Correspondence article

Circulating *emm* types of *Streptococcus pyogenes* in Scotland: 2011-2015

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Streptococcus pyogenes (GAS) can colonise skin and mucosal membranes giving rise to carriage, localised and/or systemic infection that can range in severity (Luca-Harari *et al*., 2009, Fiedler *et al*., 2015). Traditionally *S. pyogenes* isolates were serotyped using M and T factor specific anti-sera that have been replaced by M genotyping (*emm* typing) (Johnson *et al*., 2006). The M protein is a major virulence factor of GAS and is a hair-like projection from the bacterial cell surface where it facilitates resistance to complement-mediated killing and aids in bacterial evasion of phagocytosis (Bessen *et al*., 2008). The M protein is also essential for the bacterial attachment to keratinocytes that play a significant role in infections originating at the skin surface (Biswas *et al*., 2001). M genotyping has made international comparisons possible and identified a world-wide perspective of strain variation. There are over 200 recorded *emm* types and subtypes that help in the identification of outbreaks and cluster management (CDC, 2008). In Scotland, between 1999 and 2005, there was a rise in invasive GAS infections from 50 to 200 per year (HPS weekly report, 2006), a finding consistent with observations from other European countries (Luca-Harari *et al*., 2009, Koutouzi *et al*., 2015). This study reports on the distribution of GAS *emm* types and subtypes from invasive and non-invasive sites over a 4 year period and further identifies the most susceptible age/sex range for invasive disease.

GAS isolates were referred to the reference laboratory from routine Scottish microbiology laboratories between June 2011 and April 2015. All isolates were sub-cultured onto Columbia horse blood agar plates (Oxoid™) and incubated at 37˚C for 24 hours in an atmosphere of 5% CO_2. The isolates were phenotypically identified by colony morphology, beta haemolysis and Lancefield group identification using a Prolex™ Strep Grouping Latex Test Kit. DNA was prepared using an achromopeptidase (ACP) extraction method (Boujaafar *et al*., 1988). Sequencing was

performed as previously described (CDC, 2008) and sequenced on an AB 3500 xL sequencer (Molling *et al*., 2000).

The sequences were manually edited using CLC Main Workbench software and the FASTA files were uploaded to the Blast 2.0 Server on the CDC website (CDC, 2008). The assignation of type relies upon the 90 bases encoding the N terminal 30 residues but subtypes are assigned according to the exact 180 base sequences encompassing the signal peptide (10 residues) and the 50 residues of the mature M protein.

The z -test was used for statistical analysis of the data at $P < 0.01$.

In total, eight hundred and ninety two GAS isolates were typed by *emm* sequencing. Table 1 shows the distribution of the major *emm* types from all sites but throughout the study period a total of 40 *emm* types were identified. The most common *emm* types from sterile and cutaneous sites were 1, 76 and 89 and from mucosal sites were 1, 89 and 12. Over the study period, *emm* type 1 had the strongest association with invasive disease from sterile sites (n= 215, $P < 0.001$) when compared to both cutaneous and mucosal sites. The *emm* types 76 and 89 were most commonly associated with cutaneous sites ($p<0.001$) with *emm* type 89 also statistically significantly linked to mucosal sites ($p=0.007$). The *emm* types 12, 75, 28 and 6 were more commonly linked to mucosal sites ($p<0.001$, $p= 0.007$, $p<0.001$, $p=0.001$). In Table 1 the *emm* subtype 1.52 is included to show the statistical differences within an *emm* type as this subtype of 1 was more commonly associated with mucosal sites ($p<0.001$) whereas *emm* type 1 was more commonly associated with sterile sites ($p<0.001$). Four *emm* types (3, 4, 5 and 22) showed no statistical preference between acquisition sites. When the non-invasive sites alone were compared, *emm* type 76 was statistically linked to cutaneous sites ($p<0.001$). In mucosal sites, the only *emm* type that was statistically more prevalent was 12 ($p=0.007$). When age and sex distribution

were analysed (Table 2), there were statistically significant differences in sex and age related disease site acquisition. The major difference between the sexes related to the age when invasive disease became most prevalent. In the male population age groups (51-64 years and >65 years) there was a statistically significant increase in cases associated with invasive disease ($p<0.001$) compared to females when invasive disease was statistically ($p<0.001$) most prevalent in >65 year age range. Other interesting observations include the prevalence of cutaneous isolates in the female 0-4 and 5-19 year age range changing to mucosal site acquisition in the 5-19 and 20-34 year age range. In the male population, mucosal acquisition was most prevalent in the 0-4 and 5-19 year age range with cutaneous acquisition more common in the 20-34 age range.

In conclusion the most prevalent *emm* types identified were 1, 76 and 89. This is partly mirrored in other European countries, apart from *emm* type 76 that was linked to a single outbreak involving PWID. In 2008, there was a Europe wide publication on *emm* type distribution in invasive disease covering the years 2003-4 and a wide diversity of *emm* types (n = 104) were found among clinical isolates of *Streptococcus pyogenes* from 11 European countries (Lamagni *et al*., 2008). The 10 most predominant *emm* types were *emm* type 1, 28, 3, 89, 87, 12, 4, 83, 81 and 5 in descending order. At the national reference laboratory (England and Wales) *emm* strain typing on 1,271 invasive GAS isolates was undertaken from October to June 2015 (PHE, 2015). The results indicate that *emm* type 1 was the most common followed by 3, 12 and 89. The isolates identified in this Scottish cohort included both invasive and non invasive strains were in descending order *emm* type 1, 76, 89, 12, 75, 28, 4 and 3. Euro-prevalent *emm* type 83 was identified only once in Scottish isolates. We report that *emm* type 1 was most significantly associated with invasive

disease. A paediatric study (d'Humieres *et al*., 2015) showed that *emm* type 1 is also associated with the most life-threatening clinical disease manifestations. In the adult population of Europe and the USA, *emm* type 1 and 3 are strongly associated with invasive infections. This contrasts to Africa and Asia where *emm* types differ quite significantly (Steer *et al*., 2009, Efstratiou and Lamagni, 2016). The reasons for the different molecular epidemiology in these regions is not clear however it may relate to the high numbers of impetigo cases identified in Africa and Asia that are not mirrored in Europe and North America. Therefore it is important to be aware of increases in circulating *emm* types in the GAS population as between 1999 and 2005 there was a rise in iGAS infections in Scotland (HPS Weekly report, 2006) a finding consistent with observations from other European countries (Zakikhany *et al*., 2011, Koutozi *et al*., 2015, Efstratiou and Lamagni, 2016). In 2014, Scotland saw higher than expected levels of GAS and iGAS (HPS weekly report, 2015) that occurred against a backdrop of increased scarlet fever notifications in the rest of the UK. The increased iGAS cases in Scotland may be attributable to a natural cycle in disease incidence although the potential for changes in the virulence of circulating strains or increased incidence in a particular risk and age group can not be excluded and continued vigilance remains essential to spot changing disease patterns.

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Transparency Declaration

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195 **Table 1.** Prevalence of the top eleven *Streptococcus pyogenes emm* types from mucosal and cutaneous non invasive disease sites compared to
196 invasive sterile sites and between non-invasive mucosal and cutaneous sites

emm	**Cutaneous**		**Sterile site**		**Mucosal**	**Cutaneous**		**Mucosal**
type/subtype*	**N (%)**	**P**	**N (%)**	**P**	**N (%)**	**N**	**P**	**N**
1	81 (32)	**<0.001**	215 (66)	**<0.001**	47 (25.2)	81	0.184	47
76	56 (22)	**<0.001**	24 (7.4)	0.263	9 (4.8)	56	**<0.001**	9
89	41 (16)	**<0.001**	22 (6.7)	**0.007**	26 (13.9)	41	0.610	26
12	12 (4.7)	0.095	7 (2)	**<0.001**	21 (11.3)	12	**0.007**	21
75	12 (4.7)	0.095	7 (2)	**0.007**	13 (7)	12	0.280	13
28	13 (5.1)	0.032	6 (1.8)	**<0.001**	15 (8)	13	0.184	15
4	5 (2)	0.944	6 (1.8)	0.101	8 (4.3)	5	0.138	8
3	12 (4.7)	0.509	19 (5.8)	0.460	8 (4.3)	12	0.881	8
5	7 (2.8)	0.631	11 (3.4)	0.818	7 (3.7)	7	0.518	7
22	7 (2.8)	0.101	3 (0.9)	0.011	8 (4.3)	7	0.347	8
6	7 (2.8)	0.042	2 (0.6)	**0.001**	9 (4.8)	7	0.226	9
1.52*	8 (3.2)	0.119	4 (1.2)	**<0.001**	15 (8)	8	0.018	15

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199 Eighteen *emm* types had insufficient isolates (<10) for statistical calculation. These included *emm* types 2, 9, 11, 18, 44, 58, 73, 77, 78, 81, 82,
200 87, 90, 102, 103, 108, 124 and 182 that are not reported in the table. The remaining *emm* types were identified only once during the study time
201 frame and were classed as rare *emm* types. These include 8, 29, 33, 63, 68, 80, 83, 93, 94, 101 and 179.

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Table 2. The Distribution of GAS from cutaneous, mucosal and sterile site dependent on age and sex

	Female					Male				
Age group	Cutaneous (%)	P	Sterile (%)	P	Mucosal(%)	Cutaneous (%)	P	Sterile (%)	P	Mucosal (%)
0-4	27 (19)	**0.004**	11 (7)	0.327	14 (11)	26 (15)	0.017	15 (7)	**<0.001**	24 (29)
5-19	22 (15)	**0.005**	8 (5)	**<0.001**	46 (35)	21 (12)	0.019	11 (5)	**<0.001**	22 (27)
20-34	22 (15)	0.174	15 (10)	**0.005**	29 (22)	49 (28)	**<0.001**	27 (13)	0.829	10 (12)
35-50	34 (23)	0.373	29 (19)	0.920	26 (20)	48 (28)	0.036	38 (19)	0.772	14 (17)
51-64	16 (11)	0.453	21 (14)	0.09	10 (8)	18 (10)	0.046	36 (18)	**<0.001**	1 (1)
>65	25 (17)	**<0.001**	68 (45)	**<0.001**	8 (6)	12 (7)	**<0.001**	78 (38)	**<0.001**	11 (13)
Total	146		152		133	174		205		82

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