemolytic M-type 58 *Streptococcus pyogenes*. *Indian J Med Res* 2004; **119** (Suppl): 17–21.

16 Taylor MB, Barkham T. Fatal case of pneumonia caused by a nonhemolytic strain of *Streptococcus pyogenes. J Clin Microbiol* 2002; **40** (6): 2311–2.
17 Turner DP, Gunn SL. Fatal case of sepsis caused by a non-haemolytic strain of *Streptococcus pyogenes. J Clin Pathol* 2007; **60** (9): 1057.
18 Jantsch J, Gerlach RG, Ensser A *et al*. Severe soft tissue infection caused by a non-beta-hemolytic *Streptococcus pyogenes* strain harboring a premature stop mutation in the *sagC* gene. *J Clin Microbiol* 2013; **51** (6): 1962–5.
19 Datta V, Myskowski SM, Kwinn LA *et al*. Mutational analysis of the group A streptococcal operon encoding streptolysin S and its virulence role in invasive infection. *Mol Microbiol* 2005; **56** (3): 681–95.
20 Dierksen KP, Ragland NL, Tagg JR. A new alkaline pH-adjusted medium enhances detection of beta hemolytic streptococci by minimizing bacterial interference due to *Streptococcus salivarius. J Clin Microbiol* 2000; **38** (2): 643–50.
21 Darenberg J, Henriques-Normark B, Lepp T, Tegmark-Wisell K, Tegnell A, Widgren K. Increased incidence of invasive group A streptococcal infections in Sweden, January 2012–February 2013. *Euro Surveill* 2013; **18** (14): 20443.